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Briefing Book

for the

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Blood Products Advisory Committee (BPAC)

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TABLE OF CONTENTS

ABBREVIATIONS.....3
1.0 PROPOSED INDICATION 8
2.0 EXECUTIVE SUMMARY 9
3.0 INTRODUCTION 22
4.0 BACKGROUND 42
5.0 SYNOPSIS OF THE HBOC-201 IND 45
5.a Biopure Corporation's Regulatory History **45**
5.b Chemistry Manufacturing and Control (CMC) **46**
5.c *RESUS* IND Preclinical Studies **52**
5.d Prior Human Exposure **84**
Pharmacokinetic and Pharmacological Profile 84
Study Designs of Completed Studies 85
Matching Subgroup Analysis 105
6.0 THE RESUS IND 130
6.a *RESUS* Trial Abstract **130**
6.b Summary of *RESUS* Study Design **133**
7.0 RESUS IND CLINICAL HOLD ISSUES 136
7.a Vasoactivity **136**
7.b *RESUS Dosing Guidelines* Rationale **180**
7.c Benefit:Risk And Risk Mitigation **189**
Semi-Quantitative Analysis Of Benefit:Risk In RESUS Using SOC Mortality Equivalents (Analysis #3) 222
7.d *Exception from Informed Consent (EIC)* **235**
8.0 CONCLUSION 241
The Way Forward **242**
9.0 REFERENCES 243
10.0 APPENDICES 251
Appendix A: National Trauma Data Bank and UAB and UMD trauma registry mortality data .. **252**
Appendix B: Key Adverse Safety Data (HEM-0115, ISS) **252**
Appendix C: *RESUS* protocol details **252**
Appendix D: *RESUS* in-hospital trauma care guidelines **252**
Appendix E: HEM-0125 S. Africa ER Trauma Trial - Biopure Provided Documents **252**
Appendix F: Document prepared by Fred Apple, PhD re: codification of cardiac biomarker (troponin) data in diagnosis of MI **252**

Tables

Table 1: Semi-quantitative benefit:risk prediction for *RESUS* trial (Analyses #1 and #2) 19
Table 2: Summary of semi-quantitative benefit:risk analysis #3..... 20
Table 3: Robustness of *favorable* benefit:risk assessment in analysis #3 20
Table 4: HBOC-201 mortality reduction effect size in preclinical HS studies..... 24
Table 5: HBOC-201 Specifications 49
Table 6: TSE Clearance Factors 51
Table 7: Viral Clearance Factors 52
Table 8: Mortality in preclinical HS studies with HBOC-201 77
Table 9: NMRC Preclinical survival statistics..... 78
Table 10: Completed Clinical Trials Performed with HBOC-201 87
Table 11: Proportion of HBOC-201-Treated Subjects Receiving No RBC Transfusion - N (%)..... 89
Table 12: Summary of all (overall) AEs..... 90
Table 13:AEs with Statistically Significant Group Differences ($p \leq 0.1$) (HBOC-201 > Controls) in all Studies (ISS) 90
Table 14: SAE Incidence by SOC in All Studies (ISS)..... 92
Table 15: Overall SAE incidence stratified by age (ISS) 93
Table 16: Baseline Subject Characteristics in HEM-0115 94
Table 17: Blood Transfusion Avoidance and Reduction (Efficacy) in HEM-0115 94
Table 18: AEs by SOC Incidence Having a > 10% Absolute Difference Between Treatment Groups in HEM-0115 96
Table 19: SAEs by SOC and Preferred Term with Incidence ≥ 1% in Either Treatment Group in HEM-0115 96
Table 20: A-D Pivotal Phase 3 Orthopedic Trial – Stable Trauma Sub-population Data Summary..... 100
Table 21: Bias from asymmetric study design in HEM-0115 108
Table 22: Identification of matching subgroups in HEM-0115..... 109
Table 23: Medical History in the *HH* and *R*- subgroups in HEM-0115 111
Table 24: Estimated Blood Loss the Fluid Volumes Administered in *HH* and *R*- subgroups in HEM-0115 112
Table 25: Exposure to CTM in the *HH* and *R*- subgroups in HEM-0115 112
Table 26: Overall Medical Risk Assessment in the *HH* and *R*- subgroups in HEM-0115..... 113
Table 27: AEs in the *HH* and *R*- subgroups in HEM-0115 114
Table 28: SAEs in the *HH* and *R*- subgroups in HEM-0115 115

Table 29: Medical History In The HR Vs. R+ Subgroups In HEM-0115 116
Table 30: Exposure to CTM in the *HR* and *R+* subgroups in HEM-0115 117
Table 31: EBL the fluid volumes in the HR and R+ subgroups in HEM-0115 119
Table 32:Overall Medical Risk Assessment for the *HR* and *R+* subgroups in HEM-0115 119
Table 33: AEs in the *HR* and *R+* subgroups in HEM-0115 120
Table 34: SAEs in the *HR* and *R+* subgroups in HEM-0115 121
Table 35: Summary of *Matching Subgroups* Analysis of AEs in HEM-0115 122
Table 36: MAP Δ in NMRC swine HS studies 142
Table 37: Clinical parameters in NMRC swine studies - group means vs. outliers 146
Table 38*: Peak SBP responses* in overall population in HEM-0115 - first infusion 155
Table 39*: SBP Δ responses* in overall population in HEM-0115 - first infusion 155
Table 40*: Peak SBP responses* in stable trauma sub-population in HEM-0115 - first infusion 156
Table 41*: SBP Δ responses* in stable trauma sub-population in HEM-0115 - first infusion 156
Table 42: AEs and SAEs in potentially "vasoactivity-related" systems (SOC) in HEM-0115 157
Table 43: Incidence of animals reaching the *RESUS* HBOC-201 stopping criterion of 120 mm Hg in preclinical NMRC HS studies 164
Table 44: Elevations of troponin (> ROC) in HBOC-201 subjects in HEM-0115 174
Table 45: Classification of troponin elevations in HBOC-201 subjects in HEM-0115 (derived from modified *Table 1* in [111]) 175
Table 46: Patients with Troponin Elevations >ROC in Study HEM-0115 176
Table 47: Cardiac biomarkers in HEM-0115*# 177
Table 48: HEM-0115 Troponin Subgroups (troponin elevation defined as > ROC) 179
Table 49: SAEs and mortality in HEM-0115, stratified by age and dose 185
Table 50*: Peak SBP responses* in HEM-0115 subjects with maximum infusion rates > 25 ml/min 186
Table 51: AE and SAE rates in HEM-0115 subjects with maximum infusion rates > 25 ml/min 186
Table 52: Comparison Of Interim Dosing Data In HEM-0125 with RESUS Dosing Guidelines 188
Table 53: Blood transfusion avoidance in NMRC preclinical HS studies 211
Table 54: Mortality in prior HBOC-201 surgery/orthopedics trials 222
Table 55: Scoring of SOC SAEs relative to mortality 225
Table 56: Risk Scores (mortality equivalents) in HEM-0115 matching groups 227
Table 57: Risk Scores (mortality equivalents) in HEM-0115 overall population and < 70 year old sub-population 228
Table 58: Risk Scores (mortality equivalents) in HEM-0115 in HR and R+ Subgroups 229

Table 59: Effect of Error in Estimate of Mortality or Treatment Effect Size 232
Table 60: Risk Scores (mortality equivalents) in HEM-0125 233

Figures

Figure 1: Incidence of SAEs (final, interim and predicted group differences) 40
Figure 2: Hb Values in Stable Trauma Subjects in HEM-0115 103
Figure 3: Aes/Subject As A Function Of The Dose Of RBC In HEM-0115 108
Figure 4: Panel A and B - Total Hemoglobin (THb) Concentrations in HBOC-201- vs. RBC-treated subjects in HEM-0115. 126
Figure 5: MAP responses in NMRC swine HS studies 138
Figure 6: SBP in the DCLHb HOST trial 189
Figure 7: Differences in incidence of key safety signals in > 70 vs. < 70 years old subjects treated with HBOC-201 vs. RBC in HEM-0115 206
Figure 8: Dependence of BRR on Treatment Effect Size (based on HEM-0115 data) 230
Figure 9: Equipoise Lines as a Function of Risk in Terms of Control Population Mortality and Effect Size (based on HEM-0115 data) 232

Abbreviations

ACLS	Advanced Cardiac Life Support
ABCDEs	Patient assessment tool used inprehospital transport
ABG	Arterial blood gas
ADR	Adverse Drug Reaction
AE	Adverse Events
ALT	Alanine transferase
ANOVA	Analysis of variance
ARDS	Acute Respiratory Distress Syndrome
ARF	Acute renal failure
AST	Aspartate transferase
ATLS	Advanced Trauma Life Support
BD	Base deficit
BLA	Biological License Application
BP	Blood pressure
BPAC	Blood Products Advisory Committee
$brPO_2$	Brain tissue oxygenation
BRR	*Benefit:risk ratio*
BSA	Body surface area
BSE	Bovine spongiform encephalitis
BT	Bleeding Time
BUN	Blood urea nitrogen
CBF	Cerebral blood flow
CCD	*Community consultation and disclosure*
CDC	Center for Disease Control
CDP	Clinical Development Plan
CHF	Congestive Heart Failure
CI	Cardiac index
CK/CK-MB	Creatinine kinase/Creatinine kinase-MB
CL	Clearance
CMC	Chemistry Manufacturing and Controls
CO	Cardiac output
CPP	Cerebral perfusion pressure
CRF	Case Report Form
CRP	C Reactive Protein
CT	Closure time
CTM	Clinical test material
CVA	Cerebral vascular accident
CVP	Cerebral vascular pressure
DCLHb	Diaspirin Cross-linked Hemoglobin
DMC	Data monitoring committee
DO_2	Oxygen delivery
DSMB	Data Safety Monitoring Board
EBV	Estimated blood volume
ECG	Electrocardiogram
EMD	Electromechanical dissociation
ESR	*Efficacy and Safety Review*
EIC	*Exception from informed consent*
EMS	Emergency Medical Services
EMT	Emergency medical technician
EMT-I	Emergency medical technician-Intermediate
EMT-P	Emergency medical technician-Paramedic
ER	Emergency room
ESS	*Excess SAE Score*
FACS	Fluorescence activated cell sorter
GFAP	Glial fibrillary acidic protein
GCS	Glascow Coma Scale
H&E	Hemotoxylin and Eosine Staining
Hb	Hemoglobin
HBOC	Hemoglobin-Based Oxygen Carrier
HBOC-201	Hemopure® (bovine polymerized hemoglobin, Biopure)
HBOC-301	Oxyglobin® (bovine polymerized hemoglobin, Biopure)
HPH	Human Polymerized Hemoglobin (PolyHeme®)
HR	Heart rate or hazard ratio
HS	Hemorrhagic shock
HAS	Human serum albumin
IB	Investigators Brochure
IC	*Informed consent*
ICP	Intracranial Pressure
ICU	Intensive care unit
IDE	Investigational device exemption
IND	Investigational New Drug
IRB	Institutional Review Board
ITT	Intent to treat (analysis)
IV	Intravenous
LA	Lactic Acid
LD	Long delay
LDH	Lactate dehydrogenase
LFB	LFB
LFTs	Liver function tests
LR	Lactated Ringer's solution
MAP	Mean arterial pressure
metHb	Methemoglobin
MI	Myocardial infarction
MODS	Multiple organ dysfunction score
MOF	Multiple Organ Failure
MPAP	Mean pulmonary artery pressure
MVC	Motor vehicle collision
NTDB	National Trauma Data Bank
NMRC	Naval Medical Research Center (U.S. Navy)
NNH	Needed to harm
NNT	Number needed to treat
NO	Nitric oxide
NSAID	Non-steroidal anti-inflammatory drug
NTDB	National Trauma Data Bank
NS	Normal saline
OBRR	Office of Blood Research and Review
OIF	*Operation Iraqi Freedom*
O_2	Oxygen
PCPS	Pittsburg cerebral perfusion score
PCWP	Pulmonary capillary wedge pressure
PI	Principal Investigator (trauma center)
PIN	Personal Identification number
PMN	Polymorphonuclear neutrophil
PO_2	Cerebral oxygenation
Pre-ED	*Pre-enrollment disclosure*
Post-ED	*Post-enrollment disclosure/option to withdraw*
PTCI	Post-traumatic cerebral infarction
PVRI	Pulmonary vascular resistance index
QA	Quality assurance
RBC	Red blood cells
RES	Reticuloendothelial system
RESUS	*Restore Effective SUrvival in Shock* (Clinical trial)
RIND	Reversible Ischemic Neurologic Deficit

RR	Relative rate
RTS	Revised Trauma Score
SAE	Serious Adverse Event
S. Africa	South Africa
SBP	Systolic blood pressure
SD	Short delay
SEEC	Safety endpoint evaluation committee
SSO_2BD	Sagittal sinus base deficit
SSO_2LA	Sagittal sinus lactic acid
SSO_2sat	sagittal sinus oxygen saturation
STORMACT	*Strategies TO Reduce Military And Civilian Transfusions*
SVO_2	Mixed venous O_2 saturation
SVR	Systemic vascular resistance
SVRI	Systemic vascular resistance index
TBI	Traumatic brain injury
$tcPO_2$	transcutaneous tissue oxygenation
TIA	Transient ischemic attack
TSE	Transmissible Spongiform Encephalitis
UI	Unmeasured ions
USUHS	Uniformed Services University Health Services
VO_2	Oxygen consumption
VS	Vital signs
WHO	World Health Organization

1.0 PROPOSED INDICATION

HBOC-201 is indicated for the treatment of traumatic hemorrhagic shock (HS) where safe blood transfusions are unavailable.

2.0 EXECUTIVE SUMMARY

Background

Trauma is the leading cause of death and disability among young adults and military personnel, and hemorrhagic shock (HS) accounts for most of the potentially preventable deaths. Standard prehospital treatment includes basic life support, hemostasis efforts, and intravenous resuscitation with non-oxygen carrying resuscitation fluids. These fluids restore intravascular volume but dilute blood oxygen content, further contributing to tissue hypoxia and anaerobic metabolism. Blood transfusions can be life-saving but are rarely available prior to hospital arrival. Consequently, mortality remains high for patients with severe HS (~ 1 in 2) with current standard care. As 50-90% of traumatic deaths occur prior to hospital arrive, optimization of prehospital resuscitation must be paramount in public health strategies to improve the clinical outcome of patients in trauma.

HBOC-201 And *RESUS* Inception

The oxygen-carrying resuscitation fluid, HBOC-201 (Hemopure®, Biopure Corp., Cambridge, MA), is universally compatible, stored without refrigeration, and is easily administered. Thus, HBOC-201 has intuitive potential for a transformational improvement in prehospital resuscitation for traumatic HS. Supported by a large preclinical database showing improved outcome in HS, and a large clinical database in non-trauma subjects showing transfusion avoidance (efficacy) and reasonable safety, the U.S. Navy (Naval Medical Research Center [NMRC], Silver Spring, MD) submitted an Investigational New Drug (IND) application to the FDA including a protocol to compare HBOC-201 and the standard fluid, lactated Ringer's (LR) solution, for the prehospital resuscitation of patients with traumatic HS, in a randomized, controlled, single-blinded, and multicenter Phase 3 clinical trial called RESUS (Restore Effective SUrvival in Shock). The RESUS project emanated from objective and non-company affiliated consensus recommendations of a panel of civilian and military academicians meeting in symposiums with the aim of improving transfusion medicine in the aftermath of 9/11 (STORMACT I-IV [Strategies TO Reduce Military And Civilian Transfusions]). RESUS is entirely funded by the U.S. government. In concert with its steering committee of nationally-renown subject matter experts (RESUS Advisory Board), as regulatory sponsor, NMRC is responsible for all aspect of the trial, including study design, execution, adverse event reporting, and data analysis and reporting.

Trauma Trial Heterogeneity And Bimodal Mortality Distribution

Patient heterogeneity and bimodal mortality distribution have historically confounded and impeded success in trauma clinical trials. A common strategy has included targeting the general hypotensive HS population, usually defined as systolic blood pressure (SBP) < 90 mm Hg; however, reliance on hypotension alone results in a population with mortality of only ~ 20% and a bimodal (U-shaped) mortality distribution. The overwhelming majority of such subjects would have either non-salvageable injuries (will die irrespective of the novel treatment) or mild injuries (will survive irrespective of the novel treatment). In order to obtain meaningful results overcoming the low mean mortality and non-normalized distribution would require a large study with enrollment of thousands of subjects.

***RESUS* target population**

Thus with FDA's advice, NMRC's strategy has been to target a more homogeneous population with severe HS but potentially salvageable injuries. *RESUS* targets enrollment of patients with severe HS with or without blunt traumatic brain injury (TBI). Specifically, NMRC has incorporated a narrow intermediate range of the standard Revised Trauma Score (RTS) into *RESUS* inclusion criteria. Key *RESUS* inclusion/exclusion criteria include age 18 to < 70 years old; traumatic injury with known/suspected hemorrhage; SBP < 90 mm Hg; RTS 1 to < 5; absence of cardiac arrest or penetrating TBI; and unavailability of blood transfusions. As detailed below, these *RESUS* inclusion/exclusion criteria target a population with high mortality (58.1%) and a relatively homogeneous normalized (bell-shaped) mortality distribution.

***RESUS* IND Clinical Hold**

In accordance with 21 CFR 50.24, *RESUS* is an emergency medicine trial requiring an allowance for *exception from informed consent* (*EIC*). The *RESUS* IND has been placed on Clinical Hold by the FDA, mainly citing safety concerns, believing that subjects enrolled in *RESUS* would have unreasonable risk. FDA has questioned the accuracy of NMRC's predicted mortality in control subjects and predicted mortality reduction effect size; adequacy of protocol risk mitigation strategies; and whether EMS (Emergency Medical Services) personnel will be capable of administering HBOC-201 safely. NMRC believes that HBOC-201 has *prospect for direct benefit to the subjects enrolled* in *RESUS* with *reasonable* risk (21 CFR 50.24), applicable statutory requirements have been met and often surpassed, and the Clinical Hold should be lifted, allowing this vital clinical research to proceed.

***RESUS* Mortality Prediction With Standard Care**

In order to provide an evidence basis for predicted mortality in the proposed *RESUS* trial the in-hospital National Trauma Data Bank (NTDB, N = 4,568) and prehospital University of Alabama/Maryland trauma registries (UAB/UMD, N = 497) were queried incorporating the specific *RESUS* trial inclusion/exclusion criteria (Appendix A). The queries revealed similar mortality rates of 55.8% [95% CI 53.8-57.8] and 58.1% [95% CI 51.8-64.3], respectively. The UAB/UMD prehospital data, buttressed by the larger NTDB, were utilized as the basis for a 58.1% mortality estimate in subjects receiving standard care (controls) in *RESUS*. NMRC believes these data demonstrate that *human subjects* (enrolled in *RESUS*) *are facing a life threatening situation* (and) *available treatments are…unsatisfactory* (21 CFR 50.24).

***RESUS* Preclinical Database—Physiologic Benefits And Adverse Events**

HBOC-201 has been shown to transport and unload oxygen efficiently in *in vitro* experiments, animal tissue oxygenation studies, and in a human exercise study. Moreover, in numerous preclinical HS studies in swine (N = 269), including models of controlled and uncontrolled hemorrhage, with and without concomitant brain trauma, HBOC-201 significantly improved outcome in comparison with standard fluids. Consistent systemic physiologic findings have included stabilization of hemodynamics; improved tissue oxygenation and sagittal sinus oxygen saturation; decreased anaerobic metabolism, lactic acidosis, and base deficit; and diminished blood transfusion requirements. Consistent neurophysiologic findings include improved cerebral perfusion pressure, brain tissue oxygenation, cerebral autoreactivity, secondary brain injury, and contusion volume. Hemorrhage has been equivalent with HBOC-201 and control fluids and histopathologic. Cardiac troponin data reveal equivalent to possibly protective myocardial effects. Consistent but mild side effects have included lower cardiac output in less severe HS models but equivalence in severe HS, transient elevation of liver function tests, hepatobiliary histopathology, methemoglobinemia, and oxygen desaturation (due to right shift of HBOC-201's oxygen dissociation curve).

***RESUS* Preclinical Database—Mortality Reduction—Basis For *RESUS* Effect Size Estimate**

Mortality has been consistently and dramatically decreased in preclinical models of severe HS, with individual study and combined mortality reduction effect sizes of ~ 75% ($p < 0.0001$). As direct

extrapolation of these animal data to prediction of responses in humans may have limitations, a conservative mortality reduction effect size of 15% was chosen for *RESUS*, providing a 5-fold margin of error cushion. NMRC believes that the large number of studies and the redundancy of the positive results support conclusions that the *research holds out the prospect of direct benefit to the subjects* (and) *preclinical studies…support…potential…to provide a direct benefit* (21 CFR 50.24) to enrolled subjects in *RESUS.*

***RESUS* Clinical Database—Surgery/Orthopedics Trials—Efficacy And Safety In Intent-To-Treat (ITT) Population (Appendix B)**

In Phase 1-3 clinical trials, predominantly comparing HBOC-201 and gold-standard red blood cell (RBC) transfusions in older populations of surgery/orthopedics patients (mean age 61 years old, N > 800), HBOC-201 has shown high transfusion avoidance (efficacy) and equivalent mortality, but a mild adverse shift in the safety profile. Most importantly, in the HEM-0115 Phase 3 trial, a 7.7% absolute increase in incidence of overall serious adverse events [SAEs] was seen in the overall population enrolled in the HEM-0115 Phase 3 trial ($p = 0.02$). The most notable specific key adverse safety signals were cardiac SAEs (3.6% excess, $p = 0.01$) and CVA (stroke) AEs (1.7%, $p = 0.03$).

***RESUS* Clinical Database—Surgery/Orthopedics Trials—Safety In Younger Sub-Populations**

In sub-populations of < 70 and especially < 50 year old subjects, more predictive of the younger population to be enrolled in *RESUS* (expected mean age ~ 35-39 years old), group differences in key adverse safety signals were generally reduced or nonexistent and sometimes reversed. For example, in HEM-0115, in < 70 and < 50 year old sub-populations, group differences were reduced for overall SAEs to 6.0 and 6.1%, cardiac SAEs 1.6% and 2.1%, and CVA AEs to 0.8% and 0%, respectively1. These observations formed the basis for NMRC's decision to exclude the elderly from *RESUS***.**

Extrapolation Of Clinical Data From Prior Trials For Prediction Of Benefit:Risk In *RESUS*

Analogous to FDA's questioning of the accuracy of NMRC's extrapolating the *RESUS* mortality reduction effect size from preclinical animal studies, NMRC has questioned the accuracy of FDA's extrapolating safety data from prior surgery/orthopedics trials for prediction of benefit:risk in *RESUS*. NMRC believes that prior non-trauma trials are unlikely to predict benefit:risk in the

[1] The same efficacy and safety pattern was seen in Integrated Safety Summary (ISS) analyses of all HBOC-201 trials in the overall ITT population and younger < 70 and < 50 year sub-populations.

RESUS study accurately because of different clinical settings (elective surgery/orthopedics vs. acute HS); populations (older vs. younger adult populations); exposures (prolonged blood transfusion substitution vs. short oxygen bridge); physiologic states (hemodynamically stable vs. unstable); comparators (gold standard RBC transfusions vs. suboptimal crystalloid fluid); and most notably, potential benefit (transfusion avoidance vs. survival).

Benefit:Risk Prediction For Who Will Survive In *RESUS*

FDA has contended that subjects destined to survive irrespective of treatment group allocation would be exposed to HBOC-201-related risk without potential for benefit. NMRC believes that this argument is flawed because prospectively, all subjects have a > 50% mortality rate and it is unknown who will survive and who will die; additionally, preclinical studies predict that survivors will have systemic and neurophysiologic benefits as well as blood transfusion avoidance. Nevertheless, to address FDA's concern, risk was assessed in prospectively-defined matched sub-populations of subjects from the HEM-0115 trial with low risk. Specifically, risk was compared in the ~ 60% of HBOC-201 subjects (N = 211, *HH group*) which did not cross over to RBC with the ~ 68% of RBC subjects (N = 231, *R- group*) receiving $\leq$ 3 units of RBC. This analysis allowed comparison of safety signals in comparable groups with regards to need of oxygen carrying fluid (i.e., Hb load—whether HBOC-201- or RBC-derived), and revealed equivalent SAE rates (mean $\pm$ SEM: 0.14 $\pm$ 0.03 vs. 0.14 $\pm$ 0.03 per subject, respectively). Further detail will be provided later in the briefing book. These data predict that there is no excess HBOC-201-related risk in *RESUS* subjects destined to survive.

***RESUS* clinical database—interim data from S. Africa traumatic HS trial (HEM-0125)**

A recent interim analysis of safety data from an ongoing trial in S. Africa comparing HBOC-201 and RBC for resuscitation of patients with traumatic HS in the emergency room (N = 19), revealed equivalent mortality and trends to decreased numbers of AEs and SAEs per subject and fluid and blood transfusion requirements with HBOC-201.

HBOC Vasoactivity

The HBOC drug class has known inherent vasoactive properties, causing smooth muscle contraction predominantly but not entirely due to extravascular extravasation and binding of nitric oxide. Clinically, vasoactivity is manifested by vasoconstriction (e.g., elevated blood pressure [BP] responses) and gastrointestinal symptoms (e.g., abdominal cramps). It is believed that most of the clinically important side effects are related to vasoconstriction. The first generation HBOC, diaspirin cross-linked hemoglobin (DCLHb), was unpolymerized and had potent vasoactivity;

although results were equivocal in a prematurely discontinued prehospital traumatic HS trial, results showed adverse outcome in a prematurely discontinued in-hospital trauma traumatic HS trial. HBOC-201 is > 97% polymerized, resulting in less extravascular extravasation and thus mild to moderate vasoactivity. Preclinical data show that polymerization diminishes vasoactivity and strongly support a hypothesis that HBOC-201 vasoactivity is significantly decreased in comparison with DCLHb. Indeed, in preclinical HBOC-201 HS studies, BP responses have been mild to moderate. In the HEM-0115 clinical trial, mild to moderate BP elevation resulting in non-serious hypertension AEs were more common with HBOC-201 (12.3%) than RBC (5.3%) but hypertension SAEs occurred in only 0.6% of subjects. Thus, although higher BP is expected in subjects receiving HBOC-201 than LR in *RESUS*, severe responses are expected to be rare.

Vasoactivity Effects On Prehospital EMS Care

FDA has raised concern that higher BP responses could mislead health care providers (especially EMS personnel) about the fluid status of HBOC-201-treated subjects, resulting in under-resuscitation and hypoperfusion. However, in all preclinical HS studies, tissue perfusion has been either equivalent or improved with HBOC-201 in comparison with standard fluids. Standard prehospital (EMS) and trauma center practice mandates consideration of numerous physiologic parameters with minimization of the importance of BP, in the assessment of fluid status in patients with HS. Moreover, the *RESUS* protocol includes extensive training about potential for higher BP responses and secondary AEs due to under-resuscitation and hypoperfusion, and mitigation strategies; this training further ensures low risk due to elevated BP responses.

RESUS risk mitigation

Multiple strategies have been incorporated in the *RESUS* protocol to maximize potential benefit and minimize risk to subjects. In broad categories, these include optimization of target population selection, standardization of clinical care with practice guidelines and extensive training, allowance for concomitant standard care, and comprehensive surveillance methods for early detection and action regarding adverse safety signals. The specific *RESUS* risk mitigation strategies, many of which were recommended by OBRR or were in response to OBRR concerns, include the following:

1. Targeting a population with severe HS and without access to blood transfusions.
2. Exclusion of elderly subjects (≥ 70 years old).
3. Hypotension and tachycardia criteria for re-infusion of HBOC-201.
4. Thorough EMS and trauma center training.
5. Access to standard IV fluids during the prehospital period.

6. Access to standard blood transfusions immediately upon availability.
7. Improved standardization of prehospital care.
8. Standardization of in-hospital care with guidelines.
9. Prospective *increased BP* and *hypertension* coding definitions.
10. Extensive secondary outcome measurements.
11. *Elevated blood lactate* trial relative stopping criterion.
12. HBOC-201 infusion stopping criterion (SBP $\geq$ 120 mm Hg).
13. Expedited AE reporting to DMC and FDA.
14. Hypoperfusion markers reports.
15. Early *Efficacy and Safety Reviews* (interim analyses).

Semi-Quantitative Benefit:Risk Prediction For *RESUS*

Quantification of the relationship between benefit and risk derived from any new treatment is important in determining whether to go forward with a clinical trial evaluating any proposed intervention. This can prove difficult when the units of measure of benefit and risk are not equivalent. In the *RESUS* trial, the issue is one of determining how to weigh the potential benefit of saving a life against the potential risk of assuming the occurrence of intervention-associated SAEs. NMRC contends that there are no data directly predicting increased SAE risk for enrolled subjects in *RESUS* treated with HBOC-201 because: extensive preclinical HS data predict mortality reduction without harm; limited clinical HS data in HEM-0125 show a trend to lower risk of SAEs with HBOC-201; and extensive clinical data in non-trauma studies have only circumstantial relevance to prediction of risk in *RESUS*. However, the preponderance of available clinical data are derived from the Phase 3 HEM-0115 trial and FDA has based its benefit:risk assessment for *RESUS* mainly on SAE data from the HEM-0115 trial. Thus, focusing on HEM-0115 SAE data, three semi-quantitative predictions of benefit:risk to enrolled subjects in *RESUS* were completed in order to attempt to objectively estimate risk using FDA's risk assumptions.

For assessment of benefit, all three analyses utilized *RESUS* assumptions of a 58.1% mortality rate in control subjects and a 15% mortality reduction effect size. For assessment of risk, all three analyses utilized intent-to-treat (ITT) safety data from the overall population and < 70 year old sub-population in HEM-0115. In addition, Analysis #3 further assessed risk utilizing SAE data from HEM-0115 *matching subgroups* and the ongoing S. Africa ER trauma trial (HEM-0125).

Analysis #1 utilized overall SAE data from the overall population and < 70 year old sub-population in HEM-0115, which had excess SAE incidences of 7.7% and 6.0%, respectively. This analysis allowed a pure quantification of a *benefit:risk ratio* (*BRR*) in which the clinical significance of death and SAE occurrence was assumed to be equivalent. With this assumption, *BRR* > 1 predicts

favorable benefit:risk, *BRR* = 1 predicts *equipoise*, and *BRR* < 1 predicts *unfavorable* benefit:risk. As patients and physicians do not consider death and SAEs to be clinically equivalent, this analysis provided an overly conservative estimate of *BRR* and thus limited applicability to prediction of benefit:risk in *RESUS*.

Analyses #2 and #3 allowed more *RESUS*-relevant semi-quantitative assessments of benefit:risk, accounting for disparity in the clinical significance of death and SAEs. These analyses provided more applicable estimates of benefit:risk in *RESUS*; as they required clinical judgment, personal preference subjectivity may be a limitation. Nevertheless, NMRC believes that Analyses #2 and #3 provide the most accurate quantification of benefit:risk in *RESUS*.

In Analysis #2, the number of excess subjects expected to experience ≥ 1 SAE for every life saved was calculated, represented as an *Excess SAE Score* (*ESS*). NMRC assumed that a limited number of excess SAEs would be tolerable by most patients and physicians to save a life (i.e., *favorable* benefit:risk). Conversely, an indeterminate number of SAEs would eventually be questionable or intolerable even with potential to save a life (i.e., *equipoise* and *unfavorable* benefit:risk, respectively). The difficulty is in determining these levels. NMRC made the following assumptions: *ESS* < 1 = *highly favorable* benefit:risk, *ESS* 1 to < 2 = *favorable* benefit:risk, *ESS* 2 to < 3 = *probably favorable* benefit:risk, *ESS* 3 to < 4 = *possible equipoise*, and *ESS* > 4 = *possible unfavorable* benefit:risk. These assumptions are very conservative because depending on the severity of the SAEs, even 3-4 or more than 3-4 excess SAEs, would be tolerable by many patients and physicians to save a life.

Analysis #3 was the most *RESUS*-relevant assessment of benefit:risk. In order to better account for the disparity in the clinical significance of death and SAEs, System Organ Class (SOC) SAE categories were compared (by an experienced trauma surgeon) in terms of their significance vis-à-vis death in clinical scenarios with varying predicted mortality rates. The clinical significance of the SOC SAEs was represented as *mortality equivalents*, incorporated into a calculation of a *RESUS*-relevant *BRR* estimate. This analysis (detailed in Section 7.0) provided the most scientifically valid, comprehensive, and *RESUS*-relevant prediction of benefit:risk for the *RESUS* trial.

The results of Analyses #1 and #2 show that in the targeted population of 1,108 subjects, 48 fewer deaths would be expected at the possible expense of 34-43 excess subjects experiencing ≥ 1 SAE. These numbers predict that the Number Needed to Treat (NNT) is 11.5 (to save a life) and the

Number Needed to Harm (NNH) is 13-17 (to cause an additional subject to experience ≥ 1 SAE). Thus, Analysis #1, which overly conservatively equates death and SAEs as clinically equivalent, shows a *BRR* of 1.1-1.5 (i.e., *favorable* risk:benefit). Analysis #2, which partially accounts for the disparity in the clinical significance of death and SAEs, shows an *ESS* of 0.71-0.92 (i.e., *highly favorable* benefit:risk) (Table 1). NMRC strongly believes that saving a life at the possible expense of less than one additional subject experiencing an SAE predicts *highly favorable* risk:benefit.

Analysis #3 more comprehensively accounts for the disparity in the clinical significance of death and SAEs by the assigning of *mortality equivalents* for all SOC SAEs categories (in contrast, only overall SAE incidence was included in Analysis #2). The analysis demonstrated higher NNH values, 67 in the HEM-0115 overall population, and 83.3 in the < 70 year old sub-population, resulting in *BRR* values of 5.8 and 7.2, respectively. Even the most conservative worse-case estimate based on data from the high need *matching subgroups* in HEM-0115, revealed *BRR* values of 2.9-3.4. Finally, HEM-0125 data revealed *BRR* = ∞ (Table 2). NMRC strongly believes that these most *RESUS*-relevant and comprehensive semi-quantitative objective predictions, showing *BRR* values 3-7 fold higher than equipoise, strongly support *highly favorable* benefit:risk in *RESUS*.

The FDA has critiqued the robustness of such semi-quantitative predictions, questioning whether variance in one or both of the key assumptions (i.e., lower control mortality and/or effect size) could manifest in *RESUS* and result in *unfavorable* benefit:risk prediction. Hence, NNT, NNH, and *BRR* estimates were calculated with variances in both assumptions. The results of the overly conservative Analysis #1 assessment of benefit:risk in *RESUS*, show that even if control mortality turns out to be as low as 45%, the *BRR* would remain ≥ 1 for < 70 year olds (i.e., *favorable* benefit:risk); the results of Analysis #2 show that the *ESS* would still remain < 1 for < 70 year olds (i.e., *highly favorable* benefit:risk). Similarly, if the effect size turns out to be as low as 10%, Analysis #1 shows that the *BRR* would still remain ≥ 1 for < 70 year olds (i.e., *equipoise* benefit:risk); Analysis #2 shows that the *ESS* would be 1-1.3 (i.e., *favorable* benefit:risk). Analysis #3 reveals that *equipoise* (*BRR* ≥ 1.0) is predicted for *RESUS* even with absolute decreases in mortality as low as 3, 1.5, and 1.2%, based on all SOC SAE category data from the HEM-0115 high need *matching subgroups* (worse-case scenario), overall population, and < 70 year old sub-population, respectively. These benefit:risk estimates predict a 3-7 fold cushion margin of error as the absolute reduction in mortality expected in *RESUS* is > 8.7% (Table 3). Thus, analysis of the robustness of a *favorable* outcome, indicated that benefit outweighed risk over a large variation in

parameter estimates even for the worse-case scenario estimate of *net risk* based on safety data in the high needs *matching groups* in HEM-0115.

NMRC submits that these three semi-quantitative analyses robustly predict *highly favorable* benefit:risk for subjects enrolled in *RESUS*, more than exceeding the regulatory requirement of *reasonable* risk (21 CFR 50.24).

Table 1: Semi-quantitative benefit:risk prediction for *RESUS* trial (Analyses #1 and #2)

Mortality (LR → HBOC) (%)	Mortality reduction effect size (%)	Reduction in deaths (N)	Excess subjects experiencing ≥ 1 SAE (N)*	NNT (N)	NNH (N)	Analysis #1 *BRR** and quantitative assessment of benefit:risk	Analysis #2 *ESS** and semi-quantitative assessment of benefit:risk
Control mortality variance with effect size constant							
65 → 55.2	15	54	34-43	10.3	13-17	1.3-1.7 *Favorable*	0.6-0.8 *Highly favorable*
60 → 51.0	15	49	34-43	11.3	13-17	1.2-1.5 *Favorable*	0.7-0.9 *Highly favorable*
58.1 → 49.4	**15**	**48**	**34-43**	**11.5**	**13-17**	**1.1-1.5 *Favorable***	**0.71-0.92 *Highly favorable***
50 → 42.5	15	41	34-43	13.5	13-17	1.0-1.3 *Favorable (< 70 y/o) Equipoise (overall pop)*	0.8-1.0 *Highly favorable (< 70 y/o) Favorable (overall pop)*
45 → 38.3	15	37	34-43	15	13-17	0.9-1.1 *Favorable (< 70 y/o) Unfavorable (overall pop)*	0.9-1.2 *Highly favorable (< 70 y/o) Favorable (overall pop)*
Control mortality constant with effect size variance							
58.1 → 47.0	20	64	34-43	8.7	13-17	1.5-2.0 *Favorable*	0.5-0.6 *Highly favorable*
58.1 → 49.4	**15**	**48**	**34-43**	**11.5**	**13-17**	**1.1-1.5 *Favorable***	**0.71-0.92 *Highly favorable***
58.1 → 49.4	10	32	34-43	17.3	13-17	0.75-1.0 *Equipose (< 70 y/o) Unfavorable (overall pop)*	1.0-1.3 *Favorable*
58.1 → 49.4	5	16	34-43	34.6	13-17	0.4-0.5 *Unfavorable*	2.0-2.7 *Probably favorable*
Control mortality AND effect size variance							
65 → 52.0	20	72	34-43	7.7	13-17	1.7-2.2 *Favorable*	0.5-0.6 *Highly favorable*
58.1 → 49.4	**15**	**48**	**34-43**	**11.5**	**13-17**	**1.1-1.5 *Favorable***	**0.71-0.92 *Highly favorable***
50 → 45.0	10	28	34-43	19.8	13-17	0.7-0.9 *Unfavorable*	1.2-1.5 *Favorable*
50 → 47.5	5	14	34-43	39.6	13-17	0.3-0.4 *Unfavorable*	2.3-3.0 *Probably favorable (< 70 y/o) Possible equipoise (overall pop)*
45 → 40.5	10	25	34-43	22.2	13-17	0.6-0.8 *Unfavorable*	1.3-1.7 *Favorable*
45 → 42.75	5	12	34-43	46.2	13-17	0.3-0.4 *Unfavorable*	2.7-3.6 *Probably favorable (< 70 y/o) Possible equipoise (overall pop)*

* The ranges reflect calculations based on overall SAE data from the overall population and from the < 70 year old sub-population.

Table 2: Summary of semi-quantitative benefit:risk analysis #3

Populations Compared	Risk Scores (*mortality equivalents*)					
	HBOC-201	**Control**	***Net Risk***	**NNH**	**NNT**	***BRR***
ITT HEM-0115 overall pop	0.050	0.035	0.015	67	11.5	5.8
ITT HEM-0115 < 70 sub-pop	0.029	0.017	0.012	83	11.5	7.2
HEM-0115 *HH* vs *R-matching subgroups*	0.028	0.027	None	N/A	11.5	Very high
HEM-0115 HR* vs *R+ matching subgroups	0.083	0.053	0.030	33	11.5	2.9
HEM-0125	0.406	0.410	None	N/A	11.5	Very high

Table 3: Robustness of *favorable* benefit:risk assessment in analysis #3

Estimated Mortality	Effect Size %	*BRR* (Worse-Case)
60	15	3
20	15	1
30	10	1
40	7.5	1
60	5	1

Summary

In summary, redundant results from queries of NTDB and UAB/UMD databases predict mortality > 50% in the targeted severe HS population receiving standard care in *RESUS* (controls). Preclinical data from numerous HS studies, utilizing HBOC-201 doses and infusion rates similar to and higher than proposed in the *RESUS* trial, robustly predict clinically-significant physiologic benefits, blood transfusion avoidance, and a mortality reduction of 75% in subjects enrolled in *RESUS*. Extensive clinical data from non-trauma studies (with circumstantial relevance to prediction of benefit:risk in *RESUS*), utilizing HBOC-201 doses similar to those proposed in the *RESUS* trial, show blood transfusion avoidance efficacy and a reasonable safety profile particularly in younger subjects. Limited interim clinical data in traumatic HS (with direct relevance to prediction of benefit:risk in *RESUS*), utilizing HBOC-201 doses and infusion rates similar to and higher than proposed in the *RESUS* trial, predict a favorable safety profile. These preclinical and clinical data provide a strong evidence basis for *RESUS* Dosing Guidelines. Semi-quantitative analyses predict favorable benefit:risk in *RESUS* with reasonably large cushions (margins of error) in case control mortality

and/or effect size assumptions turn out to be lower than anticipated in *RESUS*. Multiple protocol risk mitigation strategies further reduce risk.

NMRC believes that when HBOC-201's overall safety profile is considered in the context of a predicted mortality of ~ 1 in 2 with standard care, a 75% mortality reduction effect size in preclinical severe HS studies, a targeted mean age of ~ 35-39 years old, and a conservatively predicted 15% mortality reduction with HBOC-201, Risks associated with the intervention are reasonable in relation to what is known about the medical condition (21 CFR 50.24) for enrolled subjects in *RESUS*. We hope that the BPAC will recommend to the FDA a lifting of the *RESUS* Clinical Hold. We thank the FDA and BPAC members for their time and effort.

3.0 INTRODUCTION

Trauma is the leading cause of death among younger adults [1-3], accounting for 109,043 deaths in the U.S. and 4,736,000 deaths globally annually. As severe hemorrhage resulting in hemorrhagic shock (HS) accounts for the preponderance (~ 60%) of deaths in patients with potentially salvageable injuries (~ 50%) [4], amounting to about one third of trauma deaths[5], the HS burden is estimated at 35,984 deaths in the U.S. and 1,562,880 deaths globally annually. Moreover, in *Operation Iraqi Freedom* (*OIF*), of the ~ 2,900 U.S. military deaths, it is estimated that 1,305-1,972 (45-68%) were likely related to severe hemorrhage[2]. Furthermore, as many as 50% of civilian urban, 80% of civilian rural, and 90% of military trauma fatalities occur prior to hospital arrival. [4-9] Thus, most HS patients do not benefit from transformational improvements in hospital trauma care that have occurred over the last decades, and improving prehospital HS resuscitation outcome is a key priority of both civilian and military traumatologists, representing a major unmet medical need.

HS Pathophysiology And Treatment

Profound hemorrhage adversely affects the individual's cardiovascular capacity leading to a *failure of adequate oxygen delivery or utilization by the tissues of the body.* The resulting condition is known as HS. Diminished tissue perfusion creates an ischemic environment resulting in anaerobic metabolism with production of lactic acid (LA) and oxygen free radicals, which in turn cause cellular damage.

Early resuscitation efforts are designed to stop further blood loss and to provide hemodynamic support. However correcting hemorrhage and restoring circulating volume alone may be insufficient for reversing the effects of a significant oxygen debt, as the release of toxic by-products upon reperfusion of ischemic vascular beds impairs systemic recovery. For this reason, early resuscitation efforts directed at tissue oxygen delivery and resolution of oxygen debt may be particularly effective in decreasing the morbidity and mortality associate with HS.

Prehospital HS Treatment

The current standard-of-care for prehospital treatment of casualties with HS relies on resuscitation with asanguinous, non-oxygen carrying crystalloid (e.g., lactated Ringer's solution [LR] or normal saline) and sometimes colloid (e.g., hetastarch) fluids. Standard resuscitation fluids restore

[2] Extrapolated from the Navy-Marine Corps Combat Trauma Registry, Naval Health Research Center, San Diego, CA (22 Feb 2005).

intravascular volume but dilute the blood's oxygen content, and hence, often fail to correct tissue hypoxia resulting in anaerobic metabolism and decompensated terminal HS. Blood transfusions can restore intravascular volume, replenish blood oxygen carrying capacity, avert or reverse tissue hypoxia and consequent anaerobic metabolism, and are consequently often live-saving in HS, but blood transfusions are rarely available in civilian or military prehospital settings. Thus, development of alternative oxygen-carrying resuscitative fluids is a logical approach towards a goal of decreasing morbidity and mortality related to HS.

HBOC-201

Hemoglobin-Based Oxygen Carrier-201 (HBOC-201, Hemopure®, Biopure Corporation, Cambridge, MA), is a second generation, bovine derived, polymerized hemoglobin (Hb), resuscitation fluid that:

- Replenishes intravascular volume similarly to standard colloid fluids
- Transports and unloads oxygen efficiently
- Is universally-compatible
- Does not require refrigeration
- Is easy to administer in field settings
- Has low infection transmission risk

Phase 1-3 human clinical trials including > 800 subjects (mainly for surgical and orthopedic indications), have demonstrated that HBOC-201 is effective in decreasing the need for blood transfusion.

Rationale

U.S. Navy (NMRC) investigators believe that sufficient preclinical and clinical data are available to hypothesize that in comparison with standard therapy, prehospital resuscitation of patients with HS with HBOC-201 will improve clinical outcome. This hypothesis is based on the following observations:

(1) In the majority of 23 animal HS studies with controlled and uncontrolled hemorrhage and HS, HBOC-201 has stabilized hemodynamics, improved tissue oxygenation, averted or reversed anaerobic metabolism, and increased survival (Table 4); and despite mild to moderate vasoactivity, increased bleeding has not been seen with hypotensive resuscitation in models of uncontrolled hemorrhage.

Table 4: HBOC-201 mortality reduction effect size in preclinical HS studies

	HBOC-201 Mortality	Control Mortality	P	Reduction in mortality (effect size)	Absolute group difference
More severe HS (uncontrolled)	7/42 (16.7%)	40/43 (93.0%)	< 0.0001	82.0%	76.3%
Less severe HS (controlled)	7/75 (9.3%)	13/69 (18.8%)	> 0.05	50.5%	9.5%
All HS (uncontrolled and controlled)	14/117 (12.0%)	53/112 (47.3%)	< 0.0001	74.6%	35.3%

(2) In Phase 2/3 clinical trials in surgical/orthopedic patients, HBOC-201 decreased blood transfusion requirements and had a *reasonable* safety profile (vis-à-vis the indication requested herein). These animal and human findings support the hypothesis that HBOC-201 holds the *prospect* for a *direct benefit* to victims of traumatic HS who are otherwise at substantial risk of death (outlined in Section 5.0).

Potential Impact

The goal of the *RESUS* (*Restore Effective SUrvival in Shock*) program is to test U.S. Navy researchers' hypothesis that prehospital resuscitation utilizing HBOC-201 will decrease mortality by 15% for both military and civilian casualties suffering severe HS, in comparison with prehospital resuscitation utilizing standard fluids. If the Navy's hypothesis is confirmed, 97 subjects will be saved in the *RESUS* trial alone. In addition, by applying *RESUS* inclusion/exclusion criteria to available databases (22% of trauma deaths), NMRC estimates the following potential annual life savings annually (*efficacy*): U.S. National Trauma Data Bank (NTDB)—41, U.S.—3,598, worldwide 156,288, and military personnel in *OIF*—96[3]. By extrapolating these estimates to the larger general traumatic HS population (33% of trauma deaths), NMRC estimates the following potential annual life savings (*effectiveness*): 62 trauma patients in the U.S. NTDB database, 5,398

[3] Potentiial efficacy calculations

- U.S. NTDB—of 6,255 trauma deaths in NTDB (2000-04) (1,251 annually), 1,363 (273 annually) met *RESUS* criteria (22%). With a 15% mortality reduction from 58.1 to 49.4%, 188 deaths might be averted (41 annually).
- U.S. total—of 109,043 annual trauma deaths [10], NMRC estimates based on the above NTDB data that 22% might meet *RESUS* criteria (23,990). With a 15% mortality reduction, 3,598 deaths might be averted. The calculations show that the NTDB represents ~ 1% of U.S. trauma deaths.
- Worldwide—of 4,736,000 annual trauma deaths [11], NMRC estimates based on the above NTDB data that 22% might meet *RESUS* criteria (1,041,920). With a 15% mortality reduction, 156,288 deaths might be averted.
- *OIF*—of ~ 2,900 deaths, NMRC estimates based on the above NTDB data that 22% might meet *RESUS* criteria (638). With a 15% mortality reduction, 96 deaths might be averted.

trauma patients in the U.S., and 234,432 trauma patients globally could potentially be saved each year. Moreover, of the ~ 2,900 U.S. military personnel deaths in *OIF* to date, 196-296 could potentially have been saved.

HBOC-201 Safety Profile In Prior Surgery/Orthopedics Clinical Trials

Given that the adverse events (AE) profile of HBOC-201 was inferior to RBC in prior Phase 2/3 surgical/orthopedic trials in populations of mainly older patients, RBC transfusion appears to remain a safer alternative for Hb replacement in such clinical settings. However, in comparison with gold standard RBC transfusion, the AE profile of HBOC-201 was only minimally inferior to RBC. Thus, even in an older patients undergoing stable surgical/orthopedic procedures, when safe and expeditious RBC transfusions are unavailable, the benefit:risk comparison may indeed be favorable for administering HBOC-201 in clinical settings associated with severe blood loss where the risk of mortality is high (Appendix B).

Specifically, in the overall population enrolled in the Phase 3 HEM-0115 trial conducted by Biopure (mean age 61 years), in which HBOC-201 and RBC were compared in subjects with perioperative anemia undergoing orthopedic procedures, the incidence of AEs and serious adverse events (SAEs) in the intent-to-treat (ITT) analyses were higher in HBOC-201 than RBC subjects for a number of key safety signals[4]: overall AEs (delta 4.3%, $p = 0.03$), overall SAEs (delta 7.7%, $p = 0.02$), cardiac SAEs (delta 3.6%, $p = 0.01$), MI AEs (delta 0.6%, $p = 0.7$), troponin elevations (delta 12%, $p = 0.0003$), heart failure/fluid overload AEs (delta 2.0%, $p = 0.04$)[5], cardiac arrest AEs (delta 1.7%, $p = 0.1$)[6], cerebral ischemic (CVA/TIA/RIND) AEs (1.4%, $p = 0.07$), CVA AEs (delta 1.7%, $p = 0.03$), hypertension AEs (delta 7.0%, $p = 0.002$), hypertension SAEs (delta 0.6%, $p = 0.5$), and mortality (delta 1.1%, $p = 0.3$)[7].

However, stratification of AE data from the HEM-0115 trial, based on age reveals that HBOC-201's AE profile was age-dependent. Specifically, adverse group differences were usually lower in < 70 than ≥ 70 year olds: overall SAEs (delta 6.0 vs. 11.7%), cardiac SAEs (delta 1.6 vs. 8.1%), MI

[4] Key adverse safety signals are those predicted to significantly affect benefit:risk prediction in the proposed high-mortality *RESUS* trial (generally but not exclusively SAEs). There were group differences in other non-serious AEs (e.g., asymptomatic elevation in LFTs and lipase—discussed below), but these were not considered key or integral to benefit:risk prediction in *RESUS*.
[5] *Combination 20* (OBRR-Biopure June 2006).
[6] *Combination 22* (OBRR-Biopure June 2006).
[7] P values reflect group comparisons (HBOC-201 vs. RBC) in the overall population (Fisher's Exact test, two-way).

AEs (delta 0 vs. 1.8%), troponin elevations (delta 9.9 vs. 21.6%), heart failure/fluid overload AEs (delta 0.8 vs. 4.5%), cardiac arrest (delta 0.8 vs. 3.6%), cerebral ischemic AEs (delta -0.1 vs. 4.5%), CVA AEs (delta 0.8 vs. 3.6%), hypertension AEs (delta 6.8 vs. 7.2%), hypertension SAEs (delta 0.8 vs. 0%), and mortality (delta 0.8 vs. 1.8%)[8]. In addition, group differences in incidence of peak *systolic blood pressure (SBP) responses* ≥ 140 mm Hg were also lower in < 70 than ≥ 70 year olds (26 vs. 35.1%, respectively). These data show that although some AEs (e.g., hypertension) were similar in subjects < 70 vs. ≥ 70 years of age, HBOC-201's overall *relative* AE profile, especially regarding key SAEs, was more favorable in subjects less than 70 years old.

U.S. Navy researchers have concluded that the HEM-0115 trial suggests that in a relatively older population of patients undergoing orthopedic surgery, overall clinical outcome is better with RBC transfusion than with HBOC-201 infusion; but remarkably, only minimally so, and where safe and expeditious transfusions are available (i.e., in-hospital setting in developed countries)[9]. Thus, HBOC-201 may have significant clinical utility in clinical settings where safe and rapidly available transfusions are unavailable (e.g., prehospital, under-developed countries, stockpiling for disaster, and military).

HBOC-201 Safety Profile in Traumatic HS

Twenty-one patients have been enrolled in the ongoing HEM-0125 ER clinical trial in South Africa (50 patient enrollment planned), comparing HBOC-201 and RBC treatment in unstable 18-65 year old patients with HS. An interim analysis of safety data from 19 evaluable subjects, with mean age 38 ± 3.3 years and mean infusion rate 73 ml/minute, reveals a favorable HBOC-201 *relative* safety profile despite an essentially high-bar comparison with gold standard RBC transfusions. There are no statistically significant group differences in mortality or in the number of AEs and SAEs per subjects. A Data Safety Monitoring Board (DSMB) reviewed these data and recommended continuation of the trial. U.S. Navy researchers have concluded that these interim data suggest that HBOC-201 may be at least as efficacious and safe as RBC transfusions in a mainly younger

[8] In comparing ≤ 50 with ≥ 70 years olds, HEM-0115 group differences were further narrowed: overall SAEs (delta 6.1 vs. 11.7%), cardiac SAEs (delta 2.1 vs. 8.1%), MI AEs (delta 0 vs. 1.8%), troponin elevations (delta 3 vs. 21.6%), cerebral ischemic AEs (delta -0.4 vs. 4.5%), hypertension AEs (delta 2.5 vs. 7.2%), hypertension SAEs (delta 1.0 vs. 0%) and mortality (delta 0 vs. 1.8%). The ≤ 50 year old sub-population was small (97 HBOC-201 vs. 69 RBC subjects), but data support the hypothesis that *relative* risk will be improved in the younger *RESUS* population.

[9] Note: although Office of Blood Research and Review (OBRR) and Biopure have recently endeavored to reconcile differences in enumeration and classification of a few AEs in the Integrated Safety Summary (ISS) database, minor differences remain. However, NMRC does not expect that these differences will significantly alter overall benefit:risk prediction for *RESUS*.

population of acute HS patients, but definitive conclusions await completion of this Phase 2 and larger Phase 3 studies (Appendix E).

HBOC-201 IND

U.S. Navy researchers submitted a human protocol and IND application to FDA's OBRR to evaluate HBOC-201 in acute trauma. The clinical study, named *RESUS*, is designed to assess the efficacy and safety of HBOC-201 as a prehospital resuscitation fluid for HS in a multi-center, randomized, controlled, and two-staged Phase 2/3 clinical trial, enrolling 50 and 1,108 subjects, respectively. The objective of *RESUS* is to test the hypothesis that in comparison with standard LR, prehospital resuscitation of non-elderly patients with *severe traumatic HS* with HBOC-201 will decrease the 28-day mortality rate from 58.1% to 49.4% (15% relative reduction) and be safe and tolerated where blood transfusions are unavailable. Being an emergency medicine trial in which prior *Informed Consent* (*IC*) will rarely be feasible, the *RESUS* trial must meet all provisions for *Exception from Informed Consent* (*EIC*) as set forth in the applicable statute, 21 CFR 50.24.

Clinical Hold Placed On The HBOC-201 IND

OBRR placed the *RESUS* IND on Clinical Hold (08 Jul 2005), primarily citing safety concerns from prior Phase 2/3 surgical trials (especially related to HBOC-201's vasoactive properties) in which HBOC-201 was generally compared with RBC transfusion with the goal of reducing or eliminating blood transfusion requirements. In the pivotal Phase 3 HEM-0115 trial, prospectively defined efficacy (59% transfusion avoidance) and safety objectives were met (Safety Endpoint Evaluation Committee [SEEC] analysis), but the AE profile in the overall population of HBOC-201 subjects was inferior to RBC. Importantly, mortality was not significantly different between HBOC-201 (10/350 [2.9%]) and RBC subjects (6/338 [1.8%]), $p = 0.3$.

OBRR's major safety concerns have been focused on observations of relatively higher blood pressure (BP) responses and other potentially "vasoactivity-related" AEs and SAEs (especially cardiac and cerebral ischemic) in HBOC-201 subjects10. OBRR has been concerned that

[10] Vasoactivity is characteristic of all HBOCs, predominantly but not entirely related to nitric oxide (NO) binding by low molecular weight (mw) components (especially tetrameric Hb), which are decreased in HBOC-201 due to $\geq$ 97% polymerization. HBOC-201 ($\leq$ 3% tetrameric Hb) is less vasoactive than other less polymerized HBOCs (HBOC-301 [32% tetrameric], Hemolink® [31% tetrameric, Hemosol Inc.], 38 and 78% tetrameric bovine HBOCs, and Baxter's prior DCLHb [100% tetrameric].[12-14] Dr. Alayash's (OBRR) slides provide data about more vasoactive HBOCs which are not being tested in *RESUS* (HBOC-301); thus, NMRC believes that they have no direct relevance to the test product proposed in *RESUS* (HBOC-201).

commonly observed higher BP responses (e.g., hypertension AEs in 38/350 [11%] HBOC-201 vs. 15/338 [4%] of RBC subjects, p = 0.002), even if not severe, could mislead ambulance personnel and physicians about subjects' fluid status, resulting in inadequate fluid resuscitation and secondary complications of hypoperfusion. OBRR has also been concerned by less commonly observed severe BP responses (i.e., hypertension SAEs in 2/350 [0.6%] HBOC-201 vs. 0/338 [0%] RBC subjects, p = 0.5).

In addition, OBRR has contended that the *RESUS* IND fails to satisfy other requirements of 21 CFR 50.24. Specifically, OBRR has critiqued the robustness of NMRC's predicted benefit from the preclinical HBOC-201 HS database and has argued that the protocol's inclusion/exclusion criteria fail to target a population with a life-threatening situation and unsatisfactory available treatment options.

NMRC's Perspective On The Clinical Hold Issues

NMRC concurs with OBRR's concern about potential risk of increased incidence of adverse safety signals in *RESUS*, based on HBOC-201's AE profile in older populations in prior surgical/orthopedic trials. Consequently, NMRC has implemented numerous protocol design optimizations to address OBRR's safety concerns. However, NMRC's most important risk mitigation strategy was originally to exclude enrollment of the elderly because of our observations that HBOC-201's relative AE profile (especially cardiac) in HEM-0115 was less favorable in ≥ 70 year old subjects, and that similar adverse cardiac AE profiles were seen in older subjects treated with other HBOCs as well (DCLHb [HemAssist®, Baxter Healthcare, Round Lack, IL [15]; *o*-raffinose polymerized Hb [Hemolink®, Hemosol Inc., Etibicoke, ON] [16]; and human polymerized Hb [PolyHeme®, Northfield Laboratories, Evanston, IL). NMRC concluded that older patients do not appear to tolerate the HBOC drug class' stereotypical property of vasoactivity as well as younger patients, and that exclusion of the elderly, in concert with the other risk mitigation strategies detailed below, would significantly shift predicted risk in *RESUS*, ensuring predicted *Risks associated with the intervention are reasonable in relation to what is known about the medical condition...*(and) *risks and benefits of standard therapy* (21 CFR 50.24). However, OBRR directed NMRC to delete the *RESUS* elderly exclusion criterion at the 14 Apr 2004 Pre-IND meeting. In further IND correspondence, NMRC requested OBRR's guidance about possible exclusion of the elderly, but a formal response has not yet been received. At a teleconference on 02

June 2006, NMRC requested guidance and OBRR stated its preference that the BPAC advise on this issue.

NMRC has also argued that the *RESUS* IND preclinical database is robust and compelling, showing significant physiologic and survival benefits in the individual indication-specific HS IND-enabling studies requested and/or previously accepted by OBRR[11], as well as in combined categorized analyses of the database (Table 4)[12]. NMRC believes that despite potential limitations of individual studies, overall, 21 CFR 50.24 requirements that *preclinical studies…support the potential…to provide a direct benefit* and *research holds out…prospect of direct benefit,* are met.

NMRC has also optimized *RESUS* inclusion/exclusion criteria to ensure enrollment of a population for whom NMRC could objectively and confidently predict that *research holds out…prospect of direct benefit*. Heeding OBRR's pre-IND meeting (14 Apr 04) concern about potential confounding due to the bimodal distribution of trauma mortality, and its suggestion to focus the target population from one that includes all hypotensive HS patients[13] to one with a more homogeneous population with severe HS and high mortality, NMRC added a *Revised Trauma Score* (*RTS*) inclusion criterion of 1 to < 6.5. [19] This modification resulted in a mortality prediction of 34%. Although OBRR initially concurred with this modification[14], it was later deemed inadequate[15], considered persistently heterogeneous. Subsequently, NMRC focused the target population further, narrowing the RTS inclusion criterion to 1 to < 5, excluding the two RTS subgroups with relatively low mortality (5-5.9 and 6-6.5) (the "right" tail of the U-shaped distribution). This protocol

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[11] 14 Apr 04 Pre-IND meeting minutes: *FDA has reviewed the raw data from your porcine liver lobe resection model of HS* (referring to [17])*…Despite the shortcomings…No additional preclinical studies will be required to address this concern…Before this trial moves forward, the sponsor will need to submit for FDA review…an animal model of traumatic brain injury + uncontrolled HS, which shows that HBOC-201 administration does not exacerbate CNS injury*.

03 Oct 05 Clinical Hold Letter: *FDA takes note of the favorable outcome in the two preclinical studies cited* (referring to [17, 18]).

[12] OBRR has critiqued the scientific basis for combining preclinical studies with varying designs. Although NMRC understands limitations of potentially arbitrary combinations, NMRC believes that its categorization includes study designs with relatively similar animal species and population characteristics, comparators, treatment guidelines, and primary outcome measurements. That mortality results in the combined analyses matches observations in most of the individual studies is supportive. NMRC believes that the combinations assist in *hypothesis generation* for prediction of potential benefit in *RESUS*, analogous to OBRR's use of combinations from HBOC-201's clinical ISS database for the same purpose.

[13] Akin to Northfield Laboratories' prehospital PolyHeme® trauma study design.

[14] 07 Jul 04 clarification letter: *Are the changes to the inclusion/exclusion criteria adequate? FDA response: The changes address FDA's concerns*.

[15] 02 Mar 06 Clinical Hold letter: *RTS criterion of 1 to ≤ 6.5 comprises a heterogeneous population….*

modification resulted in a more homogeneous population[16], an increase in predicted mean mortality from 34% to 55.8-58.1%, and a normalized (bell-shaped) N distribution. NMRC also optimized definition of the *blood transfusions available* exclusion criteria to avoid enrollment of urban trauma patients with short prehospital delay who would be predicted to have less potential benefit from the study intervention. Thus, NMRC contends that the *RESUS* protocol's inclusion/exclusion criteria, targeting a population with extremely severe HS and high mortality (greater than one in two) without access to blood transfusions, surpass 21 CFR 50.24 requirements that *subjects are facing a life-threatening situation* and *available treatments are…unsatisfactory*.

Benefit to Risk Balance

NMRC and OBRR have not yet come to a consensus about whether overall potential benefits and risks in the *RESUS* study represent a *reasonable* safety profile. It appears that NMRC and OBRR have digressed in their assessment of underlying assumptions used to reach disparate conclusions. Thus, in this submission to FDA's BPAC, NMRC will aim to provide evidence-based data to support its prediction that the benefit:risk balance is favorable for subjects enrolled in the *RESUS* study, that the *research holds out the prospect of direct benefit to the subjects*, and *Risks associated with the intervention are reasonable in relation to what is known about the medical condition...*(and) *risks and benefits of standard therapy* (21 CFR 50.24).

NMRC's Assumptions

NMRC's favorable benefit:risk prediction for subjects enrolled in the *RESUS* trial is based on the following assumptions:

Assumption #1: Predicted mortality is 58.1% in the *RESUS* target population receiving standard care, equating with a *life-threatening situation* and *available treatments are unsatisfactory* (21 CFR 50.24).

- Using the NTDB (N = 4,568) which provides hospital admission data (mortality 55.8%; 95% CI 53.8-57.8)[17] and the UAB/UMD database (N = 497) which contains prehospital data (mortality 58.1%; 95% CI 51.8-64.3), a 58.1% mortality rate is predicted in the *RESUS* target population receiving standard care (Appendix A).

[16] RTS stratification mortality ranges: 29.7-67.6% (NTDB) and 42.5-88.6% (UAB/UMD prehospital databases).
[17] The NTDB estimate is from the current 2000-2004 database, using *RESUS* age restriction (18 to less than 70 years old), SBP < 90 mm Hg, and RTS (1 to < 5) inclusion criteria. Referenced Ns are for subpopulations with *RESUS* criteria only.

Assumption #2: Preclinical HS studies with HBOC-201 show improved outcome and predict *prospect for direct benefit* and *potential to provide direct benefit to human subjects* (21 CFR 50.24).

- A wide variety of HS studies conducted in several species have demonstrated significant benefits of HBOC-201 on improving survival[18] with effect sizes of 74.6% overall with HS ($p < 0.0001$) and 82% with severe HS ($p < 0.0001$) (Table 4 and Table 8, detailed in preclinical summary, Section 5.c)[19].

- In HS studies with and without concomitant TBI, additional clinically-relevant systemic physiological benefits have been observed with HBOC-201 resuscitation (e.g., stabilization of hemodynamics, improved tissue oxygenation, reversal of anaerobic metabolism, transfusion avoidance, and ventilator weaning). [17, 18, 20, 21, 23-25, 27-32] In HS studies with concomitant TBI, additional clinically-relevant neurophysiological benefits have been observed with HBOC-201 (and HBOC-301 [Oxyglobin®]) resuscitation (e.g., improved brain tissue oxygenation [$brPO_2$] and sagittal sinus oxygen saturation [SSO_2sat], decreased sagittal sinus LA [SSLA], improved cerebral perfusion pressure [CPP], decreased secondary brain injury, decreased contusion volume, and improved autoreactivity). [18, 25, 26, 33, 34]

Assumption #3: Preclinical data from standard swine HS models predict that mortality in humans will be decreased with HBOC-201 (*prospect for direct benefit* and *potential to provide direct benefit to human subjects* [21 CFR 50.24]).

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18 OBRR previously acknowledged that HBOC-201 improves survival in animal HS studies:

- 04 May 2004: *...both studies show that HBOC-201…significantly improves survival…in the setting of uncontrolled HS in this animal model.*
- 08 Jul 2005: *We agree that the two studies in animal models of uncontrolled HS and uncontrolled HS with concomitant traumatic brain injury…show a survival benefit.*
- 03 Oct 2005: *...FDA takes note of the favorable outcome in the two preclinical studies cited.*

19 NMRC's perspective on OBRR Reviewer comments: improved outcome has been documented in many swine HS studies, including controlled and uncontrolled hemorrhage models (with and without traumatic brain injury [TBI]). Characteristics of NMRC's controlled and uncontrolled HS models included routine soft tissue injury, prehospital resuscitation that was not pressure-controlled but was bolus infusion-guided by both MAP and heart rate [HR], and subsequent in-hospital resuscitation guided by additional parameters (MAP, HR, and Hb in all studies; as well as LA and CPP in the HS/TBI model.[18] LA/BD were equivocal in the less severe HS models [12, 20, 21], but significantly improved with HBOC-201 in more severe HS models. [17, 18] UNC-CH and UCSF investigators also showed improvements in their more severe HS models submitted. [22-25] OBRR's reference to *slower LA clearance* refers to a HBOC-301 study (not HBOC-201) in a HS/TBI model in Dr. K. Proctor's laboratory. [26] In that HBOC-301 study, LA clearance was slower when additional fluids were not provided but outcome was better with HBOC-301 than standard fluids.

- Improved survival with HBOC-201 treatment has been shown in a *number of animal models* and in a *variety of species* (i.e., swine, rats, and dogs) ([Table 8] Preclinical Summary [5.c]).
- The less polymerized veterinary equivalent of HBOC-201, HBOC-301 (Oxyglobin®), is FDA-approved for treatment of anemia in dogs.
- The critical mass of HS preclinical data are from swine models, considered appropriate for prediction of human responses in HS.

Assumption #4: As efficacy data from preclinical *in vitro* and animal studies and prior clinical trials show that HBOC-201 effectively carries and transports oxygen, similar effects are predicted for subjects enrolled in *RESUS (prospect for direct benefit* and *potential to provide direct benefit to human subjects* [21 CFR 50.24]).

- *In vitro*, HBOC-201 transports oxygen more efficiently than RBC. [35, 36]
- In *in vivo* animal HS studies, HBOC-201 increases tissue oxygenation, and decreases anaerobic metabolism, blood LA, and base deficit (BD). [17, 18, 20, 21, 24, 25, 27-29, 32]
- In Phase 1 exercise clinical trials, HBOC-201 was shown to be an effective oxygen carrier[20].
- In the HEM-0115 trial, blood transfusion avoidance was observed in 59% of subjects at 42 days and in > 95% in the first 24 hours (note: HBOC-201's half-life is 19 hours).
- In the HEM-0125 trauma trial, interim analysis reveals a trend to decreased RBC transfusion requirements (5.4 $\pm$ 1.2 units/HBOC-201 subject vs. 16.8 $\pm$ 5.7 units/RBC subject, $p = 0.08$).

Assumption #5: In the prior Phase 3 HEM-0115 trial, the AE profile of HBOC-201 was inferior to that of RBC in the overall population.

- In a comparison of HBOC-201 and RBC in the overall population of subjects enrolled in the elective orthopedics HEM-0115 trial, incidences of overall AEs and SAEs, cardiac AEs and SAEs, and cerebral ischemic AEs were higher in HBOC-201 than RBC subjects (Appendix B).

[20] In a randomized, single-blind, two-way crossover study (i.e., study M9990-0062) healthy volunteers subjected to submaximal bicycle exercise stress test (BEST) to an aerobic threshold were able to perform the same level of exercise following hemodilution and after receiving one third the amount of Hb in the form of HBOC-201 compared with RBC. HBOC-201 subjects displayed consistently lower HR and LA levels than controls. [37]

Assumption #6: Safety data in overall populations in prior HBOC-201 surgery/orthopedics trials are unlikely to accurately predict benefit:risk in *RESUS* (*Risks…are reasonable in relation to what is known about the medical condition* [21 CFR 50.24]).

- Efficacy and safety data from prior HBOC-201 trials are unlikely to predict benefit:risk in the *RESUS* study accurately because of different clinical settings (elective surgery/orthopedics vs. acute HS); populations (older vs. younger adult populations); exposures (prolonged blood transfusion substitution vs. short oxygen bridge); physiologic states (hemodynamically stable vs. unstable); comparators (gold standard RBC transfusions vs. suboptimal crystalloid fluid); and most notably, potential benefit (transfusion avoidance vs. survival).

Assumption #6a: Even if one assumes prior HBOC-201 surgery/orthopedic trials accurately predict benefit:risk in the *RESUS* study (as suggested by OBRR), safety data in overall populations still predict *reasonable* risk in *RESUS* (*Risks…are reasonable in relation to what is known about the medical condition* [21 CFR 50.24]).

- Group differences in key safety signals were *relatively* low in HEM-0115 when considered in the context of high mortality in severe HS with standard care and potential for survival benefit with HBOC-201 (i.e., *reasonable in relation to what is known about the medical condition…* (and) *risks and benefits of standard therapy* [21 CFR 50.24]) (Appendix B).

Assumption #6b: Even if one assumes that prior HBOC-201 surgery/orthopedic trials accurately predict benefit:risk in the *RESUS* study (as suggested by OBRR), group differences of key adverse safety signals were narrowed or nonexistent in younger sub-populations more closely resembling the younger *RESUS* study population, further predicting *reasonable* risk in *RESUS* (*Risks…are reasonable in relation to what is known about the medical condition* [21 CFR 50.24]).

- Group differences (delta) in key adverse safety signals were generally reduced in < 70 in comparison with ≥ 70 year old subjects in HEM-0115 (Appendix B).
- As the AE profile of all HBOCs tested in advanced clinical trials has been inferior in older in comparison with younger subjects (DCLHb, HemAssist®,[15]; *o*-raffinose polymerized Hb [Hemolink®, [38]; human polymerized Hb [PolyHeme®]; and HBOC-201), exclusion of elderly subjects can be predicted to improve the potential benefit:risk equation in *RESUS*.

Assumption #7: Interim data from the ongoing HEM-0125 S. Africa trauma trial, comparing HBOC-201 and essentially "high-bar" RBC transfusions for resuscitation from HS in the ER, show equivalent mortality and a favorable safety profile, further predicting *reasonable* risk in *RESUS* (*Risks…are reasonable in relation to what is known about the medical condition* [21 CFR 50.24]).

- Mortality rates are equivalent.
- There are trends to decreased AEs/subject, SAEs/subject, and fluid and blood transfusion requirements in subjects treated with HBOC-201.
- A DSMB has recommended the continuation of the trial (Appendix E).

Assumption #8: Safety data from prior trauma trials with the first generation HBOC, DCLHb, are unlikely to accurately predict benefit:risk in *RESUS* (*Risks…are reasonable in relation to what is known about the medical condition* [21 CFR 50.24]).

- HBOC-201 can be presumed to be less vasoactive than DCLHb. [12, 13, 39]
- Different trial designs[21].
- Improved understanding of vasoactivity has led to incorporation of strategies to maximize benefit and minimize vasoactivity risk in the *RESUS* study.

Assumption #9: Preclinical and clinical data support *RESUS Dosing Guidelines* (*prospect for direct benefit* and *potential to provide direct benefit to human subjects* [21 CFR 50.24]).

- Multiple indication-specific HS preclinical studies reported improved outcome with individual and maximum doses and rates of infusions similar to or higher than proposed in *RESUS*.
- In the HEM-0115 Phase 3 orthopedics trial, the relative safety of HBOC-201 was improved in subjects receiving the maximum dose proposed in *RESUS*.

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[21] In the U.S. in-hospital trial in which DCLHb was compared with normal saline (NS) as an adjunct to blood transfusions, outcome was worse with DCLHb. [40] NMRC believes the outcome was predictable because there was minimal potential benefit with all the attendant risks of the HBOC in that trial. Because of different HBOC vasoactivity profiles and different trial designs, NMRC believes that the in-hospital trial has little relevance to prediction of benefit:risk in *RESUS*. In the European HOST trial, in which DCLHb was compared with NS for prehospital resuscitation in HS (blood transfusions unavailable), mortality was not significantly different.[41] Because of different HBOC vasoactivity profiles, the HOST trial also has little *RESUS*-relevance, but its equivocal results do not alter our prediction of *reasonable* risk in *RESUS*. The current prehospital HS trial, comparing the second generation HBOC, PolyHeme® (Northfield Laboratories, Evanston, IL), with saline, completed enrollment of > 700 subjects without reaching a safety stopping criterion. The PolyHeme® trial targeted an overall HS population. Neither the prehospital HOST nor PolyHeme® trial predicts *unreasonable* risk in *RESUS*.

- An interim analysis of indication-specific HS clinical data from the HEM-0125 S. Africa trauma trial, comparing HBOC-201 and RBC in the ER, reveals an equivalent safety profile with individual and maximum doses and rates of infusions similar to or higher than proposed in *RESUS*.

Assumption #10: Incorporation of multiple strategies to minimize risk in the *RESUS* protocol has further diminished risk to subjects in the *RESUS* study (*Risks…are reasonable in relation to what is known about the medical condition* [21 CFR 50.24]).

- Multiple strategies have been incorporated in the *RESUS* protocol design to maximize potential benefit to subjects and to minimize risk. These approaches *maximize benefit* by optimizing target population selection. These approaches *minimize risk* via standardization of clinical care with practice guidelines and extensive training; allowance for concomitant standard care; and comprehensive surveillance methods for early detection and action regarding adverse safety signals. *RESUS* risk mitigation strategies, many of which were recommended by OBRR or were in response to OBRR concerns, are summarized below:

  a. **Targeting a population with severe HS and without access to blood transfusions.**
     i. Maximizes benefit by enrolling a population with high mortality but without access to optimal resuscitation; this minimizes risk.
  b. **Exclusion of elderly subjects (≥ 70 years old).**
     i. Minimizes risk by excluding the older population which was shown to have the highest incidence of clinically significant SAEs in prior HBOC-201 trials and which had similar safety concerns in clinical trials with other HBOCs. [15, 38] Maximizes predicted benefit by targeting a younger adult population analogous to the young animals studied in preclinical HS studies.
  c. **Hypotension and tachycardia criteria for re-infusion of HBOC-201.**
     i. Maximizes benefit by including fluid re-infusion criteria demonstrated to be sensitive with HBOC-201 in preclinical HS studies. [12] This minimizes risk of inadequate fluid resuscitation and secondary hypoperfusion.
  d. **Thorough EMS and trauma center training.**
     i. Maximizes benefit, minimizes risk, and standardizes care by education about optimal practice guidelines (*prehospital fluid re-infusion guidelines*

and *in-hospital trauma care guidelines*), including risk mitigation strategies.

e. **Access to standard IV fluids during the prehospital period.**
   i. Minimizes risk of inadequate fluid resuscitation and secondary hypoperfusion.

f. **Improved standardization of prehospital care.**
   i. Maximizes benefit via optimal practice guidelines, and minimizes risk of inadequate fluid resuscitation and secondary hypoperfusion.

g. **Access to standard blood transfusions immediately upon availability.**
   i. Minimizes risk of anemia and secondary tissue hypoxia (inadequate efficacy) and side effects (safety) by *minimizing exposure time* to test drug and allowing rapid access to standard care.

h. **Standardization of in-hospital care with guidelines for fluid infusions, blood transfusions, inotropic support, and other supportive care and HBOC-201 issues (short T½, methemoglobinemia, cellular mass, elevated BP responses, colloidal properties/fluid overload, and TBI) (Appendix D).**
   i. Minimizes risk by optimizing care with standard evidence/literature-based guidelines.
   ii. Minimizes risk of inadequate fluid resuscitation and secondary hypoperfusion AEs (e.g., oliguria).
   iii. Minimizes risk of inadequate blood transfusion resuscitation and secondary anemia.

i. **Prospective *increased BP* and *hypertension* coding definitions.**
   i. Minimizes risk by promoting efficient regulatory AE reporting, allowing appropriate action.

j. **Extensive secondary outcome measurements.**
   i. Minimizes risk by comprehensively following safety signals, allowing appropriate action.

k. ***Elevated blood lactate* relative stopping criterion.**
   i. Minimizes risk by stopping the trial if there is evidence of hypoperfusion (in absence of survival benefit).

l. **HBOC-201 infusion stopping criterion (SBP $\geq$ 120 mm Hg).**
   i. Minimizes risk by diminishing likelihood of uncommon idiosyncratic severe BP responses.

m. **Expedited AE reporting to DMC and FDA.**
    i. Minimizes risk by ensuring efficient regulatory AE reporting, allowing appropriate action.
n. **Hypoperfusion markers reports.**
    i. Minimizes risk by comprehensively following *potentially vasoactivity-related* hypoperfusion safety signals (i.e., LA, BD, and fluid infusion volumes) and ensuring efficient regulatory AE reporting, allowing appropriate action.
o. **Early *Efficacy and Safety Reviews* (*ESR*s) (interim analyses).**
    i. Minimizes risk by ensuring early interim analysis of safety data and regulatory reporting, and allowing appropriate action.

Assessment of Relative Risk

NMRC maintains that data from prior surgical/orthopedics HBOC-201 trials do not accurately predict relative risk of clinically relevant SAEs in *RESUS* because anticipated physiologic and survival benefits in *RESUS* are not accounted for in surgical/orthopedic HBOC-201 trials. Consequently, consistent with statutory requirements that *Risks…are reasonable in relation to…risks and benefits of standard therapy* (21 CFR 50. 24), assessment of *relative* risk is key to NMRC's prediction of overall benefit:risk in *RESUS*. In prior HBOC-201 trials, SAE incidence was higher in subjects treated with HBOC-201 than with control fluid because the comparator was mainly RBC transfusions (a high bar) and the study population was one with known potential safety issues (i.e., mainly older adults); clearly, the group difference in SAE incidence was narrowed in younger sub-populations. Although absolute incidence of SAEs is likely to be higher in HBOC-201 (and control) patients in *RESUS* than in prior trials (due to higher patient acuity), it is reasonable to predict that group differences will be reduced or reversed in *RESUS* because preclinical studies suggest significant benefit, the comparator will be suboptimal non-oxygen carrying LR (a low bar), and the study population will be one predicted to have minimal safety issues (i.e., mainly younger adults). That the SAE profile of HBOC-201 is equivalent with RBC (a high bar) in an interim analysis of data from the ongoing HEM-0125 trauma trial (in mainly younger adults), provides compelling reassurance that relative risk is likely to be low in *RESUS*.

NMRC believes that all 21 CFR 50.24 requirements have been met, if not exceeded, notably (related to benefit:risk):

- ***human subjects are facing a <u>life-threatening situation</u>.***
    - NMRC believes that predicted mortality of more than 1 in 2 in the *RESUS* target population receiving standard care equates with a *life-threatening situation* and *unsatisfactory* treatments.
- ***<u>available treatments</u> are unproven or <u>unsatisfactory</u>.***
    - NMRC believes that predicted mortality of more than 1 in 2 in the *RESUS* target population receiving standard care equates with *unsatisfactory* treatments.
- ***research holds out the <u>prospect of direct benefit</u> to the subjects.***
    - NMRC believes that *in vitro* and *in vivo* animal studies, showing improved tissue oxygenation, decreased anaerobic metabolism, and deceased blood transfusion requirements[22], support *prospect* of *benefit*.
- ***<u>preclinical studies</u>…<u>support the potential</u>…to provide a direct <u>benefit</u>.***
    - NMRC believes that preclinical HS studies, showing high survival benefit[23], support *potential* for *benefit*.
- ***<u>Risks</u> associated with the intervention <u>are reasonable</u> <u>in relation</u> to what is known about <u>the medical condition</u>.***
    - NMRC believes that *risks…are reasonable* in the context of what is *known about the medical condition* when potential benefit and risk are considered, as shown using the following semi-quantitative assessments of risk. [45]
    - Analysis #1 utilized overall SAE data from the overall population and < 70 year old sub-population in HEM-0115 trial, overly conservatively equating death and SAE occurrence as clinically equivalent. Nevertheless, the analysis shows that a 15% mortality reduction would translate to 48 fewer deaths at the possible expense of an excess of 34-43 (6.1-7.7%) of 1,108 subjects in *RESUS* experiencing ≥ SAE. The data predict that the number needed to treat (NNT) (to save an additional life) may be 11.5, and the number needed to harm (NNH) (to cause an additional subject to experience an SAE) may be 13-17. Despite the inaccurate equating of death and SAEs as clinically equivalent, the *benefit:risk ratio* (*BRR*) is 1.2-1.5, predicting *favorable* benefit:risk.

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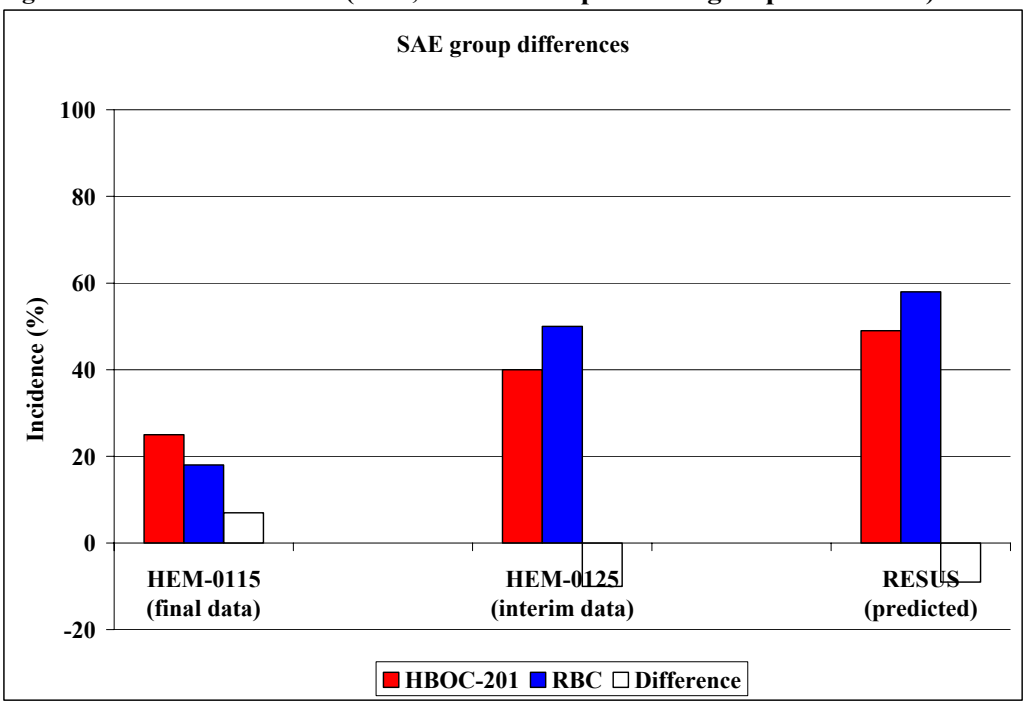
[22] Transfusion avoidance has also been documented in human clinical trials.

[23] Preclinical swine studies show survival benefit effect sizes of 74.6% overall in HS ($p < 0.0001$) and 82% in severe HS ($p < 0.0001$) (Table 6) and improved perfusion in severe HS (improved tissue oxygenation and decreased LA and BD) (vs. standard fluids). No animal model consistently predicts human responses but swine are considered appropriate models for cardiovascular physiology studies. [42-44] Individual studies have model design- and *RESUS*-relevance limitations, but in aggregate, preclinical data support our hypothesis for a 15% improvement in survival in *RESUS* with a large cushion (margin of error of ~ 5-fold).

- Analysis #2 utilized overall SAE data from the overall population and < 70 year old sub-population in HEM-0115 trial, attempting to account for disparity in the clinical significance of death and SAE occurrence by calculating an *Excess SAE Score* (*ESS*) which rates the number of excess SAEs that would be tolerable by patients and physicians in order to save a life. The analysis shows that the *ESS* is 0.71-0.92 (predicting *highly favorable* benefit:risk); that is, for every life saved in *RESUS*, 0.71-0.92 excess subjects might experience an SAE. NMRC concludes that the prospect of less than one subject experiencing an additional SAE for every life saved in a population with ~ 1:2 risk of death, exceeds the 21 CFR 50.24 requirement for *reasonable* risk[24].Analysis #3 utilized all SOC SAE category data from the overall population and < 70 year old sub-population in HEM-0115 trial, attempting to more comprehensively account for disparity in the clinical significance of death and SAEs. SOC SAE category data from *matching subgroups* in HEM-0115 and from the ongoing HEM-0125 S. Africa ER trauma trial were also assessed. The assigning of clinical relevance to the SOC SAE category datapoints in terms of *mortality equivalents* resulted in higher NNH calculations (i.e., 69 and 83.3 in the overall population and < 70 year old sub-population, respectively). The analysis showed respective *BRR values* of 5.8 and 7.2. Analysis of the worse-case scenario high hemoglobin need *matching subgroups* in HEM-0115 still revealed *BRR* values of 2.9-3.4. As there is no extrapolatable risk from the HEM-0125 trauma trial, the *BRR* is ∞. Analysis of the robustness of a *favorable* outcome (*BRR* ≥ 1.0), indicated that benefit outweighed risk over a large variation in parameter estimates even for the worse-case scenario estimate. NMRC believes that this objective, comprehensive, and *RESUS*-relevant semi-quantitative benefit:risk analysis, showing *BRR* values with robust 3-7 fold margin of error cushions above *equipoise*, demonstrates highly favorable benefit:risk prediction for the *RESUS* trial.

[24] The survival benefit estimate (and NNT) is based on preclinical HS data (i.e., 15%). The risk estimate is based on group differences (delta) in overall SAE incidence in the overall enrolled population in the Phase 3 HEM-0115 trial. We believe the NNH estimate is conservative because relative risk (delta) is expected to be lower in the *RESUS* study in which the comparator is LR and not RBC transfusions.

Figure 1: Incidence of SAEs (final, interim and predicted group differences)



Conclusion

In summary, HBOC-201 is predicted to improve clinical outcome of subjects enrolled in the *RESUS* study. This is expected because HBOC-201 efficiently transports and unloads oxygen; an extensive indication-specific preclinical database with HBOC-201 predicts high benefit:risk; clinical data from prior studies for other indications show a relatively small adverse shift in the HBOC-201 AE profile in comparison with the gold standard (RBC transfusion) even in the older enrolled overall population; this small negative difference was reduced in younger subjects more closely resembling subjects to be enrolled in the *RESUS* study; and interim safety data from the ongoing ER trauma trial in mainly younger subjects suggest at least equivalence with RBC transfusions. Even if the AE profile observed in prior HBOC-201 trials among younger subjects, in which HBOC-201 was compared with RBC is observed in the *RESUS* study, the potential benefit of increased survival outweighs risk of potentially increased incidence of common non-serious AEs and uncommon SAEs.

NMRC believes that prior HBOC-201 trials predict a real but acceptable (reasonable) level of risk in the *RESUS* study. NMRC further believes that risk has been significantly minimized by protocol design modifications made during IND discussions with OBRR; and when considered in the

context of predicted 58.1% mortality with standard therapy (i.e., LR) in the *RESUS* study, potential risks are overshadowed by potential beneficial effects on survival predicted from a wide variety of preclinical HS studies.

NMRC hopes that the BPAC will consider recommending to OBRR a lifting of the *RESUS* Clinical Hold. Alternatively, NMRC hope that the BPAC will make recommendations about protocol/IND modifications necessary for lifting of the *RESUS* Clinical Hold.

4.0 BACKGROUND

The initial physiological response to HS is a reflex release of catecholamine vascular mediators intended to redistribute blood flow to critical vascular beds such as the heart and brain at the expense of less critical vascular beds such as mesentery and integument. [46] Diminished perfusion to various tissues creates an ischemic environment resulting in the production of LA and oxygen free radicals which in turn directly cause cellular damage. Ischemic cells also release a variety of inflammatory factors: prostacyclin, thromboxane, prostaglandins, leukotrienes, endothelin complement, interleukins, tumor necrosis factor, and others.[47] A cumulative perfusion deficit and ongoing cellular damage with inadequate or no resuscitation initiates an oxygen debt that has been inversely correlated with survival.[48-50]

The current standard of care for prehospital treatment of HS continues to rely on attempts at external hemostasis in concert with rapid intravenous administration of non-oxygen carrying crystalloid fluids (e.g. LR or saline). However, decreasing further blood loss and restoring circulating volume alone may be insufficient for reversing effects of significant oxygen debt, as release of toxic by-products upon reperfusion of ischemic vascular beds impairs systemic recovery. Crystalloid resuscitation may in fact exacerbate these same metabolic problems due to hemodilution and resultant persistent tissue hypoxia, secondary anaerobic metabolism, refractory shock, and organ failure. The longer the period of shock, the greater the potential for ARDS, MOF, sepsis, and death. Blood transfusions restore blood oxygen content and may be life-saving but are usually unavailable in the prehospital setting. Thus, optimization of early resuscitation techniques, especially those aimed at restoration of tissue oxygenation, is imperative in order to decrease morbidity and mortality associated with HS.

Upon onset of the *War on Terrorism* after *911*, Navy researchers appreciated that U.S. forces would face hostile action and suffer significant numbers of combat casualties, but optimization of *Force Protection* (preventive) and field resuscitation (therapeutic) measures could minimize morbidity and mortality of injured *Warfighters*. Improved body armor increased *Force Protection*, but field resuscitation had not changed significantly since the Vietnam War. As hemorrhage accounts for the preponderance (~ 60%) of potentially salvageable combat casualty (and also civilian trauma) mortality[4], improvements in hemostatic techniques to diminish massive hemorrhage (e.g., fibrin and other bandages, recombinant factor VIIa), and resuscitative fluids to treat the consequences of massive hemorrhage, HS, were targeted. As blood transfusions improve survival in severe HS but are usually unavailable prior to evacuation to the hospital, and current standard resuscitative fluids

do not carry oxygen, alternative oxygen carrying resuscitative fluids (HBOCs, often called *blood [hemoglobin] substitutes*) were considered.

Selection of HBOC-201

A U.S. in-hospital Phase 3 trial with the first generation HBOC, DCLHb (Baxter Healthcare, Round Lake, IL), was discontinued prematurely after demonstration of worse outcome [15], but results were equivocal in the concomitant European prehospital Phase 3 HOST trauma trial where blood transfusions were unavailable.[41] Adverse outcome with DCLHb has been attributed to complications related to vasoactivity, a characteristic side effect of HBOCs, mainly although not entirely due to NO binding. Low mw components of HBOCs (especially tetrameric Hb [64 kD]) have been linked with vasoactivity properties; DCLHb is 100% tetrameric.

Second generation HBOCs have been developed using polymerization and conjugation techniques to enlarge molecular size (decreasing tetrameric Hb), thereby increasing vascular retention and decreasing extra-endothelial extravasation where NO binding occurs. As second generation HBOCs have variable tetrameric Hb levels (1-31%) and potential to be efficacious "fieldable" resuscitative fluids, an objective evaluation and selection process was completed with these fluids. Three second generation HBOCs in advanced stages of development were compared: human polymerized hemoglobin (PolyHeme®, Northfield Laboratories, Evanston, IL), *o*-raffinose polymerized hemoglobin (Hemolink®, Hemosol Inc., Etobicoke, ON), and bovine polymerized hemoglobin (HBOC-201 [Hemopure®], Biopure Corp., Cambridge, MA). HBOC-201 was selected by Navy researchers for further development and clinical evaluation for a prehospital traumatic HS indication (where blood transfusions are unavailable), based on the following rationale:

1. **Logistics:** HBOC-201 can be stored without refrigeration (having a shelf-life of 3 years at 2-30°C and 1.5 years at 2-40°C). Prior clinical trials with HBOC-201 were conducted using non-refrigerated product. HBOC-201 met military (and civilian EMS) field specifications, being a low-volume and -weight, universally compatible, and unrefrigerated resuscitation fluid.
2. **Purification/polymerization:** As tetrameric Hb content was low (< 2-3%), potentially adverse vasoactivity appeared mild to moderate in severity.
3. **Published database:** As a large preclinical and clinical database was available (published and unpublished), potential benefits and risks could be reasonably assessed.
4. **Preclinical database:** As published and unpublished preclinical indication-specific HS data suggested high survival benefit in swine models (considered reasonably predictive of human

responses), similar benefits could be predicted in humans (i.e., *preclinical studies…support the potential…to provide a direct benefit* [21 CFR 50.24]).

5. **Human experience:** Published and unpublished clinical data from ~ 800 subjects in trials for other indications showed a safety profile considered *reasonable* for prediction of benefit:risk for the planned indication (i.e., *risk is reasonable in relation to what is known about the medical condition* [21 CFR 50.24]).
6. **Independent evaluation:** Critically, the manufacturer assented to an independent *government funded, sponsored, directed, executed, analyzed, and reported trial.*

In summary, HBOC-201 has been shown to efficiently transport oxygen; improve systemic and neurophysiologic parameters and survival in preclinical severe HS studies; have a *reasonable* safety profile in prior surgery/orthopedics clinical trials vis-à-vis the indication sought in the *RESUS* IND; have a favorable safety profile in an interim analysis of the ongoing HEM-0125 S. Africa ER traumatic HS trial; and meet logistical requirements for military field and civilian ambulance deployment. Thus, U.S. Navy (NMRC) researchers hypothesize that HBOC-201 is likely to improve outcome as a prehospital resuscitative fluid for patients with HS in comparison with LR.

5.0 SYNOPSIS OF THE HBOC-201 IND

NMRC assumed responsibility as regulatory sponsor for *RESUS* and initiated Pre-IND deliberations with OBRR in Feb 2004, completed the OBRR-directed swine HS/TBI study in Apr 2005, and submitted the *RESUS* IND application (BB-IND-12504) in June 2005. In accordance with the U.S. Navy regulations, NMRC's Commanding Officer is the FDA Form 1571 signatory**.**

Test drug names and active ingredients

Hemoglobin-Based Oxygen Carrier-201 (HBOC-201); Hemopure®; Hemoglobin glutamer-250 (bovine); polymerized bovine hemoglobin.

Pharmacological Class

Hemoglobin-based oxygen carrier.

5.a Biopure Corporation's Regulatory History

Founded in 1984, Biopure Corporation utilizes proprietary protein purification processes to develop, manufacture, and market hemoglobin-based oxygen therapeutics, namely Hemopure® for use in humans and Oxyglobin® for veterinary applications. To date there have been over 200 preclinical studies in a variety of species, studying safety, pharmacology, pharmacokinetics, toxicology, and pharmacodynamics.

- The company's first IND application was submitted to the FDA in 1988. Biopure subsequently completed 22 clinical trials, mostly for the treatment of surgical anemia. Worldwide, there were 1,512 subjects enrolled of whom 836 received HBOC-201.
- HBOC-301 (Oxyglobin®) is approved in the U.S. and European Union for the treatment of anemia in dogs. The FDA and European Commission approvals were granted in Jan 1998 and Dec 1999, respectively. To date ~ 173,000 units of HBOC-301 have been sold, and ~ 90,000 animals treated.
- In April 2001, the S. African *Medicines Control Council* authorized marketing and sale of HBOC-201 for reducing or delaying need for allogenic RBC transfusion in acutely anemic adults undergoing surgery.
- In mid-2002, Biopure submitted a BLA to the OBRR, seeking U.S. regulatory approval for treatment of anemia in orthopedic surgery. OBRR responded with a number of questions.

At this time, Biopure is not pursuing an FDA approval of the BLA for the use of HBOC-201 in surgical anemia. Still, the company plans on responding to all of OBRR's questions. Resources involved with the BLA response process have been temporarily redirected towards other more time sensitive activities including support of *RESUS*, the June 2006 submission of the European marketing application, and ongoing planned communications with FDA regarding a potential ischemia trial in the U.S.

- Under a formal *Collaborative Research and Development Agreement* (*CRADA*) authorized in 2002, Biopure is supporting[25] the U.S. Navy's development of the product for prehospital treatment of trauma patients with HS (*RESUS*).
- Still ongoing and in the recruitment phase in S. Africa, is Biopure's Phase 2 single site study to assess safety and tolerability of HBOC-201 in ER trauma subjects.
- Currently ongoing in Europe and S. Africa are Phase 2 safety and feasibility studies in which HBOC-201 is being investigated as a treatment for ischemia. The following studies are actively recruiting patients:

  - *Enhancement of Tissue Preservation during Cardiopulmonary Bypass* – Regulatory and Ethics approval have been received in Greece and United Kingdom; currently under Ethics review in Switzerland.

  - *Wound Healing Patients with Peripheral Vascular Disease & Undergoing Lower Limb Amputation Due to Lower Limb Ischemia* - Regulatory and Ethics approvals have been obtained in United Kingdom and S. Africa.

5.b Chemistry Manufacturing and Control (CMC)

The active biological component in HBOC-201 is Hemoglobin glutamer – 250 a highly purified, cross-linked and polymerized bovine Hb. HBOC-201 is a solution of polymerized Hb molecules with mw ranging from 65 to > 500 kD (average 250 kD); the mw profile is consistent. The partial pressure of oxygen at which 50% of the Hb has bound oxygen (P_{50}) is ~ 40 mm Hg and the Oxygen Equilibrium Curve is hyperbolic. HBOC-201 contains ~ 32.5 g Hb glutamer 250 in modified LR solution (154 mmol/L sodium, 111 mmol/L chloride, 4.1 mmol/L potassium, 1.1 mmol/L calcium, 27 mmol/L LA, and 0.2 g/dL N-acetyl-L-cysteine (antioxidant) in 250 ml water for injection at pH 7.6-7.9.

[25] As HBOC-201 manufacturer and holder of other/prior HBOC-201 INDs, regulatory support as necessary; not financial (the trial is entirely government-financed).

Manufacturing Process

The source material, whole bovine blood is collected at Moyer Packing Company (MOPAC, Souderton, PA). Only appropriately tagged cattle from the herd management program enter the Biopure dedicated Collection Facility. Cattle are stunned using a USDA-approved non-pneumatic captive bolt method.

Up to 15 L of whole bovine blood is collected per animal in a sanitary fashion into separate citrated 20 L collection tanks. A USDA inspector inspects each carcass. After release, the blood is pooled at a nearby Biopure facility (Souderton, PA), plasma proteins are removed by 0.45 µm diafiltration, cells separated and lysed by centrifugation, and Hb separated from cell debris by 100 kD diafiltration to give a cell-free Hb solution (C-500). C-500 is filtered through a sterilizing grade 0.2 µm filter into sanitized vessels.

C-500 is purified by anion exchange chromatography with a pH gradient at the Cambridge, MA facility, the column eluate is concentrated and deoxygenated with a hollow fiber gas exchange cartridge, and then filtered through a sterilizing grade 0.2 µm filter into sanitized vessels to produce the intermediate C-800. C-800 is polymerized with glutaraldehyde, the reaction products stabilized with the reductant borohydride, diafiltered with modified LR to remove excess glutaraldehyde and borohydride, and lower mw Hb species removed by fractionation with 100 kD ultrafiltration to produce HBOC-201.

Control of Starting Materials

Cattle supplier requirements include adequate records, systems, and traceability to document compliance. Only animals < 30 months of age are eligible. Herds originate from the U.S. and animals are traced to their source farm. No animals originate from areas in the U.S. with chronic wasting disease.

Transmissible spongiform encephalitis (TSE) risk analysis has been performed using 2 methods: the BfArM (German Federal Institute for Drugs and Medical Products) and the Pharmaceutical Research and Manufacturers of America (PhRMA) BSE Committee. Using the risk assumptions, the risk per dose of HBOC-201 is calculated to be 1.3×10^{-12}, and 1.3×10^{-11} for 10 doses. PhRMA states that a value of less than 1×10^{-10} represents an insignificant risk. The German system assigns

points, where products receiving ≥ 20 points are considered safe. The score is 22 points for HBOC-201. HBOC-201 is certified by the EDQM.

Controls of Critical Steps and Intermediates

All process variables for each unit operation of the C-500, C-800, and HBOC-201 production have been examined in detail and critical processes identified and tested during validation studies across 9 lots. The basic process chemistry has remained unchanged for 10 years through clinical and manufacturing scale production. The commercial batch size is the same as that used to manufacture clinical batches. Suppliers certify that raw materials are not derived from specified risk materials.

Studies were performed to characterize the product and to confirm that the material to be produced for commercial batches is equivalent to material used in the clinical trials. The analyses included: P50, SDS polyacrylamide gradient gel electrophoresis, isoelectric focusing gel electrophoresis, high performance size exclusion chromatography, circular dichroism spectroscopy, electronic absorption spectroscopy, reverse phase HPLC, laser desorption mass spectrometry, and amino acid analysis.

Specifications

Validation of analytical methods has been performed according to FDA and ICH guidelines.

Initial specifications were chosen based on early development work. Specifications were subsequently tightened when supported by process data from 40 lots of product (mean ± 3 SDs, N = 40 lots) and stability data. Assay variability was also taken into account. Reference standards used HBOC-201 analyses have been qualified following QC testing and characterization of the substance.

Table 5: HBOC-201 Specifications

HBOC-201 Specifications		
Test	**Specification**	**Method**
Total hemoglobin	12.0 – 14.0 g/dL	Co-Oximetry/spectrophotometry
Methemoglobin	≤ 5%	Co-Oximetry/spectrophotometry
Oxyhemoglobin	≤ 10%	Co-Oximetry/spectrophotometry
Sterility Test	Meets Test	Sterility (USP)
Endotoxin	≤ 0.02 EU/ml	Endotoxins (USP 29 <85>)
Glutaraldehyde	≤ 0.1 μg/ml (100 ppb)	HPLC
N-Acetyl-L-Cysteine	0.02 – 0.22%	HPLC
Integrity/Appearance	Intact/Deep Purple	Visual
Molecular Weight Distribution*	> 500,000: ≤ 18.0%	HP-SEC
	≤ 65,000: ≤ 5.0%	
Total Elemental Boron	≤ 5 ppm	Inductive coupled plasma
pH	7.6 - 7.9 at 18-22°C	Potentiometric
Sodium	145 – 160 mmol/L	Ion selective electrode
Chloride*	105 – 117 mmol/L	Ion selective electrode
Potassium*	3.7 – 4.5 mmol/L	Ion selective electrode
Calcium*	0.8 – 1.3 mmol/L	Ion selective electrode
Osmolality	290 – 310 mosmol/Kg	Freezing point depression
P_{50}	34-46 mm Hg	Hemox analyzer
General Safety	Pass	USP

*Specifications tightened since the original BLA and IND filings.

Stability

Eleven recent lots have been on long term (36 month) storage at up to 30°C. Recent lots still on test are at 30°C/35% RH in full accordance with ICH guidelines. Four lots have also been stored for nine months at 40°C/15% RH in addition to 40°C/75% RH as well as between 2-30°C. These data support an expiry period for HBOC-201 of 36 months from the date of manufacture when stored at 2-30ºC in the infusion bag with a foil overwrap (gas impermeable pouch).

Container Closure System

The final product container for marketed product is a 250 ml sterile infusion bag from Stericon. Infusion bags meet USP physico-chemical test standards for plastics and all plastic materials meet the requirements for USP Class VI plastics. The adhesive meets requirements of 21 CFR 175.105, Adhesives. Toxicological testing performed on the Stericon 250 ml infusion bag includes USP Plastics Class VI, cytotoxicity, and hemolysis. In order to protect filled infusion bags from oxygen, a functional secondary packaging material is required. This is achieved by employing a gas impermeable pouch made from polyester/aluminum/polyethylene film.

Facilities and Equipment

The Separation Plant (~ 18,000 sq. feet) is located ~ 1 mile from the collection area at Moyer Packing Company (MOPAC) in Souderton, PA. The facility includes a clean-out-of-place area, cold storage, quality control laboratories, process area, employee locker rooms and restrooms, utility area, and warehouse areas. The Cambridge, MA, manufacturing plant is a two-story, steel I-beam constructed building. The 1st floor (21,500 sq. feet) contains chromatography columns, polymerization plant, fractionation area, fill suite, plant utilities, and Reverse Osmosis/De-ionized Water system.

Room finishes are smooth, cleanable, water and chemical resistant, and free of flaking. Lighting in the process area is recessed, clean room style, fully gasketed, and hermetically sealed. The facilities have two air handling systems. One system is dedicated to unclassified and non-process areas. The 2nd separate air handling system is dedicated to areas with a controlled classified environment. This separate dedicated air handling system for the process area is provided with HEPA filtration, high room air change rates, and differential air pressure design. Pressure distribution within the facility is designed to provide a cascade effect, preventing air from lower air classification areas from entering areas with higher air classification. The facilities have been designed with flows for materials, product, personnel, and waste to avoid the potential for cross contamination. Access is controlled by an electronic cardkey system, and personnel enter and exit the facility via separate airlocks.

Two systems are used in the cleaning of equipment, a Clean-Out-Of-Place system (COP) used to clean portable process vessels and filter skids, and a Clean-In-Place (CIP) system used to clean permanently installed process vessels such as process and buffer vessels, as well as their associated pumps and piping. The products are proteinaceous solutions produced with dedicated equipment, the purity of which increases with processing. As such, cleaning is facilitated by careful attention to measures to prevent proliferation of microbial contamination and accumulation of endotoxin residuals, carried forward in the process. The primary cleaning agent utilized is Sodium Hydroxide, known to breakdown complex proteins, without need for supplemental detergents or surfactants.

At the Cambridge site, Water for Injection (WFI) is used as the primary source of water for making buffers, direct product contact, and final rinse after cleaning. The System utilizes distillation to provide a continuous source of WFI that meets USP specifications. Hot WFI loops in both the Purification and Polymerization Process Areas are maintained at ≥ 80°C.

No other products are manufactured at the Souderton site and thus all equipment in the facility is considered dedicated to a single product. C-500 is sourced from no other location. Only HBOC-201 and HBOC-301 are manufactured at the Cambridge site.

Transmissible Spongiform Encephalitis (TSE) Agents

The capacity of the manufacturing process to clear infectious TSE particles was demonstrated in two separate studies. The original study, RDR-0113: measured individual clearance factors for murine adapted scrapie, study RDR-321: used a hamster adapted scrapie strain

Table 6: TSE Clearance Factors

TSE Clearance Factors (Log Reduction)		
Process Step	**Study # RDR - 0113**	**Study # RDR - 321**
	Removal Factor (Log_{10})	
100 kD Diafiltration	2.6	> 3.9
Anion Exchange Chromatography	≥ 3.0	> 3.0
Overall	≥ 5.6	> 6.9

Key findings:

- Significant log reduction (≥ 5.6 for RDR-0113 and > 6.9 for RDR-321).
- 2 robust clearance steps.
- No lifetime clearance degradation for 100 kD diafiltration membranes or Anion Exchange Chromatography media.
- No carryover in subsequent diafiltration cycles.
- No carryover in subsequent chromatography cycles.

Viral Adventitious Agents

The capacity of the manufacturing process to clear infectious particles has been evaluated. Biopure performed several separate viral studies. Key findings are:

- Raw Material (bovine blood) was found to be free of 7 specific viruses.
- Significant log clearance of both enveloped and non-enveloped viruses exceeding guidelines.
- Three robust clearance steps (> 4 logs clearance each).
- No lifetime clearance degradation for 100 kD diafiltration membranes or Anion Exchange Chromatography media.
- No viral carryover in subsequent chromatography cycles.
- Virus washes out in chromatography cleaning cycle.
- Virus inactivation in both cleaning solutions and the glutaraldehyde step reaches maximum value within the time period studied.
- Significant viral inactivation in filter NaOH and NaOCl cleaning solutions.

Table 7: Viral Clearance Factors

Viral Clearance Factors (Log Reduction)					
Process step	**Log Reduction Virus ***				
	BPV	Reo3	XMuLV	PRV	BVDV
100 kD	4.1	≥ 5.7	≥ 4.7	≥ 6.5	≥ 5.2
Anion Exch.	≥ 5.3	≥ 4.3	≥ 4.3	≥ 4.8	≥ 4.5
Glutaraldehyde.	4.0	≥ 4.3	≥ 3.8	≥ 5.5	≥ 6.4
Total	≥ 13.4	≥ 14.3	≥ 12.8	≥ 16.8	≥ 16.1

* Bovine Parvovirus (BPV), Reovirus type 3 (Reo 3), Xenotropic Murine Leukemia Virus (XMuLV), Pseudorabies virus (PRV), and Bovine Viral DiarrheaVirus (BVDV)

5.c *RESUS* IND Preclinical Studies

Summary of preclinical HS studies

In multiple preclinical HS studies, in comparison with crystalloid and colloid fluids, HBOC-201 resuscitation improved overall outcome. As expected, HBOC-201 increased blood oxygen content and tissue oxygenation. Consequently, HBOC-201 ameliorated anaerobic metabolic processes equivalently with asanguinous resuscitation fluids in mild to moderate HS, but better with more severe HS. Mild and probably clinically insignificant methemoglobinemia (< 5%) was seen. Mainly due to vasoactivity, physiologic effects were seen with significantly lower fluid volumes. As well, blood transfusion requirements were generally lower with HBOC-201. Organ regional blood flow was generally stable but decreased in a minority of studies. Mild to moderate vasoactivity led to more rapid resolution of systemic and pulmonary hypotension, sometimes causing mild to moderate relative systemic and pulmonary hypertension and secondary relative bradycardia and decreased CO. Although HBOC-201 resuscitation led to relatively lower CO in mild to moderate HS, this did not generally occur in severe HS, and almost invariably, CO did return to baseline. Despite HBOC-201's vasoactive properties, evidence for increased hemorrhage was not seen in uncontrolled HS studies due to severe liver injury (with or without concomitant TBI) conducted at University of North Carolina-Chapel Hill (three studies) and at NMRC (two studies). Hemorrhage was increased in pigs resuscitated with normotensive but not hypotensive strategies with HBOC-201 in an uncontrolled HS study due to iliac vessels injury conducted at USUHS, but this study was poorly conducted (had multiple protocol deviations). A few hemostasis studies have shown that HBOC-201's fluid sparing properties resulted in decreased hemodilution and relatively mild variable and probably clinically insignificant effects on coagulation and platelet function. Overall, in these

studies, mild thrombocytopathy was decreased during simulated prehospital time (mainly due to decreased fluid requirements and hemodilution), and mild coagulopathy/ thrombocytopathy was increased during simulated hospital time (mainly due to decreased blood cellular components [hematocrit] due to decreased blood transfusion requirements but possibly also intrinsic properties of HBOC-201). RBC, whole blood, and blood component transfusions after ER arrival are expected to nullify these effects. Most importantly, HBOC-201 resuscitation generally led to improved short- and long-term survival, with benefits being especially significant with more severe HS and longer simulated prehospital transportation time.

Studies on effects on morbidity have generally shown favorable or equivalent results, but some stereotypical side effects were seen. Evaluation of specific organs in HS studies (and coronary artery occlusion), have generally shown equivocal or potentially beneficial effects on myocardial histopathology with variable effects on cardiac enzymes (similar troponin but elevated CK-MB). Effects on pulmonary, jejunal, hepatic parenchymal, and renal cortical and medullary histopathology have not been different than with asanguinous resuscitation fluid. However, mild hepatobiliary changes (possibly due to biliverdin turnover) with transiently elevated LFTs and renal papillary changes (possibly due to fluid sparing and/or vasoactivity) usually with minimal elevation of BUN and creatinine and with variable effects on urine output were seen in one study. Importantly, these renal papillary changes were not reported in other histopathology studies.

HBOC-201's ability to increase tissue oxygenation has been purported to have potential to result in oxidative stress and increase risk of organ reperfusion injury. But assays for organ tissue nitrosylation (surrogate marker for peroxynitrate formation) have shown similar levels in HBOC-201 and asanguinous resuscitative fluids. Additionally, with the minor exceptions noted above, histological analyses have not shown significant worsening of organ tissue pathology. That redox stress and reperfusion injury have not been shown to be worsened by HBOC-201 may be due to measurement of insensitive surrogate markers or tissues, the hypothesis may be erroneous in the 1st place, or HBOC-201's ability to rapidly reconstitute homeostatic physiology and tissue oxygenation may preclude a sufficiently long period of tissue hypoperfusion and oxygenation necessary for development of reperfusion injury. Additionally, absence of significant immunological activation may contribute to these results. *In vitro*, HBOC-201's effects on PMN adhesion marker expression and oxidative burst were comparable to standard resuscitative fluid at usual concentrations. In mild to moderate HS, blood PMN adhesion marker expression (CD11 and CD18), lymphocytic apoptosis (annexin V/PI), and Th1:Th2 cytokines (similar IL-2 and IL-6 but elevated IL-10) were attenuated,

and CD4:CD8 ratio and lymphocyte adhesion marker expression (CD49) equivocal, in comparison with standard resuscitative fluids. Moreover, in mild to severe HS, evidence for increased apoptosis in tissues has not been found. In severe HS, PMN and lymphocytic adhesins increased but it was not possible to distinguish group differences. All in all, immunological studies have shown equivocal and possibly beneficial immunomodulation effects characterized by decreased immunocyte activation and apoptosis. At this point, it is unclear if this extends to severe HS where multiple doses of HBOC-201 are infused.

Four studies evaluated HBOC-201 in the setting of controlled (three studies) or uncontrolled HS (one study) with concomitant TBI. Another controlled HS/TBI study with HBOC-301 showed similar results. Also, in contrast with free human Hb, HBOC-201 does not appear to be toxic to neural cells *in vitro*. Outcome has been improved by HBOC-201 in these studies. Neurophysiologic parameters, including $brPO_2$, SSO_2sat and SSLA, CPP, ICP, mannitol requirements, and CO_2 reactivity, were generally improved. Effects on CBF were more variable but overall not likely to be clinically significantly different. Histopathologic and immunohistochemical evaluation of brain tissue showed equivocal (e.g., neutrophilic infiltration and brain edema) but sometimes beneficial (e.g., intracranial hemorrhage, contusion volume, secondary neuronal injury) changes with HBOC-201. In one swine study, neuronal necrosis and white matter degeneration were slightly increased with HBOC-201, an observation probably related to sufficient survival time only in that group. Overall, systemic and neurophysiology, neurohistopathology, and survival and have been improved in the HS/TBI swine and rat studies but long-term functional neurocognitive outcome was not evaluated.

In summary, in comparisons with asanguinous resuscitation fluids in preclinical trauma studies, HBOC-201 resuscitation has been shown to result in overall clinical improvements in hemodynamics, tissue oxygenation, anaerobic metabolism, neurophysiology and histopathology, fluid and blood transfusion requirements, and survival—especially with more severe HS. Results have been equivocal in terms of effects on regional blood flow, myocardial, pulmonary, jejunal, hepatic parenchymal, and renal cortical/medullary histopathology, blood cardiac biomarkers, bleeding volume in uncontrolled hemorrhage, immune activation, apoptosis, and oxidative stress. Some data suggest myocardial protection in HS, evidenced by less myonecrosis histopathologically and trends to decreased peak troponin levels. Adverse effects have included mild to moderate relative increases in systemic and pulmonary blood pressure and vascular resistance due to vasoactivity, decreased CO in less severe but not in severe HS, mild hepatobiliary changes and

transiently elevated LFTs, mild renal papillary histopathology changes, mild coagulopathic changes mainly due to blood transfusion avoidance, and mild methemoglobinemia. As aforementioned, neurophysiological parameters, neurohistopathology and survival have generally been equivocal or improved with HBOC-201 in HS/TBI studies.

Overall, HBOC-201 has shown beneficial effects on clinical outcome in preclinical animal studies of controlled and uncontrolled HS with and without TBI. Detailed descriptions and results are presented below.

Trauma studies

HS with controlled hemorrhage

1. **Improved reversal of anaerobic metabolism (swine). [29]**

After hemorrhage to MAP of 30 mm Hg and maintenance of hypotension for 45 minutes, resuscitation with HBOC-201 (target MAP 50 vs. 60 mm Hg), LR, and LR + shed blood (SB) was compared. MAP was higher and CO and PCWP lower with HBOC-201. Despite decreased resuscitation volume requirements, LA and BD were lower and more rapidly cleared with HBOC-201 (target 60 mm Hg) than with LR. Mortality was equivalent across groups. The study showed decreased LA and BD with HBOC-201 despite lower resuscitation fluid requirements.

2. Improved brain tissue oxygenation (swine). [51]

Pigs were hemorrhaged to a MAP 40 mm Hg and provided HBOC-201 (n = 6) and high flow oxygen to assess brain tissue oxygenation ($brPO_2$). $brPO_2$ decreased in response to hemorrhage but rapidly returned following resuscitation with HBOC-201 and high-flow oxygen. The authors concluded that in HS, low volume resuscitation with HBOC-201 restores cerebral oxygenation.

3. Liver and deltoid tissue oxygenation comparable to other resuscitative fluids (swine). [52]

In a similar model to Manley [51], Knudson assessed liver and deltoid muscle oxygenation after pigs were hemorrhage to MAP 40 mm Hg and resuscitated with HBOC-201, LR, or hypertonic saline dextran (HSD). HBOC-201 pigs failed to show improvement (nor detriment) in liver or deltoid muscle oxygenation (and high-flow oxygen) vs. LR or HSD (and high-flow oxygen) (N = 10/group). MAP was stabilized more effectively with HBOC-201 but CO was highest with HSD.

The authors concluded that HBOC-201 can be administered safely in small doses and compared favorably to resuscitation with the comparator fluids.

4. Reduced blood LA vs. LR, but mild hepatotoxicity (swine). [31]

After hemorrhage to MAP of 30 mm Hg, 24 pigs were maintained hypotensive for 45 minutes, and then divided into 4 groups (N = 6/group): resuscitation with shed blood to baseline MAP; resuscitation with shed blood for 4 hours to a hypotensive MAP of 60 mm Hg; resuscitation with LR up to 40 ml/kg then shed blood (normotensive); and resuscitation with HBOC-201 (hypotensive). Outcome measurements were followed for 6 hours, during which all animals survived. One animal in the LR + shed blood group died on post-operative day 1. MAP, HR, and pH were similar among groups. CO was lower throughout hypotensive resuscitation in the HBOC-201 group. Jejunal oximetry was similar in all groups. LA was lower with HBOC-201 than LR early post-resuscitation (when compared against LR alone); LA was higher in HBOC-201 than LR animals at 300 minutes (when compared against LR and blood transfusions). Histopathologic evaluation revealed mild hepatocellular damage concomitant with elevated serum AST levels. In conclusion, low volume HBOC-201 resuscitation provided adequate tissue oxygenation, decreased blood LA when compared with LR, but demonstrated potential for mild transient hepatotoxicity.

5. Low-volume reversal of anaerobic metabolism (swine). [53]

After hemorrhage to MAP 30 mm Hg, pigs (n = 6/group) were resuscitated with no fluids, low-volume HBOC-201, hypertonic saline 7.5% (HTS), hypertonic saline 7.5%/Dextran-70 6% (HSD), pentastarch 6%, hetastarch 6%, and LR to MAP 60 mm Hg after 45 minutes. HBOC-201 treated pigs required less fluids, had similar LA, base excess, oxygen consumption, but had lower CO, mixed venous oxygen saturation (SVO_2), and urine output. Survival was 0% in swine resuscitated with HTS, 17% without resuscitation fluid, 83% with HSD, and 100% with HBOC-201 or other fluids. The authors concluded that hypotensive resuscitation with HBOC-201 restored tissue oxygenation and reversed anaerobic metabolism with lower fluid volumes compared to the other fluids. Furthermore, they concluded, *It does not appear that the vasoactive effects of HBOC-201 limit its ability to deliver oxygen in regional circulatory beds during resuscitation in models of controlled HS.*

6. Low-volume reversal of anaerobic metabolism, vasoactivity due to non-NO mechanisms (swine). [54]

After hemorrhage to 20 mm Hg and hypotension maintained for 45 minutes, HBOC-201 resuscitation was compared with shed blood (SB) or LR plus SB (LRSB) in swine (n = 8/group). Hence, both comparators included blood transfusions. Survival, MAP, and MPAP were equivalent across groups, but HR, systemic and pulmonary vascular resistance, and metHb levels were higher, and CO and SVO_2 were lower, with HBOC-201. LA and BD values were not statistically different between groups (there were statistically insignificant trends to higher LA but lower BD with HBOC-201 than in the two comparators with blood transfusions). Thus, overall no significant differences in global measurements of tissue oxygenation were noted. Acetylcholine endothelial-dependent relaxation was decreased but systemic blood NO levels did not differ, suggesting non-NO binding related vasoactivity mechanisms. Low volume resuscitation with HBOC-201 resulted in equivalent outcome in "high bar" comparisons with regimens including blood transfusions.

7. Low-volume reversal of anaerobic metabolism, increased cutaneous tissue oxygenation, and mild coagulopathy (swine). [20]

This was the first NMRC study in a series of hemorrhage-severity escalation controlled HS studies with *simulated delayed prehospital transportation*. In this moderately severe HS model, after 40% EBV (volume-controlled) hemorrhage, swine were resuscitated with HBOC-201 or Hextend with a target MAP of 60 mm Hg (pressure-controlled), or not resuscitated (N = 8/group). Pigs received re-infusions for persistent hypotension or tachycardia and were monitored invasively for a 4-hour *prehospital* period after which hospital arrival was simulated; surgical sites were then repaired, blood or saline provided, and animals were recovered from anesthesia and non-invasively monitored for 72 hours. HBOC-201 resuscitation resulted in higher MAP, MPAP, and transcutaneous tissue oxygenation ($tcPO_2$), but lower CO and fluid requirements, in comparison with Hextend. LA and survival were equivalent. The authors concluded that HBOC-201 was at least as efficacious as Hextend for resuscitation of swine with moderately severe HS.

8. Low-volume reversal of anaerobic metabolism, increased cutaneous tissue oxygenation in severe HS (swine). [27]

This is the second NMRC study of controlled hemorrhage-severity escalation with *simulated delayed prehospital transportation*. In this severe 55% EBV hemorrhage model (volume-controlled), as described above, swine were resuscitated with HBOC-201 or Hextend or not resuscitated (N = 8/group) to a target MAP of 60 mm Hg. HBOC-201

resuscitation resulted in higher MAP, MPAP, and $tcPO_2$, and lower HR, urine output, and blood transfusion requirements. LA, BD, CO, and survival was similar to Hextend pigs. The authors concluded that HBOC-201 resuscitation appeared increasingly beneficial with increasing hemorrhage severity, demonstrating increased tissue oxygenation without decreased CO.

9. Low-volume resuscitation with HBOC-201 serves as an adequate bridging fluid to definitive care. [21]

This is the third NMRC study of controlled hemorrhage-severity escalation with a further extended *prehospital* transportation to 24 hours. Swine were hemorrhaged 55% EBV as described above and provided HBOC-201, Hextend, or not resuscitated (N = 8/group). HBOC-201 resuscitation increased MAP and $tcPO_2$ compared to Hextend, and decreased blood transfusion requirements in the hospital phase. The authors concluded that because HBOC-201 restored hemodynamics, maintained tissue oxygenation, and decreased blood transfusion requirements compared to Hextend, potential use of HBOC-201 as a bridging resuscitation fluid was further substantiated in HS.

10. Low-volume reversal of anaerobic metabolism and improved survival in moderate HS (swine). [55]

After hemorrhage to MAP 30 mm Hg and hypotension maintained for 45 minutes, 16 pigs were resuscitated with HBOC-201 or Hextend (N = 8/group) to target MAP 60 mm Hg. After 8 hours, both groups received LR; the Hextend group received additional shed blood. Animals were followed until day 5. HBOC-201 pigs required less volume during the initial 8 hours, and had lower CO and SVO_2; SVR returned to baseline in HBOC-201 animals but remained below baseline in Hextend animals. There were no group differences in global resuscitation markers of pH, BD, LA, and urine output. Survival was 83 and 50% in HBOC-201 and Hextend groups, respectively. Surviving animals did not display clinical or laboratory evidence of long-term organ dysfunction in either group. The authors concluded, *HBOC-201 more effectively restored and maintained perfusion pressures with lower volumes, and allowed for improved survival.*

11. Similar volume expansion to albumin in two controlled hemorrhage models (swine). [56]

Similar volume expansion (indocyanine green and hematocrit dilution), relative systemic and pulmonary hypertension, decreased CO and HR, and similar oxygen delivery, SVO_2, and LA, in

comparison with oncotically-matched albumin, in sedated splenectomized swine with mild (10% EBV) and moderate (30% EBV) controlled HS**.** Survival was not different and was 11/12 with HBOC-201 and 11/12 with albumin.

12. HBOC-201 improves cerebral oxygenation following hemorrhage in swine. [30]

Swine (N = 7) were hemorrhaged to MAP 40 mm Hg and maintained hypotensive for 20 minutes. Pigs were given high-flow oxygen for 10 minutes and provided 6 ml/kg HBOC-201. Following HBOC-201 infusion, cerebral tissue oxygenation and cerebral venous oxygen tension increased to or above baseline. The authors concluded that HBOC-201 adequately restored $brPO_2$ following severe hemorrhage.

HS with uncontrolled hemorrhage

13. Decreased blood LA and increased acute survival with severe liver laceration injury (swine). [24]

Manning and Katz (*Carolina Resuscitation Research Group*) evaluated HBOC-201's effect on acute survival in a swine model of HS with uncontrolled hemorrhage due to multiple liver lacerations, in a model simulating prehospital resuscitation with delayed evacuation. HBOC-201 and LR were infused (10 ml/kg/min) 9 minutes after injury, with target MAP 60 mm Hg. 100% (7/7) of HBOC-201 vs. 10% (1/10) of LR pigs ($p = 0.0004$) survived the 2-hour monitoring period. LA was significantly lower with HBOC-201 than hetastarch (11.2 $\pm$ 1.4 vs. 19.1 $\pm$ 2.0 at 30 minutes, respectively, $p < 0.05$). The study showed decreased LA and increased short-term survival with HBOC-201 in severe uncontrolled HS.

14. Increased return to spontaneous circulation and acute survival after liver laceration induced cardiac arrest (swine). [28]

The authors compared HBOC-201 and LR selective aortic arch perfusion after traumatic cardiac arrest due to an exsanguinating liver laceration injury. Cardiac arrest occurred at 10-13 minutes, oxygenated fluids were infused at 15 minutes, until return of spontaneous circulation or achievement MAP 60 mm Hg. Epinephrine was infused if needed every 3 minutes, starting 18 minutes post-injury. Prior to ephinephrine infusion, return of spontaneous circulation was achieved in 100% (6/6) vs. 0% (0/6) of HBOC-201 and LR pigs, respectively. No HBOC-201 pigs required

epinephrine. Return of spontaneous circulation was achieved in 33% of LR pigs after epinephrine, but was sustained for less than 10 minutes. Respective one-hour survival rates were 83% with HBOC-201 vs. 0% for LR ($p < 0.05$). The authors concluded that selective aortic arch perfusion with oxygenated HBOC-201 may provide critical oxygenation and perfusion as well as sustain short-term survival in exsanguinating cardiac arrest. The study demonstrated improved survival with HBOC-201 in n extreme model of exsanguinating severe HS.

15. Improved long-term survival after liver laceration/crush injury (swine). [23]

Katz and Manning extended prior studies to evaluate HBOC-201's effect on long-term survival in a severe uncontrolled HS model (due to liver injury). Swine underwent liver crush and laceration, 50 ml/kg initial blood loss, and followed by continuous 3 ml/kg hemorrhage during resuscitation. 100% (8/8) of HBOC-201, vs. 0% (0/8) of hetastarch, vs. 0% (0/6) of untreated pigs ($p < 0.05$) survived 60 minutes. All HBOC-201 pigs survived 24 hours and 87.5% survived 96 hours. BD was significantly lower with HBOC-201 than hetastarch (-5.0 ± 2.0 vs. -14 ± 9.0 at 30 minutes, respectively, $p < 0.05$). The study showed decreased BD and improved long-term survival with HBOC-201 in severe uncontrolled HS.

16. Improved survival without increased hemorrhage after liver laceration/crush injury (swine). [17]In the NMRC model of severe uncontrolled HS due to liver laceration/crush injury and delayed evacuation, the effects of HBOC-201 or Hextend resuscitation (pressure controlled with target MAP 60 mm Hg) or no resuscitation were compared (N = 8/group). Additional infusions were provided for persistent hypotension and/or tachycardia and invasive hemodynamics and hemorrhage volume were followed during a 4-hour *simulated prehospital delayed evacuation* phase. Upon simulated hospital arrival, pigs received blood transfusions and NS as needed and the liver injury was repaired. Noninvasive monitoring and survival were followed for 72 hours. HBOC-201 resuscitation resulted in increased MAP, MPAP, and cutaneous tissue oxygenation, equivalent LA, but decreased BD in comparison with Hextend. CO was equivalent. Fluid and blood transfusion requirements were lower. Hemorrhage volume was not increased and long-term survival was increased with HBOC-201 (87.5%) vs. HEX (12.5%) ($p = 0.01$). The study showed decreased BD and improved long-term survival in severe uncontrolled HS with prehospital delay.

17. HBOC-201 for resuscitation of uncontrolled HS due to vascular injury (swine). *USUHS* Study Summary, (Philbin, manuscript in preparation).

In a model of severe uncontrolled HS due to large vascular (iliac artery and vein) injury and delayed evacuation (allowing multiple dosing), the effects of resuscitation in 59 pigs using (A) hypotensive and (B) normotensive strategies with HBOC-201, Hextend, and NS, or no resuscitation were compared. In order to simulate ongoing hemorrhage, blood was also withdrawn from an arterial catheter. Hypotensive resuscitation had a target MAP of 50 mm Hg; normotensive resuscitation targeted baseline MAP.

(A) Hypotensive resuscitation: HBOC-201 stabilized hemodynamics and averted anaerobic metabolism resulting in similar survival. In comparison with saline, incidence of rebleeding was increased, although total blood loss was similar across all groups. In comparison with Hextend, neither rebleeding nor total blood loss was different. Due to vasoactivity, MAP, systemic vascular resistance index, and MPAP were higher. CI was decreased with HBOC-201. Urine output was not different between groups. There was no clinically significant increase in blood oxygen content (due to low HBOC-201 infusion). Hence, LA, BD, oxygen delivery, consumption and extraction ratio, and transfusion requirements were similar.

(B) Normotensive resuscitation: HBOC-201 resuscitation also stabilized hemodynamics, averted anaerobic metabolism, and resulted in similar survival across all groups. However, in comparison with NS, although the incidence of rebleeding was similar, total blood loss was increased with HBOC-201. In comparison with Hextend, neither rebleeding nor total blood loss was different. MAP and MPAP were relatively higher and CI decreased. Fluid requirements were lower than with Hextend or NS but higher than in *hypotensive* resuscitation. Thus, blood oxygen content increased and cutaneous tissue PO_2 was improved temporarily. LA, BE, oxygen delivery, consumption and extraction ratio and transfusion requirements were similar. The authors noted that there were no significant differences between HBOC-201 and Hextend but that interpretation of results from this study were limited by suboptimal design and execution.

HS with concomitant Traumatic Brain Injury (TBI)

18. Improved neurophysiological markers and ventilator weaning in controlled HS and TBI (HBOC-301) (swine). [26]

In a swine model of controlled HS (30 ml/kg) and concomitant blunt TBI (fluid percussion), the effects of resuscitation with HBOC-301 (FDA-approved veterinary HBOC), LR, and combined HBOC-301/LR were compared (n =5/group). Resuscitation targets were SBP 100 mg Hg and/or

HR 100 bpm. Re-infusions of fluids were provided for hypotension (MAP 70 mm Hg), whole blood for anemia (Hb $\leq$ 5 g/dL), mannitol for elevated ICP (20 mm Hg), and LR to maintain CPP (79 mm Hg). With HBOC-301 or HBOC-301/LR, ICP, mannitol requirements, CPP correction and maintenance, $brPO_2$, intracranial compliance ($\Delta pCO_2/\Delta$ ICP), cerebrovascular reactivity ($\Delta pO_2/\Delta CO_2$), and fluid and blood transfusion requirements, and weaning off mechanical ventilation were all improved. Pigs in the HBOC-301 alone group had higher oxygen extraction ratio and slower rates of lactate clearance. None of the LR + mannitol + RBC animals could be weaned from the ventilator. In contrast, all HBOC pigs were weaned and extubated with no detectible neurologic deficits and normal hemodynamics at 72 hours. The study showed that low-volume resuscitation with HBOC-301 improved neurophysiologic parameters and ventilator weaning in controlled HS with concomitant TBI.

19. Improved neurophysiological markers and ventilator weaning in controlled HS and TBI (HBOC-201) (swine). [34]

In a similar controlled HS/blunt TBI model (K. Proctor's laboratory, University of Miami Medical Center), animals received initial fluid bolus of 10 mL/kg followed by HBOC-201 6 ml/kg (one dose) or LR (same volume). Then, each received NS as need to maintain MAP and CPP. After 120 minutes, one group of LR animals received phenylephrine (PE) as well, while the other continued to receive NS only. The LR groups also received shed blood and mannitol as needed. ICP was significantly lower in HBOC-201 animals than in LR controls (without PE). CPP and $PbrO_2$ were significantly greater in HBOC-201 animals vs. controls; LR animals only increased $PbrO_2$ to HBOC-201 levels after PE administration. Plasma cytokines and histopathology (H&E and LFB) were not significantly different. Overall, CPP, $brPO_2$, ICP/mannitol requirements, fluid requirements, and weaning off mechanical ventilation, were improved with HBOC-201. The study demonstrated improved neurophysiology with HBOC-201 in controlled HS with concomitant TBI.

20. Low-volume resuscitation, similar brain edema, decreased contusion volume, decreased CBF, but improved CO2 reactivity in controlled HS and TBI (rats). [33]

After induction of blunt TBI (controlled cortical impact) and controlled HS to MAP 30 mm Hg, the effects of resuscitation to baseline MAP with HBOC-201, LR, and shed blood (SB) were compared. Cerebral blood flow (CBF) (tissue perfusion units [TPU]) was monitored with laser Doppler flowmetry. Response to hypercapnia was determined at 30 and 60 minutes post-resuscitation. Contusion volume was assessed by Thionin staining and image analysis software, on a separate group of animals sacrificed at 24 hours. Brain edema was assessed by weighing brains before and

after dehydration at 70oC for 48 hours. HBOC-201 rats had lower fluid requirements (3.9 + 0.1 ml) when compared to LR (45.9 + 2.7 ml) ($p < 0.001$), yet diminished acidosis (pH: HBOC-201 7.45 + 0.01, LR 7.3 + 0.04, and SB 7.41 + 0.02, $p < 0.01$) and improved bicarbonate and base excess. There were no differences in ipsilateral or contralateral brain edema. Contusion volume was lower with HBOC-201 (45.1 mm3) compared to LR (63.5 mm3) and SB (35.1 mm3) ($p < 0.01$). While CBF was diminished with HBOC-201 (70.1 + 3.8% baseline TPU) compared with LR (105.8 + 10.1% baseline TPU) and SB (96.8 + 5% baseline TPU) ($p < 0.05$), CBF autoregulation in response to hypercapnia was maintained in HBOC-201 animals. The authors concluded, …resuscitation with HBOC-201 protects autoregulatory mechanisms and may reduce secondary brain injury in TBI. The study demonstrated improved diminished systemic acidosis and improved neurophysiology and histopathology in controlled HS and concomitant TBI.

21. Improved neurophysiologic parameters and decreased contralateral brain injury in controlled HS and TBI (swine). [25]

After *controlled cortical impact*, hemorrhage to MAP 40 mm Hg, and maintenance of hypotension for 35 minutes, swine were resuscitated with HBOC-201 (6 ml/kg) vs. LR (12 ml/kg) over 10 minutes. Additional IVF were administered to maintain CPP > 60 mm Hg. At the end of the 6.5 hour observation period, brain MRI was completed followed by euthanasia and necropsy with tissue specimen harvesting for histopathology and immunohistochemistry. MAP, BD, CPP, ICP, and ipsilateral $PbrO_2$ were improved with HBOC-201. Fluoro-Jade B (early neuronal damage marker) staining and T2-weighted MRI evidence for injury volume was equivalent on the side ipsilateral to injury. However, staining was significantly lower with HBOC-201 on the contralateral side, suggesting potential to diminish secondary brain injury. The study demonstrated decreased BD and improved neurophysiology and neurohistopathology in controlled HS with concomitant TBI.

22. OBRR-directed *RESUS* IND-enabling study: Improved neurophysiological parameters without increased bleeding in uncontrolled HS and TBI (swine). [18] (Complete study report submitted with BB-IND 12504)

Resuscitation with HBOC-201 or LR or no fluids were compared in a swine model of uncontrolled HS due to liver injury and blunt TBI, with simulated short (transportation) delay (SD, 30 minutes, single dose) and long delay (LD, 75 minutes, multiple doses). With exceptions of improved contralateral pO_2, decreased tachycardia, and decreased ipsilateral CBF (doppler), in HBOC-201 pigs, survival, blood loss, and other systemic and neurophysiological parameters were not different

in the SD cohort. Overall survival to 6 hours was 67% (6/9) and 44% (4/9) with HBOC-201 vs. LR (not significant). However, in the LD cohort, overall survival to 6 hours was significantly improved with HBOC-201. Survival was 73% (8/11) with HBOC-201 and 9% (1/11) with LR. CPP, SSO_2sat, SSLA and SSBD, $tcPO_2$, systemic blood LA and BD, and blood transfusion requirements were all improved with HBOC-201; MAP, MPAP, and metHb were higher with HBOC-201 than LR, but other systemic and neurophysiological parameters were not different. ICP, mannitol requirements, and blood loss were similar in both delay cohorts. Gross and light microscopic evaluation revealed similar rates and severity of subarachnoid, subdural, and intraparenchymal hemorrhage, neutrophilic infiltration, and astrocytic injury in both delay cohorts (H&E, GFAP, and microtubule-associated protein-2 [MAP2]). In the SD cohort, neuronal necrosis and white matter degeneration were also similar. In the LD cohort, higher rates and severity of neuronal necrosis and white matter degeneration occurred with HBOC-201 than LR. In summary, in comparison with LR, in the SD cohort, minimal systemic and neurophysiologic benefits were noted, but in the LD cohort, significant systemic and neurophysiologic and survival benefits were seen with HBOC-201. Mainly due to prolonged survival and consequently increased histopathologic evidence of brain injury, neuronal necrosis and white matter degeneration were higher with HBOC-201[26]. The study showed decreased LA and BD and improved neurophysiology in severe uncontrolled HS with concomitant TBI, especially with increased prehospital delay.

Blunt Whole Body Trauma

23. Stabilization of hemodynamics and increased survival (rats). [57]

Hayward and Lefer examined whether HBOC-201 was beneficial in a 100% lethal rat model of severe trauma involving multiple tissue and organ injury. HBOC-201 normalized BP, preserved

[26] Details of histopathology results: severity scores for subdural and subarachnoid hemorrhage in the ipsilateral cortex were lower with HBOC-201 vs. LR in the LD cohort (long-term survivors only). Intraparenchymal hemorrhage (IPH) in the contralateral hippocampus incidence and severity score were higher with HBOC-201; however, severity score was only ≤ 1. IPH was lower with HBOC-201 vs. LR in the anterior thalamus in the LD group. Neuronal necrosis was slightly higher with HBOC-201 vs. LR only in the ipsilateral cortex and anterior thalamus (severity scores ≤ 1). White matter degeneration (WMD) was higher with HBOC-201 vs. LR in the ipsilateral cortex (SD only) and the anterior thalamus and ipsilateral and contralateral hippocampus (severity scores were low). Conversely, WMD incidence (but not severity score) was lower with HBOC-201 vs. LR in the pons. Astrocytic injury (GFAP) was similar with HBOC-201 and LR. MAP 2 staining (neuronal/dendritic injury) was slightly higher with HBOC-201 only in the contralateral cortex in the SD group. Conversely, in the LD group, HBOC-201 pigs had less injury vs. LR only in the contralateral cortex gray matter. Brain histology analysis may have been confounded by longer survival with HBOC-201 and short follow–up time (only 6 hours). Overall, brain histopathology results were not significantly different.

superior mesenteric artery endothelial function, had no effect on ileal peripheral mononucleocyte infiltration (myeloperoxidase), and doubled survival time. The study showed improved survival in severe HS.

Hematology Studies

Hematology/hemostasis in HS with controlled and uncontrolled hemorrhage

24. Mild delayed in-hospital coagulopathy but decreased prehospital thrombocytopathy in *moderate controlled HS* (swine). [58]

Hematology/hemostasis assays were completed on blood specimens obtained in the NMRC model of moderate controlled HS. [20] Complete blood count, *in vivo* bleeding time, and *ex vivo* bleeding time (Platelet Function Anaylzer-100 [PFA-100] Closure Time [CT]), thromboelastography (TEG), and clinical coagulation parameters are reported. As described above, HBOC-201 or Hextend or no resuscitation (N = 8/group) were compared after controlled 40% EBV hemorrhage; hematology results were followed until death or euthanasia at 72 hours. Hematocrit was similar in all groups throughout the experiment, but hemoglobin was higher in HBOC-201 pigs. Platelets diminished in both resuscitation groups during the prehospital phase, and at 24 hours, platelets were lower with HBOC-201 than Hextend. Bleeding time was similar in both resuscitation groups. PFA-100 CT increased in both resuscitated groups but less with HBOC-201 ($p = 0.02$) in the prehospital phase; this effect was reversed by 24 hours ($p = 0.02$). TEG-R increased with HBOC-201 during the prehospital phase and was higher than with Hextend or no resuscitation at 24 hours ($p = 0.03$); TEG-MA and clinical coagulation parameters were similar in all 3 groups. Electron microscopy showed no evidence of platelet/fibrin clots or microthrombi. The authors concluded that in swine with HS, HBOC-201 resuscitation induced less thrombopathy than Hextend during the prehospital phase but more thrombopathy in the hospital phase. The group differences were minimal and unlikely to be clinically significant.

25. Mild delayed in-hospital coagulopathy and thrombocytopathy *in severe uncontrolled HS in swine.* [59]

Hematology/hemostasis assays were completed on blood specimens obtained in the NMRC model of severe uncontrolled HS. [17] Experimental schedule and blood sample collections were as described previously. White blood cells, hematocrit, and platelets were similar with HBOC-201 (N = 8) and Hextend (N = 8). Throughout the prehospital phase, Hb was higher with HBOC-201 than

Hextend. Both *in vivo* and *in vitro* PFA-100 CT bleeding times and TEG-R and TEG-MA levels were statistically similar with HBOC-201 and Hextend; there was a trend to lower TEG-MA and higher PT with Hextend. During the hospital phase, group comparisons could not be made due to low survival with Hextend and no resuscitation. There were no group differences in clinical coagulation parameters (PT, PTT). Overall, clinically significant effects on coagulation were not seen in severe uncontrolled HS.

Immunology

26. PMN adhesion marker and oxidative burst increases comparable to standard IV fluids (except highest doses) (*in vitro*). [60]

Ortegon compared the effects of HBOC-201, HTS, HSD, Hextend, LR, and Pentalyte, on human peripheral mononucleocyte activation *in vitro*. Oxidative burst increased in a dose-dependent fashion but similarly to the other non-HTS containing resuscitative fluids (oxidative burst did not increase with HTS or HSD). CD11b expression was higher in peripheral mononucleocytes incubated with HBOC-201 than Hextend or LR, but only at high concentrations (50% and 75% HBOC-201). This study showed that at clinically-relevant concentrations, HBOC-201 does not increase immune activation.

27. Absence of significant immunologic activation or suppression in moderate controlled HS (swine). [61]

Immunological analysis was completed on whole blood samples collected at Time 0 (pre-hemorrhage), and 1, 4, 24, and 72 hours (surviving animals) from the NMRC's moderate controlled HS model. [20] The natural course of HS (non-resuscitated pigs) was characterized by ~ 2-fold increased expression of peripheral mononueclocytes β-2 integrins, CD11 and CD18. Resuscitation with Hextend (N = 8) increased β-2 integrin expression even higher—to 3 to 5-fold the baseline value; in contrast, HBOC-201-treatment (N = 8) averted a significant rise in peripheral mononueclocytes β-2 expression ($p = 0.001$ and 0.01, respectively, at 4 hours). Increases in lymphocytic CD49d expression levels and apoptosis occurred only in non-resuscitation and Hextend groups, respectively ($p < 0.01$). Lymphocyte percentages decreased and peripheral mononueclocytes percentages increased around 4 hours post-hemorrhage in all groups. CD3 cells decreased in all groups, but CD4 and CD8 cells decreased only in the resuscitation groups. TNF-α levels were not detectable in any groups. IL-6 levels were similar, however, IL-10 levels were

higher in HBOC pigs, as early as 1 horr post-hemorrhage (p = 0.04). In conclusion, HBOC-201 had no significant adverse or beneficial effects on immune function in moderately severe controlled HS.

28. Absence of significant immunologic activation or suppression in severe controlled HS (swine). [62]

Immunological analysis was completed on whole blood samples collected at Time 0 (pre-hemorrhage), and 1, 4, 24, and 72 hours (surviving animals) from the NMRC's severe controlled HS model.[27] There were no significant group differences in leukocyte immunophenotype (CD4:CD8 ratio), adhesion marker expression (neutrophil CD11b; monocyte or lymphocyte CD49d), apoptosis (lymphocyte and neutrophil annexin V+/PI), and cytokine elaboration (IL-6, IL-10 [there was a trend to higher plasma IL-10 in HBOC-201-treated animals]). Overall, HBOC-201 had no significant adverse or beneficial effects on immune function in severe controlled HS.

29. Increased immunocyte activation and apoptosis due to *severe uncontrolled HS* but group differences could not be compared (swine) (Hall, manuscript in preparation).

Immunological analysis was completed on whole blood samples collected at Time 0 (pre-hemorrhage), and 1, 4, 24, and 72 hours (surviving animals) from the NMRC severe uncontrolled HS model. [17] Expression of peripheral monocytes CD11b and CD18 and of lymphocytic CD49, increased ~2-3-fold in HBOC-201-pigs; CD49 remained stable. Overall, there were no significiant differences in leukocyte adhesion marker expression. CD4/CD8 ratio was similar across treatment groups (N = 8/group) but the ratio appeared to remain stable in the HBOC-201 group. Clear differences between treatment groups in IL-2, IL-6, and IL-10 were not seen but there was a trend to higher plasma levels of IL-10. A trend towards increased peripheral monocytes and lymphocyte apoptosis (Annexin-V and TUNEL) was seen in HBOC-201-pigs ($p > 0.05$). The pattern of apoptotic changes appeared similar to immunological changes. As there were few survivors in the control groups and the study was powered for physiological rather than immunological variables, accurate group treatment-effects could not be compared. Nevertheless, results of this study suggest that HBOC-201 does not cause clinically significant immune activation or suppression in severe uncontrolled HS.

Organ Function and Oxidative Stress Studies

30. HBOC-201 had equivocal effects on organ injury and did not induce oxidative stress, but was associated with increased mild hepatobiliary and renal papillary histopathology (swine). [32]

Histopathologic, clinical chemistry, cardiac enzymes, and associated clinical cardiac analysis were completed on data from three NMRC swine HS studies: (1) moderate controlled HS (40% EBV) [20]; (2) severe controlled HS (55% EBV)[27]; and (3) *severe uncontrolled HS* (liver injury). [17] Effects of HBOC-201 were compared with Hextend and no resuscitation (N = 8/group). Pathology specimens were processed (gross, H&E, and 3-nitrotyrosine [moderate controlled HS only]) by the Walter Reed Army Institute of Research (WRAIR) Pathology Department (Silver Spring, MD) and reviewed by a board certified veterinary pathologist. Cardiac findings were corroborated by a blinded *second opinion* pathologist review. Lesion severity score was based on the percent of myocardium involved and severity of cellular changes. Troponin I assays were performed by Dr. Fred Apple at Hennepin County Medical Center, Minneapolis, MN. Myocardial necrosis and fibroplasia, fluid requirements, CO, and cardiac enzymes were generally similar or lower with HBOC-201 than Hextend (except CK-MB was higher with HBOC-201 in moderate controlled HS). Alveolar and interstitial pulmonary edema was similar (except pO_2 was higher with HBOC-201 in severe uncontrolled HS). Jejunal villar epithelial and hepatocellular necrosis were similarly minimal to moderate in all groups. Minimal biliary changes occurred exclusively with HBOC-201. AST, LDH, and AP were generally higher with HBOC-201. Mild renal papillary injury occurred more frequently with HBOC-201, but consistent patterns for urine output, BUN, and creatinine were not seen.

In order to assess oxidative stress, peroxynitrate production was quantified using the surrogate marker, 3-nitrotyrosine. Tissue specimens (myocardium, lungs, liver, jejunum, and kidney) were assayed using immunofluorescent microscopy; plasma specimens were assayed using ELISA (severe uncontrolled HS only). There were no significant differences in the relative intensity of 3-nitrotyrosine staining in any of the tissues sampled in either HS model. However, plasma 3-nitrotyrosine levels were higher in HBOC-201 pigs (severe uncontrolled HS). The plasma protein assayed has not yet been confirmed, but by Western Blot, it is ~ 50 kD, suggesting the possibility that it is albumin. It is unclear why tissue levels were similar but plasma levels were higher with HBOC-201 than Hextend. Plasma proteins may be more sensitive to oxidative stress, or have exposure to higher concentrations of HBOC-201, or plasma levels may reflect global systemic levels that may not correlate with levels in specific organ systems. Despite the limitations of the 3-nitrotyrosine assay, and preliminary evidence suggesting elevated plasma levels in HBOC-201 pigs, taken in the context of the aforementioned histopathology results, these results diminish concern for significant reperfusion-related organ injury. <u>It was concluded that in comparison with Hextend, HBOC-201 resuscitation did not increase evidence of oxidative stress, and had histopathological</u>

and/or functional effects on organs that were clinically equivocal (myocardium [potentially beneficial in moderate controlled HS], lungs, hepatic parenchyma, jejunum, and renal cortex/medulla) and potentially minimal to mild adverse effects (hepatobiliary and renal papilla).

Non-trauma studies

Arterial Occlusion Models

31. HBOC-201sustained pO_2 during arterial stenosis (dogs). [63]

Horn conducted partial arterial occlusion experiments in a dog hind limb occlusion model (95% popliteal artery occlusion). Addition of as little as 5% of EBV of HBOC-201 resulted in the return of tissue pO_2 levels to baseline. Increased tissue oxygenation was associated with higher extraction ratio with HBOC-201. The authors concluded that HBOC-201 could provide oxygenation to post-stenotic tissues.

32. HBOC-201 increases skeletal muscle oxygenation during arterial stenosis (dogs). [64]

In a canine model of 95% stenosis of the popliteal artery, HBOC-201 returned tissue pO_2 to near baseline in comparison with hetastarch (N = 7/group). The results of this study illustrate the ability of HBOC-201 in plasma to *bypass* vessel constrictions and may be beneficial in tissues with compromised RBC flow as a result of arterial injury. The authors concluded, *data suggest that increased oxygen extraction in the HBOC group is associated with improved skeletal muscle tissue oxygenation during severe arterial stenosis*.

33. HBOC-201 protects again myocardial reperfusion injury (dogs). [65]

This study investigated the cardioprotective effects of HBOC-201 (N = 10) vs. NS (N = 8) in a canine model of myocardial ischemia-reperfusion injury. HBOC-201 was infused prior to ischemia. Histological analysis showed significantly reduced infarct size (56%); reduced CK levels were also observed. The authors concluded that prophylactic treatment with HBOC-201 pre-myocardial ischemia reduced myocardial inflammation and ischemia-reperfusion injury.

34. Infarct size reduced with HBOC-201 following acute myocardial ischemia (dogs). [66]

Dogs were subjected to coronary stenosis resulting in 80-95% flow reduction for 195 minutes with pacing 10% above HR, followed by 180 minutes of reperfusion with HBOC-201 (N = 6), NS (N = 6), or phenylepherine (N = 6). The area of risk : infarct size ratio was reduced with HBOC-201 (4.4

± 2.2%) vs. NS (26.0 ± 3.6%) and phenylepherine (25.7 ± 4.1%) ($p < 0.05$). In addition, regional myocardial function was restored almost to baseline with HBOC-201. In conclusion, HBOC-201 treatment post-myocardial ischemia by acute coronary stenosis reduced infarct size and improved myocardial function.

Exchange Transfusion and Hemodilution Models

35. HBOC-201 provides higher oxygen tension in skeletal muscle than RBC (dogs). [67]

Standl tested the efficacy of HBOC-201 vs. RBC to treat severe anemia following hemodilution to hematocrit 10%. Results from 12 dogs indicated that HBOC-201 was ~ 3 times more potent than stored or fresh RBC at restoring baseline tissue oxygenation to skeletal muscle following severe acute anemia. The authors concluded that compared with RBC transfusion, low doses of HBOC-201 enhanced oxygen extraction after hemodilution.

36. HBOC-201 increased oxygen extraction and oxygenation of liver and gastrocnemius muscle following isovolemic hemodilution (dogs). [68]

Dogs were hemodiluted with LR and then with HBOC and compared to a control group (no hemodilution) to determine the effects of HBOC-201 on tissue oxygenation in central organs (e.g., liver and gastrocnemius muscle). Following hemodilution, global liver and muscle oxygen extraction ratio increased with HBOC-201 in comparison with baseline and to the control group. Liver oxygenation increased from 48 ± 9 at baseline to 53 ± 10, 67 ± 11, and 68 ± 7 at 20, 60, and 100 minutes, respectively, in the hemodiluted group. The authors concluded that hemodilution with LR followed by HBOC-201 increased liver and skeletal muscle oxygenation and oxygen extraction.

37. Stable tissue perfusion in vital organ systems and absence of necrosis, apoptosis, and tissue nitrosylation in a stepwise 50% exchange transfusion model (swine). [69]

Regional blood flow (RBF) was quantified with fluorescent microspheres after HBOC-201 (N = 8) or albumin (N = 8) normovolemic exchange transfusion (10, 30, and 50% EBV), and compared with non-hemorrhaged controls (N = 4), in sedated swine. RBF remained stable and was not different in HBOC-201 and control groups (all organs except muscle), consistent with continued metabolic coupling of oxygen delivery with metabolic demand, but regional blood flow generally increased with albumin. MAP and MPAP were relatively higher; CO, oxygen delivery and consumption, LA, and BD similar; and urine output lower. There was no evidence of tissue necrosis, apoptosis (activation of poly (ADP-ribose) polymerase and DNA laddering by whole

blood), or tissue nitrosylation (3-nitrotyrosine by whole blood) in any group. In conclusion, HBOC-201 provided stable perfusion in vital organs without causing necrosis, apoptosis, or tissue nitrosylation.

38. Improved organ tissue oxygenation and reversal of anaerobic metabolism comparable to albumin in stepwise 50% exchange transfusion model (swine). [70]

Tissue pO_2 (heart oximetry probes and brain and kidney EPR oximetry), hemodynamics, and histopathology were assessed post-HBOC-201 (N = 12) or -albumin (N = 8) normovolemic exchange transfusion (10, 30, and 50% EBV), in anesthetized swine. In the HBOC-201 group, MAP and cardiac tissue pO_2 were higher; LA, BD, and brain and kidney pO_2 similar; and blood oxygen saturation lower; mild methemoglobinemia was documented with HBOC-201. The authors concluded that significant histopathologic changes were not observed.

Endotoxemia Models

39. HBOC-201 restores cardiovascular and kidney function in endotoxin-induced shock (rats). [71]

Septic shock, a systemic inflammatory disorder, is associated with high mortality and is characterized, in part, by severe hypotension. A subpopulation of trauma patients may develop sepsis. To address the effects of HBOC-201 in this subpopulation, a rat model of lipoplysaccharide (LPS/endotoxin)-induced acute septic shock was studied. HBOC-201 was compared to NS, hetastarch, N-nitro-L-arginine, or no treatment. The shock was lethal in 25% of saline-treated animals within two hours. One N-nitro-L-arginine -treated rat died and all HBOC-201 rats survived. HBOC-201 was uniquely able to normalize both cardiovascular and renal function (glomerular filtration rate, renal blood flow, renal plasma flow, and renal vascular resistance) when compared to volume replacement with hydtroxyethyl starch or N-nitro-L-arginine synthase inhibition. The authors concluded that polymerized Hb but not nitric oxide synthase inhibition or volume replacement normalized cardiovascular and kidney function in acute septic shock.

Toxicology

Reproductive toxicology/teratology

40. HBOC-201 is not expected to cause abnormal development in humans or other mammals that do not develop inverted yolk sac placentas. [72]

Reproductive toxicology studies were initially conducted in rats. Irrespective of the dosing regimen (IV route of administration) during gestation, embryo-fetal toxicity and excessive maternal toxicity were reported. Mechanistic studies revealed that the toxicity was likely due to HBOC-201 effects on visceral yolk sac histiotrophic nutritional processes. Rodents are somewhat unique in that they possess an inverted yolk sac that encompasses the entire embryo throughout gestation, and it appeared that HBOC-201 affected this endocytotic system to mediate embryo-fetal toxicity. Since dogs and humans do not utilize this inverted yolk sac during gestation, reproductive toxicology studies were subsequently conducted on the dog. Irrespective of the dosing regimen (administration of HBOC-201 on a single gestation day or repeatedly throughout gestation), up to 2-fold the MHD caused no embryo-fetal effects. Therefore, the teratological effects are thought to have been rodent species-specific and likely due to the unique developmental system (inverted yolk sac) used by rodents during gestation.

Reproductive toxicology studies were carried out in rats and dogs to examine effects of HBOC-201 and bovine polymerized hemoglobin (BPH) on embryo-fetal development (teratology studies). Three studies in the rat included administration of HBOC-201 by continuous IV administration [gestation day (GD) 6 through 18], daily continuous IV administration (GD 6 to 7, 7 to 8, 8 to 9, 9 to 10, 10 to 11, 11 to 12 and 12 to 13) or hemodilution followed by IV infusion on GD 9. An additional study was carried out in rats in which hemodiluted pregnant animals were administered BPH IV on GD 9. In dogs, HBOC-201 was administered IV on either GDs 21, 25, 29 or 33. Finally, an HBOC-201 repeat-dosing (during gestation) dog study was completed. A mechanistic study was conducted using rat embryos to examine HBOC-201-related embryotoxicity in the rat.

These data demonstrated embryo-fetal toxicity with IV administration of HBOC-201 to pregnant rats whether administered continuously or by daily IV infusion or by IV infusion on a single critical day (GD 9). However, IV infusion of HBOC-201 to pregnant dogs at critical gestation times (GD 21, GD25, GD29 or GD30), at doses 2-fold greater than the MHD on a weight-to-weight basis, did not cause any embryo-fetal toxicity. Upon repeat dosing of HBOC-201 to pregnant dogs during organogenesis, no teratological effects were reported. Mechanistic study results indicated that HBOC-201 related embryo-fetal toxic effects in rats were likely due to effects on the inverted yolk sac, a developmental system fairly unique to the rat. <u>In conclusion, since dogs and humans do not utilize such a developmental system during pregnancy and there were no embryo-fetal effects of HBOC-201 in pregnant dogs, it is likely that these teratogenic effects of HBOC-201 are unique to rats and not relevant to humans.</u>

Neurotoxicology

41. Absence of toxicity to rat fetal neural cells (*in vitro*). [73]

As free human Hb is known to be directly toxic to neural cells, the cytotoxic effects of HBOC-201 and free human Hb were compared in an *in vitro* rat neural cell culture. Cells were incubated with HBOC-201 or human Hb (0.02-6.5 g/dL) for 24 hours prior to analyses (^{3}H-thymidine uptake, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) conversion to formazan, and ^{3}H-thymidine release). Cultures incubated with human Hb had decreased proliferation and metabolic activity and increased cytolysis. In contrast, HBOC-201 cultures had stable proliferative responses except at the highest concentration (6.5 g/dL). Metabolic responses remained stable in HBOC-201 cultures at all tested concentrations. Cytolysis was not seen in any HBOC-201 cultures. In concentrations likely to be achieved clinically, HBOC-201 did not have significant neurotoxic effects *in vitro*.

Qualitative assessment of animal Models Used To Evaluate HBOC-201

Animal HS models will not reproduce entirely the broad spectrum of morbidities and injuries of human trauma patients. The majority of HS studies utilized healthy animals that were generally anesthetized, often mechanically ventilated, and sometimes splenectomized and/or breathing an enriched oxygen mixture[27]. Trauma studies in animal models of chronic disease are rarely cited and poorly characterized in the literature. In general, it is considered ethically inappropriate and often technically not feasible to perform trauma or HS experiments on conscious swine. Careful consideration is uniformly given to choice of anesthetics and level of anesthetic depth to minimize cardiovascular effects. Ventilation is necessary to offset anesthesia-induced respiratory depression, atelectasis, ventilation-perfusion mismatch, and resultant hypoxemia and hypoventilation that could not be standardized. Positive end expiratory pressure (PEEP) is avoided to prevent unnecessary cardiovascular effects. Despite variation in models (e.g. $\pm$ anesthetics, neuromuscular blocking agents, anticholinergics, ventilatatory support, and enriched inspired oxygen), overall effects observed in trauma and HS models have been accepted as appropriate for extrapolation of effects of resuscitation fluids on human trauma patients. The number and diversity of the models presented here offset the limitations of each individual model[28].

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[27] None of the NMRC studies utilized splenectomized animals. Of the five NMRC models, only one (HS/TBI model [Study 22 [18]) maintained pigs on 100% oxygen, the rest were on room air. Study #11[56] used sedated pigs which were not anesthetized.

[28] OBRR has critiqued the heterogeity of the animal HS models included in the *RESUS* IND preclinical

While no animal model can exactly simulate the spectrum of trauma in the prehospital setting, the controlled and uncontrolled HS models, with and without TBI, and the whole body of trauma models used to evaluate HBOC-201 for use as a primary resuscitation fluid in HS, collectively address a broad range of physiological perturbations that occur in the prehospital trauma setting.

Standard HS models (mainly swine) were chosen for evaluation of HBOC-201 as they are likely to predict responses in humans. Swine are preferred for this type of biomedical research because of the similarities of their cardiovascular system, particularly the distribution of myocardial blood flow and response to ischemic events, to that of humans. [42] Review articles of HS animal studies indicate that there are a wide variety of 'standard' swine models of hemorrhage and resuscitation, but these have been accepted as appropriate for extrapolation of effects of resuscitation fluids on human trauma patients. [74-77] Furthermore, this species and the specific model used were directly requested by OBRR to ascertain particular data on HBOC-201 on a number of occasions (e.g., HS/TBI [Study 22—[Biopure PV-04 [Study 11]), Biopure BF-01-04 [Study 37], and Biopure 2004 A0024 [Study 38]), adding further support to the satisfactoriness of the species in preclinical trauma models.

Summary of preclinical HS database on HBOC-201

HBOC-201 was comprehensively evaluated in 38 *in vivo* animal studies using a wide variety of animal models, as well as 2 *in vitro* studies. Specifically, trauma studies have included: HS with controlled hemorrhage (Studies 1-12), HS with uncontrolled hemorrhage (Studies 13-17), HS with controlled hemorrhage and concomitant TBI (Studies 18-21 [Study 18 evaluated HBOC-301]), HS with uncontrolled hemorrhage and concomitant TBI (Study 22), and blunt whole body trauma (Study 23). Laboratory studies on blood samples and tissues collected from completed HS trauma studies have included hematology (Studies 24 and 25), immunology (Studies 26-29), and organ function and oxidative stress studies (Study 30). Non-trauma studies have included arterial occlusion (Studies 31-34), exchange transfusion and hemodilution (Studies 35-38), as well as endotoxemia models (Study 39) and toxicology (Studies 40-41). For clarity, those completed by NMRC are Studies 7-9, 16, 17, 22, 24, 25, 27-30.

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database; on the contrary, NMRC believes that consistency of results in varied models adds strength to predictive power of the studies in terms of expected responses in humans.

Initial HBOC-201 trauma studies, using equivalent volumes of resuscitation as control fluids or using baseline values for physiologic endpoints (e.g., administering fluid until baseline BP or HR is achieved), supported the hypothesis that HBOCs could satisfy oxygen demands under conditions of normotensive resuscitation, in situations of controlled hemorrhage, or those mimicking short prehospital time to definitive care without untoward effects. These early studies demonstrated that HBOC-201 could be used as an oxygen bridge, supplying oxygen needs until RBC were transfused or regenerated.

Military concerns also questioned the smallest effective and safe volume of HBOC that could be used. Concerns also arose as to whether or not increased BP observed with HBOC-201 might be misinterpreted as acceptable resuscitation by prehospital medics. Other concerns were raised regarding the effects of HBOC-201 on sub-populations of trauma patients such as those with head trauma, blunt trauma without hemorrhage, patients with developing sepsis, and pregnant victims. The following studies describe the efficacy of HBOC formulations in both acute and survival models of controlled or uncontrolled hemorrhage with severe organ injury, head injury, lethal whole body trauma, and septic shock, as well as acute and survival studies using hypotensive or small volume resuscitation and delayed definitive care models. The goal of many of these models was to determine the threshold or limit beyond which HBOC-201 may not be effective or safe (i.e., could HBOC-201 be used without detrimental effect in a patient with TBI? Could a smaller volume of HBOC-201 be used compared to controls to achieve similar or better outcome?).

Focus on important observations from preclinical studies

1. Improved survival with HBOC-201

Preclinical HS studies with HBOC-201 have shown reduced mortality supporting *potential* to provide *direct benefit* to human subjects in *RESUS.* In a combined analysis of 14 pertinent HS studies representing data from 229 pig experiments, including severe as well as less severe HS models[29], mortality was significantly reduced with HBOC-201 in comparison with standard fluids (14/117 [12%] vs. 53/112 [47.3%] respectively, $p < 0.0001$) (Table 8). In almost all severe HS models (e.g., LD > 50%), which more closely resemble the targeted population in *RESUS*, physiologic variables have been significantly improved and mortality dramatically reduced(7/42 [16.7%] vs. 40/43 [93%], respectively, $p < 0.0001$). [17, 18, 23, 24, 34, 53]

[29] These 14 studies were chosen because survival (or surrogate of survival) was a primary outcome measurement comparing HBOC-201 and standard fluid. The studies had varying level of severity and consisted of both controlled and uncontrolled HS studies ± TBI.

Even in mild HS models alone (e.g., LD < 50%), many of which were not designed or powered to assess survival, physiologic variables have been consistently improved; a trend to reduced mortality is apparent in these studies as well (7/75 [9.3%] vs. 13/69 [18.8%], respectively, $p > 0.05$.

Table 8: Mortality in preclinical HS studies with HBOC-201

Preclinical HS studies with HBOC-201					
Reference	**HS Model**	**Mortality**			
		HBOC-201	**Control Fluid**	**Control**	**P[#]**
Less severe HS models					
McNeil J [29]	Moderate/Controlled	1/12 (8%)	0/6 (0%)	> 0.05	LR
York G [31]	Moderate/Controlled	0/6 (0%)	0/6 (0%)	> 0.05	LR + Blood
Sampson J [53]	Moderate/Controlled	0/6 (0%)	0/6 (0%)	> 0.05	LR
Knudson M [52]	Moderate/Controlled	0/10 (0%)	0/10 (0%)	> 0.05	LR
Philbin N [20]	Moderate/Controlled	0/8 (0%)	1/8 (12%)	> 0.05	Hextend
Fitzpatrick C [55]	Moderate/Controlled	1/8 (12%)	4/8 (50%)	> 0.05	Hextend
Rice J [27]	Severe/Controlled	0/8 (0%)	2/8 (25%)	> 0.05	Hextend
Philbin N [21]	Severe/Controlled	1/8 (12%)	1/8 (12%)	> 0.05	Hextend
NMRC liver injury/TBI, unpublished	Severe/Uncontrolled with TBI (SD 30 min)	4/9 (44%)	5/9 (56%)	> 0.05	LR
Mortality—less severe HS models		**7/75 (9.3%)**	**13/69 (18.8%)**	**> 0.05**	
More severe HS models					
Manning J [24]	Severe/Uncontrolled	0/7 (0%)	9/10 (90%)	0.003	LR
Manning J [28]	Severe/Uncontrolled	1/6 (17%)	6/6 (100%)	0.002	LR
Katz L [23]	Severe/Uncontrolled	0/8 (0%)	8/8 (100%)	0.00002	Hetastarch
Gurney J [17]	Severe/Uncontrolled	1/8 (12%)	7/8 (88%)	0.004	Hextend
Stern S [18]	Severe/Uncontrolled with TBI (LD 75 min)	5/13 (38%)	10/11 (91%)	0.01	LR
Mortality—more severe HS models		**7/42 (16.7%)**	**40/43 (93%)**	**< 0.0001**	
Mortality—all (less and more severe) HS models combined		**14/117 (12%)**	**53/112 (47%)**	**< 0.0001**	
Surrogate of mortality (inability to wean from ventilator)					
King D [26](**HBOC-301**)	Severe/Controlled with TBI Part 2	0/3 (0%)	3/3 (100%)	> 0.05	LR + mannitol + RBC
Patel M [34]	Severe/Controlled with TBI	1/10 (10%)	5/5 (100%)	0.002	NS + mannitol + RBC

[#] P value: Fisher's exact test (two-tailed).

In order to assess potential clinical importance of observed HBOC-201-induced physiologic effects, NMRC evaluated association of key physiological measurements with survival in HBOC-201-treated animals. For the purposes of this analysis, NMRC's 40% and 55% EBV moderate and severe controlled HS models and severe uncontrolled HS/liver injury model (Studies 7, 8, and 16) were combined as the models were similar in design; Study 22 was analyzed separately because of differing study design[30]. A Cox proportional hazards model was used to estimate association of the following physiologic variables with survival: SBP, mean MAP, HR, CI, MPAP, LA, BD, SVO_2, and $tcpO_2$. The variables were individually added to this model in a time-dependent manner. If the observed survival benefit associated with HBOC-201 was attributable to one of these variables, the p-value for the association between HBOC-201 and survival would diminish when these variables are added to the model (i.e., become less significant).

Table 9 below shows significant (p = 0.02) association between HBOC-201 and survival in absence of the other variables of interest for Studies 7, 8, and 16. The subsequent p-values in the table indicate the effect of each specified variable on the p-value for the association between HBOC-201 and survival. For example, when MAP treatment effect is included in the analysis, the p-value increases from 0.02 to 0.5, indicating that the HBOC-201 effect on MAP may partially explain the observed survival benefit. The same correlation can be observed for SBP and LA which increase and decrease, respectively, in these studies. The remaining variables did not correlate with the observed HBOC-201 associated survival benefit. A similar analysis conducted for Study 22 showed an association between HBOC-201 and survival with a p value = 0.009 (Table 9). Survival correlated with SBP, MAP, LA, and $tcpO_2$.

Table 9: NMRC Preclinical survival statistics

	P-value for Association between HBOC-201 and Survival	
	NMRC HS studies	**NMRC HS/TBI study**
HBOC-201	0.02	0.01
SBP	0.09	0.63
MAP	0.48	0.54
CI	0.01	0.01
HR	0.02	0.01
MPAP	0.02	0.02
LA	0.99	0.75
BD	0.02	0.01
SvO_2	0.04	0.03

[30] Studies 7, 8, and 9 were HS models with a 4-hour prehospital phase providing resuscitation with HBOC-201 at 10 ml/kg followed by 5 ml/kg; Study 22 (HS/TBI) had a 75-minute prehospital phase providing HBOC-201 infusions at 10 ml/kg followed by a 285 minute in-hospital phase where blood or NS was provided.

P-value for Association between HBOC-201 and Survival		
	NMRC HS studies	**NMRC HS/TBI study**
$tcpO_2$	0.04	0.11

Note: The higher the p-value, the greater the association of the variable with survival in HBOC-201 animals.

2. Improved restoration of hemodynamics with HBOC-201

In the 14 animal studies reviewed with survival as a primary endpoint (Table 8), rapid restoration and stabilization of MAP was observed with HBOC-201. In moderate/controlled or severe/uncontrolled HS models, in which animals received > 15 ml/kg HBOC-201 (Studies 7, 8, 9, 15, 16), CI was restored to baseline. In low-volume resuscitation studies in which animals received < 15 ml/kg HBOC-201, CI did not return to baseline (Studies 1, 3, 4, 5, 6, 10); however, HS-induced lactic acidosis resolved in all studies in which it was measured (Studies 1, 4, 5, 6, 10). In severe uncontrolled HS models, the HBOC-201 dose correlated with restoration of CI to baseline (i.e., Study 13 provided a low volume of HBOC-201 [6 ml/kg] and CI remained below baseline; Study 15 which provided 11 ml/kg and CI was restored to baseline). In the most severe uncontrolled HS model (Study 14), which included cardiac arrest, although CI did not return to baseline, 100% of HBOC-201 animals survived vs. 0% of controls.

3. Improved tissue oxygenation with HBOC-201.

Efficacy data from preclinical HS studies indicate that HBOC-201 increases tissue oxygenation by direct measurement methods, and averts/decreases anaerobic metabolism, resulting in improvements in indirect global measurements of tissue oxygenation such as blood LA and BD levels[31]. In the 14 HS studies with survival as a primary endpoint, in comparison with standard

[31] In OBRR's issue summary for the planned 14 July 2006 BPAC meeting, OBRR stated, *lactate levels, when measured, were often higher and lactate clearance was often slower in HBOC-201 animals*...NMRC believes this statement is incorrect, presumably based on potential miscommunications regarding the results of Drs. York and Fitzpatrick:

York G[31]:

- **FDA issue summary (14 Jul 2006, page 34):** *After fluid resuscitation to baseline in hour 5, lactate levels for HBOC-201 animals significantly higher than for other 3 groups.*
- **York [31] (page 876):** *Arterial pH…was equivalent…*
- **York [31] (page 876-877):** *lactate levels in the HBOC (60) group rose to statistically significant levels when compared with the other groups (2.3±0.9 mmol/L vs. 0.9±0.1 mmol/L for the Shed Blood [BL] group; 1.4±0.1 mmol/L for the Shed Blood [60] group; and 1.3±0.4 mmol/L for the LR + Blood group, all p < 0.05.*

fluids, HBOC-201 resuscitation resulted in improvements in tissue oxygenation in 7 of 8 (Studies 4, 7-9, 16, 22); $brPO_2$ was improved in 5 of 5 (Studies 2, 12, 19, 21, 22) (and SSO_2sat in Studies 12, 22). LA was decreased in 5 (1, 4, 13, 16, and 22) and equivalent in 7 of 12 studies (Studies 1, 3, 6-11); and BD was improved in 6 of 6 studies (Studies 1, 15, 16, 20, 21, 22). Other investigators have also shown increased tissue oxygenation and decreased or reversal of anaerobic metabolism with HBOC-201 (Studies 2, 5, 6, 10, 11, 12, 13, 37, 38).

4. Improved neurologic outcome with HBOC-201.

Even brief episodes of hypotension and hypoxia appear to be associated with increased morbidity and mortality in patients with TBI. [78-82] Although there are no controlled trials demonstrating improved outcome with prevention of hypotension and hypoxia in TBI patients, retrospective subgroup analysis of one study showed higher survival in TBI patients resuscitated with HTS (who had higher BP) vs. standard crystalloid fluid. [83] In its prehospital guidelines, The Brain Foundation's experts concluded, data *strongly suggests that elevating the BP in hypotensive severe head injury patients improves outcome*. Thus, avoidance of hypotension and hypoxia are key goals in the resuscitation of patients with TBI. As HBOC-201 effectively expands intravascular volume, rapidly stabilizes hemodynamics with low-volume resuscitation, transports and unloads O_2, and increases tissue oxygenation in HS, it has theoretical potential to particularly benefit trauma casualties with HS and concomitant TBI.

- **York [31] (page 878):** *Table 3: R + 60: Shed blood (BL) (2.7±1.4), Shed blood (60) 2.3±0.9, LR + Blood (4.4±3.6), HBOC (60) (2.6±1.4)*.
- **NMRC**: Although all comparators included blood transfusions, lactate was actually lower in the HBOC group than in the LR + Blood group during the period comparing HBOC and LR (at 60 minutes) (akin to *RESUS*); the higher lactate in the HBOC group, as compared with the blood transfusion containing-control groups, occurred at 300 minutes after prolonged withholding of blood transfusions (irrelevant to *RESUS*). For accuracy, both the acute and the delayed observations should be considered.

Fitzpatrick C[54]:

- **FDA issue summary (14 Jul 2006, page 35):** *Lactate levels corrected with resuscitation in all groups, but were higher in HBOC-201 group*.
- **Fitzpatrick [54] (page 696):** *Lactate levels (control 0.9±0.1 mmol/L; SB 0.9±0.1 mmol/L; LRSB 1.0±0.1 mmol/L; HBOC 1.7±0.4 mmol/L, p=0.16 (Fig. 2B) and base deficit (control 4.1±0.7 mmol/L, SB 6.8±0.5 mmol/L, LRSB 6.6±0.6 mmol/L, HBOC 3.1±1.0 mmol/L, p=0.18) normalized in all groups with resuscitation*.
- **Fitzpatrick [54] (page 697)**: *Figure 2…(B) All study groups had similar lactate levels in response to hemorrhage and resuscitation*.
- **NMRC:** Lactate was not *higher in HBOC group* as the p value was 0.16; this was the conclusion of the authors. Although there was a trend to higher lactate with HBOC-201 than in the blood transfusion containing control groups, the reverse was true for BD. For accuracy, both the lactate and BD data should be considered.

Four HBOC-201 and one HBOC-301 studies evaluated HBOC-201 resuscitation in animal models of HS with concomitant TBI. In comparison with standard fluids, CPP improved in all four studies in which it was evaluated (Studies 18, 19, 21, 22). ICP was lower with HBOC-201 in 3 of 4 studies in which it was evaluated (Studies 18, 19, 21); ICP was slightly higher in one study (Study 22). Increased direct measurements of $brPO_2$ demonstrated with HBOC-201 compared to standard fluid in all four studies in which it was evaluated (Studies 18, 19, 21, 22); Studies 2 and 12 also showed increased $brPO_2$ with HS alone. SSO_2sat was higher with HBOC-201 in both studies in which it was measured (Studies 12, 22). CBF was decreased in one (Study 20) and equivalent in one (Study 22) of the two studies in which it was directly measured. Intracranial compliance/cerebrovascular reactivity was equivalent in one (Study 22) and improved in two (Studies 18, 20) of three studies in which it was measured. HBOC-201 and HBOC-301 resuscitation resulted in normalization of respiratory drive and higher rates of weaning off mechanical ventilation in both studies in which it was a measured outcome measurement (Studies 18, 19). Histopathologic analyses revealed that injury/contusion volume was diminished in 1 (Study 20) and equivalent in 3 (Studies 19, 21, 22) of four studies in which it was measured; brain edema was equivalent in the one study in which it was measured (Study 20). Subarachnoid, subdural, and/or intraparenchymal hemorrhage was diminished in one (Study 22) and equivalent in one (Study 19) of two studies in which data were reported; neutrophilic infiltration and astrocytic injury (H&E, GFAP, and MAP2) were similar in the one study in which it was reported (Study 22). Ipsilateral neuronal necrosis was equivalent in two of three studies in which it was reported (Studies 19, 21, 22)[32]; contralateral neuronal injury was decreased in both studies in which it was reported (Studies 21, 22). Overall, there were no neurophysiologic or histopathologic findings suggesting that HBOC-201 (or HBOC-301) is unsafe in TBI. On the contrary, findings suggest potential for protection against secondary neuronal injury in patients with HS and concomitant TBI.

5. Equivocal myocardial effects with HBOC-201.

In HS swine models, myocardial histopathology and blood peak cardiac troponin I levels have been equivalent or decreased with HBOC-201 resuscitation in comparison with standard fluid. Myocardial necrosis and fibroplasia was decreased in one and equivalent in two of three studies

[32] Neuronal necrosis and white matter degeneration were slightly increased in a minority of sections in HBOC-201 vs. LR pigs in 1 study (Study 22); but because only HBOC-201 pigs had sufficient survival to develop such neuropathologic changes, NMRC believes these observations are related to higher survival rather than HBOC-201 neurotoxicity.

in which it was assessed (Study 30)[33]; alveolar and interstitial pulmonary edema were equivalent in all three studies. With uncontrolled HS/TBI, myocardial inflammation (lymphoplasmacytic inflammation or subepicardial inflammation [chronic-active or perivascular-acute]), myocardial necrosis/degeneration, and peak troponin I levels were not significantly different (there were trends to lower myonecrosis incidence/severity scores and troponin I levels in HBOC-201 pigs) (Study 22, LD cohort). Myocardial degeneration (± necrosis) was equivalent and generally mild (Study 10). A consistent pattern for pulmonary capillary wedge pressure (PCWP) is not apparent. PCWP was equivalent in one (Study 22) and higher in one (Study 7) of two with HBOC-201[34]. PCWP reached or slightly exceeded (Study 15) or remained below baseline (Study 5); PCWP was lower with HBOC-201 than with LR in Study 1. Overall, beneficial effects of HBOC-201 on tissue oxygenation may be myocardially protective (despite mild vasoconstrictive responses), as myocardial degeneration, cardiac biomarkers, and histopathologic changes were similar or lower with HBOC-201 vs. Hextend (Study 30). Lack of significantly increased PCWP and lack of histopathologic evidence of pulmonary interstitial and alveolar edema (by H&E or EM), suggests that pulmonary edema (whether cardiogenic or noncardiogenic) was not a significant risk with HBOC-201 in the studies above.

Adverse Events in preclinical studies

In preclinical HS models, AEs associated with HBOC-201 included mild to moderate increases in systemic and pulmonary BP (Studies 1, 7, 8, 11, 16, 17, 22, 37) and systemic vascular resistance (Studies 6, 7, 10, and 16). CI remained below baseline values in some of the studies, especially in less severe HS models with low volume pressure-controlled resuscitation (Studies 1, 3, 4-7, 9, 10, 11); CI was generally equivalent in severe HS models (8, 16, 22). Histopathologic and clinical laboratory studies have indicated mild hepatocellular (Study 4) and hepatobiliary changes (Studies 7, 8, and 16), mild renal papillary histopathology changes (Study 30), transiently elevated liver function tests (Studies 4, 7, 8, 9, and 16), mild coagulopathic changes (attributed to blood transfusion avoidance) (Studies 25 and 26), mild methemoglobinemia (Studies 6, 22), and mild blood oxygen desaturation (with similar blood PO_2) (Study 22). However, histopathologic changes were minimal or mild, liver function tests resolved to baseline, and methemoglobinemia not clinically marked.

[33] CK-MB was slightly higher with HBOC-201 vs. Hextend with moderate severity controlled HS (Study 30); as the CK-MB/CK ratio was < 2%, a skeletal muscle source was likely.
[34] PCWP and CVP were not reported for the majority of NMRC's studies as they did not differ between HBOC-201 and control fluids (Studies 8, 9, 22).

Toxicology

Results from toxicology studies in rats indicated various embryo-fetal toxicities which were interpreted to be related to the visceral yolk sac histiotrophic nutritional processes that are unique to rodent gestation. However, as no embryo-fetal effects of HBOC-201 in pregnant dogs were observed, it is likely that the teratogenic effects of HBOC-201 observed in rats are not relevant to humans (Study 40).

Conclusions

1. While the animal models do not exactly simulate the prehospital trauma setting, the controlled and uncontrolled HS, HS/TBI, and whole body trauma models in rats, swine, and dogs collectively address the broad physiological perturbations that occur with trauma in the prehospital setting.
2. HBOC-201 was found to decrease mortality in comparison with control fluids.
    a. 9.3 vs. 18.8% ($p > 0.05$) in swine with less severe HS (LD50 < 50%)
    b. 16.7 vs. 93% ($p < 0.0001$) in swine with more severe HS (LD50 $\geq$ 50%)
    c. 12 vs. 47% ($p < 0.0001$) in swine with overall HS
    d. 41 vs. 75% ($p = 0.03$) in swine with HS + TBI (NMRC studies)
3. Data from hematology, immunology, organ function, and oxidative stress studies do not suggest grounds to preclude evaluation of HBOC-201 in humans.
4. In non-trauma studies, HBOC-201 was observed to
    a. Provide oxygenation to post-stenotic tissue, increase skeletal muscle oxygenation, and reduce coronary stenosis infarct size.
    b. Increase liver and skeletal muscle oxygenation, provide stable perfusion to vital organs, and not cause significant organ damage.
5. Data from reproductive and neurotoxicology studies do not preclude evaluation of HBOC-201 in humans.
6. The majority of studies has undergone peer-review and has been published, i.e., design, statistical power, and the validity of the conclusions have been accepted by reviewers.
7. Overall benefit:risk balance in animals supports the proposed clinical research to evaluate the hypothesis that HBOC-201 will decrease mortality in HS patients by 15% under the conditions to be studied in *RESUS*.
8. The animal models used to evaluate HBOC-201 are considered appropriate and relevant to evaluate compounds for use in prehospital trauma by the medical community. The

results of 23 separate animal studies strongly support prediction of *potential* for *direct benefit* of HBOC-201 to subjects enrolled in the *RESUS* study.

5.d Prior Human Exposure

Overview

The pharmacology, efficacy, and safety of HBOC-201 were investigated in 21 completed clinical trials. The clinical program was designed to evaluate whether HBOC-201 could replace RBC transfusions to meet perioperative oxygen needs in surgical patients. There were four Phase 1 studies, eleven Phase 1/2 studies, four Phase 2 studies, and two completed Phase 3 studies. HBOC-201 was administered intravenously to subjects in single and multiple doses for up to six days in both fixed and subject weight-adjusted doses. More than 800 subjects received HBOC-201 in doses ranging from 25 to 300g (~ 1–10 units) administered over up to six days. There were 14 studies labeled as *crystalloid/colloid controlled* (see below) trials completed in 655 subjects, including normal volunteers, sickle cell anemia, and surgical subjects. There were 4 studies labeled as *RBC-controlled* trials assessing HBOC-201 for transfusion efficacy in treatment of surgical patients and three non-controlled studies in healthy volunteers, assessing immunologic effects, tolerance, and dose escalation: Two studies remain incomplete. M9990-0088, is a randomized, single-blinded, single center, *LR-controlled* study of 2 IV doses of HBOC-201 in subjects being weaned from mechanical ventilation; it was terminated after enrollment of 1 subject because of concerns about the technical feasibility of this protocol design. HEM-0125, a randomized, single-blinded, single-center, parallel group, standard therapy controlled, variable dose study to evaluate safety and tolerability of HBOC-201 in trauma patients with HS in-hospital, is still enrolling subjects at this time; however, interim findings are discussed below

Pharmacokinetic and Pharmacological Profile

The pharmacokinetics of HBOC-201 were investigated using pooled data from 18 Phase 1-3 clinical trials, including 726 patients in various surgical settings. The pharmacokinetics of HBOC-201 following IV infusion in humans is described by a one-compartment model with first-order elimination and is linear between 0.6 and 2.5 g/kg. Estimates of the pharmacokinetic parameters from population kinetic analyses are: half-life = 19 hours, clearance CL = 0.122 L/hr, and volume of distribution Vd = 3.36 L. The volume of distribution corresponds ~ to plasma volume. Clearance and volume of distribution are correlated with body weight.

The efficacy of HBOC-201 is predicated upon 2 pharmacologic properties—its ability to transport oxygen to tissues and volume expansion. The ability of HBOC-201 to efficiently transport oxygen is its most important pharmacologic property. Improved oxygen transport is due to both and diffusive oxygen delivery mechanisms. HBOC-201 has a viscosity of 1.3 centipoise, ~ 1/3 that of blood. Hemodilution with colloids has beneficial effects on tissue perfusion and oxygenation related to viscosity, a property shared with HBOC-201, to increase convective oxygen delivery and an element of volume expansion. However, the primary mechanism by which HBOC-201 enhances oxygen carrying transport is by increased diffusive flux.

Demonstration that HBOC-201 increases the diffusive flux of oxygen delivery is difficult *in vivo;* this was studied directly *in vitro* by Hellum at Rice University. [35] Study aims were to evaluate oxygen uptake and off-loading from HBOC-201 and RBC under conditions of flow and geometry that approximate capillaries. *In vitro* findings [36] demonstrated that HBOC-201 onloads and offloads oxygen more efficiently than RBC Hb. While the enhanced offloading of oxygen to tissues was anticipated based solely upon the higher P_{50} value (40 mm Hg) for HBOC-201, enhanced onloading was not. Extrapolating to humans, HBOC-201 could therefore theoretically provide superior oxygen transport and tissue perfusion under clinical conditions of compromised flow.

Study Designs of Completed Studies

Studies in healthy volunteers

Four studies were conducted in healthy volunteers, one Hespan® (HES)-controlled and three without controls. Two studies investigated pulmonary function and found that HBOC-201 increased pulmonary diffusing capacity and was well tolerated. In an exercise study, HBOC-201 improved physiological parameters. The immune effects of up to eight repeat exposures to HBOC-201 were assessed in another study; HBOC-201 did not cause significant allergic reactions, however, two subjects with previous HBOC exposure experienced mild allergic reactions that resolved promptly with standard treatment. HBOC-201 did not induce clinically significant antibodies. The effect of long-term repeat dosing has not been evaluated.

Non-surgical subject studies

HBOC-201 was investigated in subjects with sickle cell disease, ± vaso-occlusive crisis. In exercise tests, subjects with sickle cell disease demonstrated improved physiological parameters.

Those subjects studied in vaso-occlusive crisis showed that HBOC-201 was well tolerated without significant differences in outcome measurements.

Studies in surgical subjects (Table 10**)**

There were three *HES-controlled* perioperative studies, assessing the safety and efficacy of HBOC-201 in acute normovolemic hemodilution. These trials were "low dose" studies in subjects undergoing liver resection (BR-0049-BBM-0149), elective surgery for abdominal aortic aneurysms (BR-0049-BBM-0144), and orthopedic surgery (BR-0049-BBM-0153).

Seven *crystalloid-controlled* studies utilizing LR were conducted in non-cardiac surgery subjects. The dose in these single-dose studies varied from 0.5 to 244.9 g Hb/kg, with the initial dose comparable to doses tested in Phase 1 and Phase 1/2 studies, with subsequent dosing 2- to 3-fold higher. In the multiple-dose study M9990-0101, a loading dose was followed by a maintenance dose 24 hours later. The doses were weight-adjusted (loading/maintenance dose)—0.6/0.4, 0.9/0.4 and 0.9/0.6 g Hb/kg. In all studies, the goal was to achieve plasma Hb levels greater than 1 g/dL for up to 24 hours. The HEM-0118 study allowed doses of up to 300 g of Hb (10 units of HBOC-201), evaluating the clinical effects of variable doses of HBOC-201 administered perioperatively in non-cardiac surgery subjects.

There were four *RBC-controlled* studies investigating safety and efficacy of HBOC-201 in the management of surgical anemia, and assessing RBC transfusion avoidance: two Phase 3 studies (HEM-0115 and HEM-0114), and two Phase 2 studies (HEM-0107 and HEM-0075). HEM-0115 was the largest study and assessed HBOC-201 in elective orthopedic surgery. In this study, HBOC-201 was administered over a maximum of 6 days and up to a maximum dose of 300 g (10 units). Study HEM-0114 investigated doses of up to 210 g Hb (7 units) over 6 days in surgical subjects, including orthopedic surgical subjects. The two Phase 2 *RBC-controlled* studies were conducted in non-orthopedic surgical subjects (M9990-0075, M9990-0107). In study M9990-0075, the total dose and the treatment period were 120 g Hb (4 units) over 3 days. In study M9990-0107, the total dose of HBOC-201 increased to 150 g Hb (5 units) over 4 days.

A Phase 2 study (COR-0001) was performed from DEC 2003 to APR 2005 in the Netherlands, Belgium, and Germany to assess safety and feasibility of HBOC-201 in the setting of Percutaneous Coronary Intervention (PCI) for acute coronary syndrome (ACS).

The original labeling of the studies discussed above as *colloid-* or *crystalloid-controlled* was a generalization. These studies actually represent a mix of heterogenous study designs including different subject populations, dosing, data collect criteria, proportions of subjects in the treatment to control group, and number of subjects. In fact, some of these studies actually included treatment with RBC in the control group. While pooling studies in this manner provides a convenient way of discussing which studies were performed, pooling studies in a similar manner for analysis of efficacy or safety is not recommended by existing guidelines. The clinical trials literature demonstrates that valid results and data analyses can only be expected when pooling studies with similar design and reasonably homogenous populations.

Table 10: Completed Clinical Trials Performed with HBOC-201

Protocol Number **Subject**	**Study Design** **Total Dose Administered**	**N** **Age Range (Mean)** **Demographic characteristics**
M9990-**0053** Healthy Volunteers	Phase 1, single dose, randomized, single-blind, placebo-controlled, parallel group study Total Dose: 3.25 – 45g (≤ 1.5U)	N_{HBOC} = 32 (14 HSA, 18 HBOC-201)**;** N_{HSA} = 23 Age 18-45 (29.0); % M/F 100/0
M9990-**0059** Healthy Volunteers	Phase 1, single dose, randomized, single-blind, rate-escalation parallel group study Total Dose: ≤ 45g	N_{HBOC} = 18; N_{LR} = 6 Age 18-43 (29.7); % M/F 50/50
M9990-**0062** Healthy Volunteers	Phase 1, randomized, single-blind, 2-way crossover 1 infusion 45g HBOC-201 and 110 to 120g ATX Total Dose: 45g	N_{HBOC} = 6 Age 25-45 (37); % M/F 100/0
M9990-**0070** Immunology Healthy Volunteers	Open Label, 2 Doses (at T = 0 and T = 29 day) Total Dose: 45 – 90g	N_{HBOC} = 8 Age 21-39 (31.5); % M/F 100/0
M9990-**0061** Radical Prostatectomy	Phase 1/2, randomized, single-blind, placebo-controlled, parallel group study, single dose Total Dose: 0.5 – 45g	N_{HBOC} = 16; N_{LR} = 11 Age 48-73 (60.5); % M/F 100/0
M9990-**0063** Surgery Gynecological	Phase 1/2, single dose, randomized, single-blind, placebo-controlled, parallel group study Total Dose: 23.4 – 45g	N_{HBOC} = 10; N_{LR} = 8 Age 24-74 (45.1); % M/F 0/100
M9990-**0068** Surgery Orthopedic	Phase 1/2, single dose, randomized, single-blind, placebo-controlled, parallel group study Total Dose: 21.8 – 45g	N_{HBOC} = 13; N_{LR} = 10 Age 49-80 (64.4); % M/F 39/61
M9990-**0071** Surgery Gynecological (discontinued)	Phase 1/2, single dose, randomized, single-blind, placebo-controlled, parallel group study Total Dose: 27g	N_{HBOC} = 1; N_{LR} = 1 Age 31-37 (34); % M/F 0/100
M9990-**0072** Sickle Cell Non Crisis	Phase 1/2, single dose, randomized, single-blind, placebo-controlled, parallel group study Total Dose: 10.8 – 42.9g	N_{HBOC} = 12; N_{NS} = 7 Age 19-47 (30.1); % M/F 84/16
M9990-**0099** Sickle Cell in Vaso-Occlusive Crisis	Phase 1/2, randomized, single-blind, placebo-controlled, parallel group study Total Dose: 14.0 – 78.3g (2 doses at T0 and T24hr)	N_{HBOC} = 12; N_{LR} = 7 Age 7-48 (22.3); % M/F 79/21
BR-0049-BBM-**0144** Surgery Abdominal Aortic	Phase 1/2, single dose, randomized, single-blind, comparator-controlled (HES), parallel group study Total Dose: 28.5 – 97.8g	N_{HBOC} = 19; N_{HES} = 20 Age 43-75 (63.5); % M/F 74/26
BR-0049-BBM-**0149**	Phase 1/2, single dose, randomized, single-blind,	N_{HBOC} = 6; N_{HES} = 8

Protocol Number **Subject**	**Study Design** **Total Dose Administered**	**N** **Age Range (Mean)** **Demographic characteristics**
Surgery Liver Resection	comparator-controlled (HES), parallel group study Total Dose: 22.4 – 36.4g	Age 25-70 (55); % M/F 64/36
BR-0049-BBM-**0153** Surgery Orthopedic	Phase 1/2, single dose, randomized, single-blind, comparator-controlled (HES), parallel group study Total Dose: 43.2 – 66.6g	N_{HBOC} = 6; N_{HES} = 8 Age 45-76 (63); % M/F 7/93
M9990-**0100** Surgery Non-Cardiac	Phase I/II, single dose, randomized, single-blind, placebo-controlled, dose-escalation, parallel group study Total Dose: 0.7 – 244.9g	N_{HBOC} = 55; N_{LR} = 26 Age 35-86 (60); % M/F 59/41
M9990-**0101** Surgery	Phase 1/2, multiple dose, randomized, single-blind, LR-controlled, dose-escalation, parallel group study Total Dose: 0.7 – 165.8g	N_{HBOC} = 25; N_{LR} = 14 Age 36-75 (60.5); % M/F 82/18
M9990-**0075** Surgery CPB	Phase 2, multicenter, randomized, double-blind, RBC-controlled, variable-dose (3 doses over 72 hr), parallel group study Total Dose: 60 – 120g	N_{HBOC} = 50; N_{RBC} = 48 Age 44-82 (66.4); % M/F 62/38
M9990-**0107** Abdominal Aortic Reconstruction	Phase 2, multicenter, randomized, single-blind, RBC-controlled, variable-dose (4 doses over 96 hr), parallel group study Total Dose: 60 – 150g	N_{HBOC} = 48; N_{RBC} = 24 Age 41-82 (69.4); % M/F 81/19
HEM-**0118** Surgery Non-cardiac	Phase 2, multicenter, randomized, single-blind, placebo-controlled, parallel group study, 3 doses over 72 hr Total HBOC Dose: 90 – 300g	N_{HBOC} = 26; N_{LR} = 25 Age 24-83 (58.2); % M/F 84/16
COR-**0001** Acute Coronary Syndrome	Phase 2, multicenter, randomized, single–blind, placebo-controlled, 3 arm parallel group study Total HBOC Dose:15-30g	N_{HBOC}=29; $N_{Placebo}$=16 Age 38-74 (60.07); % M/F (73/27)
HEM-**0114** Surgery Non-cardiac	Phase 3, multicenter, randomized, single-blind, RBC-controlled, parallel group study, 6 doses over 6 days Total Dose: 60 – 210g	N_{HBOC} = 83; N_{RBC} = 77 Age 21-86 (61.0); % M/F 56/44
HEM-**0115** Surgery Orthopedic	Phase 3, multicenter, randomized, single-blind, RBC-controlled, parallel group study, 9 doses over 6 days Total Dose: 60 – 300g	N_{HBOC} = 350; N_{RBC} = 338 Age 18-95 (60.8); % M/F 45/55

1 – R = randomized, PG = parallel group, SB = single blind.
2 – One unit of HBOC ~ 30 g/Hb.
3 – HSA = Human Serum Albumin, LR = lactated Ringer's solution, HES = Hespan®.

Efficacy of HBOC-201 in the treatment of surgical anemia

In the four *RBC-controlled* clinical trials designed to assess safety and efficacy of HBOC-201 for treatment of acute surgical anemia, both total dose and duration of potential exposure were investigated. In studies HEM-0075, HEM-0107, HEM-0114, and HEM-0115, HBOC-201 eliminated RBC transfusion in 34%, 27%, 43%, and 59% of subjects, respectively (Table 11) As with the labeling of *crystalloid-* and *colloid-controlled* studies, the *RBC-controlled* label was also a generalization. Studies HEM-0114 and HEM-0115 were clearly *RBC-controlled* with blood avoidance and reduction of RBC transfusion clearly defined as study endpoints. However, this was not true for studies HEM-0075 and HEM-0107. In study HEM-0075, subjects received RBC

intraoperatively following bypass surgery, after which HBOC-201 was administered; transfusion avoidance was then measured postoperatively. Similarly, in study HEM-0107, subjects were re-infused with autologous blood and only after all autologous blood was re-infused were subjects randomized to HBOC-201 or heterologous RBC.

Table 11: Proportion of HBOC-201-Treated Subjects Receiving No RBC Transfusion - N (%)

Study Number	N_{HBOC}	Maximum Dose (U)	Day 1	Day 7	Day 28	Day 42
HEM-0115	350	10	337 (96%)	246 (70%)		208 (59%)
HEM-0114	83	7	68 (82%)	46 (55%)	36 (43%)	
M9990-0075	50	4			17 (34%)*	
M9990-0107	48	5			13 (27%)*	

* Day 21-28 (3-4 weeks after first dose of HBOC-201)

Evaluation of Safety

The initial evaluation for safety signals was assessed by pooling results of all completed trials, including 797 subjects exposed to ≥ 1 dose of HBOC-201. This approach represents an integrated tabulation of all the safety signals observed in all the clinical trials, but fails to account for differences in study design (including asymmetrical aspects and repeat and multiple treatment exposures) and use of different comparators (None, HES, LR, and RBC), in different randomization schemes. The pooling of this non-homogenous data, while appropriate for initial identification and detection of safety signals, is inadequate for comprehensive quantitative analysis of safety data.

Integrated Safety Summary (ISS)—detection of safety signals (all studies)

Seven hundred forty (740) subjects (93%) in the HBOC-201 group and 581 subjects (88%) in the comparator control group experienced ≥ 1 AE. The absence of 1:1 randomization in some trials may have influenced the discrepancies in event frequency. A total of 9,828 AEs were reported in 21 trials, 6,204 and 3,624 AEs in the HBOC-201 and control groups, respectively. 187 subjects (23%) in the HBOC-201 group and 120 subjects (18%) in control groups experienced SAEs for a total of 434 SAEs reported in the 21 trials, 269 SAEs in HBOC-201 group and 165 SAEs in control groups (Table 12).

Table 12: Summary of all (overall) AEs

Number of Subjects	Number (%) of subjects with AEs			Total number of AEs		
	Total N = 1,458	HBOC-201 N = 797	Control N = 661	Total N = 1,458	HBOC-201 N = 797	Control N = 661
Treatment-emergent AEs	1,321 (91%)	740 (93%)	581 (88%)	9,828	6,204	3,624
Treatment-emergent SAEs	307 (21%)	187 (23%)	120 (18%)	434	269	165

N = number of subjects.

In addition to the data summarized in **Error! Reference source not found.**, there is AE data from COR-0001 as yet to be integrated into the overall safety signal detection analysis. In this PCI study, there were 23 AEs (1.9/subject) reported in the HBOC-201 group receiving 30 g, 19 AEs (1.1/subject) in the HBOC-201 group receiving 15 g, and 15 AEs (0.93/subject) in the control group. In the ongoing HEM-0125 S. Africa ER trauma trial, 21 subjects have been enrolled (1 protocol enrollment violation dropped from study) and treated with up to 10 units HBOC-201. Mortality was 4 of 10 (40%) in both study arms. There were 10 SAEs in HBOC-201 subjects (10/10 [1.0 SAE per subject]) and 13 SAEs in RBC subjects (13/9 [1.4]). Nine of 19 evaluated subjects experienced an SAE, 4 (40%) in HBOC-201 subjects and 5 (55%) in control subjects. An interim analysis of the first 21 patients has been completed and the DSMB has given approval to continue the trial without modification.

Adverse Events by System Organ Class (SOC)

AEs with a statistically significant (at the level of $p \leq 0.1$) higher frequency in the HBOC-201 group, occurred in the Blood/Lymphatic, Cardiac, Gastrointestinal, Investigations, Hepatobiliary, Metabolism/Nutrition, Renal/Urinary, Respiratory, Skin, and Vascular systems (Table 13).

Table 13:AEs with Statistically Significant Group Differences ($p \leq 0.1$) (HBOC-201 > Controls) in all Studies (ISS)

System Organ Class (SOC)/Preferred Term	HBOC-201 N (%)	Control N (%)
Blood and Lymphatic System Disorders	**111 (14%)**	**42 (6%)**
Anemia	63 (8%)	23 (3%)
Methemoglobinemia	18 (2%)	0 (0%)
Cardiac Disorders	**216 (27%)**	**140 (21%)**
Cardiac Failure Congestive	15 (2%)	3 (0%)
Myocardial Infarction	13 (2%)	4 (1%)
Eye Disorders	**27 (3%)**	12 (2%)

System Organ Class (SOC)/Preferred Term	HBOC-201 N (%)	Control N (%)
Scleral discoloration	12 (2%)	0 (0%)
Gastrointestinal Disorders	**505 (63%)**	**336 (51%)**
Nausea	226 (28%)	120 (18%)
Vomiting	129 (16%)	67 (10%)
Constipation	125(16%)	127 (19%)
Diarrhea	63 (8%)	32 (5%)
Abdominal pain	58 (7%)	11 (2%)
Abdominal pain, upper	42 (5%)	13 (2%)
Dyspepsia	56 (7%)	32 (5%)
Flatulence	52 (7%)	14 (2%)
Dysphagia	48 (6%)	7 (1%)
Abdominal distension	42 (5%)	18 (3%)
General Disorders	**415 (52%)**	**313 (47%)**
Fatigue	43 (5%)	17 (3%)
Unevaluable edema	23(3%)	9 (1%)
Hepato-biliary Disorders	150 (19%)	14 (2%)
Jaundice	135 (17%)	6 (1%)
Infections and Infestations	**152 (19%)**	**120 (18%)**
Sepsis NOS	**10 (1%)**	**2 (0%)**
Injury and Poisoning	**83 (10%)**	**55 (8%)**
Abrasions	8 (1%)	1 (0%)
Investigations	**294 (37%)**	**164 (25%)**
Aspartate aminotransferase increase	34 (4%)	12 (2%)
Blood creatinine phosphokinase MB increased	7 (1%)	1 (0%)
Blood potassium decreased	16 (2%)	5 (1%)
Lipase increase	48 (6%)	12 (2%)
Blood pressure increased	35 (4%)	7 (1%)
Oxygen saturation decreased	38 (5%)	15 (2%)
Metabolism and Nutrition Disorders	**137 (17%)**	**79(12%)**
Dehydration	11 (1%)	0 (0%)
Nervous System Disorders	**270 (34%)**	**199 (30%)**
Cerebrovascular accident	9 (1%)	1 (0%)
Taste disturbances	5 (1%)	0 (0%)
Renal/Urinary Disorders	**203 (25%)**	**115 (17%)**
Oliguria	95 (12%)	42 (6%)
Hematuria	47 (6%)	17 (3%)
Urinary hesitation	6 (1%)	0 (0%)
Respiratory Thoracic and Mediastinal Disorders	**212 (27%)**	**144 (22%)**
Hypoxia	24 93%)	10 (2%)
Skin/Subcutaneous Tissue Disorders	**278 (35%)**	**129 (20%)**
Dermatitis	60 (8%)	27 (4%)
Ecchymosis	26 (3%)	9 (1%)
Skin discoloration	52 (7%)	23 (3%)
Sweating increased	30 (4%)	11 (2%)
Face oedema	8 (1%)	0 (0%)
Petechiae	6 (1%)	0 (0%)
Purpura NOS	5 (1%)	0 (0%)
Skin disorder NOS	9 (2%)	6 (1%)
Urticaria NOS	5 91%)	0 (0%)
Yellow skin	19 (2%)	0 (0%)
Vascular Disorders	**243 (30%)**	**139 (21%)**
Hypertension	120 (15%)	48 (7%)
Hematoma	30 (4%)	14 (2%)

System Organ Class (SOC)/Preferred Term	HBOC-201 N (%)	Control N (%)
Phlebitis NOS	7 (1%)	1 (0%)

There was a significant group difference in HBOC-201 vs. comparator groups at the level of SOCs for cardiac SAEs (p = 0.07) (Table 14). At the level of individual Preferred Terms, SAEs occurring more often at the $p \leq 0.05$ level of significance in the HBOC-201 group were post-operative bleeding (0.9 vs. 0%, p = 0.02) and CVA (1.0 vs. 0.2%, p = 0.05).

Table 14: SAE Incidence by SOC in All Studies (ISS)

	Incidence (%) of treatment-emergent SAEs				
	HBOC-201	Control	P value	Odds ratio	95% CI on the odds ratio
SOC/Preferred Term	**N (%)**	**N (%)**			
Blood and Lymphatic System Disorders	5 (1%)	1 (0%)	0.23	4.2	0.5;35.7
Cardiac Disorders	46 (6%)	26 (4%)	0.07	1.6	1.0;2.8
Congenital and Familial/Genetic Disorders	1 (0%)	0 (0%)	1.00	2.5	0.1;61.3
Endocrine Disorders	1 (0%)	0 (0%)	1.00	2.5	0.1;61.3
Gastrointestinal Disorders	22 (3%)	15 (2%)	0.62	1.2	0.6;2.4
General Disorders and Administration Site Conditions	17 (2%)	10 (2%)	0.44	1.4	0.6;3.1
Hepato-Biliary Disorders	8 (1%)	2 (0%)	0.12	3.3	0.7;15.8
Immune System Disorders	2 (0%)	0 (0%)	0.50	4.1	0.2;86.7
Infections and Infestations	23 (3%)	23 (3%)	0.55	0.8	0.4;1.5
Injury and Poisoning	13 (2%)	11 (2%)	1.00	1.0	0.4;2.2
Investigations	7 (1%)	1 (0%)	0.08	5.8	0.7;47.6
Metabolism and Nutrition Disorders	5 (1%)	4 (1%)	1.00	1.0	0.3;3.9
Musculoskeletal, Connective Tissue and Bone Disorders	5 (1%)	2 (0%)	0.47	2.1	0.4;10.7
Neoplasms Benign & Malignant (include Cysts & Polyps)	1 (0%)	0 (0%)	1.00	2.5	0.1;61.3
Nervous System Disorders	8 (1%)	5 (1%)	0.78	1.3	0.4;4.1
CVA*	8 (1%)	1 (0%)	0.05	6.7	0.8;53.6
Psychiatric Disorders	4 (1%)	3 (0%)	1.00	1.1	0.2;5.0
Renal and Urinary Disorders	10 (1%)	8 (1%)	1.00	0.9	0.3;2.4
Reproductive System and Breast Disorders	0 (0%)	1 (0%)	0.45	0.3	0.0;6.8
Respiratory, Thoracic and Mediastinal Disorders	20 (3%)	11 (2%)	0.28	1.5	0.7;3.2
Skin & Subcutaneous Tissue Disorders	2 (0%)	0 (0%)	0.50	4.1	0.2;86.8
Surgical and Medical Procedures	21 (3%)	15 (2%)	0.74	1.2	0.6;2.3
Post-operative Hemorrhage*	7 (1%)	0 (0%)	0.02	12.6	0.7;220.2
Vascular Disorders	30 (4%)	19 (3%)	0.38	1.3	0.7;2.4

* Difference between treatments exists at preferred term level, but not at SOC level.

The increased incidence of reported postoperative bleeding in the HBOC-201 group is unexplained as coagulopathy was not identified by clinicians and could be an artifact of reporting. Eight HBOC-201 subjects experienced a CVA; all seemed to have had an embolic event and most had evidence of underlying disease. Two-thirds of the subjects had the onset of CVA within 5 days of exposure to CTM, not clearly related to evidence of HBOC-201 being present. When CVA is combined with other clinically relevant neurologic syndromes (TIA and RIND) the incidence is not statistically significantly different between the 2 groups (1 vs. 0.2%, p = 0.4). 10/797 (2.5%) vs. 3/661 (0.45%)

SAEs as a function of age

The overall incidence of SAEs shows a clear relationship with age in both the HBOC-201 and control groups (Table 14). Trends observed in both the HBOC-201 and control groups are best explained by noting that elderly subjects have more concomitant disease and less physiologic reserve, and may be more sensitive to fluid shifts, changes in oxygen carrying capacity and impaired oxygen delivery, and even the mild to moderate vasoactive properties of HBOC-201.

Table 15: Overall SAE incidence stratified by age (ISS)

	SAE incidence - % (95% CI)				
Age Group sub-population	**HBOC-201 N = 797**		**Control N = 661**		**Difference in % Incidence**
≤ 50	N = 245	36 (14.7% 10.2; 19.1)	N = 158	19 (12% 7; 17)	2.7%
> 50-75	N = 457	105 (23.0% 19; 27)	N = 414	70 (16.9% 12.9; 20.9)	6.1%
> 75	N = 95	46 (48.4% 38.4; 58.4)	N = 89	31 (34.8% 25.8; 43.8)	13.6%

Summary of Pivotal Phase 3 Orthopedic Trial (HEM-0115)

Studies HEM-0114 and HEM-0115 had similar designs and populations and were considered appropriate for pooled safety analysis. However, problems created by differences in the methods for data collection including coding of AEs (Costart vs. MedDRA), prospectively defined measurement time points, and differences in maximum dose and duration of treatment period limit the accuracy of pooling of the full set of data for analysis.

Study HEM-0115 was the largest (N = 688) individual study performed with HBOC-201, and included the most exhaustive data collection; it provided the basis for comprehensive analysis,

including thorough root cause analysis of SAEs. In addition, none of the previously identified safety signals from the review of the ISS database was omitted. This is consistent with the fact that HEM-0115 was the only clinical study sufficiently prospectively powered to detect AEs and true safety signals. Accordingly, the most reliable quantitative analysis of expected risk for benefit:risk assessment can be derived from the this homogeneous population. In this large multinational, randomized, *RBC-controlled*, single blind, parallel-group study, subjects undergoing elective orthopedic surgery at 46 hospitals and medical centers in the U.S., Europe, Canada, and S. Africa were randomized to receive either HBOC-201 or allogeneic RBC at the first clinical transfusion decision. Baseline characteristics were essentially the same in the two treatment arms as shown in Table 16.

Table 16: Baseline Subject Characteristics in HEM-0115

Baseline characteristics	HBOC-201 (n = 350)	RBC (n = 338)	P
Demographics			
Age (mean)	60.3	61.4	0.4
Male (%)	46.9	43.2	
White (%)	82.6	83.4	
Hematologic			
Total Hb (g/dL)	9.1	9.2	0.8
Hematocrit (%)	28	28	1.00
Surgery			
Back (%)	13.1	14.2	0.2
Non-back (%)	86.9	85.8	

Efficacy

The primary efficacy endpoint was the incidence of the elimination of allogeneic RBC transfusions at follow-up day 42. The primary endpoint would be met if the final incidence of elimination was ≥ 35% (lower 95% confidence limit ≥ 30%). The majority of HBOC-201 subjects avoided allogeneic blood transfusion throughout the 6-week study period: 96.3% in the first 24 hours, 70.3% at 1 week, and 59.4% at 6 weeks (Table 17). The primary safety endpoint of the trial was to observe that subjects treated with HBOC-201 have outcomes no worse than subjects treated with allogeneic RBC.

Table 17: Blood Transfusion Avoidance and Reduction (Efficacy) in HEM-0115

Efficacy	HBOC-201 (n = 350)	RBC (n = 338)	P
RBC transfusion avoidance (%)			
Post-operative day 1	96.3	N/A*	
Post-operative day 7	70.3	N/A	
Post-operative day 42	59.4	N/A	

Efficacy	HBOC-201 (n = 350)	RBC (n = 338)	P
# RBC units transfused (mean)	1.4	3.1	< 0.001

* All subjects randomized to this study required transfusion according to inclusion criteria.

Safety

The primary safety endpoint analysis in HEM-0115 was based upon statistical analysis of safety outcomes performed on the results by an independent SEEC blinded review of each subject's CRFs and medical records, including AEs, vital signs (VS), electrocardiogram (ECG) results, clinical laboratory tests, and physical and neurological examination results. The Mann-Whitney estimate of the probability of a more severe overall medical risk in the HBOC-201 group was p = 0.561 (upper 95% confidence limit 0.594), demonstrating that the probability of a more severe AE profile in the HBOC-201 treatment group was within a prospectively defined (upper 95% confidence limit 0.600) acceptable range.

In the HBOC-201 group, 95.4% (344) of subjects experienced ≥ 1 AE compared with 91.1% (308) in the RBC group (p = 0.024). In this study, 2,964 AEs (8.47/subject) were reported for the HBOC-201 group vs. 1,987 AEs (5.88/subject) for the RBC group. More subjects in the HBOC-201 group (N = 266) than in the RBC group (N = 206) experienced ≥ 1 moderate-to-severe AE (p < 0.001). In the HBOC-201 group, 25.1% of the subjects experienced ≥ 1 SAE vs. 17.5% in the RBC group. A > 10% absolute difference in the incidence of AEs (HBOC-201 > RBC) was seen in the following body systems (Table 18): Cardiac Disorders, GI, Hepato-biliary, Investigations, Skin and subcutaneous tissue, and Vascular disorders. Individual AEs across system organ classes (SOC) showed a > 5% difference between treatment groups for the following preferred terms: anaemia, tachycardia, abdominal pain, constipation, diarrhea, dysphagia, nausea, vomiting, pyrexia, jaundice, increased lipase, oliguria, and hypertension. A larger proportion of AEs were associated with exposure to HBOC-201 (31%) than to RBC (12%). The major contribution for this imbalance came from AEs in the GI, Hepato-biliary, Investigations, Skin and subcutaneous tissue, and Vascular disorders SOCs. This observation was expected since many of the AEs such as dysphagia, yellow skin (jaundice), transient increases in alanine transferase (ALT) and aspartate transferase (AST) activities, skin discoloration, tachycardia, and transient hypertension are known to be associated with HBOC-201 infusion.

Table 18: AEs by SOC Incidence Having a > 10% Absolute Difference Between Treatment Groups in HEM-0115

SOC	HBOC-201 (N = 350) N (%)	RBC (N = 338) N (%)
Cardiac Disorders	101 (29)	57 (17)
Gastrointestinal Disorders	257 (73)	195 (58)
Hepato-biliary Disorders	90 (26)	8 (2)
Investigations	138 (39)	84 (25)
Skin and Subcutaneous Tissue Disorders	142 (41)	96 (28)
Vascular Disorders	101 (29)	51 (15)

SAEs

In the HBOC-201 group, 88 (25.1%) subjects reported 118 SAEs and 83 (17.5%) subjects in the RBC group reported 59 SAEs (p = 0.01). Cardiac disorders was the only SOC showing a statistically significant (p = 0.01) difference between groups. The treatment groups did not differ in the number of subjects experiencing ≥ 1 SAE resulting in death (HBOC-201 2.9%, RBC 1.8%; p = 0.5). SAEs with an incidence rate in either the body system or preferred term ≥ 1% (i.e., ≥ 2 subjects) in either treatment group are presented in Table 19.

Table 19: SAEs by SOC and Preferred Term with Incidence ≥ 1% in Either Treatment Group in HEM-0115

System Organ Class/Preferred Term	HBOC-201 (N = 350) N (%)	RBC (N = 338) N (%)
Blood and Lymphatic System	2 (1)	1 (0)
Anemia NOS	2 (1)	0
Cardiac Disorders	22 (6)	9 (2)
Cardiac Arrest	5(1)	2 (1)
Cardio-Respiratory Arrest	3 (1)	0
Myocardial Infarction	4 (1)	2 (1)
Cardiac Failure Congestive	3 (1)	1 (0)
Pulmonary Edema	4 (1)	0
Gastrointestinal Disorders	6 (2)	7 (2)
General Disorders	8 (2)	4 (1)
Hemorrhage NOS	2 (1)	0
Multi-organ Failure	2 (1)	2 (1)
Hepato-biliary Disorders	5 (1)	0
Cholecystitis	2 (1)	0
Infection and Infestation	11 (3)	12 (4)
Cellulitis	1 (0)	2 (1)
Colitis Pseudomembranous	0	2 (1)
Osteomyelitis	0	2 (1)
Pneumonia	3 (1)	1 (0)
Wound Infection	4 (1)	5 (1)
Injury and Poisoning	10 (3)	11 (3)

System Organ Class/Preferred Term	HBOC-201 (N = 350) N (%)	RBC (N = 338) N (%)
Femur Fracture	1 (0)	2 (1)
Joint Dislocation NEC	3 (1)	8 (2)
Investigations	3 (1)	0
Metabolism and Nutrition Disorders	2 (1)	1 (0)
Musculoskeletal, Connective Tissue, Bone	3 (1)	1 (0)
Nervous System Disorders	5 (1)	2 (1)
Cerebrovascular Accident NOS	5 (1)	0
Psychiatric Disorders	2 (1)	1 (0)
Renal And Urinary Disorders	7 (2)	4 (1)
Renal Failure Acute	5 (1)	2 (1)
Respiratory, Thoracic, and Mediastinal	7(2)	3 (1)
Pneumonia Aspiration	2 (1)	0
Respiratory Failure (Exc Neonatal)	4 (1)	0
Surgical/Medical Problems	5 (1)	8 (2)
Postoperative Hemorrhage	2 (1)	0
Seroma	2 (1)	1 (0)
Wound Dehiscence	0	2 (1)
Vascular Disorders	14 (4)	11 (3)
Deep Vein Thrombosis NOS	0	2 (1)
Hematoma NOS	3 (1)	4 (1)
Hypertension NOS	2 (1)	0
Pulmonary Embolism	3 (1)	3 (1)
Venous Thrombosis Deep Limb	6 (2)	3 (1)

Although acute renal failure was recorded as an AE with approximately equal incidence between the HBOC-201 (N = 5 [1.4%]) and RBC (N = 4 [1.2%]) groups, it was reported as an SAE in 5 HBOC-201 (1.4%) vs. 2 RBC (0.6%) subjects. There were 22 subjects (6%) in HBOC-201 and 9 (3%) in the RBC group with reported cardiac SAEs. The cardiac/cardiopulmonary arrest SAEs were not associated with CTM treatment in five subjects and reported as unknown for the remaining subjects. Although elevations of cardiac troponin were noted in a greater number of HBOC-201 subjects, these elevations were not correlated with incidence of MI or ischemic cardiac AEs, whereas there was a strong correlation in the control group.

There were 5 subjects with nervous system disorders SAEs in the HBOC-201 group and 2 in the RBC group with 5 vs. 0 CVAs. One subject in the RBC group had a TIA SAE and 1 subject had a RIND SAE. The association between HBOC-201 administration and the incidence of CVA was adjudicated as *not associated* for 4 subjects and *unknown* in 1 subject. An in-depth root cause analysis seeking evidence to support the diagnosis and reasons behind this difference found that

3 of the 4 purported CVAs in the HBOC-201 group could not be supported by evidence and were considered *misclassified*.

Seven subjects had pulmonary SAEs (2%) in the HBOC-201 group and 3 (1%) in the RBC group. Four subjects with SAEs of respiratory failure and 2 with SAEs of aspiration pneumonia were reported in the HBOC-201 vs. none in the RBC group. Three of the 4 subjects who had respiratory failure were involved in accidents and sustained crush injuries; the fourth subject received a large volume (20 L) of perioperative fluids, a major contributor to the pulmonary edema. Other subjects sustained fat embolism syndrome related to their injury. Subjects ranged in age from 20 to 62 years and all had evidence of underlying cardiac and/or pulmonary disease. Only one of the four SAEs was observed within 5 days of exposure to HBOC-201.

Two subjects experienced aspiration pneumonia and had evidence of underlying cardiac and pulmonary disease; the aspiration was preceded by cardiac complications, including atrial fibrillation. One subject had ECG evidence of an MI and the other subject had a possible small posterior wall MI. The onset of aspiration pneumonia in both of these cases was within 5 days of exposure to HBOC-201; in one subject, the SAE occurred after the subject received RBC.

Root Cause Analysis

Approximately 40% of the subjects allocated to the HBOC-201group crossed over to treatment with RBCs (*HR* subgroup) while 60% completely avoided RBC (*HH* subgroup). Thus, 40% of subjects in the HBOC-201 treatment arm HR were exposed to the potential for AEs associated with either treatment. This asymmetry significantly confounds assessment of the intrinsic safety of HBOC-201 as compared to RBC. Resolution of this issue necessitated stratification of subjects in the R group into two subgroups on the basis of clinical need, defined in terms of the number of RBC units received (i.e. ≤ 3 units [*R*- subgroup] vs. > 3 units [*R*+ subgroup]). Such stratification provides a method to evaluate the extent and nature of potential bias created by the asymmetrical study design. Specifically, this allowed for comparison of *matching subgroups* in the HBOC-201 (*H*) vs. RBC (*R*) arms of this study. This is discussed in greater detail in Section 5.0. One paired comparison comprised subgroups that received treatment with only one of the two possible therapies, *HH* vs. *R*-. The *matching groups* comparison permitted identification of the intrinsic safety side effect profile of HBOC-201. Of note, the side effect profile is consistent with the known pharmacology of HBOCs and observations from previous clinical trials with HBOC-201.

A second paired comparison examined the cross-over subgroup (*HR*) in the HBOC-201 arm with a matching subgroup in the R arm (*R+*). This *matching subgroup* comparison permitted complete root cause analysis of the most significant AE profile in HEM-0115, since the primary discrepancy in SAEs was revealed on comparison of the *HR* vs. *R+* subgroups. This analysis indicated that several factors contributed to the elevated incidence of AEs and SAEs, including events related to limitations of the current formulation of HbOC-201 coupled with exaggerated expectations, patient management issues, and inadequate training on all of the above. These manifested in a variety of observations such as under and delayed effective treatment and volume management issues, including oliguria and volume overload with pulmonary edema and congestive heart failure. These observations suggested that the predisposition to avoid treatment with RBC in HBOC-201 subjects for whom clinical need exceeded ability to manage the anemia with HBOC-201 alone, was a major contributor to the delay in effective treatment with RBC.

The *matching subgroup* analyses permit a more accurate assessment of the risk associated with exposure to HBOC-201, applicable to quantitative benefit:risk analysis in *RESUS*. Similarly, the root cause analysis data defined the framework for proper bridging therapy and specification of mitigation methods to minimize SAE risk (applied and incorporated into the design of *RESUS*).

Pivotal Phase 3 Orthopedic Trial - Stable Trauma Subjects Sub-population

Sixty two stable trauma subjects were enrolled in the pivotal orthopedic Phase 3 trial—(Table 20 A-D). Thirty-four received HBOC-201 and 28 received usual care with RBC. Up to 2 units of HBOC-201 was administered peri-operatively after 24 hours of stabilization. Mean age was ~ 40 years old, ~ 70% were male, and ~ 70% were caucasian. The indication for the 1st transfusion was Hb $\geq$ 6 and < 10.5 g/dL. This criterion was observed in 98.4% of subjects, as well as one of the following: tachycardia (54.8%), hypotension (9.7%), ECG evidence of myocardial ischemia (3.2%), acute blood loss > 7 ml/kg (30.6%), oliguria (1.6%), and restricted subject activity due to weakness/dizziness (33.9%). Estimated blood loss was ~ 1 liter (~ ATLS Class II hemorrhage) and the preponderance (~ 2/3) of surgical procedures was fracture repairs (e.g., open reduction and internal fixation).

Stable trauma HBOC-201 subjects had RBC avoidance rates of 88.2 and 44.1% on days 1 and 42, respectively (similar to the overall study population). AEs (*all*) were reported in 100% of HBOC-201 vs. 89% of RBC subjects, of HBOC-201 and RBC subjects. However, *associated* or

association unknown AEs were less frequent (41 vs. 32%). GI (e.g., abdominal distension and dyspepsia), pyrexia, and surgical procedures AEs occurred more frequently in HBOC-201 treated subjects, but none were assessed as *associated* with CTM administration. Jaundice, assessed as *associated* or *association unknown*, occurred in 6% and 0% of HBOC-201 and RBC treated subjects, respectively. There was one death SAE in an HBOC-201 treated subject, occurring > 200 hours after CTM administration (assessed as *not associated*). Analysis of LFTs revealed temporary relative elevations of bilirubin, AST, ALT, as well as amylase/lipase, but not alkaline phosphatase, in HBOC-201 subjects. Although creatinine was similar in the two groups, peak BUN was higher in HBOC-201 subjects, presumably due to the protein load of the solution. CK-MB was similar in the two groups. Expectedly, mild methemoglobinemia was seen in HBOC-201 subjects.

Unexpectedly, total Hb, measured at day 1, did not increase vs. baseline in HBOC-201 subjects (Figure 2). Possible explanations include short half-life due to rapid elimination, extravasation due to capillary leakage, hemodilution due to the oncotic properties of HBOC-201, or differences in asanguinous fluids administered. Finally, because the dose administered was low (2 units), any of these effects may have resulted in this significant laboratory finding. That the free Hb rose to 1.47 g/dL on day 1 refutes elimination and leakage, as likely explanations. Thus, low dose and hemodilution (due to either reason) are plausible explanations for the persistent low Hb.

Table 20: A-D Pivotal Phase 3 Orthopedic Trial – Stable Trauma Sub-population Data Summary

A - Demographic and Procedure Summary

Phase 3 orthopedic trial Stable trauma subjects data summary	**HBOC-201 (N = 34)**	**RBC (N = 28)**
Demographics		
Age (mean)	39.8	41.8
Male (%)	70.6	71.4
Caucasian (%)	67.6	75
Hb at transfusion trigger (g/dL)	7.9	10.7
Estimated blood loss (ml)	1,088	906
Procedure (%)		
Total hip arthroplasty	3.2	5.9
Fracture repair	69.4	64.7
Spinal	14.5	11.8
Other	12.9	17.6
Efficacy		
RBC avoidance (%)		
Day 1	88.2	-

Phase 3 orthopedic trial Stable trauma subjects data summary	HBOC-201 (N = 34)	RBC (N = 28)
Day 7	55.9	-
Day 42	44.1	-
Number of RBC units transfused	2.4	2.6

B - Lab Value Summary (peak values are presented in bold type)

Lab parameters	Lab values (mean)				
	Baseline	**Day 1**	**Day 4**	**Day 5**	**Day 42**
Bilirubin total (umol/l)					
HBOC-201 (N = 34)	13.2	**15.9**	-	-	6.8
RBC (N = 28)	**13.8**	10.7	-	-	6.6
Lipase (U/L)					
HBOC-201 (N = 34)	66	**228**	117	192	34
RBC (N = 28)	26	39	**83**	62	37
AST (U/L)					
HBOC-201 (N = 34)	77	125	**404**	93	28
RBC (N = 28)	61	**83**	60	67.3	20.1
ALT (U/L)					
HBOC-201 (N = 34)	63	53	**202**	104	40
RBC (N = 28)	50	59	64	**67**	22
Creatinine (umol/l)					
HBOC-201 (N = 34)	69.9	63.4	76.6	**79.6**	64.7
RBC (N = 28)	61.9	60.2	63.2	66.1	**70.7**
BUN (umol/L)					
HBOC-201 (N = 34)	4.5	5.6	7.3	**8.1**	4.8
RBC (N = 28)	4.8	4.6	4.7	**5.3**	4.5
CK-MB (ng/ml)					
HBOC-201 (N = 34)	8.7	**11.2**	5.1	4.4	1.6
RBC (N = 28)	6.8	**8.8**	3	2.2	1.1
Hb (g/dL)					
HBOC-201 (N = 34)	9	8.6	**8.5**	8.9	10
RBC (N = 28)	**8.6**	10.1	10	10.6	10.6
metHb (%)					
HBOC-201 (N = 34)	0.76	2.8	**4.7**	2.8	0.85
RBC (N = 28)	0.71	0.91	**0.93**	0.73	0.67
Hb plasma (g/dL)					
HBOC-201 (N = 34)	0.05	**1.47**	0.96	0.32	0.01
RBC (N = 28)	0	0	0	0	**0.01**

C - Vital Signs Summary

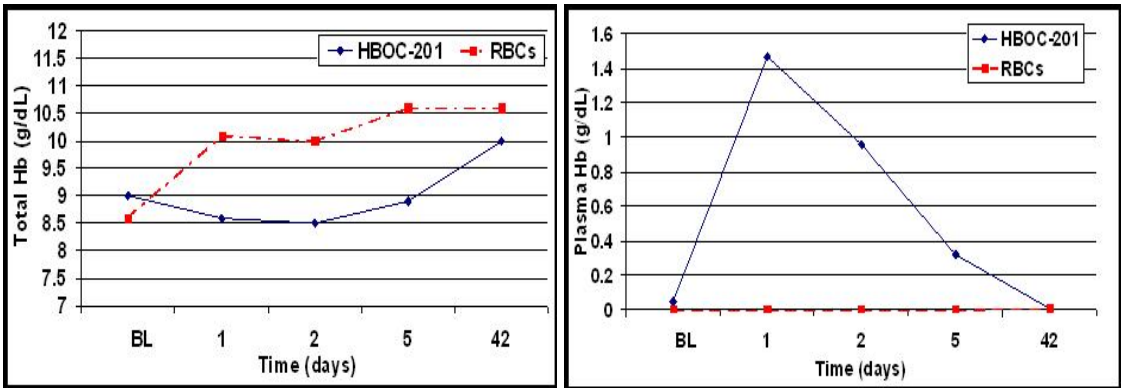
Vital Signs (mean)	Pre-Infusion	30 minutes Post-infusion	Day 2 Post-infusion
SBP (mm Hg)			
HBOC-201 (N = 34)	128	138	141
RBC (N = 28)	129	134	128
HR (beats/min)			
HBOC-201 (N = 34)	106	99	-
RBC (N = 28)	92	95	-

Vital Signs (mean)	Pre-Infusion	30 minutes Post-infusion	Day 2 Post-infusion
RR (resp/min)			
HBOC-201 (N = 34)	18.7	18.4	-
RBC (N = 28)	17.5	19.4	-
Temp (°C)			
HBOC-201 (N = 34)	37.3	37.3	-
RBC (N = 28)	37.4	37.8	-
O_2 saturation (%)			
HBOC-201 (N = 34)	98.3	97.9	-
RBC (N = 28)	97.54	97.6	-

D - Safety Summary

Safety	HBOC-201	RBC	HBOC-201	RBC	Comments
Adverse events	**All AEs**		**Associated or unknown association**		**Excludes unrelated AEs**
AEs (%)	100	89	41	32	
Cardiac	15	18	0	0	
GI	62	54	**24**	**7**	None "associated"
Abd distension	15	4	9	4	
Dyspepsia	12	4	**12**	**0**	
Investigations	44	29	6	11	
Inc amylase	44	29	3	0	
Inc lipase	21	0	3	0	
Jaundice	12	4	**6**	**0**	3% "associated"
Pyrexia	18	4	**6**	**0**	None "associated"
Renal/urinary	12	21	0	1	
Respiratory	38	32	3	0	
Skin	32	36	3	7	
Surgical/medical procedures	26	14	**6**	**0**	None "associated"
Vascular	32	11	3	4	
HTN	12	7	0	0	
DVT	9	0	0	0	
SAEs (%)	18	3	1	0	Acute resp failure (assoc unknown)
Death	2.9	0	0	0	1 death 226.5 hrs post-1st infusion

Figure 2: Hb Values in Stable Trauma Subjects in HEM-0115



Summary of HEM-0115 Stable Trauma Sub-Population Analysis

In a subgroup of 62 stable adult trauma subjects with ATLS class II hemorrhage, HBOC-201 efficacy was demonstrated (RBC transfusion avoidance), although 2 units of HBOC-201 failed to increase blood total Hb levels at 24 hours. The HBOC-201 AE profile included pyrexia, jaundice, GI complaints, mild hypertension, and temporary asymptomatic elevations of LFTs, amylase/lipase, BUN, and metHb.

Summary

The results of the Phase 3 HEM-0115 trial indicate that the OR for AEs and SAEs following treatment with HBOC-201 (H) as compared with RBC (R) was ~ 1.5**.** These findings raised concern regarding the intrinsic safety of HBOC-201 based on the use in treatment of acute surgical anemia compared to the established gold standard, RBC transfusions. As the proposed *RESUS* study requires *EIC*, it is important to carefully assess the risk associated with treatment with HBOC-201 that is non-separable (intrinsic) from exposure to this drug. Several initial approaches to addressing this question were considered. The first approach involved pooling AE data from all studies conducted to date with HBOC-201 (i.e., the ISS database). While this approach is adequate for searching for safety signals, ISS pooling of the various studies does not support a scientifically sound quantitative risk assessment. This approach was ruled out on the basis of the heterogeneity with regard to study population, design, dosing, and endpoint assessments. The second approach was to use the largest (44% of all subjects) and most homogeneous population from which data was obtained in a randomized and controlled clinical trial setting for HBOC-201, study HEM-0115.

HEM-0115 was a randomized single-blinded study of 688 acutely anemic orthopedic surgery patients. Sixty percent of subjects in the HBOC-201 arm completely avoided RBC (*HH* subgroup), while 40% crossed-over to treatment with RBC (*HR* subgroup). Thus, 40% of the subjects in the HBOC-201 arm were exposed toAEs associated with treatment with HBOC-201 and RBC. This asymmetry significantly confounded the assessment of the intrinsic safety of HBOC-201 as compared to RBC, necessitating stratification of subjects in the R arm into two subgroups on the basis of clinical need (defined in terms of the number of RBC units received: ≤ 3 units (*R*- subgroup) vs. > 3 units (*R*+ subgroup). Such stratification provided a method to evaluate the extent and nature of potential bias created by the asymmetrical study design. Specifically, this allowed for comparison of *matching subgroups* in the H vs. R arms in HEM-0115. The first paired comparison comprised subgroups receiving treatment with only one of the two possible therapies, *HH* vs. *R*-; this permitted identification of the intrinsic safety (side effect) profile of HBOC-201.

A second paired comparison examined the cross-over subgroup (*HR*) in HBOC-201 arm vs. the *R*+ subgroup. This *matching subgroups* comparison permitted complete root cause analysis of the most significant AE profile in this study since the primary discrepancy in SAEs was revealed on comparison of the *HR* vs. R+. This analysis indicated that several factors contributed to the elevated incidence of AEs and SAEs, including events related to limitations of the current HBOC-201 formulation coupled with exaggerated expectations, patient management issues, and inadequate training on all of the above. These manifested in a variety of observations such as under and delayed effective treatment and volume management issues including oliguria, volume overload with pulmonary edema, and congestive heart failure. These observations suggested that the predisposition to avoid treatment with RBC in subjects, in whom clinical need exceeded the ability to manage the anemia with HBOC-201 alone, was a major contributor to the delay in effective treatment with RBC

The results from the *matching subgroups* analyses permitted more accurate assessment of the risk associated with exposure to HBOC-201, applicable to quantitative benefit:risk analysis for *RESUS*. Similarly, this also permitted root cause analysis which defined the framework for proper bridging therapy and mitigation against the risk of SAEs (applied in the *RESUS* protocol design).

Matching Subgroup Analysis

Introduction

Evaluation of the intrinsic safety profile is required for accurate assessment of the risk of AEs following exposure to HBOC-201 when treatment with RBC is the comparator group. Although RBC are not available for use in the out-of-hospital setting of *RESUS*, comparison to RBC provides the most stringent assessment of *net* (intrinsic) *risk*. The results of study HEM-0115 provides the largest homogeneous patient sample with the greatest sensitivity to identify relatively rare events associated with exposure to HBOC-201. However, the asymmetric study design and cross-over in one arm of the study only, confounded ability to directly compare the safety of HBOC-201 to RBC. The subjects that crossed-over from initial treatment with HBOC-201 were also exposed to RBC and thus the side effects associated with exposure to both treatments.

Only 60% of subjects in the H group were exposed to HBOC-201 only (*HH*) and were appropriate for comparison with subjects randomized to treatment with RBC only (*R*). However, comparison of *HH* to *R* is a highly biased comparison in favor of HBOC-201. This bias is illustrated by examination of the dose response relationship between the number of units of RBC received and either AEs or SAEs, which shows a positive correlation. Although some of this relationship reflects intrinsic AEs associated with treatment with RBC, a significant component of this dose-response relationship is due to underlying medical status and corresponding requirement for treatment. That is, the higher doses of RBC were required by "sicker" subjects, those more likely to experience AEs because of underlying medical status. The *HH* subgroup received on average a lower dose (3.3 vs. 5.5 units) of HBOC-201 compared to *HR* subjects. That is, *HR* subjects were, on average, the "sicker" subjects of those randomized to treatment with HBOC-201. Therefore, comparison of *HH* to *R* subgroups represents a comparison of the less "sick" *HH* subjects to the entire population of *R* subjects, a highly biased comparison. It is not surprising that the incidence of AEs is more favorable for comparison of the *HH* vs. *R* subgroups. This comparison offers little or no insight into the relative intrinsic safety profile of HBOC-201 compared to RBC.

To accurately assess the intrinsic safety profile of HBOC-201 compared with RBC, it is important to compare equivalent subgroups of subjects treated with HBOC-201 alone to subjects treated with RBC alone. As suggested above, one of the differences between the *HH* and *HR* subgroups

is related to medical need, that is, the amount of oxygen carrying Hb required by these subjects. This suggested an approach which could be used to define appropriate *matching subgroups* for comparison. This *matching subgroups* comparison was based on identification of an equivalent population of subjects in the *R* group that were stratified by medical need for treatment with RBC. As described in detail below, this stratification resulted in identification of the population of subjects in the *R* group that received ≤ 3 units of RBC which very closely matched the medical need (Hb required) in the *HH* subgroup. The natural consequence of this dichotomy based on medical need was the correspondence between the *HR* subgroup and the subjects that received >3 units of RBC in the *R* group. This approach and the results of the comparison of these subgroups are discussed in detail alone with definition of the intrinsic safety profile of HBOC-201.

The HR subgroup accounted for the primary difference in the incidence of SAEs vs. the control population. These findings contributed to an impression that HBOC-201 was less safe than treatment with RBC. Application of root cause analysis to the *matching subgroup* comparison with the *R*+ subgroup identified the risk factors contributing to this increased incidence of both AE and SAEs.

Matching subgroup analysis

Randomization to treatment with HBOC-201 resulted in the generation of two treatment subgroups: subjects who avoided RBC transfusion (HBOC-only group, *HH* subgroup) and subjects who required treatment with RBC prior to the 6 week follow-up assessment (cross-over group, *HR* subgroup). These subgroups were prospectively defined in the statistical analysis plan for this study in the section describing the primary efficacy endpoint analysis.

Justification for matching group analysis

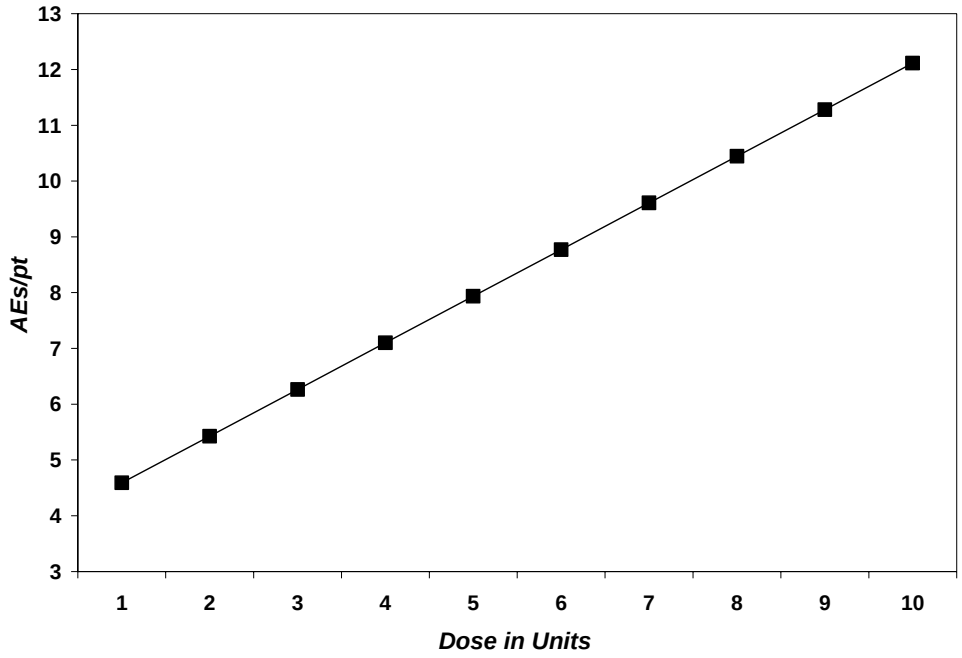
Table 21: Bias from asymmetric study design in HEM-0115summarizes the possible group comparisons in HEM-0115. The study design may have resulted in bias against HBOC-201 when comparing treatment arms, *H* vs. *R*. Comparison of the subgroups *HH* or *HR* against *R* are both biased in opposite directions.

Table 21: Bias from asymmetric study design in HEM-0115

Possibly biased against HBOC-201 group	***H* vs. *R* subgroups**
Biased against RBC group	***HH* vs. *R* subgroups**
Biased against HBOC-201 group	***HR* vs. *R* subgroups**
Unbiased	***HH* vs *?***
Unbiased	***HR* vs . *?***

The problem with comparison of non-matching subgroups is illustrated by the data in Figure 3: Aes/Subject As A Function Of The Dose Of RBC In HEM-0115. The number of AEs and SAEs/subject are plotted as a function of the number of RBC units received in study HEM-0115. This shows a clear and significant dose-response relationship. The increase in the rate of AEs and SAEs is probably dependent on subjects' increasing need for treatment. That is, the subjects receiving the higher doses were probably "sicker" with the greatest need for therapy.

Figure 3: Aes/Subject As A Function Of The Dose Of RBC In HEM-0115



Analysis of the dose response relationship using a general linear model showed a high degree of correlation with $p < 0.0001$. Results were just as significant for the HBOC-201 arm of this study. Analysis of AEs/subject for all subjects in the study gave the glm with the following equation:

$$\text{AEs/subject} = 3.8 + 0.75*[\text{HBOC-201}] + 0.83*[\text{RBC}]$$

Determination of the matching groups

Proper comparison of the safety profiles of these distinct subgroups with the control population required identification of corresponding or *matching subgroups* in the RBC arm. Examination of the *HH* and *HR* subgroups reveals that the *H* arm was separated naturally on the basis of need (Table 22: Identification of matching subgroups in HEM-0115)The *HR* subgroup represent subjects whose need exceeded that which could be provided by HBOC-201. This and data in Figure 1 suggested that it might be possible to identify a level of need that will result in a dichotomy defining equivalent subgroups in the two treatment arms.

Table 22: Identification of matching subgroups in HEM-0115

***H* Group**	***R* Group**
***HH* Subgroup: low needs: best group, 60%**	**?**
***HR* Subgroup: high needs, worst group, 40%**	**?**

Identification of this equivalent dichotomy seen in the H group was accomplished in the RBC arm by retrospective analysis. That is, by answering the question; "on the basis of clinical need (number of RBC administered), what group of randomized subjects in the RBC group corresponds to the HBOC-only subgroup?" Subjects in the RBC group were stratified on the basis of the actual number of RBC units received. The idea was to define the equivalent dichotomy in the two treatment arms based on clinical need determined by study outcome. On the basis of pharmacokinetics and Hb content of HBOC-201 supported by frequentist statistical analysis, the most reasonable dichotomy was predicated on division into subjects receiving < 3 vs. > 3 units of RBC. This dichotomy, based upon observed clinical need, resulted in corresponding populations representing similar fractions of their respective populations and showing nearly identical baseline and surgery-emergent characteristics. The results of paired comparison of these matching subgroups are described below.

The Hb content of HBOC-201 and RBC provides the most fundamental measure by which to assess equivalence. HBOC-201 is 13 g Hb/dL vs. RBC which is at ~ 26g Hb/dL. On this basis, two units of HBOC-201 are equivalent to one unit of RBC in terms of total Hb, ~ 60g in each case. Thus, a subject requiring treatment with four units of RBC requires at least eight units of HBOC-201. This does not take into consideration the 19 hour half-life of HBOC-201 which, at a minimum, would require additional dosing with at least four units of HBOC-201. This brings the

total to twelve units (i.e., two units more than the maximum number of units allowed in study HEM-0115). In contrast, assuming a requirement for treatment with three units of RBC, this would be equivalent to six units of HBOC-201 plus the equivalent of three units of HBOC-201 to maintain Hb levels following the initial dosing and within the ten unit limit.

These data suggest that the four unit RBC requirement would exceed the ten unit HBOC-201 limit whereas the requirement for three units of RBC might be manageable with HBOC-201 alone. This iterative process suggested that acutely anemic patients requiring a maximum of three units of RBC could be treated with HBOC-201 alone based on the maximum of ten units of HBOC-201. Accordingly, using three units as the criteria for creating the dichotomy in the population of subjects treated with RBC, should identify the subpopulations equivalent to the *HH* and *HR* subgroups in terms of need. The following discussion compares the subpopulations of subjects in the two treatment arms to assess whether or not this approach to stratification of the RBC treatment arm identified subpopulations equivalent to the *HH* and *HR* subgroups in the *H* treatment arm.

HBOC-201-Only Subgroup (HH) vs. ≤ 3 RBC Subgroup (R-)

The matching subgroup inside *R* corresponding to the *HH* subgroup was determined by selecting the subjects in this arm receiving up to three units of RBC. 60% (211) of subjects in the H arm were represented in the *HH* subgroup and 68% (231) of the R arm was represented in the *R*-subgroup.

Medical History

Table 23: Medical History in the *HH* and *R*- subgroups in HEM-0115summarizes baseline medical history of subjects in the two subgroups. The baseline characteristics were very similar in these subgroups. However, a history of immunologic (9.2 vs. 4.4%) events was more common in the *HH* subgroup than the *R*- subgroup.

Table 23: Medical History in the *HH* and *R*- subgroups in HEM-0115

	***HH* subgroup N (%)**	***R*- subgroup N (%)**
Allergic	77 (36.5)	98 (42.4)
Cardiovascular	139 (66.5)	173 (74.9)
Dermatologic	37 (17.9)	42 (18.5)
Gastrointestinal	120 (56.9)	137 (59.3)
HEENT/Mouth	106 (50.7)	119 (52.0)
Hematologic	35 (16.8)	34 (15.0)
Hepato-biliary	31 (14.9)	42 (18.5)
Immunologic	19 (9.2)	10 (4.4)
Metabolic/Endocrine	81 (38.6)	91 (40.3)
Musculoskeletal	201 (95.3)	228 (98.7)
Neoplastic	27 (13.1)	38 (16.8)
Neurologic	64 (30.5)	72 (31.3)
Psychiatric	48 (23.2)	64 (27.8)
Pulmonary	94 (44.5)	112 (48.5)
Renal/Urinary Tract	67 (32.1)	83 (36.2)
Reproductive/Genitalia	97 (47.3)	116 (51.8)
Transfusion	31 (15.2)	41 (18.6)

Source: Data Table 14.1.21.8

Estimated Blood Loss (EBL) and Fluid Volumes in the HH and R- subgroups

The results summarized in Table 24 indicate that the EBL in the *HH* subgroup was 537 ± 35 mL vs. 525 ±31 mL in the *R*- subgroup. Subjects in the *HH* and *R*- subgroups received nearly identical mean (± SEM) total fluid (7,700 ± 256 vs. 7,542 ± 224 mL), crystalloid (7,261 ± 245 vs. 7,177 ± 215 mL), and colloid (452 ± 65 vs. 381 ± 52 mL) volumes.

Table 24: Estimated Blood Loss the Fluid Volumes Administered in *HH* and *R*- subgroups in HEM-0115

Treatment	Measure	N	Mean (mL)	SEM	Min (mL)	Max (mL)
***HH* subgroup**						
	EBL	200	537	35	0	2,600
	Volume Crystalloid	210	7,261	245	140	24,574
	Volume Colloid	204	452	65	0	5,500
	Total volume Fluids	210	7,700	256	210	25,074
***R*-subgroup**						
	EBL	222	525	31	0	3,000
	Volume Crystalloid	229	7,178	215	500	17,560
	Volume Colloid	219	381	52	0	6,500
	Total volume Fluids	229	7,542	224	1,450	17,560

Extent of Exposure

Subjects in the *HH* subgroup received larger volume of CTM than in the *R*- subgroup (Table 25). *HH* subjects received an average 3.29 ± 0.14 units of HBOC-201 and *R*- subjects received an average of 1.96 ± 0.05 units of RBC. The average rate of infusion was similar, 3.96 ± 0.28 vs. 3.69 ± 0.50 mL/min, respectively. These data indicate that 1.96 units of RBC were replaced with 3.29 units of HBOC-201 resulting in a RBC replacement ratio of 1.6.

Table 25: Exposure to CTM in the *HH* and *R*- subgroups in HEM-0115

Parameter	***HH* subgroup**	***R*- subgroup**
Total Volume of CTM (mL)		
N	211	231
Mean ± SE	821.13 ± 34.39	578.73 ± 17.04
Median	500	580
Range	200 to 2500	100 to 1,650
Number of HBOC-201-Only Units		
N	211	
Mean ± SE	3.29 ± 0.14	
Median	2	
Range	1 to 10	
Number of RBC Units		
N	0	231
Mean ± SE	0.00 ± 0.00	1.96 ± 0.05
Median	0	2
Range	0-0	1 to 3
Average Rate of Infusion (mL/min)		
N	209	230
Mean ± SE	3.96 ± 0.28	3.69 ± 0.50
Median	2.38	1.82
Range	0.37 to 25.00	0.75 to 99.17

Source: Data Table 14.1.35.8

SEEC Data Analysis for the Matching Subgroups

The overall medical risk was analyzed and the MW estimate of the probability of more severe overall medical risk in the HH subgroup relative to the R- subgroup was p = 0.519 (95% CL 0.481-0.558). These data indicate that the probability of the SAE profile in the HH subgroup was similar to the R- subgroup (Table 26).

Table 26: Overall Medical Risk Assessment in the *HH* and *R*- subgroups in HEM-0115

Overall Medical Risk	***HH* subgroup N (%)**	***R*- subgroup N (%)**
None or Minimal	127 (60.2)	147 (63.6)
Mild	62 (29.4)	64 (27.7)
Moderate	19 (9.0)	16 (6.9)
Severe	1 (0.5)	1 (0.4)
Outcome is Death	2 (0.9)	3 (1.3)

Source: Data Table 14.3.7.8

Examination of AEs that were statistically significantly different between the *HH* and *R*-subgroups (Table 27) revealed that there were differences between treatment groups in cardiac disorders, GI disorders, hepato-biliary disorders, investigations, skin and subcutaneous disorders, and vascular disorders (P <0.001 for all SOCs). Tachycardia accounted for nearly 70% of all cardiac AEs. Constipation, nausea, and vomiting accounted for the majority of the GI AEs. Jaundice accounted for 95% of the hepato-biliary AEs, and BP, body temperature and lipase elevations accounted for the majority of investigations AEs. Hypertension was the major contributor to vascular AEs. There were no statistically significant differences in the incidence of SAEs when analyzed according to individual preferred terms of SOC. There were 0.14 SAEs/subject in both groups whereas 6.42 vs. 4.53 AE/subject were observed in the *HH* vs. the *R*- subgroups, respectively.

Table 27: AEs in the *HH* and *R*- subgroups in HEM-0115

System Organ Class (SOC) Preferred Term	*HH* subgroup		*R*- subgroup		Fisher's 2-tail
	N	%	N	%	
Blood and Lymphatic System Disorders	13	6	15	6	1
Cardiac Disorders	46	22	25	11	0.0018
Ear and Labyrinth Disorders	2	1	3	1	1
Endocrine Disorders	0	0	1	0	1
Eye Disorders	5	2	3	1	0.4874
Gastrointestinal Disorders	146	69	118	51	0.0001
General Disorders and Administration Site Conditions	111	53	117	51	0.7038
Hepato-biliary Disorders	38	18	4	2	< 0.0001
Immune System Disorders	3	1	0	0	0.108
Infections and Infestations	29	14	34	15	0.787
Injury and Poisoning	29	14	25	11	0.3846
Investigations	71	34	47	20	0.0018
Metabolism and Nutrition Disorders	22	10	20	9	0.6266
Musculoskeletal, Connective Tissue and Bone Disorders	36	17	38	16	0.8989
Nervous System Disorders	82	39	72	31	0.1097
Psychiatric Disorders	36	17	38	16	0.8989
Renal and Urinary Disorders	42	20	34	15	0.1659
Reproductive System and Breast Disorders	2	1	8	3	0.109
Respiratory, Thoracic and Mediastinal Disorders	42	20	41	18	0.6261
Skin & Subcutaneous Tissue Disorders	77	36	53	23	0.0024
Surgical and Medical Procedures	37	18	43	19	0.8054
Vascular Disorders	47	22	19	8	< 0.0001

SAEs in the HH and R- subgroups in HEM-0115

The SAEs observed in both *HH* and *R*- subgroups are summarized in Table 28. There was no statistical difference for SOC as the rate of SAEs was comparable in both of these subgroups.

Table 28: SAEs in the *HH* and *R*- subgroups in HEM-0115

System Organ Class (SOC) Preferred Term	*HH* subgroup		*R*- subgroup		Fisher's 2-tail
	N	%	N	%	
Cardiac Disorders	5	2	3	1	0.4874
Gastrointestinal Disorders	3	1	4	2	1
General Disorders And Administration Site Conditions	0	0	2	1	0.4999
Hepato-biliary Disorders	1	0	0	0	0.4774
Infections And Infestations	5	2	6	3	1
Injury And Poisoning	4	2	4	2	1
Metabolism And Nutrition Disorders	1	0	1	0	1
Nervous System Disorders	0	0	1	0	1
Psychiatric Disorders	1	0	0	0	0.4774
Renal And Urinary Disorders	0	0	2	1	0.4999
Reproductive System And Breast Disorders	0	0	1	0	1
Respiratory, Thoracic And Mediastinal Disorders	0	0	1	0	1
Surgical And Medical Procedures	2	1	3	1	1
Vascular Disorders	6	3	5	2	0.7637

Source: Data Table 14.3.10.8

Cross-Over Subgroup (*HR*) And > 3 RBC Subgroup (*R*+)

The matching RBC subgroup for *HR* was determined by selecting subjects in this subgroup who received more than three units of RBC. 40% (139) of subjects randomized to the *H* arm were represented in the *HR* subgroup and 32% (107) of subjects randomized to the *R* arm were represented in the *R*+ subgroup.

Medical history

The overall medical history for the *HR* and *R*+ subgroups is shown in Table 29; the only significant difference between the two groups was a history of hematologic events, ~ 13% higher in the *HR* subgroup.

Table 29: Medical History In The HR Vs. R+ Subgroups In HEM-0115

	***HR* subgroup N (%)**	***R+* subgroup N (%)**
Allergic	59(42.4)	42 (39.3)
Cardiovascular	96 (69.1)	77 (72.0)
Dermatologic	41 (29.9)	27 (25.5)
Gastrointestinal	90 (64.7)	70 (65.4)
HEENT/Mouth	69 (49.6)	47 (44.3)
Hematologic	45 (32.6)	20 (18.9)
Hepato-biliary	29 (21.3)	20 (19.0)
Immunologic	8(5.9)	5(5.0)
Metabolic/Endocrine	51(37.6)	40(37.4)
Musculoskeletal	133 (95.7)	103 (96.3)
Neoplastic	16 (11.9)	13 (12.5)
Neurologic	53 (38.4)	35 (33)
Psychiatric	48 (34.8)	33 (30.8)
Pulmonary	70 (50.7)	46 (43.0)
Renal/Urinary Tract	50 (36.2)	34 (31.8)
Reproductive/Genitalia	48 (35.0)	38 (36.5)
Transfusion	43 (31.9)	27 (26.7)

Exposure to CTM

The extent of exposure to HBOC-201 and RBC is summarized in Table 30. Subjects in the *HR* subgroup were exposed to an average of 5.5 units of HBOC-201 and 3.5 units of RBC. Treatment with HBOC-201 reduced the total number of RBC required to treat subjects in the *HR* subgroup. The total volume of ctm required in the *HR* subgroup was substantially higher than in the *R+* subgroup.

Table 30: Exposure to CTM in the *HR* and *R+* subgroups in HEM-0115

Parameter	*HR* subgroup	*R+* subgroup
Total Volume of CTM (mL)		
N	139	107
Mean ± SE	1,367.2 ± 63.60	1,401.7 ± 62.6
Median	1,250	1,250
Range	50 to 2,750	225 to 4,450
Number of HBOC-201-Only Units		
N	139	107
Mean ± SE	5.5 ± 0.25	0.00 ± 0.00
Median	5	0
Range	1 to 11	0 to 0
Number of RBC Units		
N	139	107
Mean ± SE	3.51 ± 0.21	5.37 ± 0.24
Median	3	4
Range	1-14	4 to 22

Source: Data Table 14.1.35.8

Assuming that one unit of HBOC-201 is equivalent to ~ 30 g Hb and one unit of RBC is ~ 60g Hb, the average number of grams of Hb administered to the HR and R+ subgroups was estimated at 375 and 324g, respectively. There were 5.5 units of hboc-201 or 165 g Hb in the form of HBOC-201 administered in the HR subgroup. This compensated for the 5.4-3.5 = 1.9 unit difference with the replacement ratio of 5.5/1.9 = 2.9.

EBL and fluid volume administration in the HR and R+ subgroups in HEM-0115

The results summarized in

Table 31indicate that ebl was similar in the two groups with 1,043 ± 111 ml vs. 1,140 ± 117 ml in the *HR* and *R+* subgroups, respectively. Subjects in the *HR* and *R+* subgroups received similar mean (± sem) total fluid (9,734 ± 482 vs. 9,055 ± 575 ml) and crystalloid volumes (9,105 ± 464 vs. 8,103 ± 376 ml), whereas the colloid (658 ± 89 vs. 979 ± 143 ml) volumes were different.

Table 31: EBL the fluid volumes in the HR and R+ subgroups in HEM-0115

Treatment	Measure	N	Mean (mL)	SEM	Min (mL)	Max (mL)
***HR* subgroups**						
	Estimated Blood Loss	134	1,043	111	0	7,100
	Volume of Crystalloid	138	9,105	464	890	27,821
	Volume of Colloid	132	658	89	0	5,500
	Total volume of Fluids	138	9,734	482	890	27,821
R+ subgroup						
	Estimated Blood Loss	103	1,140	117	0	5,300
	Volume of Crystalloid	106	8,104	376	575	18,000
	Volume of Colloid	103	979	143	0	7,983
	Total volume of Fluids	106	9,055	575	1,450	20,888

SEEC Data Analysis for the Matching Subgroups

The overall medical risk was analyzed and the MW estimate of the probability of more severe overall medical risk in the HR subgroup vs. the R+ subgroup was p = 0.605 (95% CL 0.550-0.662). These data suggest that the probability of a more severe AE profile was greater in the HR subgroup (Table 32).

Table 32:Overall Medical Risk Assessment for the *HR* and *R+* subgroups in HEM-0115

Overall Medical Risk	*HR* subgroup N (%)	*R+* subgroup N (%)
None or Minimal	44 (31.7)	52(48.6)
Mild	45(32.4)	34(31.8)
Moderate	36(25.9)	16(15.0)
Severe	5 (3.6)	2(1.9)
Life-Threatening/and/or Persistent	3(2.2)	0(0.0)
Outcome is Death	6(4.3)	3(2.8)

Source: Data Table 14.3.7.11

AEs in the *HR* and *R+* subgroups in HEM-0115

In light of the relatively small number of subjects in these subgroups, the SOCs with incidences that differed at the p < 0.1 level were considered significant. Examination of the AEs (Table 33) revealed that differences existed between treatment groups in blood/lymphatic disorders, hepato-

biliary disorders, investigations, metabolism, reproductive and respiratory disorders SOCs. In addition, significant differences were observed at the level of preferred terms including, nausea, vomiting, diarrhoea, dysphagia, GI pain/distension, fatigue, jaundice, increased metHb, decreased potassium, magnesium, Hb, increased lipase, ECG abnormalities, fluid overload, muscle cramps, CVA, headache, ecchymosis, and skin lesions.

Table 33: AEs in the *HR* and *R+* subgroups in HEM-0115

System Organ Class (SOC) Preferred Term	*HR* subgroup		*R+* subgroup		Fisher's 2-tail
	N	%	N	%	
Blood and Lymphatic System Disorders	48	35	7	7	7.00E-08
Cardiac Disorders	56	40	32	30	0.1078
Congenital and Familial/Genetic Disorders	0	0	1	1	0.435
Ear and Labyrinth Disorders	3	2	2	2	1
Eye Disorders	4	3	3	3	1
Gastrointestinal Disorders	111	80	77	72	0.1733
General Disorders and Administration Site Conditions	97	70	72	67	0.6801
Hepato-biliary Disorders	52	37	4	4	4.60E-11
Immune System Disorders	3	2	3	3	1
Infections and Infestations	43	31	28	26	0.4785
Injury and Poisoning	23	17	17	16	1
Investigations	67	48	37	35	0.0375
Metabolism and Nutrition Disorders	41	29	19	18	0.0368
Musculoskeletal, Connective Tissue and Bone Disorders	35	25	36	34	0.1579
Neoplasms Benign and Malignant (Including Cysts and Polyps)	0	0	1	1	0.435
Nervous System Disorders	71	51	50	47	0.5221
Psychiatric Disorders	53	38	33	31	0.2808
Renal and Urinary Disorders	45	32	30	28	0.4878
Reproductive System and Breast Disorders	15	11	4	4	0.0528
Respiratory, Thoracic and Mediastinal Disorders	55	40	29	27	0.0432
Skin & Subcutaneous Tissue Disorders	65	47	43	40	0.3644
Social Circumstances	1	1	0	0	1
Surgical and Medical Procedures	42	30	30	28	0.7779
Vascular Disorders	54	39	32	30	0.1775

SAEs in the *HR* and *R+* subgroups in HEM-0115

Significant differences in the incidence of SAEs were observed for cardiac disorders (p = 0.04) SOC only (Table 34). Although a significant difference was not noted for the nervous system disorders SOC, a difference (p = 0.07) at the preferred term level for CVA was observed.

Consistent with observations from analysis of incidence, comparison of the overall rate of AEs and SAEs in these matching subgroups indicated there were 11.58 vs. 8.79 AEs/subject and 0.63 vs. 0.47 SAEs/subject in the *HR* vs. *R*+ subgroups, respectively.

Table 34: SAEs in the *HR* and *R*+ subgroups in HEM-0115

System Organ Class (SOC) Preferred Term	*HR* subgroup		*R*+ subgroup		Fisher's 2-tail
	N	%	N	%	
Blood and Lymphatic System Disorders	2	1	1	1	1
Cardiac Disorders	17	12	5	5	0.0441
Gastrointestinal Disorders	3	2	3	3	1
General Disorders And Administration Site Conditions	8	6	2	2	0.1935
Hepato-biliary Disorders	4	3	0	0	0.1347
Infections And Infestations	6	4	6	6	0.7677
Injury And Poisoning	6	4	7	7	0.5676
Investigations	3	2	0	0	0.2597
Metabolism And Nutrition Disorders	1	1	0	0	1
Musculoskeletal, Connective Tissue and Bone Disorders	3	2	1	1	0.6347
Nervous System Disorders	5	4	1	1	0.2369
Psychiatric Disorders	1	1	1	1	1
Renal And Urinary Disorders	7	5	2	2	0.3061
Respiratory, Thoracic And Mediastinal Disorders	7	5	2	2	0.3061
Skin & Subcutaneous Tissue Disorders	1	1	0	0	1
Surgical And Medical Procedures	3	2	5	5	0.2999
Vascular Disorders	8	6	6	6	1

Source: Data Table 14.3.10

Paired comparisons indicate that the incidence and rate of AEs and SAEs was greater in the *HR* subgroup. The increased incidence of AEs was anticipated based upon the *HH* vs. the *R*-comparison. While the incidence and rate of SAEs (0.63 vs. 0.47 SAEs/subject) was higher in the *HR* subgroup, to some degree, this is not surprising. As noted previously, subjects in the *HR* subgroup were exposed to treatment with HBOC-201 and RBC and their attendant side effects and risks. Other factors, including inadequate dosing with HBOC-201 and problems with volume management, probably also contributed to the elevated rate of SAEs.

Summary of AEs and SAEs in *matching subgroups* in HEM-0115

The baseline and surgery-emergent characteristics were similar for the paired comparisons. The fraction of the treatment arms associated with the paired subgroups (*HH* vs. *R*- and *HR* vs. *R*+)

was not exactly the same (60 vs. 68% and 40 vs. 32%). These differences are thought to be related to the fact that the theoretically estimated cut point corresponding to the *HH* subgroup was about 2.8 units RBC. However, whole units and not fractions of units were administered and do not permit the level of precision of the dichotomy needed to produce exactly equivalent paired subgroups. Thus, from this perspective, it is not possible to produce an exact match because of the mismatch in precision of the stratification possible in the RBC arm. Nonetheless, the paired subgroups were closely matched in terms of medical history, EBL, and volumes of fluids administered. These results confirmed the close correspondence of these subgroups with regard to measures of clinical need associated with surgical anemia. Comparison of these subgroups compensates for some of the bias introduced by cross-over to treatment with RBC in the *H* arm.

Comparison of the AE profile for the *HH* and *R-* subgroups indicates that there were no significant differences in the rate of SAEs (Table 35). However, the rate of AEs was higher in the *HH* subgroup than the *R-* subgroup (6.42 vs. 4.53, respectively). Examination of SOCs and preferred terms where significant differences occurred, reveals the fundamental difference between treatment groups and this net difference reflects the side effect profile of HBOC-201. This is characterized by GI side effects (dysphagia, nausea, vomiting, etc.), hypertension, jaundice, skin discoloration, and transient elevation of liver and pancreatic enzymes. Thus, this analysis has delineated the side effect profile of HBOC-201 in acutely anemic orthopedic surgery patients estimated to require treatment equivalent to ≤ 3 U RBC.

Table 35: Summary of *Matching Subgroups* Analysis of AEs in HEM-0115

Group	N	Mean ±SEM
***HH* subgroup**		
AEs/subject	211	6.42 ± 0.31
SAEs/subject	211	0.14 ± 0.03
***R-* subgroup**		
AEs/subject	231	4.53 ± 0.27
SAEs/subject	231	0.14 ± 0.03
***HR* subgroup**		
AEs/subject	139	11.58 ± 0.64
SAEs/subject	139	0.63 ± 0.07
***R+* subgroup**		
AEs/subject	139	8.79 ± 0.64
SAEs/subject	139	0.47 ± 0.08

Analysis of the differences in AEs in the *HR* vs. *R*+ subgroups showed that same profile of specific AEs seen in the *HH* vs. *R*- subgroups comparison. The SAEs/subject in the *HR* subgroup was significantly higher than that observed in the *R*+ subgroup. As noted above, this can be understood, in part, based upon exposure to both HBOC-201 and RBC in the *HR* subgroup coupled with delay in administration of RBC.

It is important to consider what happened in the *HR* subgroup with respect to the effectiveness of treatment with HBOC-201. This subject population required treatment with 34% fewer (3.5 ± 0.2 units) RBC units than in the *R*+ subgroup (5.4 ± 0.2 units); thus, treatment with HBOC-201 showed a small but significant reduction in the need for RBC in the *HR* subgroup. This appears consistent with the observed benefit in the *HH* subgroup where the use of RBC was eliminated. Comparison of the total Hb levels prior to the first treatment with HBOC-201 and just after the last treatment, showed that the mean ± SEM level of total Hb was 8.6 ± 0.1 g/dL and 8.2 ± 0.1 g/dL , respectively. These results suggest that on the average, subjects treated with HBOC-201 received treatment with RBC after sufficient delay such that patient Hb levels returned to pre-treatment or lower levels.

A key concept suggested by these data is that effective bridging requires that treatment with RBC occurs before the effectiveness of HBOC-201 is completely lost due to elimination. Thus, extended bridging (days vs. hours) with HBOC-201, when the requirements for treatment are > 3 units of RBC, is not justified when blood is available. That is, management of anemia is a continuum and should not be delayed until total Hb levels fall and symptoms return. In the subject population requiring treatment with > 3 units of RBC, there will be need for cross-over to treatment with RBC and this crossover should occur without significant delay. The results shown in Figure 4 (discussed below) indicate that total Hb levels were allowed to fall well below the average starting Hb levels and the greater the delay the lower the Hb levels. There was no evidence of carry-over benefit in the *HR* subgroup reflected in the total Hb levels when RBC were administered. This situation likely contributed to the significantly elevated rate of AEs and SAEs observed in the *HR* compared to *R*+ subgroups.

Root Cause Analysis

The matching group comparison of the *HR* vs. *R+* subgroups provides opportunity to examine the major factors contributing to the increased incidence of AEs and SAEs in the HEM-0115 study. Examination of the data from these subjects indicates that there are several major root causes for many of the AEs. The first is the intrinsic sides effects associated with exposure to HBOC-201 that were discussed above for the *HH* vs. *R-* subgroups comparison. These AEs were expected to occur at a higher incidence because of the higher average exposure (3.3 vs. 5.5 units HBOC-201). In addition, there were AEs that occurred because of product limitations, exaggerated expectations, and inadequate training with regard to the proper use of HBOC-201.

Product limitations

HBOC-201 contains 13 g/dL of Hb compared to 26 g/dL Hb in one unit of RBC. Consequently, one unit of HBOC-201 produced a 0.16 g/dL increase in total Hb concentration rather than the 0.9 g/dL observed with RBC. This observed difference, based on study results, was predicted based on simple modeling of the increases in total Hb based on the known differences in concentration of these two Hb solutions. In the HEM-0115 protocol, investigators were told to expect the same increase in total Hb as seen with one unit of RBC. This exaggerated expectation resulted in investigators chasing total Hb concentrations. This was a recipe for increasing the likelihood of volume overload. The observed higher incidence of volume overload, pulmonary edema, and heart failure were manifestations of this phenomenon. Expert clinical cardiology review also identified that this was coupled with inadequate use and dosing with diuretics to properly manage volume status.

In the *HR* subgroup, it was clear that the need for oxygen carrying Hb solution treatment over six days exceeded what could be handled with ten units of HBOC-201 alone. As discussed above, subjects in this group required > 3 units of RBC up to a maximum of 22 units of RBC. Unfortunately, no instructions were provided to investigators as to how to deal optimally with this scenario. The decrease in total Hb concentration at the end of treatment with HBOC-201 (Figure 4) suggested that there was delay in the adequate treatment of anemia.

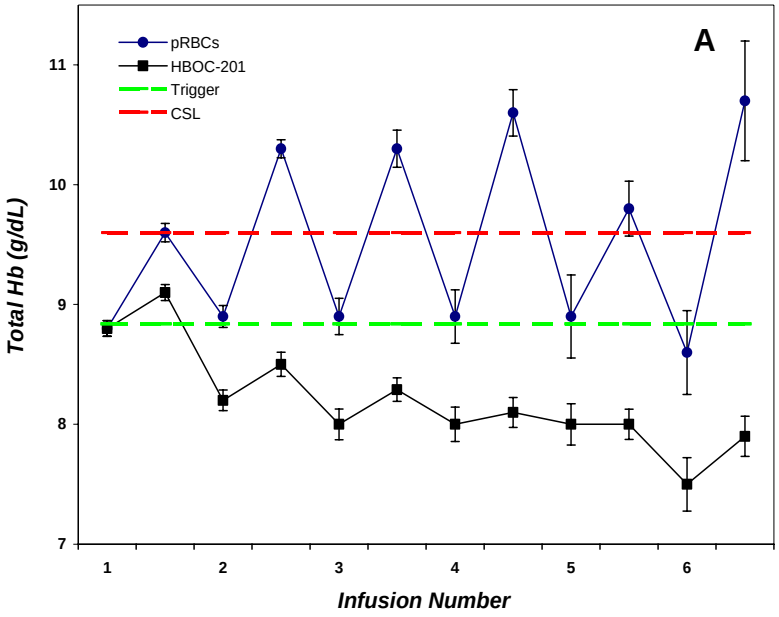
The first indication that anemia was not managed adequately in HBOC-201 subjects was revealed on comparison of dynamic total Hb (THb) concentrations in the two treatment arms in study HEM-0115 by transfusion number (Figure 4). Two horizontal lines are shown, the bottom dashed line identifies the pre-CTM THb transfusion trigger and the top dashed line identifies the median

clinically significantly low (CSL) Hb. For this analysis, the CSL Hb was defined as 0.8 times the lower limit of normal (0.8*LLN) Hb concentration with the LLN determined (and adjusted for age and gender) by the individual laboratories.

The data indicate that patients requiring ongoing treatment with HBOC-201 experienced lower THb concentrations than the patients treated with RBC. Of critical importance is the observation that THb concentrations of the HBOC-201 subjects remained below the original THb transfusion trigger throughout the treatment period, with the exception of the first post-HBOC-201 THb (Figure 4). In contrast, the RBC subjects had THb concentrations that increased above the transfusion trigger and the CSL Hb threshold after each transfusion. These data indicate that treatment with HBOC-201 did not result in correction of the subject's anemia in a significant portion of the population. Furthermore, the average THb levels were highest for subjects treated with RBC, followed by the *HH* subgroup, and lowest for the *HR* subgroup (Figure 4, Panel B).

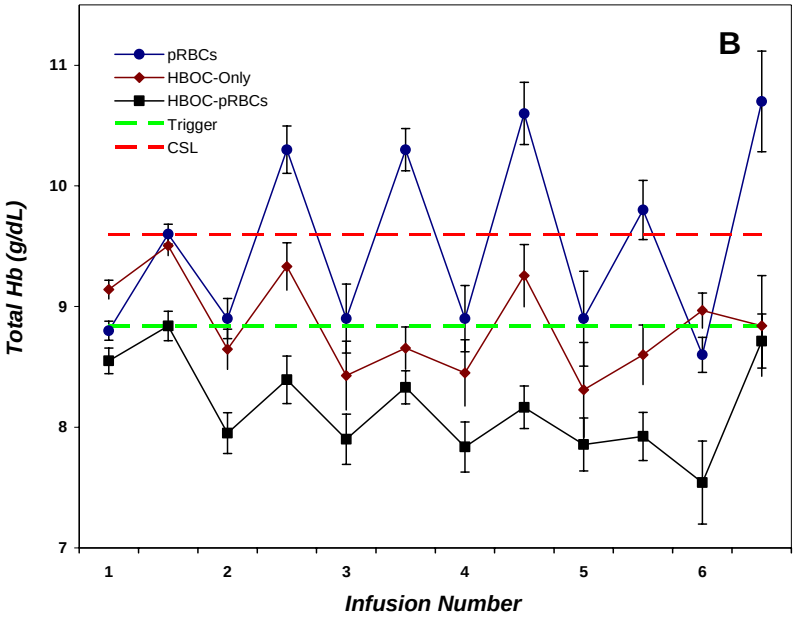
Figure 4: Panel A and B - Total Hemoglobin (THb) Concentrations in HBOC-201- vs. RBC-treated subjects in HEM-0115.

Figure 4: ***Panel A*** *shows THb concentrations (mean ± SEM) before and after infusions for subjects requiring treatment with either RBC (circles) or HBOC-201 (squares).*



The twelve data points represent pre- and post-infusion measurements for each of six infusions. The top horizontal dashed line represents the median clinically significantly low (CSL) Hb concentration. The bottom horizontal dashed line represents the average (N = 648) initial transfusion trigger. Data are presented for measurements of THb obtained ≤ 30 min before or after CTM infusions. The symbols are connected to facilitate comparison of the groups, however, the number of subjects decreases with increasing infusion number. Data for infusions where N was < 3 were not included.

Figure 4: Panel B *plots the same pre- and post-THb values for RBC subjects but the HBOC-201 data is divided into subjects receiving HBOC-201 only (diamonds) and HBOC-201 plus subsequent RBCs (squares) during the treatment period.*



Evidence that there was inadequate training of medical personnel regarding how to most effectively administer HBOC-201 is seen in the exaggerated expectation for improvements in THb. In addition, there was inadequate appreciation of the impact that chasing THb (with a solution that contained half the concentration of Hb than that of RBC) with regard to subject volume status and the obvious increased potential for volume overload, heart failure, and pulmonary edema. This is reflected in the less than adequate frequency and level of dosing with diuretics. In addition, there was an absence of discussion in the protocol of the dosing schedule(s) needed to adequately bridge the oxygen carrying needs over the six day treatment period. Investigators were left on their own to figure this out. In this special case where treatment with a new therapy having very different characteristics from RBC, very explicit instructions were needed to help ensure optimum therapy and safety.

In addition to the need for more comprehensive training on how to dose and manage THb concentrations and patient volume status, there was the need for instructions in the event of "treatment failure". That is, how to recognize as well as deal most effectively with situations

where the need outstripped the capacity for HBOC-201 to provide effective management of anemia. In retrospect it is clear that protocols for short term management, bridging, were needed to avoid delayed effective treatment as occurred in the *HR* subgroup.

Summary of *matching subgroups* analysis

The design of study HEM-0115 included a cross-over arm for subjects randomized to treatment with HBOC-201. This created an asymmetry between study arms given that no similar cross-over arm was in place for the RBC group (*R*). This asymmetry confounded the analysis and understanding of safety data. The natural separation of the HBOC-201 group (*H*) into avoidance (*HH*) and crossover (*HR*) subgroups necessitated stratification of subjects in *R* into two subgroups. That is, matching subgroups in *R* corresponding to the blood avoidance (*HH*) and crossover (*HR*) subgroups in the *H* group were identified on the basis of low and high needs, respectively. This was accomplished on the basis of clinical need defined in terms of the number of RBC units received (i.e., ≤ 3 units [*R*-] vs. > 3 units [*R*+]). This allowed for comparison of *matching subgroups* in the HBOC-201 and RBC arms of HEM-0115. In contrast to the confounded ITT comparisons (*H* vs. *R* groups), the *matching groups* comparisons (*HH* vs. *R*- and *HR* vs. *R*+) provided opportunity to identify the major factors contributing to the increased incidence of AEs and SAEs in the HEM-0115 study and facilitated root cause analysis. This analysis placed the observed AEs into clinical context and identified specific root causes contributing to excess SAEs. The majority of the excess non-serious AEs were considered intrinsic side effects associated with exposure to HBOC-201. In addition, these analyses identified AEs that occurred because of product limitations, exaggerated expectations, and inadequate training with regard to the proper use of HBOC-201.

In conclusion, the results of *matching subgroups* analysis permits accurate assessment of risk associated with exposure to HBOC-201 that can be applied to quantitative benefit:risk analysis in *RESUS*. Similarly, this also permitted root cause analysis which defined the framework for proper bridging therapy and mitigation against the risk of SAEs that have been applied to *RESUS* design.

South Africa - Initial Post-Approval Experience With HBOC-201

Dr. Lewis Levien, MD, Specialist Surgeon, Milpark Hospital, Johannesburg, S. Africa, and Dr. Reynhardt Van Rooyen, MD, Pretoria Heart Hospital, Preotoria, S. Africa, presented initial post-approval experience from S. Africa (Biopure Conference, Miami, FL, June, 2003). Eighty

subjects received HBOC-201 with surveillance under a Regulatory Surveillance and Education Program and 110 general application clinical cases were reported. The 80 intensive surveillance subjects had a blood transfusion avoidance rate > 90% and a lower SAE rate than that observed in clinical trials, 20 SAEs in 15 subjects, approximately 18% vs. the 25% overall observed in the HEM-0115 trial; 18 of the 20 SAEs were adjudicated as unrelated to HBOC-201 administration. In the subsequent 110 subjects, blood transfusion avoidance remained > 90% and few HBOC-201 related SAEs were reported.

The overall SAE rate in all 190 subjects was also lower than that observed in HEM-0115, ~ 10%.. There were 3 deaths possibly related to sudden withdrawal of the HBOC-201 without infusion of RBC. There was one case of pancreatitis that occurred in a subject, status post-pancreatic surgery. There were four cases of renal failure in subjects with preexisting renal disease and multiple renal insults. Volume overload was the most common related AE. Notably, there were no MIs or CVAs. Four subjects in whom blood transfusion was not an option had severely low Hb.

6.0 THE RESUS IND

6.a *RESUS* Trial Abstract

Hypotheses	**General:** That as a prehospital resuscitative agent for HS, HBOC-201 will decrease morbidity and mortality, in comparison with LR **Specific:** That for subjects in traumatic HS, in comparison with prehospital resuscitation with LR, HBOC-201 will decrease 28-day mortality, will be safe, and will improve other clinical parameters
Objectives	**General:** 1. To decrease morbidity and mortality of subjects with traumatic HS. 2. To test our hypotheses that in comparison with prehospital resuscitation of patients in HS with LR, HBOC-201 will decrease morbidity & mortality & will be safe & well tolerated. **Specific:** To test our hypotheses that in comparison with prehospital resuscitation of subjects in HS with LR, that HBOC-201 will: **Primary:** 1. **Efficacy:** Decrease the 28-day relative rate (RR) of mortality (and) 2. **Safety:** Be safe & well-tolerated **Secondary:** 1. Decrease the prehospital RR of mortality (through hospital arrival) 2. Improve key & other clinical parameters & decrease blood transfusion exposures **Tertiary:** 1. Improve the Composite Surrogate Score (CSS)
Trial design	Single-blinded, randomized, controlled multicenter Phase 2b trial leading into a pivotal Phase 3 trial
Trial execution	[1] Stage I (Phase 2 trial)— ~ 50 subjects (~ 25 HBOC-201 [study Group 1]) (~ 25 LR [control Group 2]) in ~ 10-15 level I trauma centers [2] DMC review decision (prospectively defined)—data integration into Stage II or amend/restart Stage II trial [3] Stage II (Phase 3 trial)—up to ~ 1,108 evaluable subjects (~ 554 HBOC-201, ~ 554 LR) in ~ 40 level I trauma centers
Population	Prehospital subjects with traumatic HS
# to enroll	Expected: ~ 1,130 (1,108 evaluable). Maximum: ~ 1,180
Consent	*Exception from Informed Consent*—1. *Community Consultation & Disclosure*; 2. *Informed Consent* or *Pre-Enrollment Disclosure*, when feasible; 3. *Exception from Informed Consent*; 4. *Post-Enrollment Disclosure/Option to Withdraw*
Enrollment inclusion criteria (at time of enrollment)	[1] Age 18 to less than 70 years old [2] Injury with obvious/suspected massive bleeding [3] SBP less than 90 mm Hg [4] RTS 1 to less than 5 [5] Expected transport to participating hospital [6] IV access secured
Enrollment exclusion criteria	[1] Penetrating TBI [2] Paralysis [3] Pregnancy—known/suspected [4] Cardiac arrest (absence of spontaneous circulation) [5] Known allergy to HBOC-201 [6] Known opposition to HBOC-201 [7] Burn > 20% BSA (partial or full thickness) [8] Blood transfusion available (guideline: expected < 10-15 minutes to hospital arrival)
Interventions	**Intervention:** CTM IV infusion usually over ~ 10 minutes **CTM dose:** HBOC-201 (500 ml) or LR (1,000 ml for 1st infusion and 500 ml for 2nd infusion) **Maximum total dose:** 3 HBOC-201 (total 1,500 ml) or 2 LR doses (total 1,500 ml) **CTM stopping criterion:** SBP ≥ 120 mm Hg **CTM initial infusion criteria:** Inclusion (including SBP < 90 mm Hg) and exclusion criteria met **CTM re-infusion criteria:** SBP < 90 mm Hg; or SBP 90-99 mm Hg and HR ≥ 100 bpm **Standard IV fluids:** Usually indicated for other persistent signs of HS where CTM re-infusion

	criteria not met.
Outcome measurements	**Primary (acute mortality & safety)** 1. 28-day RR of mortality 2. Safety & tolerability—SAEs **Secondary (key clinical parameters, morbidity, long-term survival, & safety)** 1. Hemodynamics—MAP, HR, RR, O_2 saturation, Swan Ganz parameters 2. Tissue oxygenation—BD, LA, pH, UI, BD/LA/UI clearance, TI 3. Renal function—urine output, urinalysis, BUN/creatinine 4. O_2 content—Hb/Hct, free Hb, metHb 5. Organ function—MODS ARDS score 6. Trauma scores—RTS, ISS, TRISS 7. Infectious complications—SIRSS, CRP, specific 8. Abdominal complications—compartment syndrome 9. Length of stay (LOS)—ICU, hospital 10. Ventilator support—duration 11. Hemostasis—PT/PTT, platelets, TEG, PFA-100, time to definitive control of bleeding 12. Blood transfusions—blood transfusion avoidance, reticulocytes 13. Fluid requirements—qualitative, quantitative 14. Immunologic activation—IL-6, IL-10 15. Neurocognitive function—GCS, PCPS, CPC 16. Neurophysiology (subjects with TBI)—ICP and CPP (when available), and mannitol requirements, seizures 17. Safety labs—CBC, electrolytes, BUN, creatinine, LFT, lipase, troponin 18. Clinical safety—medical/surgical complications, AEs 19. Disposition—home, rehabilitation, hospital 20. Survival—prehospital RR of mortality (hospital arrival) **Tertiary** 1. Composite Surrogate Score (CSS)
Sample size	To decrease the 28-day RR of mortality from 58.1% in LR to 49.4% in HBOC-201 subjects (Δ = 15%, α = 0.045, power = 0.80, drop out 2%): Stage II sample size = 1,130 subjects (1,108 evaluable subjects)
DMC reviews	After accrual of: 50 (~ 5%), 222 (20%), 554 (50%), 1,108-1,158 (100%) evaluable subjects

Stopping criteria	**Efficacy stopping criteria:**
	• **Absolute:** Significantly decreased 28-day RR of mortality (intent-to-treat [ITT])
	Safety stopping criteria:
	• **Absolute**: Increased ($p \leq 0.05$) SAEs in HBOC-201 subjects, as compared to controls, that:
	  • Result in death
	  • Result in persistent or significant disability
	  • Are congenital anomalies or birth defects
	• **Relative**:
	  o Increased ($p < 0.05$-0.1) SAEs in HBOC-201 subjects, as compared with controls that:
	  • Are life-threatening without survival benefit ($0.05 < p < 0.1$)
	  • Require expedited reporting without survival benefit ($0.05 < p < 0.1$)
	  i. Cardiac—acute coronary syndrome, congestive heart failure
	  ii. Pulmonary—acute respiratory failure, pulmonary edema, pneumonia
	  iii. Neurologic—cerebral vascular accident, transient ischemic attack
	  iv. Renal—acute renal failure
	  • Require in-patient hospitalization or prolongation of existing hospitalization without survival benefit ($p < 0.05$)
	  • LSI or DMC consider to be "medically significant events"
	  i. Diminish expected efficacy/toxicity ratio to a threshold not considered likely to be ethically and/or medically beneficial to participating subjects
	  • Absolute safety stopping criteria ($0.05 < p < 0.1$)
	  • Significant worsening of key surrogate measurements without survival benefit ($p < 0.05$)
	  o MAP, LA, BD at hospital arrival

Data analysis	ITT, efficacy significance (O'Brien-Fleming boundary adjustment) $p \leq 0.045$; safety significance $p \leq$ 0.05-0.1
Statistics	Two-sided. Categorical data: 2x2 table Chi Square, Fisher's exact test. Continuous, ordinal, and injury score data: Repeated measures ANOVA and/or individual t tests or trapezoidal AUC and/or Wilcoxon rank sum; Global or Bonferroni adjustment. Survival: Chi Square or Fisher's exact test for RR; Wilcoxon rank sum for duration. Confounding parameters: Cox proportional hazard, multiple logistic regression

6.b Summary of *RESUS* Study Design

In this single-blind, randomized, and controlled multicenter Phase 2b/3 trial, the efficacy and safety of HBOC-201 and LR will be compared for the prehospital resuscitation of HS casualties. The primary aim of the trial is to test the hypothesis that 28-day relative rate of mortality will be reduced by 15% in HBOC-201- in comparison of with LR-resuscitated subjects (from 58.1% to 49.4%). The trial will be conducted in two stages. Stage I will enroll 50 subjects in ~ 10-15 trauma centers and is designed as a *pilot study* to allow optimization of the study design and sample size, if necessary, prior to completion of the pivotal Stage II (Phase 3) study in which up to 1,108 evaluable subjects will be enrolled in ~ 40 trauma centers.

Upon completion of Stage I, the *RESUS Advisory Board* will complete a *Program Review* and submit a *Data Integration Disposition* summary report to the respective IRBs, DMC, and FDA. The *Data Integration Disposition* summary will report the DMC's recommendation about whether to integrate Stage I data into Stage II or to terminate the Phase 2 trial and restart a separate Phase 3 trial subsequently. The decision will depend on the finding (recommend against data integration) or the absence of finding (recommend data integration) of significant *trial conduct* issues. If the *RESUS Advisory Board* recommends against data integration, Stage II will not resume until noted QA issues and necessary study modifications are addressed and approved by the DMC, IRBs, and FDA. To ensure absence of bias, the *Data Integration Decision* will be made prior to and independent of the first *ESR* by the independent DMC. In addition, upon completion of Stage I, a revised sample size calculation will be conducted based on aggregate mortality in the first 50 subjects; if the *RESUS* mortality prediction of ~ 58% is materialized, the 95% CI will be ~ 44% to 71%. Using Bayesian principles, the DMC will reevaluate the target sample size and make recommendations to the *RESUS Advisory Board* for sample size (and *ESR* time points) adjustment as necessary. At its discretion, the DMC may make additional recommendations, including additional *down-the-road* sample size re-analyeses (and adjustments as needed).

Inclusion and exclusion criteria will target salvageable 18 to less than 70 year old subjects with severe HS, for whom blood transfusions are unavailable. Key inclusion criteria include SBP < 90 mm Hg; RTS 1 to less than 5; expected transport to a participating hospital; and IV access. Key exclusion criteria include penetrating TBI, cardiac arrest, known/suspected pregnancy, and rapid access to blood transfusion (i.e, expected arrival at a hospital within 10-15 minutes).

EMS interventions will include: (1) brief screening of patients with HS to assess their need for fluid resuscitation by review of inclusion/exclusion criteria; (2) obtaining *IC if feasible*, alternatively providing *Pre-ED* (right of refusal), or enrollment with *EIC* when other efforts are not feasible; (3) opening a *randomization study envelope*; (4) infusion of CTM—HBOC-201 (500 ml) or LR (1,000 ml) as determined by randomization number/study envelope; (5) re-infusion of the study drug or control fluid (maximum 1,500 ml) for persistent severe hypotension, or for moderate hypotension with associated tachycardia (per *fluid re-infusion guidelines*); (6) infusion of standard fluids for signs of hypoperfusion without hypotension; and (7) completion of *Screening* and *Resuscitation CRF*s.

After hospital arrival, no additional infusions of CTM (HBOC-201 or LR) will be initiated; subjects will receive standard care including blood transfusions and/or IV fluids as needed (per *in-hospital trauma care guidelines;* Appendix D), and will be routinely followed for 60 days, 90 days for clinically significant unresolved SAEs, and through delivery in subjects determined to be pregnant after enrollment.

The objectives of the study are to compare the relative effects of prehospital resuscitation with HBOC-201 and LR on subjects' 28-day mortality (primary efficacy and safety end-point), and other safety and tolerability variables. Secondary measurements will include a wide variety of morbidity and mortality parameters. Prospectively defined key surrogates include SBP, LA, and BD at hospital arrival, and survival up to the time of arrival at the hospital.

The sample size of 50 subjects for Stage I was chosen as a pilot study. The sample size of 1,130 subjects for Stage II (Phase 3) is based on predicted prehospital mortality of 58.1% and 49.4% in LR control and HBOC-201 groups, respectively, with $\Delta = 15\%$, $\Delta \leq 0.045$, 80% power, and 2% dropout rate (i.e., 1,108 evaluable subjects). The absolute efficacy stopping-criterion requires a statistically significant decrease in the 28-day relative rate (RR) of mortality with O'Brien-Fleming boundary adjustment and ITT analysis.

The predefined safety criteria for stopping the *RESUS* trial include absolute and relative criteria requiring significance levels of $p < 0.05$ and $p < 0.05$-0.1, respectively. The *absolute safety stopping criterion* is a statistically significant increase in mortality, with consideration of SAEs by the DMC. The *relative safety stopping-criteria* will be established by the DMC and will be based in part on SAEs identified by OBRR as always requiring *expedited* reporting (reported by

the principal investigator to the *RESUS Drug Safety Officer* no later than 7 calendar days of the investigator's learning of an "always expedited" AE and by the *RESUS Drug Safety Officer* to the FDA no later than 15 calendar days after the sponsor's initial receipt of the information) in the following organ systems: cardiovascular, pulmonary, neurologic, and renal.

ESRs will occur after accrual of 50, 222, 554, and 1,108-1,158 evaluable subjects. Trauma centers will submit SAE reports to the *RESUS* Drug Safety Officer (unblinded data). Trauma center staff will have access only to the unblinded data generated at their trauma center. The *RESUS* Drug Safety Officer will have access to individual subject unblinded data only as required for SAE reporting to the DMC and FDA. *RESUS Advisory Board* members will have access only to trial conduct data and aggregate efficacy/safety data. Three DMC reports (i.e., open session, closed session, and code) will be prepared for *ESR* timepoints and submitted to the DMC. An independent statistician will analyze and collate interim data from the database and prepare DMC closed session and code reports and provide input for DMC open session reports.

Prior to the start of subject enrollment, a comprehensive *CCD* process will occur to provide *the opportunity for discussions with, and soliciting opinions from the communities in which the study will take place and from which the study will be drawn.* [84] Adequacy of the *CCD* process in each community will be confirmed by NMRC and local trauma center IRBs prior to subject enrollment. In accordance with 21 CFR 50.24 (*EIC*), *IC* will be obtained (or alternatively *Pre-ED* will be provided) when feasible. However, in most cases, due to the subject's condition and short therapeutic window, neither *IC* nor *Pre-ED* will be feasible. Therefore, most subjects will be enrolled per *EIC*. Once a subject reaches the hospital, study personnel will initiate the *Post-ED* process, ideally to the subject and alternatively to a Legally Authorized Representative or family member, as soon as feasible. The process includes disclosure of *RESUS* study interventions, potential benefits and risks, information about rights of human research subjects, and established processes in event of suspected research-related injury. Although *Post-ED* does not provide the subject an opportunity for prospective *IC*, it does allow him/her to withdraw from the study and thus, refuse further interventions (e.g., blood drawing for research evaluations related to the study).

7.0 RESUS IND CLINICAL HOLD ISSUES

The *RESUS* IND was placed on Clinical Hold by OBRR mainly based on safety concerns arising from prior Phase 2/3 surgery/orthopedics trials with HBOC-201, primarily related to the vasoactive properties of HBOC-201 and consequent potential for vasoconstriction to affect AE incidence. When considering the importance of previous studies and their AE profiles, NMRC believes that one should keep in mind that in the majority of subjects included in these trials, HBOC-201 was compared with RBC transfusion with the aim of reducing blood transfusion requirements. The potential risk (prolonged CTM exposure in an older overall population) and benefit (RBC transfusion avoidance) of HBOC-201 in these studies contrasts with potential risk in *RESUS* (short CTM exposure [oxygen bridge] in a younger population) and benefit in *RESUS* (improved survival). Thus, previous safety profiles, while important, do not directly apply to this discussion without consideration of differences in potential risks and benefits. The overriding issue is whether predicted benefit mainly from preclinical HS studies supports prospect of benefit and reasonable risk despite safety concerns from prior non-trauma surgery/orthopedics trials. Thus, the following section concentrates on key issues important in prediction of potential benefit and risk in *RESUS*.

The several issues related to the Clinical Hold can be divided into 4 main areas as listed below.

- Vasoactivity
- Dosing
- Benefit:Risk and Risk Mitigation
- *Exception from Informed Consent*

7.a Vasoactivity

Elevated BP/HTN (HEM-0115 and COR-0001)

HBOC-201 has vasoconstrictive properties that can manifest as elevated BP, but relative potency of HBOC-201's vasoactivity should be put into perspective as it relates to prediction of benefit:risk in *RESUS*. NMRC believes that risk due to potentially vasoactivity-related elevated BP responses and secondary AEs is *reasonable* in comparison with potential benefit because polymerization has reduced vasoactivity (in comparison with prior first generation HBOCs); data from preclinical HS and prior surgery clinical studies with HBOC-201 and other HBOCs suggest that risk of adverse outcome due to elevated BP is *relatively* low; in prior HBOC-201 trials, BP responses were lower in HBOC-201 subjects with prior hypotension, stable trauma, or younger

age; and extensive mitigation strategies have been incorporated into the *RESUS* protocol to reduce risk *a priori*.

Polymerization attenuation of HBOC-201 vasoactivity

As the second generation HBOC, HBOC-201, contains ≤ 2-3% vs. 32% tetrameric Hb in HBOC-301, and 100% tetrameric Hb in the prior first generation HBOC, DCLHb, the magnitude of vasoactivity is decreased in comparison with these HBOCs. [12]

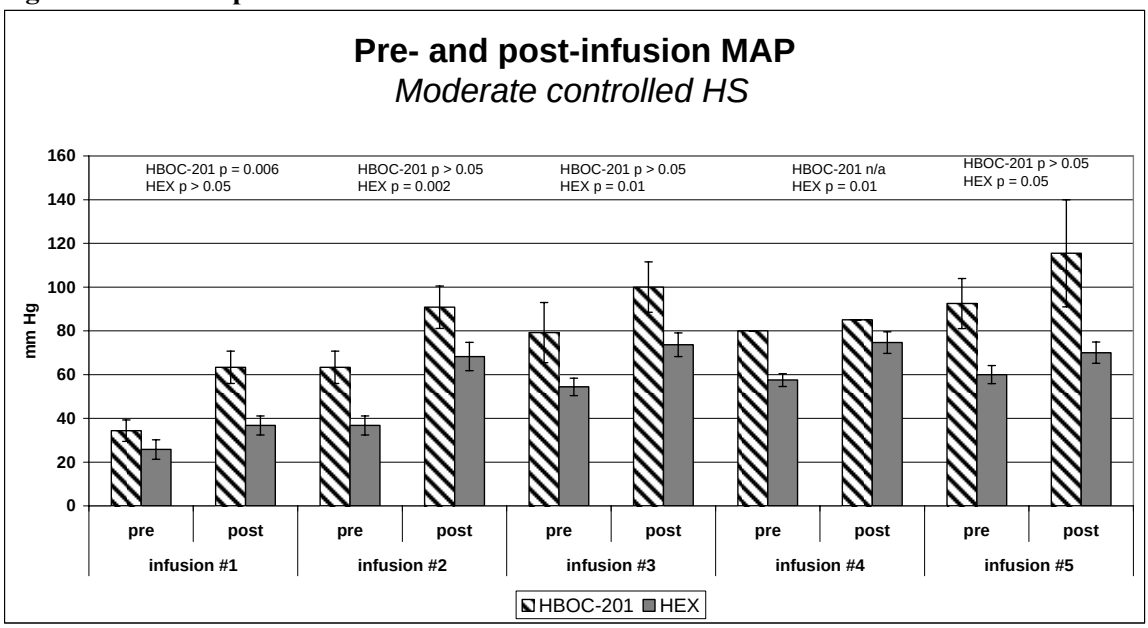
HBOC-201 BP responses in preclinical HS studies

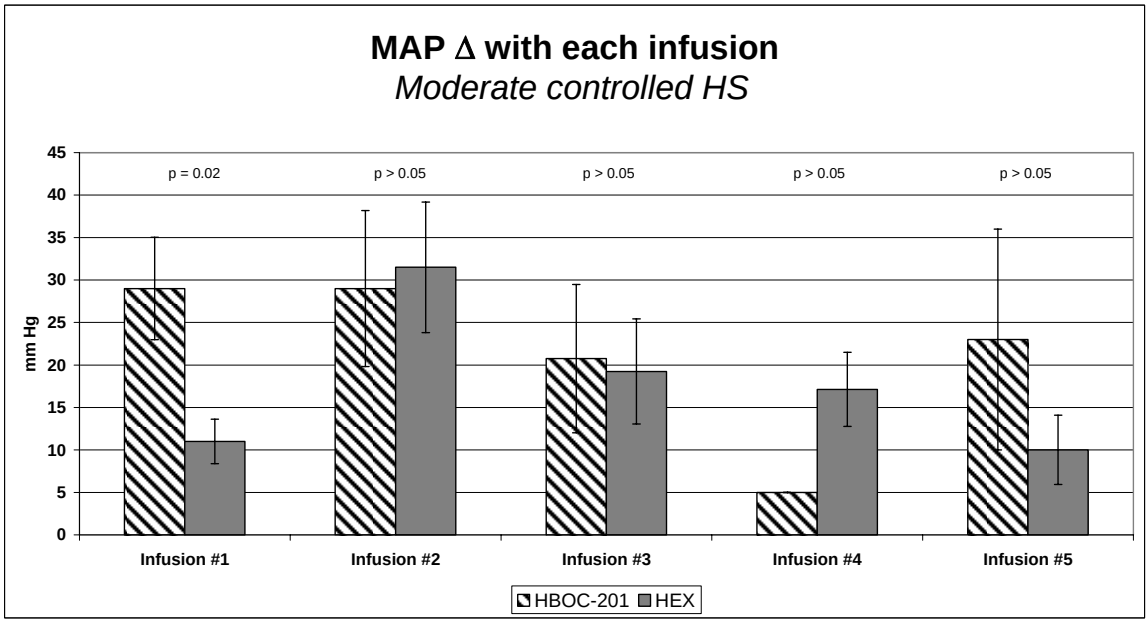
The section below details data showing that in NMRC's swine HS studies, although mild to moderate *peak SBP responses* occurred, more severe responses were uncommon, and clinical outcome was beneficial even in individual pigs with higher *peak SBP responses*.[12] Also, in other OBRR-directed animal studies, although overall BP was higher in HBOC-201 than control groups, adverse BP responses were not seen.

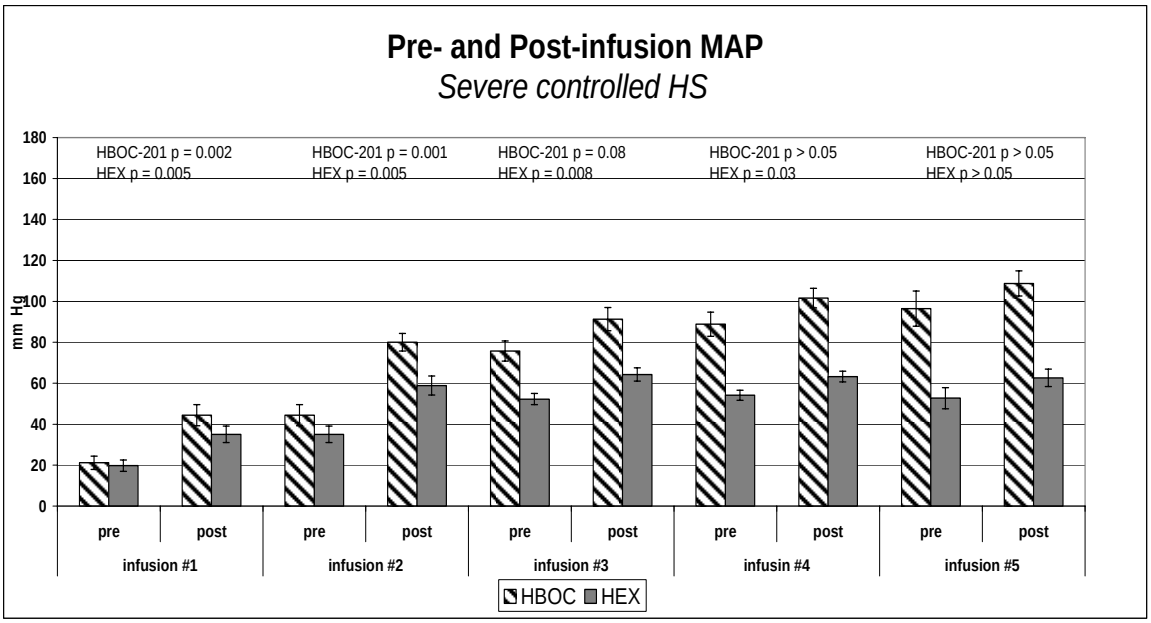
Figure 5 and Table 36 show pre- and post-infusion MAP (± SEM), MAP Δ, and MAP ranges for HBOC-201 vs. control[35] swine in four NMRC studies and in all combined. These preclinical data in HS models suggest that in similar hypotensive subjects in *RESUS*, HBOC-201-induced BP responses are likely to be mild to moderate and probably clinically insignificant, and that severe BP responses are unlikely. These conclusions are based on the following observations from these preclinical HS studies.

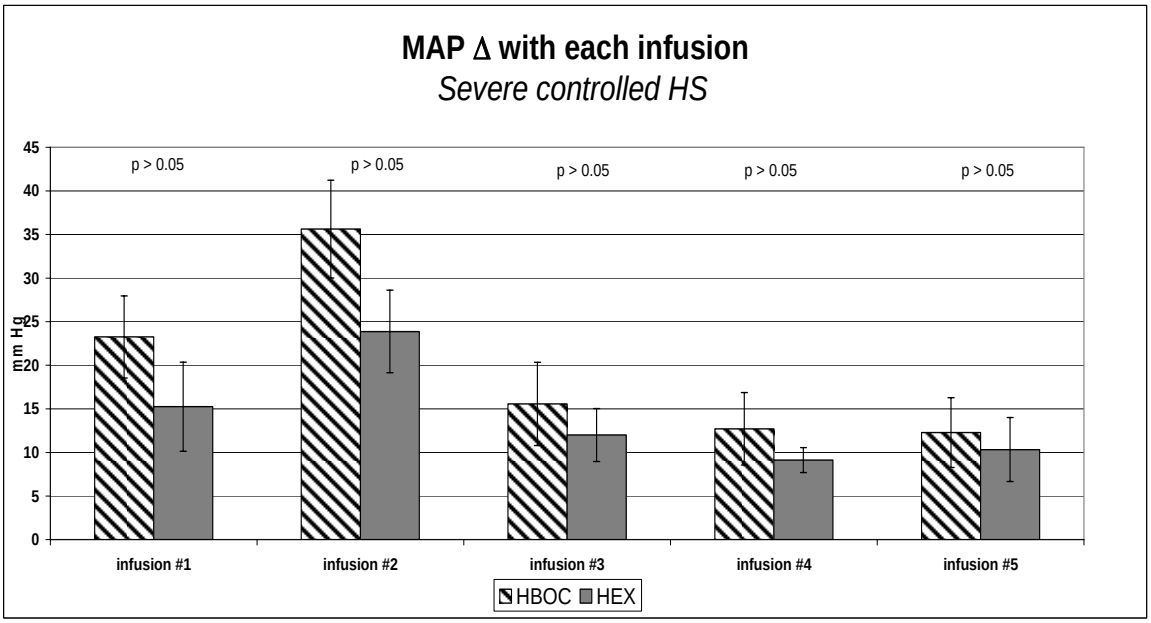
[35] Hextend in all studies except LR in *severe uncontrolled HS/TBI*.

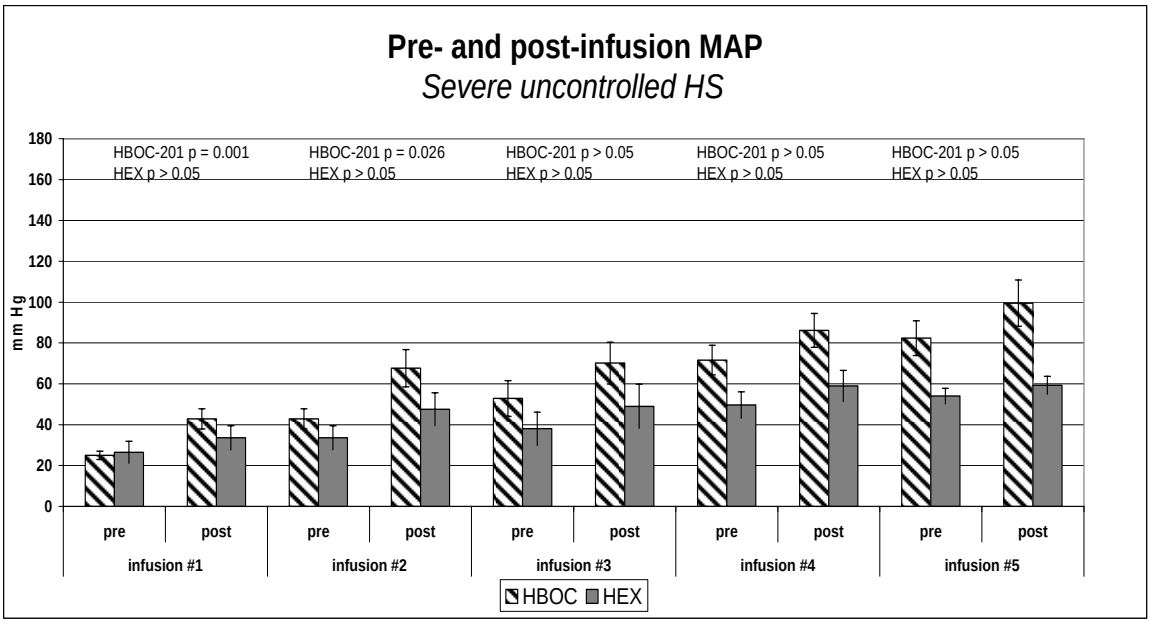
Figure 5: MAP responses in NMRC swine HS studies

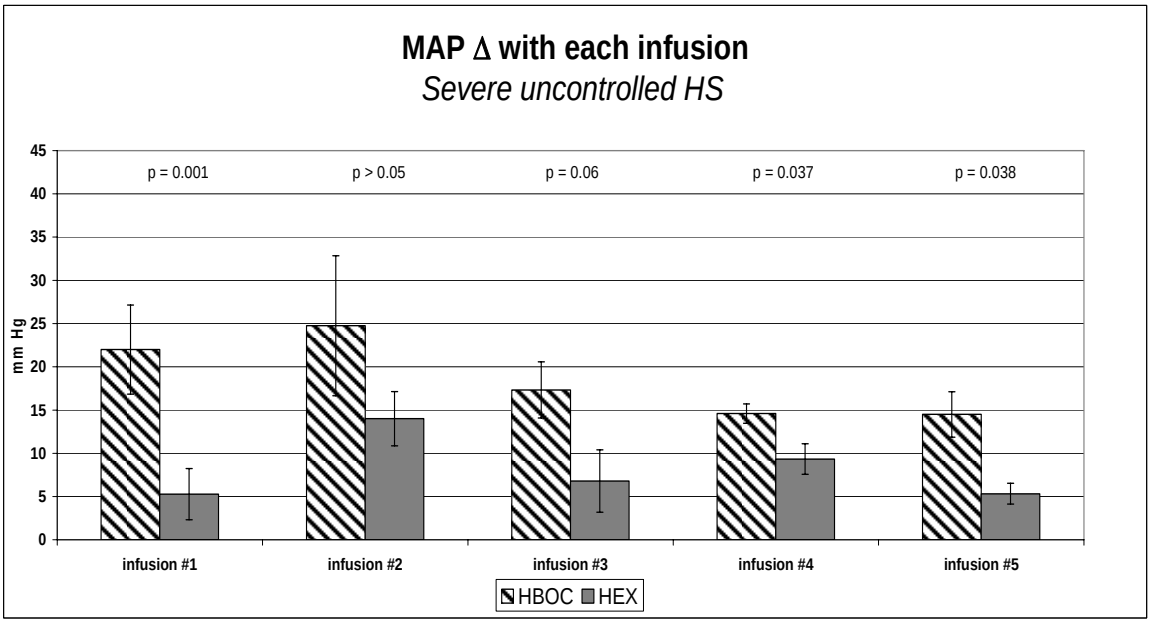





Pre- and Post-infusion MAP
Severe controlled HS
HBOC-201 p = 0.002
HEX p = 0.005
HBOC-201 p = 0.001
HEX p = 0.005
HBOC-201 p = 0.08
HEX p = 0.008
HBOC-201 p > 0.05
HEX p = 0.03
HBOC-201 p > 0.05
HEX p > 0.05
mm Hg
180
160
140
120
100
80
60
40
20
0
pre
post
infusion #1
infusion #2
infusion #3
infusin #4
infusion #5
HBOC
HEX


MAP Δ with each infusion
Severe controlled HS
p > 0.05
mm Hg
45
40
35
30
25
20
15
10
5
0
infusion #1
infusion #2
infusion #3
infusion #4
infusion #5
HBOC
HEX


Pre- and post-infusion MAP
Severe uncontrolled HS
HBOC-201 p = 0.001
HEX p > 0.05
HBOC-201 p = 0.026
HEX p > 0.05
HBOC-201 p > 0.05
HEX p > 0.05
HBOC-201 p > 0.05
HEX p > 0.05
HBOC-201 p > 0.05
HEX p > 0.05
mm Hg
180
160
140
120
100
80
60
40
20
0
pre
post
infusion #1
infusion #2
infusion #3
infusion #4
infusion #5
HBOC
HEX


MAP Δ with each infusion
Severe uncontrolled HS
p = 0.001
p > 0.05
p = 0.06
p = 0.037
p = 0.038
mm Hg
45
40
35
30
25
20
15
10
5
0
infusion #1
infusion #2
infusion #3
infusion #4
infusion #5
HBOC
HEX

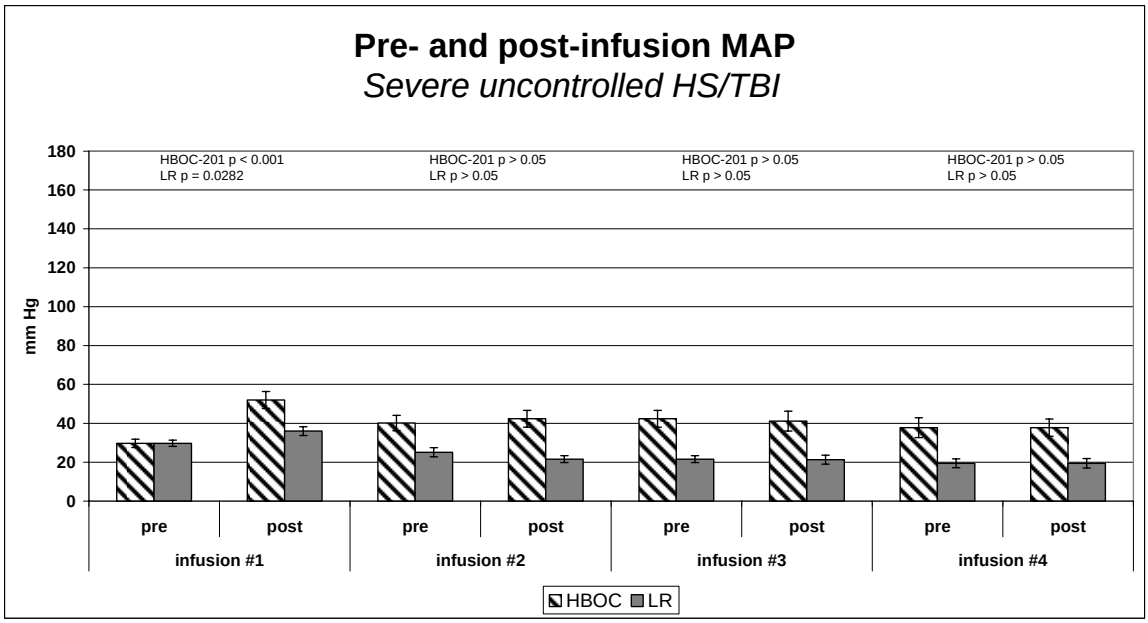

Pre- and post-infusion MAP
Severe uncontrolled HS/TBI
mm Hg
180
160
140
120
100
80
60
40
20
0
HBOC-201 p < 0.001
LR p = 0.0282
HBOC-201 p > 0.05
LR p > 0.05
HBOC-201 p > 0.05
LR p > 0.05
HBOC-201 p > 0.05
LR p > 0.05
pre
post
pre
post
pre
post
pre
post
infusion #1
infusion #2
infusion #3
infusion #4
HBOC
LR

Table 36: MAP Δ in NMRC swine HS studies

Infusion 1 (15 or 20 min)	**≥ 70 mm Hg severe MAP Δ response**		**60-69 mm Hg moderately severe MAP Δ response**		**50-59 mm Hg moderate MAP Δ response**		**40-49 mm Hg mild MAP Δ response**		**< 40 mm Hg no MAP Δ response**	
	HBOC-201	**Standard Fluid^**	**HBOC-201**	**Standard Fluid^**	**HBOC-201**	**Standard Fluid^**	**HBOC-201**	**Standard Fluid^**	**HBOC-201**	**Standard Fluid^**
Moderate Controlled Hemorrhage	0/8 (0%)	0/8 (0%)	0/8 (0%)	0/8 (0%)	0/8 (0%)	0/8 (0%)	1/8 (12.5%)	0/8 (0%)	7/8 (87.5%)	8/8 (100%)
Severe Controlled Hemorrhage	0/8 (0%)	0/8 (0%)	0/8 (0%)	0/8 (0%)	0/8 (0%)	0/8 (0%)	0/8 (0%)	0/8 (0%)	8/8 (100%)	8/8 (100%)
Severe Uncontrolled Hemorrhage	0/8 (0%)	0/8 (0%)	0/8 (0%)	0/7 (0%)	0/8 (0%)	0/7 (0%)	1/8 (12.5%)	0/7 (0%)	7/8 (87.5%)	7/7 (100%)
Severe Uncontrolled Hemorrhage + TBI	0/31 (0%)	0/28 (0%)	1/31 (3.2%)	0/28 (0%)	2/31 (6.5%)	0/28 (0%)	2/31 (6.5%)	0/28 (0%)	26/31 (83.9%)	28/28 (100%)
Combined	0/55 (0%)	0/52 (0%)	1/55 (1.8%)	0/51 (0%)	2/55 (3.6%)	0/51 (0%)	4/55 (7.3%)	0/51 (0%)	44/55 (87.1%)	51/51 (100%)
Infusion 2 (30 min)	**≥ 70 mm Hg severe MAP Δ response**		**60-69 mm Hg moderately severe MAP Δ response**		**50-59 mm Hg moderate MAP Δ response**		**40-49 mm Hg mild MAP Δ response**		**< 40 mm Hg no MAP Δ response**	
	HBOC-201	Standard Fluid	HBOC-201	Standard Fluid	HBOC-201	Standard Fluid	HBOC-201	Standard Fluid	HBOC-201	Standard Fluid
Moderate Controlled Hemorrhage	0/8 (0%)	0/8 (0%)	1/8 (12.5%)	1/8 (12.5%)	0/8 (0%)	2/8 (25%)	1/8 (12.5%)	0/8 (0%)	6/8 (75%)	5/8 (62.5%)
Severe Controlled Hemorrhage	0/8 (0%)	0/8 (0%)	1/8 (12.5%)	0/8 (0%)	1/8 (12.5%)	0/8 (0%)	0/8 (0%)	2/8 (25%)	6/8 (75%)	6/8 (75%)
Severe Uncontrolled Hemorrhage	0/8 (0%)	0/8 (0%)	1/8 (12.5%)	0/7 (0%)	1/8 (12.5%)	0/7 (0%)	0/8 (0%)	0/7 (0%)	6/8 (75%)	7/7 (100%)
Severe Uncontrolled Hemorrhage + TBI	0/15 (0%)	0/14 (0%)	0/15 (0%)	0/14 (0%)	0/15 (0%)	0/14 (0%)	0/15 (0%)	0/14 (0%)	15/15 (100%)	14/14 (100%)
Combined	0/39 (0%)	0/38 (0%)	3/39 (7.7%)	1/37 (2.7%)	2/39 (5.1%)	2/37 (5.4%)	1/39 (2.5%)	2/37 (5.4%)	33/39 (84.6%)	32/37 (86.5%)

^Hextend or LR

No significant differences between groups ($p > 0.05$)

First, mild to moderate vasoactive responses occurred, manifested by overall higher BP curves in HBOC-201 than control pigs (Figure 5). Second, significantly higher BP responses were seen in HBOC-201 than control pigs only after the first infusion; subsequent infusion responses were equivalent. For example, after the first infusion, when all the models were combined (for simplicity of presentation), MAP Δ for HBOC-201 vs. control pigs was (20.6 ± 2.4 vs. 5.5 ± 2.0 mm Hg, $p < 0.001$). After the second infusion, respective responses were 13.1 ± 3.5 vs. 8.2 ± 2.6 mm Hg ($p = 0.3$). Third, although mild to moderate BP responses were more common with HBOC-201 than controls, no severe BP responses occurred. Fourth, BP responses in HBOC-201 swine were higher with less severe HS. For example, for the first infusion, MAP Δ was 29.0 ± 6.0 mm Hg in *moderate controlled HS* (40% EBV); 23.3 ± 4.7 mm Hg in *severe controlled HS* (55% EBV); 17.9 ± 4.6 mm Hg in *severe uncontrolled HS*; and 16.6 ± 3.8 mm Hg in *severe uncontrolled HS/TBI*. Fifth, in both *severe uncontrolled HS* models, in contrast to control swine which had insignificant BP responses that were incompatible with life, mild beneficial BP responses resulting in high survival were seen with HBOC-201.

Organ blood flow in NMRC swine HS studies

Cerebral and renal blood flow rates were quantified in the OBRR-requested *severe uncontrolled HS/TBI* study. Despite OBRR's concern for decreased organ blood flow, blood flow was not adversely affected in either organ. In the additional OBRR-requested sedated swine study (30-50% EBV isovolemic exchange), except for muscle (trend for kidneys), there were no significant differences in blood flow in any of the organs analyzed[36]. In summary, there are no preclinical data to suggest that in similar hypotensive patients in *RESUS*, organ blood flow will be compromised by HBOC-201's mild to moderate vasoactivity.

Tissue oxygenation in NMRC swine HS studies

$tcPO_2$, indirect measures of oxygenation (i.e., LA and BD), and organ 3-nitrotyrosine staining intensity (surrogate marker for peroxynitrate [oxidative potential]) were assessed in NMRC's models. All of these parameters should be adversely affected by vasoactive BP responses sufficiently significant to cause tissue hypoperfusion, as suggested by the OBRR. However, in all four models, $tcPO_2$ was improved by HBOC-201. In the severe HS models, LA and BD were lower with HBOC-201 (insignificant differences in less severe HS models). Moreover, 3-nitrotyrosine staining intensity was similar in HBOC-201 and control resuscitated swine in

[36] Mongan P, USUHS, Bethesda, MD (Biopure research report SN 356 to BB-IND 2935, 29 Nov 2004).

cardiac, pulmonary, jejunal, hepatic, and renal tissue. In summary, there are no preclinical data to suggest that in similar hypotensive patients in *RESUS*, higher rates of hypoperfusion will occur due to HBOC-201's mild vasoactivity[37].

Hemorrhage in NMRC swine HS studies

Hemorrhage was assessed in both *severe uncontrolled HS* models (± TBI).[17, 18] As hemorrhage volume was not increased in either of these studies, there are no objective preclinical data to suggest that in similar hypotensive patients in *RESUS*, higher hemorrhage volumes will occur due to HBOC-201's mild vasoactivity. In the USUHS *severe uncontrolled HS* model (due to vascular injury), total hemorrhage volume was not increased with hypotensive resuscitation with HBOC-201 either.

"Clinical" outcome in NMRC swine HS studies

Higher BP responses in HBOC-201 pigs should not be construed as implying adverse responses as the overwhelming majority of pigs faired well after HBOC-201 resuscitation, resulting in combined survival rates (NMRC studies only) of 78% (36/46) for HBOC-201 vs. 43% (19/44) for control pigs ($p = 0.001$). In fact, in both *severe uncontrolled HS* models (± TBI), control fluids were *failed resuscitative fluids* (failing to restore hemodynamics compatible with life, and resulting in almost uniform mortality). NMRC assessed outcome in pigs experiencing "higher" BP responses (MAP $\Delta \geq 50$ [at least moderately severe]).**Error! Reference source not found.**Table 37 summarizes clinical outcome of pigs with these higher BP responses, comparing

[37] NMRC's perspective on OBRR's BPAC package about preclinical data: York and Fitzpatrick

1. Signs of hypoperfusion: It is stated that LA and the rate of LA clearance were diminished with HBOC-201, but the opposite was actually documented. In all animal models in which cutaneous tissue oxygen tension was evaluated, it increased with HBOC-201. In less severe controlled HS swine models, LA and BD were equivocal in HBOC-201 vs. control fluid animals (e.g., [12, 20, 29]; but in severe uncontrolled HS models, LA and BD were consistently lower with HBOC-201.[17, 18, 23, 24, 85] King's paper [26]is repeatedly referenced but it reports results with HBOC-301, not HBOC-201. LA clearance was slower in the controlled HS/TBI model with isolated HBOC-301 resuscitation without concomitant standard fluids, but the weaning surrogate of survival was higher with HBOC-301. In any case, King's results apply to a different HBOC that has been shown by NMRC to be significantly more vasoactive than HBOC-201[12]; moreover, withholding of clinically-indicated standard fluids would not occur in *RESUS* in which prehospital and trauma center guidelines dictate fluid infusions in the presence of symptoms/signs of occult HS (e.g., persistently elevated LA). Thus, King's data has only circumstantial relevance to the *RESUS* IND.

2. Dr. Alayash's vasoactivity data: The slides summarize a number of *in vitro* and animal experiments evaluating microvascular effects of HBOC-301, not HBOC-201. As HBOC-301 is more vasoactive than HBOC-201, the results have only circumstantial relevance to the *RESUS* IND.

3. Functional capillary density: Data showing decreased functional capillary density in exchange transfusions in animals are circumstantial and largely irrelevant to the *RESUS* IND as they report data with the more vasoactive HBOC-301, not HBOC-201. [86, 87]

peak values with same group means. Individual pigs were classified as having better or worse individual parameter outcome if the peak value was ≥ 2 SDs from the same group mean (note values with *). No pattern could be ascertained in these pigs, in comparison with the overall population of HBOC-201 and control pigs. Additionally, comprehensive organ function and histopathologic evaluation from these studies did not reveal significant adverse safety signals other than mild papillary necrosis in 1 of 4 studies and minimal biliary histopathologic changes; in fact, myocardial histopathology and peak troponin levels were either improved with HBOC-201 or equivalent to standard fluids. [32] It is apparent that in these four preclinical HS studies, overall BP responses were higher with HBOC-201 than with standard fluids, but severe BP responses did not occur, moderately severe responses were uncommon (1.1% post-first and 7.7% post-second infusion), and higher BP responses did not adversely affect clinical outcome.

Table 37: Clinical parameters in NMRC swine studies - group means vs. outliers

Moderate controlled HS [20]	Group	MAP Δ subgroup	Trough TCOM, mm Hg	Peak LA, mmol/L	Peak BE, mmol/L	*Peak Troponin-I, U/L	LV myonecrosis, severity score	LV fibroplasia, severity score	Fluid requirements, ml/kg	Survival time, hours
HBOC-201			2.19±0.5	4.44±0.6	6.67±1.7	1.44±0.1	0.25±0.3	0.0±0.0	18.76±1.8	72±0.0
HEX			0.81±0.4	6.34±1.1	4.58±1.1	5.69±4.9	1.6±0.6	1.4±0.7	30.58±1.1	63.6±8.4
Pig 781	HBOC	40-49	2.5	4.1	17*	0.2	0	0	15.0	72
Pig 949	HBOC	40-49	1.75	8.1*	3	1.4	0	0	25.0	72
Pig 792	HBOC	60-69	2	3.1	5.9	2	0	0	20.0	72
Pig 747	HBOC	60-69	0.75	3.8	6.3	2.3	0	0	25.0	72
Pig 785	HEX	50-59	0	7.9	2.2	12.3	2	0	35.6	5*
Pig 937	HEX	50-59	3.75*	5.1	5	12.1	4	4	30.0	72
Pig 1023	HEX	60-69	1	2.5	6.9	2.7	1	0	30.0	72
Severe Controlled HS [27]	Group	MAP Δ subgroup	Trough TCOM, mm Hg	Peak LA, mmol/L	Peak BE, mmol/L	*Peak Troponin-I, U/L	LV myonecrosis, severity score	LV fibroplasia, severity score	Fluid requirements, ml/kg	Survival time, hours
HBOC-201			3.47±1.6	6.08±0.4	4.16±0.5	Not done	0.1±0.1	0.1±0.1	27.5±1.9	72±0.0
HEX			1.13±0.6	6.64±0.5	3.28±2.4	Not done	0.1±0.1	0.1±0.1	28.6±0.8	54.9±11.2
Pig 186	HBOC	50-59	0.5	7.1	4.4	Not done	0	0	25.0	72
Pig 216	HBOC	60-69	0.5	5.4	4.3	Not done	0	0	30.0	72
Pig 141	HEX	40-49	0.25	8.2	-3.8	Not done	0	0	30.0	72
Pig 184	HEX	40-49	0.5	6.6	0	Not done	0	0	30.0	72
Severe Uncontrolled HS [17]	Group	MAP Δ subgroup	Trough TCOM, mm Hg	Peak LA, mmol/L	Peak BE, mmol/L	*Peak Troponin-I, U/L	LV myonecrosis, severity score	LV fibroplasia, severity score	Fluid requirements, ml/kg	Survival time, hours
HBOC-201			0.75±0.3	3.43±0.4	5.94±2.1	2.64±0.2	0.3±0.3	0.3±0.3	24.9±2.1	63.2±8.8
HEX			2.84±2.4	5.30±1.3	2.81±1.5	4.39±0.6	0.9±0.4	0.0±0.0	21.4±2.8	11.0±8.8
Pig 81	HBOC	40-49	2.75*	4.1	1.9	2.4	0	2*	25.0	72
Pig 943	HBOC	50-59	0	3.2	1.1	3.4	2*	0	30.0	72
Pig 814	HBOC	60-69	0.75	3	3.2	2.3	0	0	15.1	72
Severe Uncontrolled HS/TBI^ [18]	Group	MAP Δ subgroup	Trough TCOM, mm Hg	Peak LA, mmol/L	Peak BE, mmol/L	*Peak Troponin-I, U/L	LV myonecrosis, severity score	LV fibroplasia, severity score	Fluid requirements, ml/kg	Survival time, hours
HBOC-201			0.40±0.1	2.40±0.3	4.77±0.9	Not done	Not done	Not done	10.0±0.0 (30 min delay) 35.9±8.7 (75 min delay)	4.3±0.5
LR			0.34±0.1	4.73±0.6	5.33±0.4	Not done	Not done	Not done	20.0±0.0 (30 min delay) 77.1±7.3 (75 min delay)	2.6±0.5
Pig 908304	HBOC	40-49	1.49	2.2	5.8	Not done	Not done	Not done	10	2.5
Pig 1011305	HBOC	40-49	0.06	1.6	8.4	Not done	Not done	Not done	40	5.5
Pig 1033305	HBOC	50-59	0.48	1.3	8.3	Not done	Not done	Not done	20	6
Pig 1028005	HBOC	60-69	1.84	1.6	5.2	Not done	Not done	Not done	10	6

OBRR has contended that anesthesia may have confounded ability to detect adverse hypertensive responses in these animal studies, but in the OBRR-requested regional blood flow study [69] in

which swine were sedated (not anesthetized), adverse hypertensive responses were also not seen. Peak MAP ≥ 130 mm Hg was not reached by any pigs treated with HBOC-201 or human serum albumin (HSA); peak MAP 120-129 mm Hg was reached by 2/8 HBOC-201 vs. 0/8 HSA pigs; and peak MAP 110-119 mm Hg by 3/8 HBOC-201 vs. 3/8 HSA pigs (baseline mean MAP 104 ± 7.5 mm Hg for HBOC-201 and 98.1 ± 2.5 mm Hg for HSA). Similarly, in the OBRR-requested volume expansion study in which HBOC-201 and albumin were compared in sedated (not anesthetized) swine with minimal (10% EBV) and mild (30% EBV) controlled HS [56], although mild to moderate vasoactivity resulted in higher systemic and pulmonary BP, adverse hypertensive responses were not seen either. For example, with 10% hemorrhage (minimal), peak MAP ≥ 130 mm Hg was reached by 1/6 HBOC-201 (i.e., MAP 130 mm Hg) vs. 0/8 HSA pigs; and peak MAP 120-129 mm Hg was reached by 1/6 HBOC-201 vs. 0/6 HSA pigs. With 30% hemorrhage (mild), peak MAP ≥ 130 mm Hg was reached by 3/6 HBOC-201 (i.e., MAP 130, 135, and 168 mm Hg) vs. 0/6 HSA pigs; and peak MAP 120-129 mm Hg was reached by 1/6 HBOC-201 vs. 1/6 HSA pigs (survival was 100% [6/6] for HBOC-201 vs. 67% [4/6] for HSA).

Thus, in these minimally to mildly hemorrhaged sedated swine (less than expected in *RESUS*), only 1/16 (6.3%) HBOC-201 pigs had an outlier BP response (outcome was good in this pig). Second, the preponderance of subjects to be enrolled in *RESUS* will also be anesthetized and/or sedated/paralyzed during operative interventions and/or ICU ventilator care, making comparisons with anesthetized animal studies relevant. In summary, there are no objective preclinical data to suggest that in similar hypotensive subjects in *RESUS*, higher rates of roller coaster BP fluctuations and severe BP responses will occur due to HBOC-201's mild to moderate vasoactivity. These preclinical BP response and outcome data support the *RESUS Dosing Guidelines* (Appendix C).

HBOC-201 BP responses in prior clinical trials

Clinical data from HEM-0115 and other clinical trials demonstrated mild hypertensive effects in a significant number of the subjects treated with HBOC-201, but hypertensive SAEs were not common. Fifteen percent of 797 HBOC-201 vs. 7% of 661 control mainly hemodynamically stable subjects had hypertension AEs ($p < 0.0001$), but only 2 of the 797 HBOC 201 subjects (0.3% [3 in 1,000]) (vs. 0 of 661 control subjects, $p = 0.5$) had hypertensive responses classified as SAEs. Including 2 SAEs that occurred in Biopure's European PCI (percutaneous coronary intervention) trial (COR-0001), a total of 4 hypertension SAEs have occurred out of a total of 842 subjects (0.48% [< 5 in 1,000]).

Analysis of SBP responses and AE/SAE rates in HEM-0115 (Table 38**,** Table 39**,** Table 40**,** Table 41**, and** Table 42**)**

Overall population

After the first CTM infusion, mean *peak SBP responses* were ~ 17 mm Hg higher with HBOC-201 (143 ± 23 mm Hg) than RBC (126 ± 22 mm Hg) ($p < 0.0001$) in the overall population (mean age 60.3 years old). Subsequent responses were less, such that overall *peak SBP responses* (for all infusions) were ~ 9 mm Hg higher with HBOC-201 (160 ± 21 vs. 151 ± 19 mm Hg, respectively) ($p < 0.0001$). In contrast, *peak SBP responses* to RBC were cumulative. This resulted in a higher rate of *peak SBP responses* classified as ≥ mild (above normal) with HBOC-201 (179/319 [56%]) than RBC (70/246 [28%]) ($p < 0.0001$).

Most HBOC-201 *peak SBP responses* were < moderate (299/319 [94%]); *severe responses* were uncommon. For example, SBP > 200 mm Hg *peak SBP response* rates were 2/319 (0.6%) in HBOC-201 (only in subjects with pre-infusion SBP > 90 mm Hg) vs. 0/246 (0%) in RBC subjects ($p = 0.5$). *Moderately severe peak SBP responses* (SBP 181-200 mmHg) were more common, but also infrequent (18/319 [5.6%] vs. 3/246 [1.2%], respectively ($p = 0.006$).

SBP Δ response rates were also higher with HBOC-201 (70/319 [22%]) than RBC (7/246 [3%]) ($p < 0.0001$). Among HBOC-201 subjects, most (287/319 [90%]) were < mild; severe *SBP Δ response* rates were low (15/319 [5%]) but higher than with RBC (0/246 [0%]) ($p = 0.0002$). More detailed stratification of HBOC-201 subjects based on pre-infusion SBP, showed that SBP Δ > 60 mmHg (severe) was seen in 4/25 (16%) hypotensive subjects (≤ 90 mm Hg), 9/153 (6%) subjects with pre-infusion SBP 91-120 mm Hg, 2/119 (2%) subjects with pre-infusion SBP 121-150 mmHg, and 0/22 (0%) subjects with pre-infusion SBP > 150 mm Hg ($p = 0.01$). Thus, 13/15 (87%) of severe *SBP Δ responses* occurred in subjects with lower SBP (≤ 120 mm Hg) vs. 2/15 (13%) in subjects with higher SBP (> 120 mm Hg) ($p = 0.001$).

Stable trauma sub-population

Peak SBP response rates were not significantly different in HBOC-201 and RBC subjects in the younger stable trauma sub-population (10/27 [37%] vs. 3/17 [18%], respectively, $p = 0.2$) (mean age 39.8 years old). But among HBOC-201 stable trauma subjects, rates trended to be lower than in the overall population. For example, among HBOC-201 subjects, all *peak SBP responses* classified as ≥ mild, occurred in 10/27 (37%) trauma subjects vs. 179/319 (56%) of the overall

population (p = 0.07). 89% of *peak SBP responses* were < mild and all were < moderate. *Severe* and *moderately severe peak SBP responses* were not seen at all, neither among pre-infusion hypotensive nor non-hypotensive subjects. All hypotensive stable trauma subjects in both treatment groups (n = 2 each) had normal *peak SBP responses*.

SBP Δ responses (≥ mild) were equivalent in HBOC-201 (0/27 [0%]) and RBC (0/17 [0%]) subjects with trauma (p > 0.05). Among HBOC-201 subjects, the *SBP Δ response* rate was lower in subjects with stable trauma than in the overall population (70/319 [22%], p = 0.002).

Hypotensive sub-population

Peak SBP response rates trended to be higher among hypotensive HBOC-201 (7/25 [28%]) than RBC (1/20 [5%]) subjects (p = 0.06); but akin to the stable trauma subjects, rates in hypotensive HBOC-201 subjects were lower than in the overall population (179/319 [56%]) (p = 0.01). All *peak SBP responses* were < mild (none severe). Predictably, among HBOC-201 subjects, *peak SBP responses* were lower in subjects with pre-infusion hypotension (SBP < 90 mm Hg) than without hypotension (7/25 [28%] vs. 172/294 [59%], respectively (p < 0.004).

HBOC-201 subjects with pre-infusion hypotension had lower *peak SBP responses* than those without hypotension (124 + 16 vs. 160 + 21, respectively, p < 0.01); among non-hypotensive subjects, those with higher pre-infusion SBP had higher mean *responses*. More detailed stratification showed that responses classified as normal (< 140 mm Hg) occurred in 18/25 (72%) of hypotensive subjects, 84/153 (55%) of subjects with SBP 91-120 mm Hg, 34/119 (29%) of subjects with SBP 121-150 mm Hg, and 4/22 (18%) of subjects with SBP > 150 mm Hg (p < 0.02 [Fisher's Exact test, p = 0.003 [trend test]).

There was a trend to higher *SBP Δ responses* in hypotensive HBOC-201 (9/25 [36%]) vs. hypotensive RBC (2/20 [10%]) subjects (p = 0.08); the incidence in hypotensive HBOC-201 subjects was similar to that in HBOC-201 subjects in the overall population (70/319 [22%]) (p = 0.14).

< 70 year old sub-population

In < 70 year olds, *peak SBP response* rates were higher with HBOC-201 (108/216 [50%]) than RBC (38/158 [24%]) (p < 0.0001). Because this sub-population included most of the overall

population, HBOC-201 *peak SBP response* rates were similar to those in the overall population (179/319 [56%]) (p = 0.19); 79% were ≤ mild (none severe).

SBP Δ response rates were also higher with HBOC-201 (38/216 [18%]) than RBC (3/158 [2%]) (p < 0.0001). HBOC-201 *SBP Δ response* rates were similar to those in the overall population 70/319 [22%]) (p=0.2); 91% were classified as ≤ mild (4% severe). 6/24 (30%) HBOC-201 vs. 0/13 (0%) RBC subjects with hypotension had *SBP Δ responses* (p = 0.07).

< 50 year old sub-population

Peak SBP response rates were also higher with HBOC-201 (25/69 [36%]) than RBC (4/40 [10%]) in < 50 years old subjects (p = 0.003). In contrast with < 70 year olds, HBOC-201 *peak SBP response* rates were significantly lower than in the overall population (179/319 [56%]) (p = 0.003); 93% were < mild, and all were < moderate (none severe). HBOC-201 *peak SBP response* rates were similar among hypotensive < 50 year old (0/6 [0%]) and hypotensive subjects in the overall population (7/25 [28%]) (p = 0.3).

SBP Δ responses were insignificantly different in < 50 year old HBOC-201 (5/69 [7%]) and RBC (0/40 [0%]) subjects (p = 0.2). Among HBOC-201 subjects, 97% were ≤ mild (1.5% severe); these rates were lower than in the overall population (70/319 [22%]) (p = 0.004). None of 6 HBOC-201 and 5 RBC hypotensive < 50 years olds had *SBP Δ responses* (≥ mild).

Subjects with rapid infusions

In the 17 HBOC-201 and 14 RBC subjects with rapid infusion (≥ 25 ml/min) first infusion, mean post-infusion peak SBP was 163 ± 5.4 vs. 151±6.8 mm Hg, respectively (p = 0.2). In the 30 HBOC-201 and 17 RBC subjects with any rapid infusion, SBP was 166 ± 4.3 vs. 157 ± 6.7, respectively (p = 0.3).

AEs/SAEs

Overall population

Non-serious AEs were common in the evaluated systems (range 12-44% in HBOC-201 subjects), and were significantly more common with HBOC-201 than RBC (especially cardiovascular) in all SOC categories (except trend for renal/urinary). HTN AEs were also more common with HBOC-201 (43/350 [12%]) than RBC (18/338 [5%]) (p = 0.002). HBOC-201 subjects with lower

(< 40 mm Hg) vs. higher (≥ 40 mm Hg) *SBP Δ responses* had similar all cardiac (69/249 [28%] vs. 23/70 [33%], p>0.05) and ischemic AE rates (31/249 [12%] vs. 10/70 [14%], p > 0.05), respectively.

SAEs were uncommon in the evaluated systems (range 1-6%) and similar in the two treatment groups in all SOC categories, except cardiac SAE rates were higher with HBOC-201 (22/350 [6%]) than RBC (9/338 [3%]) (p = 0.03). HTN SAEs were rare and equivalent in both groups (2/350 [0.6%] vs. 0/338 [0%], respectively (p = 0.5).

Stable trauma sub-population

AEs were common in the evaluated systems (range 12-47% in HBOC-201 subjects). There were no significant group differences in AE rates in any SOC category; there was a trend to a higher vascular AE rate in HBOC-201 subjects (p = 0.07). In contrast to the overall population, the incidence of cardiac AEs in the trauma sub-population was similar in HBOC-201 and RBC subjects (all cardiac AEs: 5/34 [15%] vs. 5/28 [18%], respectively, p = 0.7; ischemic cardiac AEs only: 4/34 [12%] and 5/28 [18%], p = 0.7). Cardiac AE rates were not significantly different in HBOC-201 subjects in the trauma sub-population (5/34 [15%]) and the overall population (101/350 [29%]) (p = 0.1), but renal AE rates trended to be lower in trauma (4/34 [12%]) vs. overall population subjects (87/350 [25%]) (p = 0.09).

SAEs were generally uncommon in the evaluated systems (range 0-12% in HBOC-201 subjects), insignificantly different in the two treatment groups, and generally similar in HBOC-201 trauma and overall population subjects (except respiratory rates were higher [p = 0.05] and vascular rates trended to be higher [p = 0.06] in trauma subjects).

Hypotensive sub-population

AEs were common in the evaluated systems (range 3-48% in HBOC-201 subjects), but significant group differences were not detected in subjects with pre-infusion hypotension (although they were presumably a sicker sub-population). None of the evaluated AE rates were significantly different than for the overall population. HTN AE rates were similar in sub-populations of subjects with hypotension (1/29 [3%]) vs. trauma (4/34 [12%]) (p = 0.4), and vs. the overall population (38/350 [11%]) (p = 0.4).

SAEs were generally uncommon in hypotensive subjects in the evaluated systems (range 0-7% in HBOC-201 subjects) and insignificantly different between groups. There were no HTN SAEs in subjects with pre-infusion hypotension. There were no differences between SAE rates in hypotensive vs. overall population subjects.

< 70 year old sub-population

AEs were common in < 70 year olds in the evaluated systems (range 13-46% in HBOC-201 subjects), but only vascular group differences were significant—due to more frequent hypertension in HBOC-201 (29/239 [12%]) than RBC (13/227 [6%]) subjects (p = 0.02). There was a trend to more frequent nervous system AEs in HBOC-201 subjects (110/239 [46%] vs. 86/227 [38%], p = 0.09), but cerebral ischemic (CVA/RIND/TIA) AE rates were similar in the 2 treatment groups (2/239 [0.8%] vs. 2/227 [0.9%], respectively, p > 0.05). Among HBOC-201 subjects, cerebral ischemic AE rates were lower in < 70 year olds (2/239 [0.8%]) than in the overall population (7/350 [2%]).

SAEs were rare in < 70 year olds in the evaluated systems (range 1-4% in HBOC-201 subjects) and insignificantly different between groups.

< 50 year old sub-population

AEs were common in < 50 year olds in the evaluated systems (range 8-48% in HBOC-201 subjects), and were similar in frequency in the two treatment groups. But among HBOC-201 subjects, respiratory AEs were less common in < 50 year olds (13/84 [15%]) vs. the overall population (97/350 [28%]) (p = 0.02); a similar trend was apparent for renal/urinary SAEs (13/84 [15%] vs. 87/350 [25%], respectively (p = 0.08), and possibly for cardiovascular SAEs. Cerebral ischemic AEs were similar in the two treatment groups (0/85 [0%] vs. 1/65 [1.5%], respectively, p > 0.05); but among HBOC-201 subjects, cerebral ischemic AE rates were lower < 50 year olds than in the overall population (0/85 [0%] vs. 7/350 [2%]).

SAEs were rare in the evaluate systems (range 0-5% in HBOC-201 subjects) and similar in both treatment groups in all categories. Among HBOC-201 subjects, cardiac SAEs appeared less common (trend) in < 50 year olds than the overall population: 1/84 (1%) vs. 22/350 (6%) (p = 0.06).

Subjects with rapid infusions

AEs were not uncommon in subjects with rapid infusion rates in the evaluated systems (range 6-57% in HBOC-201 subjects), but were not significantly different between groups. AE rates in HBOC-201 subjects (including cardiac AEs) were similar to the overall population. In contrast, AEs tended appeared more common in RBC subjects in the high infusion rate sub-population than the overall population (e.g., cardiac AEs: 6/17 [35%] vs. 57/338 [17%] ($p = 0.09$).

Most evaluated systems had low SAE rates without significant group differences, but cardiac SAEs appeared more frequent in both HBOC-201 and RBC subjects in high infusion rate sub-populations (up to 18% in both groups) than the overall population (HBOC-201 6% vs. RBC 2%); a similar trend was apparent for respiratory SAEs.

Conclusions of HEM-0115 sub-populations analysis

The key findings of our analysis of SBP responses and potentially "vasoactivity-related" AEs in HEM-0115 are that in comparison with RBC transfusions, HBOC-201 infusions were associated with increased rates of frequent mild to moderate BP elevations and AEs and infrequent SAEs. However, these rates were generally lower and sometimes equivalent in the two treatment groups in sub-populations of subjects more closely resembling those expected to be enrolled in *RESUS*. This analysis suggests that in a relatively older population undergoing orthopedic surgery, overall clinical outcome is better with RBC than HBOC-201, but only minimally so, and where safe and expeditious transfusions are available (i.e., in-hospital setting in developed countries).

Specifically, mild to moderate *SBP responses* were common in HBOC-201 subjects and more frequent than with RBC, but severe *SBP responses* were rare in both groups (< 1%). *SBP responses* were lower in HBOC-201 subjects with pre-infusion hypotension, lower pre-infusion SBP even among non-hypotensive subjects, and in the younger stable trauma and < 50 year old sub-populations. Although the trends were the same as in the overall population, group differences in *SBP responses* were narrowed in these sub-populations.

SBP Δ responses were also more common with HBOC-201 than RBC in the overall population and in < 70 year olds, but not in trauma and < 50 year old subjects, in whom rates were lower than in the overall population. Clearly, HBOC-201 subjects with lower age (stable trauma and < 50 year olds) had lower rates than those with higher age (overall population, < 70 year olds, and hypotensive subjects). As most severe responses (87%) occurred in subjects with low pre-

infusion SBP, these should not be construed as adverse but *higher* and physiologically appropriate. Interestingly, no subjects with hypotension and younger age (stable trauma and < 50 year old sub-populations) had significant *SBP Δ responses* (although Ns were small). As HBOC-201 subjects with lower vs. higher responses had similar cardiac AE rates, *SBP Δ responses* did not predict outcome.

In the overall population, AEs were more common with HBOC-201 than RBC in key cardiac, vascular, nervous, respiratory, and renal/urinary SOCs (as well as HTN). SAEs were more common with HBOC-201 than RBC, but only in the cardiac SOC analysis.

In contrast, there were no significant group differences in AE or SAE rates in any SOC in sub-populations of stable trauma, hypotensive, and < 50 year old subjects. Overall, AEs were clearly less common in these sub-populations than in the overall population. For example, rates of respiratory and renal/urinary AEs, cerebral ischemic AEs, and cardiac SAEs were significantly less in < 50 year olds than the overall population; HTN AEs also appeared less common in the hypotensive sub-population.

In summary, our analysis of safety data in HEM-0115 trial, comparing HBOC-201 and RBC in subjects undergoing orthopedic surgery, shows that *SBP response* rates, all potentially "vasoactivity-related" system AE rates, and cardiac system SAE (and troponin elevation) rates were higher with HBOC-201 than RBC in the overall population. Thus, HBOC-201 has potentially adverse vasoactive properties. But, individual system SAEs were uncommon (≤ 6%) and severe *SBP responses* were rare (< 1%); as noted above, troponin elevation was unrelated to ACS or mortality. Moreover, in sub-populations of HBOC-201 subjects with stable trauma, hypotension, and younger age, rates were often lower than in HBOC-201 subjects in the overall population; as well, group differences were usually narrowed and uniformly insignificant. These data suggest that outcome is better with well-screened and expeditiously available RBC than HBOC-201 in stable orthopedic surgery patients, but not dramatically so. The data suggest that when safe and rapidly available blood transfusions are not an option, HBOC-201 may be a *reasonable* alternative. Our finding of an improved safety profile in sub-populations of subjects more closely resembling younger subjects who would be enrolled in *RESUS*, strongly supports a hypothesis that the *relative* safety of HBOC-201 will be improved in *RESUS*.

Table 38*: Peak SBP responses* in overall population in HEM-0115 - first infusion

Treatment Group	Pre-infusion SBP* mm Hg	Sample Size* N	Post-infusion Groups by SBP: Incidence (%) ≤ 140	141-160	161-180	181-200	> 200
HBOC-201	≤ 90	25	18 (72)	7 (28)	0 (0)	0 (0)	0 (0)
	91-120	153	84 (55)	50 (33)	15 (10)	4 (3)	0 (0)
	121-150	119	34 (29)	37 (31)	39 (33)	8 (7)	1 (1)
	> 150	22	4 (18)	3 (14)	8 (37)	6 (27)	1(5)
	Overall	319	140 (44)	97 (30)	62 (19)	18(6)	2(1)
RBC	≤ 90	20	19(95)	1(5)	0 (0)	0 (0)	0 (0)
	91-120	102	93(1)	8 (8)	1 (1)	0 (0)	0 (0)
	121-150	101	60 (59)	34 (34)	6 (6)	1 (1)	0 (0)
	> 150	23	4 (17)	12 (52)	5 (22)	2 (9)	0 (0)
	Overall	246	177 (72)	55 (22)	12 (5)	3 (1)	0 (0)

* Sample size was less than the total number of patients because analysis was possible only for patients with both pre- and post-infusion SBP.

Table 39*: SBP Δ responses* in overall population in HEM-0115 - first infusion

Treatment Group	Pre-infusion SBP mm Hg	Sample Size* N	SBP Δ: Incidence (%) ≤ 40	41-50	51-60	> 60
HBOC-201	≤ 90	25	16 (64)	2 (8)	3 (12)	4 (16)
	91-120	153	110 (72)	24 (16)	10 (7)	9 (6)
	121-150	119	102 (86)	12 (10)	3 (3)	2 (2)
	> 150	22	21 (95)	1 (5)	0 (0)	0 (0)
	Overall	319	249 (78)	39 (12)	16 (5)	15 (5)
RBC	≤ 90	20	18 (90)	1 (5)	1 (5)	0 (0)
	91-120	102	99 (97)	2 (2)	1 (1)	0 (0)
	121-150	101	99 (98)	1 (1)	1 (1)	0 (0)
	> 150	23	23 (100)	0 (0)	0 (0)	0 (0)
	Overall	246	239 (97)	4 (2)	3 (1)	0 (0)

* Sample size was less than the total number of patients because analysis was possible only for patients with both pre- and post-infusion SBP.

Table 40*: Peak SBP responses* in stable trauma sub-population in HEM-0115 - first infusion

Treatment Group	Pre-infusion SBP* mm Hg	Sample Size* N	Post-Infusion Groups by SBP: Incidence (%) ≤ 140	141-160	161-180	181-200	> 200
HBOC-201	≤ 90	2	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)
	91-120	10	9 (90)	1 (10)	0 (0)	0 (0)	0 (0)
	121-150	9	4 (44)	4 (44)	1 (11)	0 (0)	0 (0)
	> 150	6	2 (33)	2 (33)	2 (33)	0 (0)	0 (0)
	Overall	**27**	17 (63)	7 (26)	3 (11)	0 (0)	0 (0)
RBC	≤ 90	2	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)
	91-120	10	9/0.90	1 (10)	0 (0)	0 (0)	0 (0)
	121-150	3	3/1.00	0 (0)	0 (0)	0 (0)	0 (0)
	> 150	2	0 (0)	2 (100)	0 (0)	0 (0)	0 (0)
	Overall	**17**	14 (82)	3 (18)	0 (0)	0 (0)	0 (0)

* There were a total of 62 trauma subjects, however, pre and post infusion SBP data were available for only 44 of the 62 patients.

Table 41*: SBP Δ responses* in stable trauma sub-population in HEM-0115 - first infusion

Treatment Group	Pre-infusion SBP mm Hg	Sample Size* N	SBP Δ: Incidence (%) ≤ 40	41-50	51-60	> 60
HBOC-201	≤ 90	2	2/1.00	0	0	0
	91-120	10	10/1.00	0	0	0
	121-150	9	9/1.00	0	0	0
	> 150	6	6/1.00	0	0	0
	Overall	**27**	**27/1.00**	**0**	**0**	**0**
RBC	≤ 90	2	2/1.00	0	0	0
	91-120	10	10/1.00	0	0	0
	121-150	3	3/1.00	0	0	0
	> 150	2	2/1.00	0	0	0
	Overall	**17**	**17/1.00**	**0**	**0**	**0**

* There were a total of 62 trauma subjects, however, pre and post infusion SBP data were available for only 44 of the 62 patients.

Table 42: AEs and SAEs in potentially "vasoactivity-related" systems (SOC) in HEM-0115

	CARDIAC		VASCULAR		NERVOUS		RESPIRATORY		RENAL/URINARY		HYPERTENSION	
	HBOC	RBC	HBOC	RBC	HBOC	RBC	HBOC	RBC	HBOC	RBC	HBOC	RBC
AEs												
Overall	102/350 (29%)	58/338 (17%)	101/350 (29%)	51/338 (15%)	153/350 (44%)	122/338 (36%)	97/350 (28%)	70/338 (21%)	87/350 (25%)	64/338 (19%)	43/350 (12%)	18/338 (5%)
Stable trauma	5/34 (15%)	5/28 (18%)	11/34 (32%)	3/28 (11%)	16/34 (47%)	14/28 (50%)	13/34 (38%)	9/28 (32%)	4/34 (12%)	6/28 (21%)	4/34 (12%)	3/28 (11%)
Hypotensive (< 90 mm Hg)	11/29 (38%)	5/26 (19%)	6/29 (21%)	4/26 (15%)	14/29 (48%)	7/26 (27%)	8/29 (28%)	9/35 (26%)	10/29 (34%)	4/26 (15%)	1/29 (3%)	1/26 (4%)
< 70 y/o	60/239 (25%)	33/227 (15%)	63/239 (26%)	35/227 (15%)	110/239 (46%)	86/227 (38%)	55/239 (23%)	41/227 (18%)	50/239 (21%)	42/227 (19%)	30/239 (13%)	13/227 (6%)
< 50 y/o	18/84 (21%)	10/65 (15%)	17/84 (20%)	6/65 (9%)	40/84 (48%)	23/65 (35%)	13/84 (15%)	14/65 (22%)	13/84 (15%)	12/65 (18%)	7/84 (8%)	3/65 (5%)
SAEs												
Overall	22/350 (6%)	9/338 (3%)	14/350 (4%)	11/338 (3%)	5/350 (1%)	2/338 (1%)	7/350 (2%)	3/338 (1%)	6/350 (2%)	4/338 (1%)	2/350 (1%)	0/338 (0%)
Stable trauma	1/34 (3%)	1/28 (4%)	4/34 (12%)	0/28 (0%)	0/34 (0%)	0/28 (0%)	3/34 (9%)	0/28 (0%)	1/34 (3%)	0/28 (0%)	1/34 (3%)	0/28 (0%)
Hypotensive (< 90 mm Hg)	2/29 (7%)	0/26 (0%)	2/29 (7%)	0/26 (0%)	0/29 (0%)	0/26 (0%)	0/29 (0%)	1/26 (4%)	1/29 (3%)	1/26 (4%)	0/29 (0%)	0/26 (0%)
< 70 y/o	8/239 (3%)	4/227 (2%)	10/239 (4%)	7/227 (3%)	2/239 (1%)	2/227 (1%)	5/239 (2%)	3/227 (1%)	5/239 (2%)	2/227 (1%)	2/239 (1%)	0/239 (0%)
< 50 y/o	1/84 (1%)	0/65 (0%)	4/84 (5%)	2/65 (3%)	0/84 (0%)	1/65 (2%)	2/84 (2%)	1/65 (2%)	3/84 (4%)	1/65 (2%)	1/84 (1%)	0/65 (0%)

Hypertension SAEs in Biopure's COR-0001 trial

In Biopure's COR-0001 trial, two of 29 HBOC-201-treated subjects had hypertension SAEs. Thus, NMRC appreciates OBRR's concern about risk of rare but real idiosyncratic *unpredictable BP responses* with HBOC-201; but NMRC believes the context of the cited hypertensive SAEs should be taken into account in order to logically extrapolate their significance vis-à-vis risk in the *RESUS* trial. NMRC agrees with OBRR that the SAEs occurred in *hemodynamically stable subjects*, analogous to those in *top load* experimental protocols, but this clinical context is the essential fact that makes direct extrapolation to *RESUS* scientifically questionable. NMRC reviewed the two COR-0001 trial SAEs in detail in order to better understand their significance in terms of prediction of risk in *RESUS*. A report summarizing these SAEs, conclusions by the PI and DSMB, and NMRC's conclusions and extrapolation to *RESUS* risk, were submitted to the *RESUS Advisory Board*. The *Board* unanimously concurred with NMRC's conclusions.

Regarding subject 4002, NMRC and the *Board* agreed that the hypertensive SAE was related to HBOC-201 infusion, but appreciated that the AE was categorized as serious due to prolongation of intensive care rather than harm to the subject. Regarding subject 4007, NMRC and the *Board* appreciated the seriousness of the subject's hypertensive SAE, which was so classified by the PI on the bases of prolongation of hospitalization and being life threatening. NMRC and the *Board* noted that hypertension (probably HBOC-201-related) was under control and that the electrical mechanical dissociation (EMD) SAE was temporally related to catheter balloon inflation and contrast infusion. The subsequent occurrence of MI and watershed CVA were consistent with hypotension/hypoxemia secondary to the cardiac arrest. It was noted that the subject's clinical condition improved significantly upon follow-up. Although HBOC-201-causality cannot be eliminated entirely, NMRC and the *Board* believe that it was unlikely that the HBOC-201-related hypertension was causally related to the EMD arrest, as the only biologically plausible connection for the hypertensive reaction and EMD would be a massive MI causing LV failure causing EMD—a clinical scenario that did not occur. Thus, NMRC and the *Board* agree with the Neurology and Cardiology consultants who thought that the EMD arrest and secondary complications of MI and CVA were unlikely to be related to HBOC-201 treatment.

In summary, in evaluating these hypertension SAEs for prediction of risk in *RESUS*, NMRC notes that outcome was good in the first example and relatedness unlikely in the second. It is theoretically possible that similar serious adverse hypertensive responses will occur in the severe hypovolemic, hypotensive, and younger patient population to be enrolled in *RESUS*; but it is clinically logical to

hypothesize that this risk will be mitigated by the fact that enrollment in *RESUS* requires severe hypotension, and therefore the risk will be low. Supporting this hypothesis, analysis of data from HEM-0115 revealed that SBP responses were indeed lower in subjects with pre-infusion hypotension (detailed above). That severe BP responses were not reported in animal models of anesthetized or sedated swine, an animal species with significant vasoactive response characteristics, also suggests that this risk is low (detailed above). That serious hypertension events occurred in only four of 842 relatively stable subjects dosed with HBOC-201 in prior trials, suggests a low *a priori* risk in any case. NMRC believes that mitigation strategies incorporated in the *RESUS* protocol further mitigate this risk (especially exclusion of the elderly who may be more sensitive to vasoactivity), such that risk is *reasonable in relation to what is known about this class of subjects.*

BP responses of other HBOCs

OBRR has asserted that HBOC-201's vasoactive properties are similar to that of DCLHb, in contradistinction with Northfield Laboratory's purportedly less vasoactive PolyHeme®. Thus, a review of key DCLHb and the ongoing PolyHeme® trauma trials is indicated. OBRR suggested that the negative results of the in-hospital DCLHb trial provide a glimpse into likely outcome in *RESUS*, especially in reference to vasoactivity and hypertensive responses. However, as noted above, DCLHb is 100% tetrameric (vs. < 2-3% for HBOC-201) and likely significantly more vasoactive than HBOC-201.

In the in-hospital DCLHb Phase 3 trial [15], efficacy and safety were assessed in conjunction with blood transfusions in the hospital—a setting where the relative consequences of adverse reactions would be high in comparison with incremental or minimal improvement in blood oxygen content provided by the HBOC. This contrasts with the *RESUS* trial, in which the comparator LR is asanguinous, and thus the consequences of adverse reactions are likely to be low in comparison with the significant improvement in blood oxygen content provided by the HBOC. Nevertheless, Sloan reported, *neither uncontrolled bleeding nor higher BPs were systematically demonstrated in patients who received DCLHb*. *Similarly, neither accelerated hemorrhage nor uncontrolled hypertension were demonstrated in perioperative patients treated with DCLHb or in those treated in the prehospital traumatic hemorrhagic shock clinical trial conducted in Europe*.

In the parallel European *HOST* prehospital trial [41] in which infusion of DCLHb was compared with standard fluids, 28-day mortality was not different (41% [22/52] vs. 35% [22/58], p = 0.4). The study was terminated early after enrollment of only 121 subjects, and the primary efficacy endpoint, organ

failure, was not different between the two treatment groups. SBP was statistically higher in the DCLHb than control group at 60 minutes (median 110 vs. 100 mm Hg, $p = 0.02$), but probably not clinically significantly different. Kerner did not report *peak SBP responses* but P_{25-75} were 100-138 and 76-109 mm Hg, respectively. Cumulative blood product infusion volumes were lower in the DCLHb than control group at 24 hours (median 1,595 vs. 3,716 ml, $p = 0.007$) and 7 days (3,139 vs. 4,746 ml, $p = 0.06$) (and a trend at 14 days [3,300 vs. 4,746 ml, $p = 0.098$]. There was an insignificant trend to a higher AE rate in the DCLHb than control group (90 vs. 70%). Kerner reported that except for a higher incidence of elevated lipase (55 vs. 14%, $p < 0.001$), adverse effects on hepatic, renal, hematology, ABG, chemistry, and urinalysis laboratory tests were not seen. The median time (27 trauma centers) from injury until DCLHb infusion was 41 minutes (P_{25-75} 30-65 minutes). The authors stated, *the European study was terminated because data indicated no evidence of efficacy…termination…however, may well have been affected by the unfavorable results of the U.S. trauma study with DCLHb, which we did not observe*. They concluded, the study...*demonstrates that early administration of DCLHb has the potential to reduce the utilization of blood products without increasing morbidity or mortality*.

The authors speculated about *possible explanations for the lack of effect of DCLHb…* in the prehospital *HOST* trial. First, they wondered if a single intervention of briefly maintaining oxygen content was sufficiently robust to affect organ failure and mortality, especially potentially with an inadequate dose. Second, they wondered if direct pharmacologic effects of DCLHb, NO binding related vasoactivity, may be related. Third, they noted that the trauma bimodal distribution of survival *had not been adequately addressed in the design of the study*.

In comparing the *HOST* trial with *RESUS*, a few points are noteworthy. First, despite the study design's shortcomings, early termination, and known potent vasoactivity of this first generation 100% tetrameric HBOC, in this sole published prehospital trial evaluating an HBOC for prehospital resuscitation of HS casualties, mortality and organ failure rates were similar, exposure to blood transfusions was decreased, and overall safety was similar, in a comparison of DCLHb and standard care. These data suggest that resuscitation with HBOC-201, which is less vasoactive than DCLHb, can be predicted to result in improved and almost certainly not worse outcome in comparison with this DCLHb prehospital trial. These data should be particularly reassuring in assessing the benefit:risk

ratio for the *RESUS* trial[38]. Second, DCLHb was administered until control of bleeding in the *HOST* trial. In contrast, in *RESUS*, HBOC-201 infusions are not started after hospital arrival. Although Kerner did not report time to control of bleeding, time from injury to OR arrival has been reported as 110-161 minutes [88, 89], a prolonged period during which DCLHb may have been infused instead of blood products. Thus, blood transfusion avoidance may be lower in *RESUS*, but conversely, critical subjects in the first hours after hospital admission, are more likely to receive gold standard treatment with blood transfusions. Fourth, that the median time to infusion of DCLHb was 41 minutes in the *HOST* trial, suggests a pattern of delayed hospital arrival in a significant percentage of subjects.

That Northfield's prehospital Phase 3 trial testing of PolyHeme®, also a mild to moderately vasoactive HBOC [90][39];[91], for a similar traumatic HS indication, has been completed without

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[38] In Aug, 2005, NMRC personnel (CDR D. Freilich and Dr. M. Handrigan) reviewed unpublished data from both prior DCLHb trauma clinical trials (prehospital *HOST* and inhospital trials) with Dr. Sloan. This blinded review of Baxter summary data from the prehospital *HOST* trial included detailed presentation of fluid infusion volumes, hemodynamics, and markers of organ perfusion. The data were so similar, that is was impossible to discern treatment assignment, until presentation of hemoglobin and blood transfusion data at the end of the review. The data review showed that these clinical parameters were similar in both groups. Specifically, BP curves could be superimposed on each other; increased roller coaster-like BP responses did not occur; hypertensive responses were uncommon (e.g., peak SBP > 150 mm Hg in < 5% of subjects in both groups, and SBP Δ > 70 mm Hg in ~ 10% of subjects in both groups); and fluid infusion volumes, LA, and BD were the same. As reported by Kerner,[41] organ failure scores were similar in the 2 treatment groups. From the prehospital trial data review, NMRC personnel concluded that increased hypertensive responses, roller coaster BP responses, decreased fluid infusion volumes, and increased organ hypoperfusion, are likely to occur uncommonly, if at all, with the less vasoactive HBOC-201 in *RESUS*.

The review of data from the inhospital trial (unblinded) included the research report previously submitted by Baxter to OBRR (*THS 95.1 Trial Final Report*, Statistical Data Analysis Center, University of Wisconsin, Madison, WI, 08 Oct, 1998). Overall SBP responses, fluid infusion volumes, and markers of perfusion (LA and BD) were similar in DCLHb and control subjects. However, *post hoc* stratification based on survival (< 24 vs. > 24 hours) suggested that fluid infusion volumes may have been lower with DCLHb than control fluid in subjects with < 24 hour survival. Similar *post hoc* stratification based on mechanism of injury did not reveal group differences. Thus, despite known DCLHb vasoactivity, NMRC did not detect definitive evidence of vasoactivity in this trauma trial, although the *post hoc* stratified analysis suggests that fluid infusion volumes may have been lower in DCLHb-treated subjects with poorer outcome. Although randomization and protocol violations contributed to outcome differences, NMRC did not find clear evidence suggesting that vasoactivity was the culprit for the disparity in survival, and generally, could not find a biologically plausible explanation for the trial's adverse outcome.

Overall, NMRC did not find evidence of clinically significant vasoactivity in the prehospital DCLHb trial, and found suggestion but not definitive evidence of clinically significant vasoactivity in the in-hospital DCLHb trial. These clinical DCLHb data suggest that risk of clinically significant vasoactivity in *RESUS* is low, and that protocol design strategies to further mitigate this risk are likely to effectively manage this risk.

[39] Although not statistically significant, there were consistent trends suggesting systemic and pulmonary vasoactivity in this small trauma trial (N = 13) comparing PolyHeme® and RBC. For example, at 4 hours, SVRI appeared higher with PolyHeme® (1,725 $\pm$ 153 vs. 1,396 $\pm$ 191, respectively), PVRI appeared higher PolyHeme® (288 + 34 vs. 233 + 60, respectively), and CI was lower (~ 4.5 vs. ~ 4.8, respectively). At 8 hours, SVRI appeared higher with PolyHeme® (1,994 $\pm$ 245 vs. 1,279 $\pm$ 155, respectively), PVRI appeared higher

reaching a safety stopping criterion, also provides indirect data suggesting that HBOC-201 will be safe in the similar *RESUS* trial.

Expectation of BP responses in *RESUS*

Regarding BP responses, NMRC expects that in the overwhelming majority of *RESUS* subjects, BP responses will be mildly to moderately higher with HBOC-201 than LR, without clinical significance. This conclusion is mainly based on three important observations:

1. **Preclinical HBOC-201 data:** 94% of *peak MAP responses* were ≤ mild in mild and responses were lower in animals with severe HS in NMRC's preclinical swine HS studies. [12] In these preclinical studies, NMRC found that the *RESUS* prehospital HBOC-201 stopping criterion of 120 mm Hg was reached infrequently in HBOC-201 animals in models simulating hospital arrival at 30 minutes (HBOC-201 2.5%, Hextend 12.5%, LR 0%, and NON 2.1%); even with repeat infusions in models simulating hospital arrival at 75 minutes, the stopping criterion was reached in a minority of animals (HBOC-201 21.25%, Hextend 12.5%, LR 0%, and NON 2.1%) (

PolyHeme® (311 ± 35 vs. 282 ± 44, respectively), and CI was lower (~ 4.5 vs. ~ 4.8, respectively). The authors acknowledged, *The limited sample size in this analysis does not allow us to exclude small increases in vascular resistance*.

Table 43). These data predict that high SBP responses, requiring cessation of HBOC-201 infusion will be uncommon in *RESUS*.

2. **Clinical HBOC-201 data:** 94% of *peak SBP responses* were ≤ mild to moderate and responses were lower in subjects with hypotension in HEM-0115.
3. **Clinical prehospital DCLHb data:** *SBP responses* were similar in HBOC and control subjects with the more vasoactive HBOC, DCLHb, in the prehospital HOST trial (Figure 6: SBP in the DCLHb HOST trial).

Table 43: Incidence of animals reaching the *RESUS* HBOC-201 stopping criterion of 120 mm Hg in preclinical NMRC HS studies

	N	SBP ≥ 120 mm Hg		SBP ≥ 130 mm Hg		SBP ≥ 140 mm Hg		SBP ≥ 150 mm Hg	
Prehospital time frame		**30 min N (%)**	**75 min N (%)**	**30 min N (%)**	**75 min N (%)**	**30 min N (%)**	**75 min N (%)**	**30 min N (%)**	**75 min N (%)**
HBOC-201	**80**	2 (2.5)	17 (21.25)	2 (2.5)	9 (11.25)	0 (0)	1 (1.25)	0 (0)	0 (0)
HTS	**32**	0 (0)	0 (0)	0 (0)	2 (6.25)	0 (0)	0 (0)	0 (0)	0 (0)
LR	**28**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NON	**48**	1 (2.1)	1 (2.1)	1 (2.1)	1 (2.1)	0 (0)	0 (0)	0 (0)	0 (0)

The table summarizes number of animals/resuscitation group reaching SBP 120, 130, 140, or 150 mm Hg within 30 or 75 min prehospital time.

Synthesis/extrapolation of potentially "vasoactivity related" BP and AE/SAE preclinical and clinical data for risk:benefit prediction in the *RESUS* trial

NMRC does not expect to see more than mild to moderate BP elevations in the overwhelming majority of *RESUS* patients. In our preclinical studies, HBOC-201-related increases in SBP were mild to moderate and did not affect clinical outcome. Preclinical HS studies are the best simulation of *RESUS* conditions and deserve significant weight. Although mild to moderate BP responses were more common in HBOC-201 than control pigs, no severe BP responses (MAP ≥ 70 mm Hg) occurred in either group (as detailed below). Moderately severe BP responses (MAP 60-69 mm Hg) occurred in 1.8% (1/55) HBOC-201 pigs vs. 0% of the control pigs after the first infusion ($p > 0.05$), and 7.7% (3/39) vs. 2.7% (1/37) after the second infusion ($p > 0.05$). Data on BP responses from HEM-0115 are not inconsistent with the findings in our preclinical studies. It seems reasonably clear that where HBOC-201 demonstrated a hypertensive effect, in most cases, it was mild to moderate and not severe (only 2/319 [< 1%] evaluable subjects in HEM-0115 had severe hypertensive responses [SBP > 200 mm Hg][40]; only 4/842 [< 1%] subjects in all HBOC-201 trials had hypertension SAEs).

The greater concern is not with the SBP responses themselves, but rather with SAEs to which SBP elevations may have contributed or may contribute. OBRR has raised several concerns in this regard, but as aforementioned, in our preclinical studies, SBP increases were not linked to adverse clinical

[40] The number of evaluable subjects in HEM-0115 (N = 319) is less than the total number of HBOC-201 subjects (N = 350) because pre- and post-infusion SBP was not always available. *All Biopure trials* includes COR-0001.

outcomes. Pigs with moderately severe BP increases fared as well as those with lower responses; the higher BP responses did not affect clinical outcome. Further, vasoactivity did not compromise organ blood flow or tissue oxygenation or increase hemorrhage.

The HEM-0115 trial shows higher rates of possibly "vasoactivity-related" SAEs than subjects administered RBC. Hypertensive SAEs were relatively uncommon. Severe *SBP responses* (> 200 mm Hg) were 2/319 (0.6%). Moderately severe *SBP responses* (181-200 mm Hg) were 18/319 (5.6%) as compared to 3/246 [1.2%] with RBC. There were no *SBP responses* > 140 mm Hg in hypotensive subjects in either the stable trauma or < 50 year old sub-populations, which more closely resemble subjects to be enrolled in *RESUS*.

Although HEM-0115 HBOC-201 cardiac system SAE rates (22/350 [6%]) were higher than with than RBC (9/338 [3%]) ($p < 0.05$), HBOC-201 absolute rates and group differences (delta) were generally less in younger sub-populations more closely resembling *RESUS* subjects. For example, respective cardiac SAE rates were 8/239 (3%) vs. 4/227 (2%) ($p > 0.05$) in the < 70 year old sub-population, 2/97 (2%) vs. 0/69 (0%) ($p > 0.05$) in the ≤ 50 year old sub-population, and 1/34 (3%) vs. 1/28 (4%) ($p > 0.05$) in the young stable trauma sub-population. Although NMRC recognizes that the sample sizes of these HEM-0115 sub-populations were small, decreased absolute HBOC-201 cardiac system SAE rates and group deltas were seen in younger sub-populations in similar analyses combining all prior HBOC-201 trials (ISS data) (providing larger Ns):

- **Overall population:** HBOC-201 46/797 (5.8%) vs. RBC 26/661 (3.9%) ($p > 0.05$)
  → delta 1.8%.
- **< 70 year old sub-population:** HBOC-201 19/564 (3.4%) vs. RBC 12/465 (2.6%) ($p > 0.05$)
  → delta 0.8%. The logistic OR for the < 70 vs. 70 year old sub-population was 0.80, demonstrating significantly lower risk of cardiac SAEs in younger subjects.
- **< 50 year old sub-population:** HBOC-201 3/245 (1.22%) vs. RBC 2/158 (1.27%) ($p > 0.05$)
  → delta -0.04%. The logistic OR for the < 50 year old sub-population vs. the overall population was 0.66, demonstrating significantly lower risk of cardiac SAEs in younger subjects.

Other potentially "vasoactivity-related" SAEs were similar in HBOC-201 and RBC subjects in HEM-0115. For example, vascular SAEs occurred in 14/350 (4%) of HBOC-201 vs. 11/338 (3%) of RBC subjects ($p > 0.05$). Respective nervous system rates were 5/350 (1%) vs. 2/338 (1%) ($p > 0.05$),

respiratory rates were 7/350 (2%) vs. 3/338 (1%) ($p > 0.05$), and renal/urinary rates were 7/350 (2%) vs. 4/338 (1%) ($p > 0.05$).

Further, NRMC believes it is inaccurate to assume that there will be greater or even similar *relative*[41] SAE profiles in *RESUS* in comparison with prior surgery/orthopedics HBOC-201 trials. One important difference is the patient populations. Unlike most subjects in prior HBOC-201 elective surgery/orthopedics trials, *RESUS* subjects will enter with hypotension. Because SBP will be low at entry and the protocol includes the CTM stopping criterion of SBP $\geq$ 120 mm Hg, even relatively large SBP increases are unlikely to put subjects at risk of hypertensive SAEs. Moreover, trauma patients are a younger and healthier group, having significantly less co-morbid cardiovascular disease, thus at less risk for the cardiovascular and cerebral ischemic SAEs about which OBRR has expressed particular concern—thus, lowering both *absolute* and *relative* risk. For example, data from the NTDB shows cardiovascular history in only 6.5% of the trauma patients [92] vs. 70.7% among subjects in the HEM-0115 trial. These data, supported by our observations that group differences in BP responses and key AE/SAE rates were lower in younger subjects in HEM-0115, suggests that younger trauma subjects in *RESUS* are likely to be less sensitive to HBOC-201's vasoactive effects.

HEM-0115 data support these hypotheses: group differences in SAEs that are possibly "vasoactivity-related" were fewer in sub-populations closest to those NMRC intends to study (i.e., stable trauma, hypotensive, < 70 years old, and < 50 years old subjects). NMRC notes that there were still group differences, but these were usually narrowed and sometimes lost or reversed. NMRC also notes that some of the subgroups are not large, but they are at least as large as the groups in some Phase 2 studies. Similar trends were apparent in analyses combining all prior HBOC-201 trials (ISS analyses—allowing for large sample sizes). These results should not be dismissed as meaningless, but rather should provide significant comfort that differences in incidence of SAEs are unlikely to be as high (much less higher) in *RESUS* as were observed in prior HBOC-201 surgical/orthopedics clinical trials which compared HBOC-201 with RBC.

OBRR has posited that differences in the study populations may lead to higher SAE rates in *RESUS*. However, preclinical studies demonstrated that the more severe the HS, the lower the BP response. For example, for the first infusion, MAP increase was 29 mm Hg in moderately controlled HS, but 16.6 mm Hg in severe uncontrolled HS with TBI. The higher risk and less stable pigs showed lower

[41] *Relative* refers to group differences.

BP responses. [12] The preclinical data should also help allay concern that HBOC-201-related BP increases will increase hemorrhage. Hemorrhage volume was quantified in two severe uncontrolled HS models (± TBI), and did not increase in either preclinical study.[17, 18][42] In a separate study, the USUHS severe uncontrolled HS model (due to vascular injury), total hemorrhage volume was not increased with hypotensive resuscitation with HBOC-201.

NMRC recognizes that *RESUS* subjects will be less stable and more severely injured than in prior HBOC-201 trials. In HEM-0115, however, there were no significant group differences in the incidence of cardiac, vascular, nervous system, respiratory, and renal/urinary AE/SAE rates in sub-populations with stable trauma or pre-infusion hypotension. HEM-0115 sub-populations most closely resembling *RESUS* subjects did better than the overall group with respect to AE/SAEs.

Thus, absence of HBOC-201 adverse BP responses in preclinical studies, decreased HBOC-201 BP responses and key AE incidence in comparison with RBC in younger sub-populations in HEM-0115, and equivalent or improved outcome with HBOC-201 in comparison with RBC in the ongoing HEM-0125 trauma trial, should help mitigate OBRR's concern that because *RESUS* subjects will be at high risk and unstable, they will be at greater risk of AEs due to BP responses.

An important difference between *RESUS* and HEM-0115 that may well affect the *relative* risk of SAEs rates is the comparator. In *RESUS*, it is logical to hypothesize that beneficial effects of HBOC-201, such as improved tissue oxygenation, will result in decreased *relative* risk of SAEs when compared to LR, which does not carry oxygen. For example, the beneficial consequences of increased myocardial and cerebral tissue oxygenation may well outweigh the potentially adverse consequences of vasoactivity and result in decreased ACS and cerebral ischemic (e.g., CVA) AE rates. Thus, the *relative* SAE profile of HBOC-201 when compared to LR may be very different than when compared to RBC.

OBRR has also expressed concern about higher rates of non-serious AEs in HBOC-201 patients in prior HBOC-201 surgery/orthopedics trials. That issue would need to be resolved for an indication relating to reduced need for RBC, but need not be considered meaningful in a study in which the

[42] NMRC's perspective on OBRR's BPAC package: noting that there were imbalances (although statistically insignificant) in post-injury hemorrhage volume in NMRC's first uncontrolled HS swine study due to liver injury [17], one BPAC pre-reviewer dismissed the results as unreliable. However, the results were confirmed in the follow-up similar and tightly controlled similar model with concomitant TBI. [18]

primary endpoint is mortality. For example, AEs such as confusion or oliguria do not loom very large against an advantage in mortality. Even SBP increases to 141-160 mm Hg count less vs. mortality than they do vs. transfusion avoidance. Allowing such AEs to prevent a study of reduced mortality would be akin to preventing a cancer chemotherapy trial due to nausea or diarrhea side effects. Rather, they should be considered a small price to pay for preserving life, and as long as comprehensive mitigation strategies are included in the protocol to minimize their clinical significance, they should not significantly affect the overall benefit:risk equation.

In short, the mildly adverse shift in the AE profile seen in prior HBOC-201 surgery/orthopedics trials in comparison to RBC is insufficient to predict worse adverse outcome in *RESUS*, especially with exclusion of the elderly in *RESUS* and with favorable interim results from the ongoing HEM-0125 trauma trial, and is not predictive of *unreasonable* risk in a trial evaluating reduced mortality.

Vasoactivity risk mitigation by EMS and trauma center training

Another cited OBRR concern, that HBOC-201's vasoactive properties could lead EMTs and physicians to administer inadequate volume, leading to hypoperfusion and under-resuscitation is substantially mitigated by the trial's design and participant training. With respect to EMTs, NMRC believes that EMT-Ps and EMT-Is are more than adequately trained to appropriately and safely administer HBOC-201 in *RESUS* despite its mild to moderate vasoactive properties. Standard EMT training includes use of numerous clinical parameters in addition to BP in assessing intravascular volume, oxygen content, and tissue oxygenation in HS patients (*National Highway Traffic Safety Administration's National Standard Curriculum*). It includes comprehensive HS pathophysiology training, and especially training regarding multiple and staged (based on severity—akin to ATLS HS classes) clinical parameters to be used in assessing HS patients (including BP, HR, pulse pressure, respiratory rate, renal output, pallor, cool skin, diaphoresis, and mental status). It also includes training regarding multiple types of *intravenous volume expanders* (including *blood substitutes*).

EMT-P and EMT-I level prehospital care is delivered by highly trained and experienced medical professionals with a wide range of skills, including ability to rapidly assimilate new information and techniques. The use of HBOC-201 in the field falls well within the scope of their practice. Thus, it is reasonable to assume that these advanced health care providers are capable of safely administering HBOC-201 in the field with appropriate training[43].

[43] The *RESUS* protocol EMS training module includes detailed information regarding potential HBOC-201

The same is true of trauma center medical staffs. At Level I-II trauma centers, surgeons, emergency room and critical care physicians, and anesthesiologists will be more than adequately trained to provide optimal medical care to HBOC-201-resuscitated subjects in *RESUS*, despite its mild to moderate vasoactive properties. Even minimal standard (e.g., ATLS) and specialty Board training/testing include standard paradigms for use of numerous clinical parameters in addition to BP to assess adequacy of intravascular volume, oxygen content, and tissue oxygenation in volume depleted patients. Also, physicians have learned from prior experience with vasoactive resuscitative fluids (i.e., DCLHb). One good example is the traumatic HS chapter of the standard *ATLS for Doctors Student Course Manual* (1997). This is a minimum standard and includes only a small fraction of HS evaluation and treatment methodologies taught to physicians who care for patients at trauma centers (i.e., Board certifications).

In addition, the *RESUS* protocol includes a detailed trauma center training module and *in-hospital trauma care guidelines*. Training includes information on HS pathophysiology, BP and AE/SAE responses in prior clinical trials, reinforces diagnostic concepts, and educates personnel about *in-hospital trauma care guidelines* and about potential HBOC-201-specific vasoactivity and mitigation strategies.

Relevance of non-serious AEs/laboratory abnormalities in predicting benefit:risk in *RESUS*

In a trial with predicted mortality of 58.1%, NMRC has maintained that non-serious AEs essentially account for background noise (akin to nausea AEs in chemotherapy trials), and to accurately predict benefit:risk, they must be considered in context of high predicted mortality in control subjects and mortality reduction in HBOC-201 subjects in *RESUS*. Thus, NMRC has argued that as long as strategies are included in the protocol design to minimize risk and consequences of non-serious AEs, they are less important in *RESUS* and that mainly SAEs and survival should be key safety data points for prediction of benefit:risk in *RESUS*. Oliguria and troponin elevations are illustrative examples.

Oliguria

Although oliguria AEs were more frequent with HBOC-201 than RBC (39/350 [11%] vs. 16/338 [5%], respectively, p = 0.002) in HEM-0115, more relevant acute renal failure AEs were similar in the two treatment groups (5/350 [1.4%] vs. 4/338 [1.2%], respectively, p = 1.0). Thus, NMRC has not

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vasoactivity risk and mitigation strategies (e.g., comprehensive fluid *Dosing Guidelines* [Appendix C – Protocol details]).

disagreed with OBRR's contention that extrapolation of safety data from HEM-0115 to *RESUS* (despite limitations of such extrapolation) might predict *potential* for increased risk of renal AEs. NMRC has argued that the extrapolation is circumstantial and may be inaccurate; even if one makes the extrapolation, risk is relatively low when considered in the context of *RESUS*; awareness of oliguria and other possibly "vasoactivity-related" AE risks has led to incorporation of training and practice guidelines to minimize the likely culprit—fluid under-resuscitation (i.e., detailed EMS and trauma center training about risks and mitigation strategies, and detailed *EMS prehospital fluid re-infusion guidelines* (Appendix C – *RESUS* protocol details) *and in-hospital trauma care guidelines* ([Appendix D); and theoretical risk of increased rates of oliguria does not significantly affect overall benefit:risk prediction for *RESUS*.

Cardiac troponin elevations (Appendix F)

In HEM-0115, incidence of investigator-reported MI AEs was similar in HBOC-201 and RBC subjects (4/350 [1.1%] vs. 2/338 [0.9%], respectively, p = 0.7), but cardiac troponin elevation was more common with HBOC-201 (18/136 [13.2%] vs. 2/122 [1.6%], respectively, p = 0.0003)[44]. Clearly, there were no group differences in investigator-reported MIs. Three of the HBOC-201 subjects experienced troponin elevations prior to CTM infusion, in 1 the troponin elevation was related to ARF, and one subject experienced the troponin elevation only at 6 week follow up. Thus, 5 of the 18 troponin elevations cannot be attributed to cardiac injury related to HBOC-201 infusion. Only one experienced a documented MI. Six subjects were anemic (a possible explanation for the elevated troponin; but possibly also related to HBOC-201 treatment as per the HEM-0115 protocol). In the remaining 7 subjects, the cause could not be elucidated, and thus may be directly related to a relatively higher rate of elevated troponin in HBOC-201 than RBC subjects. Mean age ($\pm$ SEM) of subjects with elevated troponin was similar in the 2 treatment groups (i.e., 64.9 $\pm$ 2.6 vs. 66.0 $\pm$ 2.0 in HBOC-201 and RBC groups, respectively, p = 0.90).

OBRR's perspective on troponin elevations

OBRR has noted that even isolated troponin elevations, in the absence of a clinical syndrome consistent with ACS or ischemic ECG changes, equate with MI; thus, OBRR has argued that the true rate of MI in HBOC-201 subjects in HEM-0115 was 13.2%, not 1.1%. But elevation of cardiac biomarkers (troponin or CK-MB) in isolation is neither sensitive nor specific for myocardial injury in the absence of other evidence of ACS. At the 28 Nov 2005 Type A meeting, OBRR provided NMRC

[44] Troponin elevation defined as > ROC [receiver-operator curve].

with a listing of *clinically important AEs in HEM-0115* (per OBRR analysis) which included enumeration of 18 MIs (by troponin elevation in HBOC-201 subjects in HEM-0115. This point is important because an 11.6% difference in incidence of MI AEs (if diagnosed by troponin elevation alone) would affect predicted benefit:risk more significantly than a 0.2% difference (as was investigator-reported).

NMRC perspective on troponin elevation

Criteria published in the European Society of Cardiology/American College of Cardiology (*ESC/ACC*) *consensus document* [93] require that troponin elevation be associated with an acute coronary syndrome (ACS) or ECG changes in order to diagnose MI because troponin can be elevated for multiple non-ischemic and cardiac reasons. Isolated elevated troponins drawn routinely and not associated with a clinical ACS suggest some degree of myocardial injury, but causality (and therefore, prognostic implications) may be diverse (e.g., tachycardia, exercise, CHF, pulmonary embolism, COPD, subarachnoid hemorrhage, CVA, sepsis, trauma, renal failure, and myocarditis. [94-96] NMRC submits that the following quote from the cardiology literature describes what NMRC believes is OBRR's misinterpretation: *...there has been widespread misinterpretation of the new definition, and troponin concentrations are frequently assumed to reflect myocardial infarction without corroborative evidence from the patient's history or ECG.* [97]

NMRC concurs with OBRR that the literature supports the importance and prognostic implications of troponin evaluation in the setting of ACS. [98] In addition, troponin elevation appears to correlate with higher mortality in medical ICU, general medical, and surgical patients. [96, 99-103] In fact, the definition of MI was revised in 2000 by the *ESC/ACC* in order to emphasize the importance of troponin. [93] However, it is inappropriate to define MI based on biomarker elevation alone (whether cardiac troponin or CK-MB). Simply put, currently accepted guidelines do not include diagnosis of MI by isolated troponin (or CK-MB) elevation without associated ECG and/or clinical symptoms/signs. Some direct quotes from the literature are illustrative:

<u>*ESC/ACC Consensus* Document. (Alpert, 2000) [93]</u>

MI is diagnosed when blood levels of sensitive and specific biomarkers...are increased in the clinical setting of acute ischemia. These biomarkers reflect myocardial damage but do not indicate its mechanism. Thus, an elevated value in the absence of clinical evidence of ischemia should prompt a search for other causes of cardiac damage, such as myocarditis…

Definition of MI. *Criteria for acute, evolving or recent MI. Either one of the following criteria satisfies the diagnosis for an acute, evolving or recent MI:*

1) Typical rise and gradual fall (troponin) or more rapid rise and fall (CK-MB) of biochemical markers of myocardial necrosis with at least one of the following:

a) Ischemic symptoms;
b) development of pathologic Q waves on the ECG;
c) ECG changes indicative of ischemia (ST segment elevation or depression); or
d) coronary artery intervention (e.g., coronary angioplasty).

2 Pathologic findings of an acute MI.

Jaffe, 2000 [104]

Detectable increases in the biomarkers of cardiac injury are indicative of injury to the myocardium, but elevations are not synonymous with an ischemic mechanism of injury. Therefore, increases do not now and did not in the past mandate a diagnosis of MI...The term 'MI' should be used when evidence of cardiac damage exists, as detected by marker proteins in a clinical setting consistent with myocardial ischemia...One should not make the diagnosis of infarction predicated solely on the presence of increased marker protein values.

Wong, 2005 [105]

In 2000, the ESC/ACC promulgated a new definition of MI...the definition is based mainly on biochemical evidence of myocardial necrosis (rising and falling levels of cardio-specific biomarkers preferably troponin T or I), in an appropriate context of a clinical ACS or PCI...any increase in the marker...is regarded as an acute MI provided the clinical context is compatible.

Lim, 2005 [106]

Elevated troponin levels indicate myocardial injury but may occur in critically ill patients without evidence of myocardial ischemia. an elevated troponin alone cannot establish a diagnosis of MI...it is generally considered inappropriate to use elevated troponin levels as the only diagnostic criterion for MI.

ESC/ACC *Consensus* Requirement For *Typical Rise And Gradual Fall* (Of Troponin)

The *ESC/ACC* consensus requirement for *typical rise and gradual fall (troponin)* must also be taken into account in evaluating cardiac biomarkers. Isolated troponin elevation, in the absence of demonstration of the dynamic pattern stated in the *Definition of MI*, would not meet this requirement. As shown in Table 44, 3/18 troponin elevations in HEM-0115 (> ROC) were pre-existing (documented prior to CTM infusion); 4/18 were isolated elevations; 6/18 were isolated elevations

with accompanying detectable levels but ≤ ROC; and only 5/18 were serially elevated (conservatively defined as ≥ 2 elevations). Therefore, 5/18 likely met the *ESC/ACC* definition for troponin elevation; when the 6 subjects with isolated elevations > ROC with accompanying low level detectable elevations ≤ ROC are included, then a total of 11/18 possibly met the *ESC/ACC* definition for troponin elevation. It is worth noting that spurious troponin elevations have been reported to be frequently isolated elevations in the literature (e.g., [107]). Thus, NMRC clearly agrees with OBRR that the overall incidence of troponin elevation > ROC was higher in HBOC-201 than RBC subjects in HEM-0115. As previously noted, 18/136 [13.2%] vs. 2/122 [1.6%] (p = 0.0003), respectively, had any troponin elevation > ROC. But more accurate defining of elevated troponin (per *ESC/ACC consensus*), results in smaller group differences, with conservatively estimated maximum rates of 11/136 (8.1%) vs. 2/122 (1.6%) (p = 0.02) and less conservatively estimated minimum rates of 5/136 (3.7%) vs. 2/122 (1.6%) (p = 0.5), respectively.

***AHA Scientific Statement* About Elevated Troponins**

In order to address confusion regarding interpretation and over-emphasis of cardiac biomarker elevations in clinical trials since the *ESC/ACC consensus* [108-110], the *AHA Scientific Statement* was published in 2003.[111] Specific cardiac biomarker definitions were included, as well as guidelines for classification of MI based on these definitions. NMRC used the AHA *Scientific Statement*'s case-definitions to assess troponin elevations in HEM-0115 and to classify MI incidence. As shown in Table 45, 14/18 troponin elevations > ROC should be classified as *equivocal biomarkers*, and only 1/18 should be classified as *diagnostic biomarkers* (3/18 with pre-existing elevations are not included). Only the one subject with *diagnostic biomarkers* (#1922) met criteria for *definite MI*; two subjects with *equivocal biomarkers* and nonspecific ECG changes (#4201) or nonspecific ECG changes and atypical symptoms and (#4317) conservatively (considered non-ischemic by the investigator) met criteria for *possible MI*; the remainder of 12 subjects with *equivocal biomarkers* met criteria for *no MI* (3 had pre-existing elevations prior to CTM administration and were excluded *a priori*)[45]. Thus, the incidence of *definite* MI in HBOC-201 subjects in HEM-0115 remains 4/350 (1.1%); with a maximally conservative estimate (including *definite* as well as *possible* MIs), the cumulative incidence would still be only 6/350 (1.7%), not significantly different from RBC controls (3/338 [0.9%]) (p = 0.3).

[45] Categorization of 2 of the 18 troponin elevations as *possible MI* was deliberatively conservative; in fact, ECG changes and clinical symptoms were considered non-ischemic by the investigator (strictly speaking, these should be categorized as *no MI*).

Table 44: Elevations of troponin (> ROC) in HBOC-201 subjects in HEM-0115

Subject #	Serially (≥ 2)	Isolated, then detectable low level elevations (≤ ROC)	Isolated alone	Pre-CTM	Comment	ESC/ACC criterion 1 met (troponin elevation)	ESC/ACC criteria 1a-1d met
209				X			
915				X			
929			X		At 6 wk follow-up		
1113				X			
1122	X					X	
1130		X					
1255			X				
1509			X				
1523		X					
1606		X					
1617		X					
1618	X				ARF	X	
1627	X					X	
1905			X				
1922	X				MI	X	X
4201	X					X	
4312		X					
4317		X					
Total	**5**	**6**	**4**	**3**		**5**	**1**

Table 45: Classification of troponin elevations in HBOC-201 subjects in HEM-0115 (derived from modified *Table 1* in [111])

	Biomarker finding			
	Cardiac symptoms or signs present		Cardiac symptoms or signs absent	
ECG findings	**Diagnostic**	**Equivocal**	**Diagnostic**	**Equivocal**
Evolving diagnostic	***Definite*** *1922*	***Definite***	***Definite***	***Definite***
Positive	***Definite***	***Probable***	***Definite***	***Probable***
Nonspecific	***Definite***	***Possible*** *4317*	***Definite***	***Possible*** *4201*
Normal or other ECG findings	***Definite***	***Possible***	***Definite***	***No*** *929* *1122* *1130* *1255* *1509* *1523* *1606* *1617* *1618* *1627* *1905* *4312*
Total	***1***	***1***	***0***	***13***

Association Of Elevated Troponin With Investigator-Reported ACS AEs

Only one of the 18 HBOC-201 subjects with elevated troponin had an investigator-recognized and -reported MI AE. Two subjects had nonspecific ECG changes and/or chest pain, which were not considered ischemic. Thus, elevated troponin in HBOC-201 subjects did not correlate with ACS. In fact, in a prior Biopure safety report to BB-IND-2935, it was shown that troponin elevations $\geq$ 2.5 LLD had no correlation with cardiac SAEs: in the 56/146 (38.4%) HBOC-201 subjects with elevated troponin, the incidence ($\pm$ SEM) of cardiac SAEs was 0.07$\pm$0.03; in the 90/146 (61.6%) HBOC-201 subjects without elevated troponin, the incidence was 0.06$\pm$0.02 (not significantly different [2 tailed t test, p = 0.77]). In contrast, in the 26/130 (20%) of RBC subjects with elevated troponin, the incidence was 0.08 $\pm$ 0.06; in the 104/130 (80%) of RBC subjects without elevated troponin, the incidence was 0.01 $\pm$ 0.01 (significantly different [two tailed t test, p = 0.05]). <u>These data confirm the above observation that elevated troponin in HBOC-201 subjects did not predict increased risk of ACS in HEM-0115.</u> This is in contrast with the literature in various patient categories, showing an

association between elevated troponin and increased risk of ACS, and similar findings in RBC subjects in HEM-0115.

Low Level Troponin Elevations

As shown in Table 46, most troponin elevations were low level elevations, akin to troponin leaks in PCI, which may not be associated with adverse outcome.[112]

Table 46: Patients with Troponin Elevations >ROC in Study HEM-0115

N	Patient Number	ng/L*	*AHA Scientific Statement* Criteria [111]
1	- ----	0.11	Isolated value
2	- ---- -	0.12	
3	- ---- -	0.12	
4	- ----	0.12	
5	- ---- -	0.12	
6	- ----	0.12	Isolated value
7	- ---- -	0.13	
8	- ----	0.16	↑ Pre-CTM
9	- ----	0.17	↑ Pre-CTM
10	- ----	0.17	
11	- ----	0.18	Isolated value
12	- ----	0.24	Isolated value
13	- ----	0.26	
14	- ----	0.26	
15	- ----	0.31	
16**	- ----	0.58	
17	- ----	0.89	
18	- ----	5.6	↑ Pre-CTM Trop I

* Troponin T only, mean = 0.24ng/L, range:0.11-0.89ng/L, 75th percentile <0.26ng/L.
** Diagnosed with MI.
# Single value >ROC.

Elevated Troponin In < 50 Year Old Sub-Populations

Among HBOC-201 subjects only, rates of troponin elevation in the overall population vs. the < 50 year old sub-population were not significantly different (18/136 [13.2%] vs. 1/32 [3.1%], p = 0.13). But there was a trend to a lower rate of troponin elevation in the < 50 year old sub-population vs. the ≥ 50 year old sub-population (1/32 [3.1%] vs. 17/104 [16.4%], p = 0.07). There was no significant difference in rates of troponin elevation in the 2 treatment groups in the < 50 year old sub-population (HBOC-201 1/32 [3.1%] vs. RBC 0/24 [0%], p = 1.0).

Elevation of troponin (when defined as ≥ 2.5 LLD [lower limit of detection]) occurred in 56/136 (41.2%) HBOC-201 vs. 22/122 (18.0%) RBC subjects in the overall population (p = 0.0001). Mean age (±SEM) was similar in subjects with elevated troponin the two treatment groups (i.e., 62.3 ± 1.9 vs. 67.7 ± 2.7 in HBOC-201 and RBC groups, respectively, p = 0.12). Among HBOC-201 subjects only, rates of troponin elevation were lower in the < 50 year old sub-population (7/32 [21.9%]) vs. the overall population (56/136 [41.2%], p = 0.045) or vs. the ≥ 50 year old sub-population (49/104 [47.1%], p = 0.01). There was no significant difference in rates of troponin elevation in the 2 treatment groups in the < 50 year old sub-population (HBOC-201 7/32 [21.9%] vs. RBC 3/24 [12.5%], p = 0.5) (**Error! Reference source not found.**).

Table 47: Cardiac biomarkers in HEM-0115*#

		HBOC-201	RBC	P^
Overall population	**CK-MB**			
	ULN – 5 x ULN	83/250 (33.2%)	74/246 (30.1%)	0.5
	> 5 x ULN	16/250 (6.4%)	14/246 (5.7%)	0.85
	> 3% of CK	87/249 (34.9%)	88/244 (36.1%)	0.85
	> 5% of CK	29/249 (11.7%)	26/244 (10.7%)	0.78
	Trop I+T			
	> 2.5 x LLD	56/136 (41.2%)	22/122 (18.0%)	0.0001
	> 2.5 x LLD – optimal ROC	38/136 (27.9%)	20/122 (16.4%)	0.036
	> optimal ROC	18/136 (13.2%)	2/122 (1.6%)	0.0003
< 50 year old sub-population	> 2.5 x LLD	7/32 (21.9%)	3/24 (12.5%)	0.49
	> 2.5 x LLD – optimal ROC	6/32 (18.8%)	3/24 (12.5%)	0.71
	> optimal ROC	1/32 (3.1%)	0/24 (0%)	1
≥ 50 year old sub-population	> 2.5 x LLD	49/104 (47.1%)		
	> 2.5 x LLD – optimal ROC	38/104 (36.5%)		
	> optimal ROC	17/104 (16.4%)		

* Highest reported values post-CTM start for each subject
# Only subjects with available measurements were reported
^ Fisher's Exact test

Extrapolation To Risk Of Elevated Troponins In *RESUS*

NMRC appreciates OBRR's concern that the troponin elevations seen in HBOC-201 subjects in HEM-0115 could predict increased risk ACS in HBOC-201 subjects in *RESUS*. In fact, the literature

supports a conclusion that elevated troponins might predict increased risk in ACS, however the use of this clinical test is predicated on the need to characterize and or risk stratify a patient presenting with symptoms or signs of ACS and/or abnormal ECG findings. The finding of random troponin elevations in isolation of a clinical question is of questionable clinical significance. [96, 98, 100-103] In any case, in HBOC-201 subjects in HEM-0115, troponin elevation did not predict ACS occurrence[46]. Where NMRC disagrees with OBRR is in extrapolation of risk in the context of overall benefit:risk prediction in *RESUS*. NMRC's assessment is that the HEM-0115 troponin data do not significantly affect overall predicted benefit:risk in *RESUS* with the following rationale:

1. **Lower troponin elevation risk:** Based on *ESC/ACC* criteria, the true rate of troponin elevations in HBOC-201 subjects in HEM-0115 was as low as 5/136 (3.7%) or as high as 11/136 (8.1%), but not 18/136 (13.2%); hence, the true group delta likely ranges between 2.1 and 6.5%, not 11.6%. Thus, the adverse safety signal is less significant *a priori*.
2. **Lack of association with ACS:** Only 1 of the 18 (5.6%) troponin elevations (whether meeting or not meeting *ESC/ACC* criteria) in HBOC-201 subjects was associated with a clinical diagnosis of an ACS (one MI). In HBOC-201 (but not RBC subjects), there was no association between troponin elevation and ischemic AEs. Thus, troponin elevations represented a laboratory abnormality without clinical significance.
3. **Lack of association with other AEs:** There were no differences in potentially "vasoactivity-related" AE and SAE rates in OBRR's *systems of concerns* in HBOC-201 subjects with or without troponin elevations (again showing that the elevated troponin rates did not correlate with adverse outcome) (Table 48).
4. **Absence of group differences in MIs:** There was no difference in investigator-reported MI AEs, showing that irrespective of troponins, rates of clinically recognized MIs were not increased.
5. **Diminished or absent group differences in younger subjects:** Troponin elevation was clearly age-dependent with group deltas of 21.6% in $\geq$ 70 year olds, 11.6% in the general population, 9.9% in < 70 year olds, and 3.1% in < 50 year olds. There was no difference in troponin elevations in HBOC-201 (1/32 [3.1%]) vs. RBC (0/24 [0%]) subjects in the more *RESUS*-relevant

[46] For an analysis based on troponin elevation defined as $\geq$ 2.5 LLD: in HBOC-201 subjects, cardiac SAE incidence ($\pm$ SEM) was 0.07 $\pm$ 0.03 in subjects with troponin elevation vs. 0.08 $\pm$ 0.06 in subjects without troponin elevation ($p > 0.05$). In contrast, in RBC subjects, respective incidence was significantly higher with troponin elevation (0.06 $\pm$ 0.02 vs. 0.01 $\pm$ 0.01, $p < 0.05$). Using the much smaller sample size of troponin elevation defined as > ROC, there were no significant differences in SAE incidence in either group (HBOC-201 0.11 [2/18] vs. 0.07 (8/118); RBC 0 [0/2] vs. 3/120 [3%], respectively).

sub-population of < 50 year olds, p = 1.0. Thus, despite limitations of extrapolating HEM-0115 risk to *RESUS*, the data suggest a lower risk of elevated troponins in the younger *RESUS* population (especially since elderly subjects will be excluded).

6. **Low level elevations:** Most troponin elevations were low level, which may not be associated with adverse outcome.
7. **Absence of risk in preclinical HS studies: NMRC assessed myocardial histology and cardiac biomarkers in its swine HS studies.** HBOC-201 resuscitation resulted in lower rates and severity scores of myocardial necrosis and myocardial fibroplasia/histiocytic infiltration than with standard control fluid in *moderate controlled HS*; myocardial histopathology was similar in both groups in *severe controlled HS*, *severe uncontrolled HS,* and *severe uncontrolled HS/TBI*. [18, 32] There were no group differences in peak troponin levels in any of the studies.[18, 32]. In addition, HBOC-201 has been shown to be myocardial protective in models of acute coronary occlusion. [66] Thus, preclinical HS studies suggest that in comparison with asanguinous standard fluids, HBOC-201 resuscitation is unlikely be harmful and may be myocardial protective in HS[47].

Table 48: HEM-0115 Troponin Subgroups (troponin elevation defined as > ROC)

N of at least one AE or SAE per subject by Group and SOC														
Group	**Cardiac**		**Nervous System**		**Renal**		**Respiratory**		**Vascular**		**Vascular (HTN)**		**Investigations (Elevated/ Increased BP)**	
	AE	**SAE**	**AE**	**SAE**	**AE**	**SAE**	**AE**	**SAE**	**AE**	**SAE**	**AE**	**SAE**	**AE**	**SAE**
Elevated Troponin Subgroup														
HBOC-201	7/18 (39%)	2/18 (11%)	9/18 (50%)	2/18 (11%)	6/18 (34%)	2/18 (11%)	5/18 (28%)	1/18 (6%)	7/18 (39%)	1/18 (6%)	5/18 (28%)	0/18 (0%)	0/18 (0%)	0/18 (0%)
RBC	0/2 (0%)	0/2 (0%)	1/2 (50%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	1/2 (50%)	0/2 (0%)	1/2 (50%)	0/2 (0%)	0/2 (0%)	0/2 (0%)
Non-Elevated Troponin Subgroup														
HBOC-201	48/118 (41%)	8/118 (7%)	62/118 (53%)	1/118 (1%)	40/118 (34%)	2/118 (2%)	29/118 (25%)	4/118 (3%)	40/118 (34%)	4/118 (3%)	18/118 (15%)	1/118 (1%)	3/118 (3%)	0/118 (0%)
RBC	24/120 (20%)	3/120 (3%)	42/120* (35%)	1/120 (1%)	31/120* (26%)	2/120 (2%)	19/120 (16%)	1/120 (1%)	22/120* (18%)	3/120 (3%)	12/120 (10%)	0/120 (0%)	1/120 (1%)	0/120 (0%)

* Includes AEs that were not initially designated as an AE or SAE, and were later determined to be an AE.

Summary Of Troponin Data

It is clear that in comparison with gold standard RBC transfusion, HBOC-201 was associated with an increased rate of troponin elevation in HEM-0115, but unlike the case in RBC-treated subjects, troponin elevation was unrelated to ACS or mortality in HBOC-201 subjects. Thus, prognostic

[47] NMRC's perspective on OBRR's BPAC package: one reviewer's assessment of potential cardiac risk in *RESUS* included a statement that myocardial histopathology was worse with HBOC-201, when in fact, it was uniformly better or equivocal with HBOC-201 (depending on the study). [32]

implications in HBOC-201 subjects are unclear. Moreover, troponin elevations were significantly less common in the younger < 50 year old sub-population (also lower in < 70 year olds) than either the older overall population or ≥ 50 year old sub-population, suggesting that rates will be low in *RESUS* as well, and that subjects will not be placed at *unreasonable* risk for troponin elevations either. Finally, the accuracy of extrapolation of HEM-0115 troponin data to prediction of similar risk in comparison with asanguinous LR in *RESUS* is limited. It is especially unlikely that any such risk (i.e., of increased troponin elevation) can predict an association with increased risk of ACS or mortality in *RESUS*, especially with exclusion of the elderly. Thus, NMRC submits that HEM-0115 troponin data do not significantly affect the overall benefit:risk equation in *RESUS*, with maintenance of equipoise.

7.b *RESUS Dosing Guidelines* Rationale

Preclinical Data

NMRC Swine HS Studies

NMRC completed three swine HS studies in which HBOC-201 was compared with Hextend with a 4-hour simulated prehospital time and 72-hour simulated in-hospital follow-up: moderate controlled HS (40% EBV), severe controlled HS (55% EBV), and severe uncontrolled HS due to liver injury. [17, 20, 27, 32] Animals received initial resuscitation fluid doses of 10 ml/kg over 10 minutes and subsequent doses of 5 ml/kg over 10 minutes (at 30, 60, 120, and 180 minutes). These numbers correspond to 500 and 250 ml individual doses; maximum total of ~ 30 ml/kg or ~ 2,000 ml total dosing (67% of EBV); and 1 and 0.5 ml/kg/min infusion rates, respectively.

The initial dose of 10 ml/kg in these swine studies is a little higher (1.4-fold) than the *RESUS* dose of ~ 7 ml/kg; the 5 ml/kg re-infusion doses are a little lower (0.71 ml/kg/min). The subtotal doses of 15 and 20 ml/kg over 30 and 60 minutes in the swine studies, respectively (corresponding to and more relevant to expected "short" and "long" prehospital times in *RESUS*), were similar to 2 (14 ml/kg) and 3 (21 ml/kg) analogous doses in *RESUS*. The maximum dose of 30 ml/kg in the swine studies is 1.4-fold higher than the 21 ml/kg maximum *RESUS* total dose. These HBOC-201 doses and infusion rates, similar to or exceeding those in *RESUS*, resulted in improved hemodynamics and tissue oxygenation in all HS models, and decreased LA and improved survival in severe HS models, without causing severe BP responses, compromising organ blood flow, or increasing hemorrhage (Preclinical Section 5.c).

NMRC also evaluated HBOC-201 in a multitrauma model (severe uncontrolled HS due to liver injury with concomitant blunt parietal cerebral fluid percussion injury), simulating blunt trauma (e.g., MVA) with prehospital delays simulating common civilian scenarios. [18] After instrumentation, injuries, and a 15 minute delay, HBOC-201 was infused at 10 ml/kg and LR at 20 ml/kg. In the short delay cohort (simulating hospital arrival at 30 minutes), animals received a single infusion (17% of EBV) (akin to the extreme of short prehospital delay with some urban trauma). In the long delay cohort (simulating hospital arrival at 75 minutes), animals received three additional infusions (at 30, 45, and 60 minutes) (total of 40 ml/kg, 67% of EBV) for hypotension and/or tachycardia (akin to longer prehospital delay with some urban and rural civilian trauma). The two prehospital delay cohorts provided opportunity to assess potential dose responses in a clinically significant manner. Upon hospital arrival, further fluid/transfusions/mannitol were provided if needed based on standard ER, then OR, and then ICU measurements (e.g., vital signs, LA, Hb, CPP, and ICP). At hospital arrival, animals were administered the NSAID, ketorolac, to minimize blood transfusion-related pulmonary hypertension seen in both HBOC-201 and LR animals. A limitation of this study is its short follow-up (6 hours).

The individual infusion dose (10 ml/kg) and infusion rate (1 ml/kg/min) in this swine HS/TBI study were similar (1.4-fold higher) to the 7 ml/kg dose and 0.71 ml/kg/min infusion rate in *RESUS*. The total dose was less in the swine short delay cohort than the maximum dose in *RESUS* (10 vs. 21 ml/kg [0.48 lower]) but higher in swine in the long delay cohort than the maximum dose in *RESUS* (40 vs. 21 ml/kg [1.9-fold higher])—corresponding to 67 vs. 30% of EBV, respectively (2.2-fold higher).

In comparison with LR, in the short delay cohort, minimal systemic and neurophysiologic benefits were noted as well as trends to improved survival with HBOC-201 (similar to other lower severity models) (survival rates: HBOC-201 5/9 [56%] vs. LR 4/9 [44%], $p > 0.05$; survival times: 4.0 ± 0.8 vs. 3.2 ± 0.9, $p > 0.05$); but in the long delay cohort, significant systemic benefits (e.g., decreased LA), neurophysiologic benefits (e.g., improved CPP), and survival benefits were seen with HBOC-201 (survival rates: HBOC-201 8/11 [73%] vs. LR 1/11 [9%], $p = 0.008$; survival times: 4.2 ± 0.6 vs. 2.1 ± 0.8, $p < 0.05$). Mainly due to prolonged survival and consequently increased histopathologic evidence of brain injury, NN and WMD were higher with HBOC-201. NMRC does not believe

ketorolac confounded the results because many of the physiologic benefits seen with HBOC-201 were already apparent by simulated hospital arrival (prior to ketorolac administration)[48].

Thus, in uncontrolled HS with combined TBI, with doses and infusion rates exceeding those in *RESUS*, HBOC-201 improved systemic and neurophysiology ans survival without causing severe BP responses, compromising organ blood flow, or increasing hemorrhage (Section 5.c).

Other Swine HS Studies

Katz reported results of an uncontrolled HS model due to liver injury, in which HBOC-201 and hetastarch were compared with 1-hour simulated prehospital delay, and then followed up to 96 hours.[23] After injury and hemorrhage, animals were volume-resuscitated at rates of 6 ml/kg/min for 15 minutes (individual initial infusion dose of 90 ml/kg ~ 2,700 ml or ~ 150% of EBV; rate of 180 ml/min) and then 3 ml/kg/min for 30 minutes (individual re-infusion dose of 45 ml/kg ~ 1,350 ml or ~ 75% of EBV; rate of 90 ml/min) (maximum total dose of 135 ml/kg or ~ 133% of EBV).

The individual initial dose of 90 ml/kg in the swine study is 12.8-fold higher than the *RESUS* default dose of 7 ml/kg. The total maximum dose of 135 ml/kg over 45 minutes in the swine study is 6.4-fold higher than the maximum *RESUS* dose of 21 ml/kg. The infusion rates of 3-6 ml/kg/min are 4.2-8.4-fold higher than the *RESUS* default infusion rate of 0.71 ml/kg/min. These doses and infusion rates with HBOC-201 in a model of severe uncontrolled HS, significantly exceeding those in *RESUS*, improved hemodynamics, decreased blood LA, and increased survival.

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[48] NMRC's perspective on comments in OBRR's BPAC package: OBRR has dismissed NMRC's uncontrolled HS/TBI study, stating that ketorolac confounded the results. However, because HBOC-induced systemic and pulmonary vasoconstriction is mainly due to NO binding [32, 113] and to a lesser extent due to vascular response to increased oxygen unloading [114], endothelin and adrenergic activation [115, 116], and arachidonic acid metabolites (e.g., thromboxane [14], the preponderant vasoconstrictive effect of HBOC-201 (NO binding) would have been unaffected by ketorolac in our HS/TBI swine study.

More specifically, many of the physiologic benefits shown with HBOC-201 were already evident at simulated hospital arrival, prior to any potential confounding of ketorolac (which was administered prior to blood transfusions during the simulated hospital phase). Specifically, in the SD cohort, significant group differences in physiologic parameters were seen at hospital arrival as follows: MAP, MPAP, and SVRI, $tcPO_2$, CPP, L brain PO_2, Hb, $MVSO_2$, and SSO_2sat were higher with HBOC-201; arterial oxygen saturation was lower with HBOC-201. In the LD cohort, MAP, MPAP, CPP, BE, and Hb were higher with HBOC-201; HR, arterial oxygen saturation, LA, and SSLA were lower with HBOC-201.

In summary, significant systemic and neurophysiologic benefits were seen with HBOC-201 prior to ketorolac administration. That similar results were demonstrated in a rat HS/TBI animal study with HBOC-201 [33], a swine HS study with HBOC-301[26], and two swine HS studies with HBOC-201 [25, 34] without cyclo-oxygenase inhibition, is supportive of the validity of NMRC's study results.

Manning reported results of an uncontrolled HS model due to liver injury, in which HBOC-201 and LR were compared with 2-hour simulated prehospital delay. [24] After injury and hemorrhage, animals were initially volume-resuscitated at a rate of 10 ml/kg/min aiming to restore MAP to 60 mm Hg. The animals received ~ 7,200 ml of HBOC-201 over 2 hours, corresponding to a total dose of 313 ml/kg or ~ 500% of EBV, and a cumulative infusion rate of 2.6 ml/kg/min.

The initial individual dose of 10 ml/kg in the swine study is 1.4-fold higher than the *RESUS* default dose of 7 ml/kg. The total maximum dose of 313 ml/kg in the swine study is 15-fold higher than the maximum *RESUS* dose of 21 ml/kg. The infusion rates of 2.6-10 ml/kg/min are 3.7-14-fold higher than the *RESUS* default infusion rate of 0.71 ml/kg/min. <u>These doses and infusion rates with HBOC-201 in severe uncontrolled HS, significantly exceeding those in *RESUS*, improved hemodynamics, decreased blood LA, and increased survival.</u>

Fitzpatrick reported results of a controlled HS swine model, comparing resuscitation with HBOC-201 and Hextend and subsequent follow-up to 5 days. Initial fluid resuscitation with 12 ml/kg is 1.7-fold higher than in *RESUS.*[55] Similar to NMRC's and other less severe controlled HS models, hemodynamics were restored more rapidly with HBOC-201, but cardiac output was lower, markers of tissue perfusion were equivocal[49], and survival trended to be higher with HBOC-201 than Hextend animals (7/8 [88%] vs. 4/8 [50%]).

Patel reported results of a controlled HS/blunt TBI model in which after a 10 ml/kg bolus of saline, a single dose of HBOC-201 (6 ml/kg) was compared with standard fluids/care (rate of infusion not reported). [34] Subsequently, maintenance fluids were administered to maintain adequate MAP and CPP. In comparison with standard care, animals treated with HBOC-201 had higher BP, improved CPP and $brPO_2$, lower ICP, and equivocal CO and LA; 9/10 (90%) HBOC-201 animals vs. 0/5 (0%) standard care animals could be weaned from the ventilator (maintained adequate blood oxygen saturation). <u>In this study, a single 6 ml/kg HBOC-201 dose (similar to the *RESUS* dose of 7 ml/kg) significantly improved outcome in a model of combined HS and TBI.</u>

[49] Actually, blood LA was lower with HBOC-201 than control crystalloid fluid during simulated prehospital time; only control animals received blood transfusions during simulated in-hospital time, resulting in lower LA in controls by 300 minutes. [55]

Rosenthal reported results of a similar controlled HS/blunt TBI model in which after a single dose of HBOC-201 (6 ml/kg over 10 minutes) was compared with LR (12 ml/kg over 10 minutes) followed by LR maintain adequate CPP and replete intravascular volume. [25] Animals were followed for 6.5 hours. In comparison with LR, MAP, CPP, $brPO_2$ and Fluoro-Jade evidence of secondary brain injury was decreased with HBOC-201. In this study, a single 6 ml/kg HBOC dose (similar to the *RESUS* dose of 7 ml/kg) at a rate of 0.6 ml/kg/min (similar to the *RESUS* infusion rate of 0.71 ml/kg/min) significantly improved outcome in a model of combined HS and TBI.

In other published studies, infusion volumes were not always detailed but HBOC-201 infusions of 6 ml/kg over 5 minutes (i.e., 1.2 ml/kg/min) was reported with beneficial results. [30, 52].

Clinical dosing data

HEM-0115 - Maximum Dose Rationale

Justification for the proposed maximum dose of HBOC-201 in the *RESUS* trial should be predicated upon previous experience with HBOC-201 at that dose. In response to OBRR's questions, NMRC re-evaluated available clinical data regarding the proposed maximum dose. The majority of data with clinical use of moderate to high doses of HBOC-201 have been derived from the studies HEM-0114 and HEM-0115. In these studies, 2-6 and 2-10 unit doses of HBOC-201 were studied, respectively. In aggregate, there were 433 patients treated with HBOC-201 of which 85% of these subjects were treated with < 6 units of HBOC-201. Thus, only 15% of the total population was exposed to doses higher than 6 units, reflecting a relatively limited sample size for assessment of the impact of high dose HBOC-201. In HEM-0115 alone, 285/350 (81%) subjects were treated with < 6 units; only 65 (19%) of subjects received > 6 units[50]. For *RESUS*, NMRC has reasoned that if key adverse safety signals were similar or better in the HEM-0115 database of subjects treated with < 6 units, especially < 70 year olds, reasonably large databases, then sufficient safety data would be available to justify a maximum dose of 6 units in the proposed < 70 year old population to be enrolled in *RESUS*.

As shown in Table 49below, in support of our hypothesis, NMRC found that incidences of overall SAEs and SAEs in OBRR's "systems of concern" occurred in the following pattern:

[50] Lipase elevations were also lower in subjects receiving ≤ 6 vs. > 6 units of HBOC-201 (table below).

HBOC-201	N elevated Lipase	Mean	SEM	P
≤ 6 units	19/285	7%	0.01	0.003
> 6 Units	13/65	20%	0.05	

overall population was greater than the < 70 year old sub-population which was greater than the < 70 year old/< 6 unit maximum sub-population. Table 49 shows overall SAE and mortality incidence with further stratification based on age and dose; the data show that < 70 year old subjects receiving < 6 units of HBOC-201 consistently had similar or lower incidences of these adverse safety signals than subjects with older age and/or receiving higher doses. These data suggest that incorporating a maximum dose of 6 units in the *RESUS* protocol will minimize potential risk and maximize the predicted benefit:risk equation.

Table 49: SAEs and mortality in HEM-0115, stratified by age and dose
a.

	OVERALL	CARD	VASC	NERVOUS	RESP	RENAL/ URINARY	HTN
Overall pop	88/350 (25%)	22/350 (6%)	14/350 (4%)	5/350 (1%)	7/350 (2%)	6/350 (2%)	2/350 (1%)
< 70 y/o subpop	49/239 (21%)	8/239 (3%)	10/239 (4%)	2/239 (1%)	5/239 (2%)	5/239 (2%)	2/239 (1%)
< 70 y/o subpop and ≤ 6 U	33/192 (17%)	5/192 (3%)	6/192 (3%)	1/192 (1%)	1/192 (1%)	3/192 (2%)	1/192 (1%)

b.

Dose	> 6 U	Overall	≤ 6 U	> 6 U	Overall	≤ 6 U
	OVERALL SAEs			MORTALITY		
≥ 70 y/o subpop	9/18 (50%)	39/111 (35%)	30/93 (32%)	1/18 (5.6%)	8/111 (7.2%)	7/93 (7.5%)
Overall pop	25/65 (38%)	88/350 (25%)	66/285 (23%)	2/65 (3%)	10/350 (2.9%)	8/285 (2.8%)
< 70 y/o subpop	16/47 (34%)	49/239 (21%)	33/192 (17%)	1/47 (2.1%)	2/239 (0.8%)	1/192 (0.5%)

HEM-0115 - Rate Of Infusion Rationale

In order to provide clinical data regarding predicted safety of *RESUS Dosing Guidelines*, NMRC assessed dosing and rates of administration in HEM-0115 (for the first HBOC-201 administration including back-to-back infusions with up to 10 units). NMRC stratified subjects in the overall study population (N = 344 [data were unreliable for 6 subjects and were excluded]) and sub-populations of trauma (N = 32) and hypotensive (N = 29) subjects based on number of units administered, duration (hours), average rate (ml/min), and maximum rate at any time (ml/min); the maximum rate duration was not always ascertainable from the database. NMRC was not able to accurately define *rapid blood loss*; nevertheless, for the purpose of these analyses, NMRC assumed that subjects with more rapid infusion rates may have had *rapid blood loss.* NMRC compared *peak SBP responses* and AE/SAE rates in HBOC-201 and RBC subjects with higher maximum infusion rates during the first CTM administration in the overall HEM-0115 population.

HEM-0115 - Subject-Based Analysis

NMRC found that 79% (272/344) of subjects in the overall population in HEM-0115 received 2 units, akin to the planned initial dosing in the *RESUS* trial. In these subjects, the average infusion rate was 5.5 ± 0.42 ml/min, and the maximum infusion rate was 50.0 ml/min. Ten percent of subjects (N = 34) received > 4 units. In these subjects, average infusion rates were 7.4-14.1 ml/min; the maximum rate was 62.5 ml/min. Although only four HBOC-201 subjects had maximum infusion rates ≥ 40 ml/min (akin to default *RESUS* dosing of 50 ml/min [500 ml over 10 min]), there were 17 HBOC-201 and 14 RBC subjects who had maximum rates ≥ 25 ml/min during the first administration; and 30 and 17 subjects, respectively, during any CTM infusion. These sub-populations, with rapid maximum infusion rates, provided reasonable Ns (although small) on which limited safety analyses could be performed.

In these "rapid infusion" sub-populations, NMRC found that *peak SBP responses* were similar in the two treatment groups (Table 50). There were insignificant trends to higher *peak SBP responses* in HBOC-201 than RBC groups, but the difference (8.8-11.3 mm Hg) was similar in scope to (or possibly less than) the difference previously reported (17 mm Hg after the first infusion) for the HEM-0115 overall population. NMRC also found that potentially "vasoactivity-related" AE and SAE rates were not significantly different in the two treatment groups (Table 51).

Table 50*: Peak SBP responses* in HEM-0115 subjects with maximum infusion rates > 25 ml/min

	N (subjects)	Post-infusion peak SBP (mm Hg)			
		Mean	Maximum	SE	P
First Infusion					
HBOC	17	162.5	204.0	5.37	0.20
RBC	14	151.2	200.0	6.76	
All Infusions					
HBOC	30	165.7	216.0	4.34	0.26
RBC	17	156.9	200.0	6.66	

Table 51: AE and SAE rates in HEM-0115 subjects with maximum infusion rates > 25 ml/min

AEs	CARD		VASC		NERV		RESP		REN/URIN		HTN	
	HBOC	RBC	HBOC	RBC	HBOC	RBC	HBOC	RBC	HBOC	RBC	HBOC	RBC
First infusion	4/17 (24%)	4/14 (29%)	4/17 (24%)	3/14 (21%)	9/17 (53%)	5/14 (36%)	7/17 (41%)	2/14 (14%)	5/17 (29%)	4/14 (29%)	1/17 (6%)	2/14 (14%)
any time	7/30 (23%)	6/17 (35%)	9/30 (30%)	4/17 (24%)	17/30 (57%)	6/17 (35%)	12/30 (40%)	4/17 (24%)	7/30 (23%)	5/17 (29%)	4/30 (13%)	3/17 (18%)

SAEs	**CARD**		**VASC**		**NERV**		**RESP**		**REN/URIN**		**HTN**	
	HBOC	**RBC**	**HBOC**	**RBC**	**HBOC**	**RBC**	**HBOC**	**RBC**	**HBOC**	**RBC**	**HBOC**	**RBC**
First infusion	3/17 (18%)	2/14 (14%)	0/17 (0%)	0/14 (0%)	0/17 (0%)	0/14 (0%)	2/17 (12%)	1/14 (7%)	0/17 (0%)	0/14 (0%)	0/17 (0%)	0/14 (0%)
any time	3/30 (10%)	3/17 (18%)	1/30 (3%)	0/17 (0%)	1/30 (3%)	0/17 (0%)	2/30 (7%)	1/17 (6%)	0/30 (0%)	0/17 (0%)	0/30 (0%)	0/17 (0%)

HEM-0115 - Infusion-Based Analysis

NMRC noted that a limited number of subjects had repeated recordings of maximum infusion rates $\geq$ 25 ml/min. In order to maximize capture of available safety data, NMRC also compared the difference between pre- and post-infusion SBP values for all rapid infusions. This provided data on 168 HBOC-201 vs. 94 RBC *rapid infusions*. Some subjects contributed to this analysis more than once. NMRC found that mean pre-infusion SBP (131 vs. 115 mm Hg, $p < 0.0001$), immediate post-infusion SBP (136 vs. 118 mm Hg, $p < 0.0001$), and SBP Δ (5.5 vs. -1.6 mm Hg, $p = 0.02$), were all higher in HBOC-201 than RBC groups (Student t tests). But as noted above for the SBP response analysis in subjects with rapid infusion rates, this difference between HBOC-201 and RBC subjects was similar in scope to that seen in the overall population.

NMRC recognizes that these analyses have significant scientific and *RESUS*-relevance limitations (although they provide the best available *RESUS*-relevant dosing safety data). First, *rapid* bleeding could not be accurately defined from the HEM-0115 database. Second, the actual duration of rapid infusion rates could not always be identified. Third, analyses are based on small Ns (although similar to typical Phase 2 trial data). Fourth, these analyses are *post hoc*. Fifth, the group comparisons are against gold standard RBC rather than crystalloid solution.

Nevertheless, these data suggest that *peak SBP responses* and rates of potentially "vasoactivity related" AE and SAE rates were not different in the sub-population of subjects with more rapid infusions than in the overall HEM-0115 sub-population. These data confirm our prior clinical HEM-0115 and preclinical HS study observations—that the preponderance of the HBOC-201 *SBP response* occurs after the first infusion, with limited additional effects subsequently (~ dose-independent).

HEM-0125 - Interim dosing data from the S. Africa traumatic HS trial

Interim analysis of safety data, comparing HBOC-201 and RBC transfusions in the S. Africa HEM-0125 trauma trial (Appendix E), reveals equivalent mortality (4/9 [44%] vs. 4/10 [40%],

respectively), and trends to decreased incidence of AEs/subject (9.1 vs 17, respectively [p = 0.12]), SAEs/patient (1.0 vs. 1.4, respectively [p = 0.58]), asanguinous fluid requirement/subject (15,716 ± 2,570 vs. 30,242 ± 8,088, respectively [p = 0.12]), and RBC transfusion requirement/subject (5.4 ± 1.2 vs. 16.8 ± 5.7, respectively [p = 0.08]). As shown in Table 52, dose and rate of HBOC-201 infusion were similar to *RESUS Dosing Guidelines*. These results in patients with severe traumatic HS, suggesting improved outcome in a high-bar comparison with gold-standard RBC transfusions with individual and total doses and infusion rates, similar to those proposed in *RESUS*, provide additional clinical support for *RESUS Dosing Guidelines*.

Table 52: Comparison Of Interim Dosing Data In HEM-0125 with RESUS Dosing Guidelines

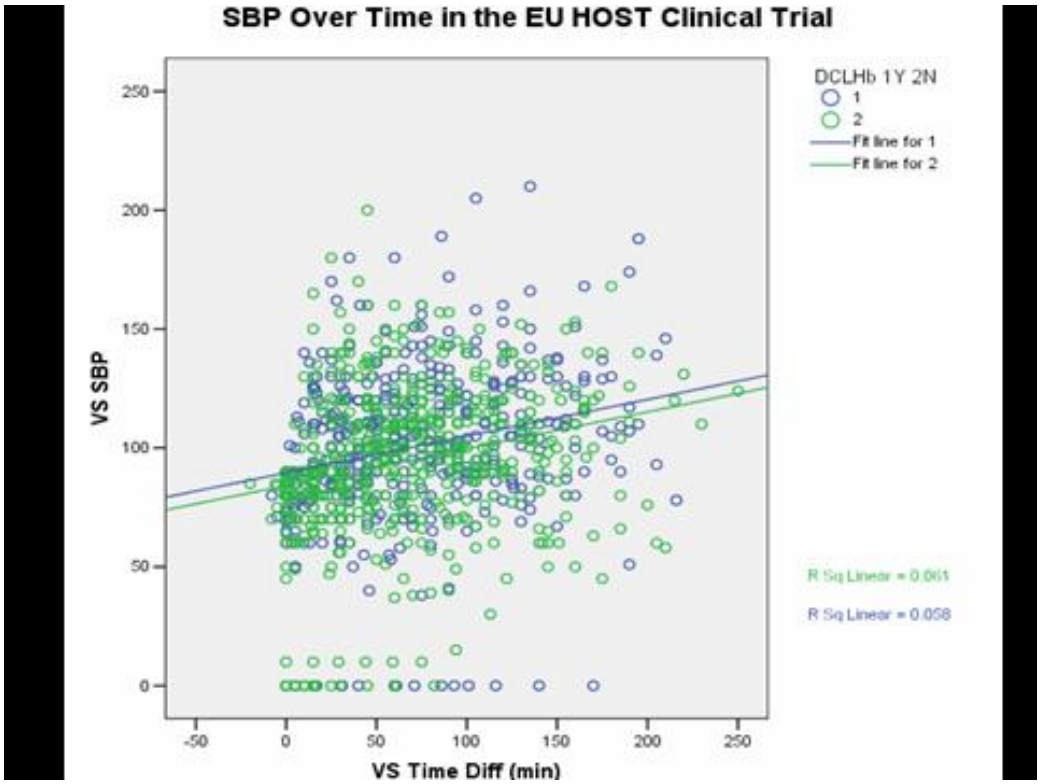
	HEM-0125	***RESUS***
Volume (ml)	304.7± 54.7	500
Duration (min)	17.7 ± 4.1	10
Rate (ml/min)	73.1 ± 14.6	50

Dosing Rationale Summary

These analyses of HEM-0115 infusion data provide clinical safety data supporting the *RESUS Dosing Guidelines* recommendation of two units of HBOC-201 with default infusion duration of 10 minutes. The trend to an improved safety profile in HBOC-201 vs. RBC subjects receiving high doses of HBOC-201 at rapid rates in the ongoing HEM-0125 ER trauma trial in S. Africa provides additional supportive data. But it should be noted that the rationale for *RESUS Dosing Guidelines* was mainly based on efficacy and safety data from indication-specific preclinical HS swine studies, in which infusion rates of 0.5-1.0 ml/kg/min and much higher infusion rates improved hemodynamics, tissue oxygenation, and survival without causing severe BP responses, compromising organ flow, or increasing hemorrhage.

Moreover, best-fit BP curves from the DCLHb and saline groups in the HOST trial (in which up to 1,000 ml of DCLHb was administered in the prehospital setting) could almost be superimposed on each other, suggesting absence of adverse vasoactive responses even with this highly vasoactive HBOC (below, slide # 23 of Dr. Sloan's slide presentation in NMRC's Pre-Meeting Information Package, 14 Nov 2005).

Figure 6: SBP in the DCLHb HOST trial



Thus, a reasonable amount of HBOC-201 clinical data (mostly non-trauma data in HEM-0115 and some trauma data from the HEM-0125 S. Africa ER trial), extensive HBOC-201 preclinical data in HS, and prehospital clinical data with the more vasoactive HBOC, DCLHb, suggest that rapid infusions of HBOC-201 are likely to be efficacious and *relatively* safe in the *RESUS*.

7.c Benefit:Risk And Risk Mitigation

RESUS Survival Benefit Estimate

OBRR has raised concern about the accuracy of the predicted survival effect size (benefit) for subjects enrolled in *RESUS*. The effect size was estimated integrating four datasets: (1) clinical Phase 1-3 clinical surgery/orthopedics non-trauma trial data (mainly HEM-0115); (2) interim clinical Phase 2 trauma data from HEM-0125; (3) recognized databases of trauma epidemiology; and (4) preclinical animal data in HS studies. (1) Knowing that the prior surgery/orthopedics trials show *reasonable* risk

in the context of the high-mortality *RESUS* trial, and (2) that interim safety data from the S. Africa trauma trial are favorable, NMRC believes that available clinical data do not conclusively predict mortality benefit or harm in *RESUS*[51]. With these observations in mind, and (3) confidence that *RESUS* inclusion/exclusion criteria ensure enrollment of a relatively homogeneous trauma population (NTDB and UAB/UMD databases), (4) preclinical HS data were used to predict *RESUS* effect size after conservative reduction in estimated benefit.

Specifically, the 15% effect size was conservatively estimated with a 5-fold margin of error, based on data from multiple preclinical swine HS studies [12, 17, 18, 20, 23, 24, 28], showing highly significant reduced mortality benefits in HBOC-201 vs. standard fluid-resuscitated animals (overall HS effect size = 74.6%, $p < 0.0001$; severe HS effect size 82%, $p < 0.0001$). Despite limitations of extrapolation from animal studies, the numerous HS models studied, primarily in swine, are standard models in the literature used to study HS. These models are considered appropriate and reasonably predictive of cardiovascular human responses in HS [42, 74-77]. Although precise extrapolation cannot be expected, it is predicted that the animal results will translate to similar responses in humans in the *RESUS* study.

***RESUS* Protocol Risk Mitigation Strategies**

Numerous risk mitigation strategies have been incorporated into the *RESUS* protocol (Appendix C and Appendix D). Some, in fact, were specifically recommended by OBRR. These approaches are intended to maximize benefit by optimizing target population selection and to minimize risk via standardization of clinical care with practice guidelines and extensive training; allowance for concomitant standard care; and surveillance methods for early detection and action regarding adverse safety signals (Appendix C: *RESUS* protocol details; Appendix A: mortality analysis and risk mitigation by age; and Appendix D: the *in-hospital trauma care guideline*).

1. **Target population with severe HS (maximize safety):** As preclinical studies demonstrate that HBOC-201 has the highest effect on survival in severe HS models [17, 18, 23, 24], *RESUS* enrollment criteria target patients with severe HS (but potentially survivable injuries) using hypotension (SBP < 90 mm Hg) and RTS (1 to less than 5) inclusion criteria. The RTS criterion was initially added at OBRR's recommendation to optimize enrollment of an intermediate

[51] That interim analysis of the ongoing S. Africa ER trauma trial does not show group differences in mortality despite HBOC-201 comparison with gold standard RBC transfusions provides circumstantial support for a prediction of decreased mortality with HBOC-201 in comparison with LR in *RESUS*.

severity HS population, and excluding subjects likely to die (RTS < 1) or live irrespective of the study intervention. [117] NMRC thanks OBRR for that protocol optimization.

2. **Exclusion of elderly:** As group differences in key adverse safety signals (especially cardiac and cerebral ischemic) were highest in ≥ 70 year old subjects in HEM-0115, and lower or inapparent in < 70 and < 50 year old sub-populations, potential risk has been minimized in *RESUS* by excluding the population predicted to have the highest *a priori* risk (due to higher incidence of pre-existing atherosclerotic disease). By excluding the elderly, it can be predicted with more certainty that *relative* risk of cardiac and cerebral ischemic AEs will be significantly lower than in HEM-0115, nonexistent, or reversed.
3. **Target population without access to blood transfusions (maximize safety):** As preclinical studies demonstrate that HBOC-201 improves physiologic parameters and survival in comparison with asanguinous resuscitation fluids (comparisons with blood transfusions have not been systematically done for HS), NMRC's prediction of favorable benefit:risk is predicated on absence of availability of blood transfusions. Thus, *transfusions are available* is included as a *RESUS* exclusion criterion, HBOC-201 infusions are to be started only during the prehospital setting (EMS systems carrying blood transfusions are excluded). In response to OBRR's request that the *transfusions are available* exclusion criterion be more specifically defined, a default recommendation of ≥ 10-15 minutes *expected transport time* was incorporated in the protocol (sufficient time for ≥ 1 CTM dose), effectively shifting prehospital time in *RESUS* from mostly rapid transport in urban trauma to a subset of more delayed urban as well as rural trauma. Inclusion of the *transfusions are available* exclusion criterion is in contradistinction to prior HBOC-201 Phase 2/3 trials (in which HBOC-201 and RBC transfusions were compared), the U.S. in-hospital Phase 3 DCLHb trial (in which HBOC was infused as an adjunct to blood transfusions) [40], and the recently completed PolyHeme® Phase 3 trial (in which HBOC was continued to the exclusion of blood transfusions for 12 hours post-hospital arrival).
4. **Fluid re-infusion criteria (maximize safety):** Preclinical HS studies at NMRC demonstrated that with HBOC-201, hypotension and tachycardia are sensitive markers of initial fluid infusion requirements, but tachycardia is a more consistent marker than hypotension for fluid re-infusion requirements.[12] Moreover, outcome has not been improved in preclinical HS HBOC studies which used exclusively BP-controlled models. Consequently, in *RESUS*, hypotension alone will suffice for initial CTM infusion, but either severe hypotension alone (SBP < 90 mm Hg) or moderate hypotension (SBP 90-99 mm Hg) and concomitant tachycardia (HR ≥ 100 bpm) will meet fluid re-infusion criteria. This approach is supported by published observations that the

combination of hypotension and tachycardia predicts outcome in trauma patients. [118] NRMC thanks OBRR for their critiques that led to addition of the tachycardia re-infusion criterion, further diminishing risk of fluid under-resuscitation due to vasoactivity.

5. **Thorough training (maximize safety):** Comprehensive training of EMS and trauma center personnel regarding risks of potentially "vasoactivity-related" hypertensive AEs and SAEs, and hypoperfusion-related AEs (especially cardiac system, cerebral ischemic, and oliguria), with specific warnings to avoid *being fooled* by potentially higher BP responses in HBOC-201 subjects, and to use all available markers of tissue perfusion to guide fluid therapy in accordance with *prehospital fluid infusion guidelines* (Appendix C: *RESUS* protocol details) and *in-hospital trauma care guidelines* (Appendix D).
6. **Standard IV fluids (minimize risk):** In order to further minimize risk of under- resuscitation due to EMTs potentially being misled by higher BP responses in HBOC-201 subjects, EMS training also includes specific delineation about other clinical signs of HS EMS personnel should use to guide standard fluid infusions (when neither hypotension/tachycardia criteria are met for CTM infusion per standard PHTLS/ATLS).
7. **Standardization of prehospital care (minimize risk):** In order to standardize EMS care across EMS systems and maximize adherence to the risk mitigation strategies described herein, *prehospital fluid infusion guidelines* have been developed and incorporated into the EMS training program. NMRC thanks OBRR for their critiques that led to incorporation of the *prehospital fluid infusion guidelines* in the *RESUS* protocol.
8. **Standardization of in-hospital trauma care (minimize risk) (proposed):** In order to standardize in-hospital trauma care across trauma centers, *in-hospital trauma care guidelines* have been developed. These guide transfusion, fluids, and inotrope use in *RESUS*. As lower relative Hb levels (anemia) were seen in HBOC-201 subjects in HEM-0115, and anemia appeared to be associated with increased rates of cardiac AEs, standardization of transfusion triggers and frequent assessment of blood oxygen content (Hb/hematocrit/metHb) will diminish risk due to inadequately corrected anemia. Similarly, standardization of fluid resuscitation endpoints (and rare inotrope use) use will diminish risk due to potentially "vasoactivity-related" under-resuscitation (hypoperfusion) and fluid overload (CHF/pulmonary edema). NMRC thanks OBRR for their critiques that led to incorporation of the *in-hospital trauma care guidelines* in the *RESUS* protocol.
9. **Prospective coding definitions (minimize risk):** In order to ensure accurate potentially "vasoactivity-related" safety reporting, prospectively defined coding criteria were defined for

increased BP and *hypertension* (SBP ≥ 150 mm Hg). This will allow prompt response (if needed), further diminishing potential risk.

10. **Secondary outcome measurements (minimize risk):** In order to maximally understand the clinical impact of HBOC-201's vasoactivity, the *RESUS* protocol includes detailed assessment of clinical markers directly or indirectly related to hypoperfusion (including standard LA, BD, LA clearance, urine output, PCWP and CVP measurements (when available), fluid volumes, ECG, and organ specific labs (e.g., troponin-I, creatinine); and non-standard $tcPO_2$ monitoring and unmeasured ions). This will allow prompt response (if needed), further diminishing potential risk.
11. **Trial stopping criteria (minimize risk):** In order to further diminish risk related to potential hypoperfusion, increased rates of elevated blood LA (in absence of increased survival) is included as a prospectively defined *RESUS* safety stopping criterion.
12. **CTM stopping criteria (minimize risk):** In response to OBRR's concern about potential for severe BP responses and to diminish risk of hypertension SAEs (occurred in 2/350 [0.6%] of HBOC-201 subjects in HEM-0115), individual subject BP stopping criterion (SBP ≥ 120 mm Hg) was included in the *RESUS* protocol[52].
13. **Expedited AE reporting (minimize risk):** As per OBRR's request, all related AEs in potentially "vasoactivity-related" *organ systems of interest* will be reported as *expedited AEs* to the DMC and OBRR. This will allow prompt response (if needed), further diminishing potential risk.
14. ***Hypoperfusion markers reports* (minimize risk)**: In response to OBRR's concerns and to further diminish risk of potentially "vasoactivity-related" hypoperfusion, serial *hypoperfusion markers reports* will be provided to the DMC and FDA within ~ 1 month of enrollment of every ~ 10-12 HBOC-201 subjects to (until the second *ESR* [222 subjects]), reporting key potentially hypoperfusion-related clinical markers (i.e., LA, BD, and prehospital fluid infusion volume)[53].
15. ***ESRs* (minimize risk):** Early *ESRs* (interim analyses) will occur after only 50 and 222 total subjects (25 and 111 HBOC-201 subjects). This will allow prompt response (if needed), further diminishing potential risk.

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[52] The CTM stopping criterion was decreased from ≥ 150 to ≥ 120 mm Hg in response to critiques by pre-BPAC reviewers (Jul 2006) that 150 mm Hg may be too high to prevent spurious BP elevations, and potentially allowing disruption of evolving clots in uncontrolled hemorrhage.

[53] LA and BD are validated and well accepted clinical surrogates for prediction of outcome in HS. [119-121] Preclinical studies suggest similar associations with HS resuscitation with HBOC-201 (Section 5.c).

Integrated Assessment Of Benefit:Risk In RESUS

Introduction/Background

The overriding concern raised by OBRR is related to the vasoactive properties of HBOC-201 and the potential for HBOC-201 to cause relatively higher BP responses and other potentially "vasoactivity-related" AEs and SAEs. OBRR has noted that commonly seen higher BP responses (e.g., hypertension AEs in 43/350 [12.3%] HBOC-201 vs. 18/338 [5.3%] RBC subjects, p = 0.002), even if not severe, might mislead ambulance personnel and physicians about their patient's fluid status, and result in inadequate fluid resuscitation, resulting in secondary complications of hypoperfusion. OBRR has also raised concern about less commonly observed severe BP responses (i.e., hypertension SAEs in 2/350 [0.6%] HBOC-201 vs. 0/338 [0%] RBC subjects, p = 0.5).

Specifically, OBRR has noted signals of cardiac system AEs (102/350 [29%] HBOC vs. 58/338 [17%] RBC subjects, p = 0.0002), vascular system AEs (101/350 [29%] HBOC vs. 51/338 [15%] RBC subjects, p = 0.0001), respiratory system (97/350 [28%] vs. 70/338 [21%], p = 0.03), and renal system AEs (87/350 [25%] vs. 64/338 [19%], p = 0.07), cardiac system SAEs (22/350 [6%] vs. 9/338 [3%], p = 0.03), cerebral ischemic AEs (7/350 [2%] vs. 2/338 [0.6%], p = 0.07), heart failure/fluid overload AEs (8/350 [2.3%] vs. 1/338 [0.3%], p = 0.04), cardiac arrest AEs (8/350 [2.3%] vs. 2/338 [0.6%], p = 0.1), and cardiac troponin elevations (18/136 [13.2%] vs. 2/122 [1.6%], p = 0.0003). OBRR has concluded that these adverse signals are likely related to HBOC-201 vasoactivity and BP effects; and that higher relative incidences of these adverse signals are likely to be seen in the *RESUS* trial as well.

Despite completion of OBRR-directed IND-optimizing preclinical studies; extensive modifications of the protocol, IND, and IB; and addition of extensive risk mitigation strategies, NMRC and OBRR have not yet been able to come to a consensus about the predicted benefit:risk balance for subjects in the proposed *RESUS* trial.

Adequacy Of Information To Assess Benefit:Risk In *RESUS*

In Mar 2006, OBRR informed NMRC, *Your proposed study under this IND remains on Clinical Hold because (1) there is inadequate information to assess whether the risks and benefits associated with HBOC-201 administration in the ambulance setting are reasonable in relation to what is known about the risks and benefits of standard therapy [21CFR 50.24(a)(3)(iii)].*

NMRC disagrees with OBRR's conclusion that there is inadequate information to predict benefit:risk in the *RESUS* trial. HBOC-201 will be compared to LR where blood transfusions are unavailable. In addition to numerous indication-specific preclinical studies in HS showing significant survival benefits, these same studies consistently demonstrated important physiologic benefits that should be considered in the overall benefit analysis [12, 17, 20, 23, 24, 31, 55] (Table 8). NMRC submits that the potential benefit of survival improvement in comparison to the risk of adverse events related to increases in BP can be adequately assessed given the currently available data.

Preclinical Database

In Mar 2006, OBRR further stated, *Your proposed study under this IND remains on Clinical Hold because…(2) information derived from preclinical studies do not support the potential for the intervention to provide a direct benefit to subjects enrolled in RESUS [21CFR 50.24(a)(3)(ii),…*

NMRC disagrees with OBRR's conclusion because HBOC-201 resuscitation has improved physiologic variables and/or survival in almost all preclinical HS studies in comparison with standard fluids. Moreover, OBRR has previously acknowledged benefits seen with HBOC-201 in preclinical HS studies[54]. NMRC agrees that overall prediction of benefit:risk in *RESUS* requires a balance assessment of preclinical and clinical data in the context of the medical condition of subjects. However, NMRC asserts that the preclinical HS database sufficiently supports the 21 CFR 50.24 requirements that *animal and other preclinical studies…support the potential…to provide a direct benefit to…subjects*[55]:

- *In vitro* studies demonstrated that HBOC-201 efficiently transports oxygen. [35, 36]

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[54] **04 May 2004:** *…both studies show that HBOC-201 administration significantly improves survival compared to asanguinous/no fluid administration in the setting of uncontrolled HS in this animal model.*
08 Jul 2005: *We agree that the two studies in animal models of uncontrolled HS and uncontrolled HS with concomitant traumatic brain injury…show a survival benefit.*
03 Oct 2005: *…FDA takes note of the favorable outcome in the two preclinical studies cited.*

[55] NMRC maintains that the preclinical efficacy/safety database in HS is likely to predict outcome in human subjects with HS in *RESUS* because most of the data are from swine, considered an appropriate animal model. As reported by the United States Department of Agriculture and experts in the field of swine biomedical research, swine are recognized as a *suitable animal model for human disease based upon their comparative anatomy and physiology.[42-44]* It is also acknowledged that large animal (e.g. swine and sheep) models are more suited for pre-clinical evaluation of resuscitation product research than small animal (e.g. rat and mice) models. [76] Swine are used extensively in biomedical research (e.g. cardiovascular) because they share *anatomic and physiologic characteristics with humans that make them a unique and viable model for biomedical research.* [44, 122-124]

- *In vivo* indication-specific animal HS studies redundantly confirmed beneficial effects on outcome in multiple animal models (Table 37).
    - The efficacy/safety database of HBOC-301 (a less polymerized but related HBOC) was sufficiently persuasive for prior FDA approval for treatment of anemia in dogs.
    - The main body of evidence demonstrating beneficial effects on outcome in preclinical HS studies is in *swine* models, considered appropriate for prediction of human responses in cardiovascular studies such as HS.[76]

An assessment of HBOC-201 effects on the most important outcome measurement in preclinical HS studies, survival, is illustrative. In a combined analysis of pertinent HS studies (representing 229 pigs), including severe as well as less severe HS models, survival was significantly improved with HBOC-201 in comparison with standard fluids (103/117 [88%] vs. 59/112 [53%] respectively, $p < 0.0001$). Even in mild HS models alone (e.g., LD < 50%), many of which were not designed or powered to assess survival, physiologic variables have been consistently improved, typically with associated trends to improved survival. [20, 27, 29-31, 52, 54] In a combined analysis of only mild HS models, there was still a trend to improved survival with HBOC-201 (68/75 [91%] vs. 56/69 [81%], respectively, $p = 0.14$). In almost all severe HS models (e.g., LD > 50%), which more closely resemble the targeted population in *RESUS*, physiologic variables and survival have been significantly and dramatically improved. [17, 18, 23, 24, 53] In a combined analysis of severe HS models, survival differences were highly significant and dramatic (35/42 [83%] vs. 3/43 [7%], respectively, $p < 0.0001$).

Moreover, in preclinical HS studies comparing HBOC-201 and standard resuscitative fluids, in addition to survival benefits, animals resuscitated with HBOC-201 had consistent systemic and neurophysiologic benefits with minimal adverse safety signals (clinically insignificant or individually addressed by mitigation strategies in the *RESUS* protocol).

Summary Results Of NMRC Preclinical HS Studies (HBOC-201 Vs. Standard Fluids)

- **Efficacy**
    - **All models**
        - Improved hemodynamics, cutaneous tissue oxygenation, and blood transfusion requirements.
    - **Severe HS models**
        - Decreased LA and BD, and improved survival (as below).
- **Safety**
    - **Overall outcome**
        - Improved survival.

        - Equivocal activity and feeding scores.
- **Hemodynamics and physiology**
    - Mild to moderately elevated MAP, MPAP, and SVR; mildly decreased HR.
    - Mildly decreased fluid requirements, CO[56], and urine output mainly in less severe HS.
        - Equivocal with more severe HS. CO improved with severe HS (HS/TBI)
    - Equivocal effects on MVO_2, DO_2, VO_2, O_2ER.
    - No oliguria.
    - Mildly decreased oxygen desaturation (< 5%) (due to right shift of HBOC-201 dissociation curve)[57].
- **Blood chemistry**
    - Transiently increased LFTs.
    - Slightly increased BUN and creatinine.
    - Equivocal cardiac troponin.
- **Hematology**
    - **CBC:** Increased Hb, low hematocrit (due to blood transfusion avoidance), equivocal WBC and platelets, mild methemoglobinemia (≤ 5%)[58].
    - **Hemostasis/coagulation:** Equivocal PT, PTT, bleeding time, TEG, PFA-100 (*in vitro* bleeding time), and platelet aggregation.
        - Mildly decreased prehospital hypocoagulation (due to decreased hemodilution).
        - Mildly increased in-hospital hypocoagulation (due to decreased blood transfusion requirements).
- **Immunology**
    - Equivocal plasma cytokines (IL-1, IL-2, IL-4, IL-6, and IL-10), immunophenotype (CD4, CD8, and CD4:CD8 ratio), and neutrophilic and lymphocytic adhesion markers (β-1 integrins [CD11 and CD18] and α-4 integrins [CD49]).
- **Histopathology**
    - Equivocal (2 models) or improved (2 models) myocardium, equivocal pulmonary edema.
    - Equivocal lung, hepatic parenchyma, jejunum, and renal cortex/medulla.
    - Mild hepatobiliary changes. Mild renal papillary necrosis (1 of 3 models only).
- **Oxidation potential**
    - Equivocal tissue peroxynitrate production (3-nitrotyrosine staining) in myocardium, lungs, liver, jejunum, and kidneys.
- **Neurophysiology and histopathology (HS/TBI only)**
    - Increased CPP, $brPO_2$, and SSO_2sat; slightly increased ICP; equivocal CBF; decreased SSLA; decreased intracranial hemorrhage; other histopathology equivocal.

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[56] NMRC's perspective on OBRR's BPAC package: in preclinical HS studies, CO was usually lower in HBOC-201 than control animals in less severe HS models in which fluid volumes tended to be lower. In severe HS models, CO was usually not significantly different and sometimes higher with HBOC-201. [12, 17, 18] Fluid volumes were lower in less severe HS models mainly because of artificial model constraints added to maximize reproducibility of the data (standardization). In *RESUS*, fluid infusion volume will be dictated by multiple clinical parameters without specific constraints (prehospital *fluid re-infusion guidelines* and *in-hospital trauma care guidelines*). Thus, risk of clinically significant decreased CO in *RESUS* is low.

[57] NMRC's perspective on OBRR's BPAC package: in preclinical HS studies, oxygen desaturation was minimal (< 5%) as long as supplemental oxygen was administered. Potentially clinically significant oxygen desaturation (~ 20%) only occurred when supplemental oxygen was withheld. As supplemental oxygen is routine in trauma [125], risk of clinically significant oxygen desaturation is low in *RESUS*. Nevertheless, *RESUS* EMS and trauma center training modules educate practitioners about this risk, risk mitigation strategies, and methods to clarify potential diagnostic ambiguity.

[58] Methemoglobinemia has been low and clinically insignificant in preclinical and clinical studies (≤ 5%). But there is a theoretical risk that intravascularly depleted subjects receiving large doses of HBOC-201 in *RESUS* will have higher metHb levels. Thus, in *RESUS*, metHb levels will be closely monitored, and a separate and comprehensive education section focusing on evaluation/treatment of methemoglobinemia is included in the trauma center training module.

In summary, preclinical HS studies are reasonably predictive of beneficial effects on systemic physiology and neurophysiology as well as high survival benefit with an overall survival effect size of 74.6% and 82% in severe HS studies*;* thus, the *RESUS* estimated 15% effect size is conservatively estimated. Mortality in the *RESUS* targeted population with current standard of care is unacceptably high (*RESUS* prediction: ~ 1 in 2). Even if the *RESUS* mortality prediction for patients with severe HS is inaccurate and mortality is lower, for example 1 in 3 or 4, it is still unacceptably high and confirms need for improved therapy. Prior HBOC-201 trials in other clinical settings have demonstrated efficacy and a *reasonable* safety profile (especially with exclusion of the elderly) when considered in the context of potential predicted benefit:risk in HS where blood transfusions are unavailable.

NMRC's Prediction Of Benefit:Risk In *RESUS*

NMRC appreciates that there are significant risks associated with participation in the proposed *RESUS* trial and these risks are especially important considerations in an *EIC* trial. In particular, NMRC agrees with OBRR that the aforementioned adverse safety signals from prior HBOC-201 trials are concerning. However, NMRC contends that non-serious AEs should not be weighted significantly in overall consideration of benefit:risk prediction in the context of very high mortality in the *RESUS* trial. In this context, NMRC maintains that *risk is reasonable* (21 CFR 50.24) in *RESUS*. NMRC's assessment is that because indication-specific HS preclinical studies have shown overwhelmingly beneficial effects on survival (rationale for conservative prediction of 15% mortality reduction), *relatively* minor increases in SAE rates and larger increases in non-serious AE and laboratory abnormality rates seen in non-elderly subjects in prior HBOC-201 trials do not outweigh predicted benefits for subjects enrolled in *RESUS*[59]. These issues are addressed in detail below.

HBOC-201's Safety Profile In Previous Surgery/Orthopedics Clinical Trials

For the purpose of predicting benefit:risk in *RESUS*, HBOC-201 AEs can be categorized as likely or unlikely to be clinically significant in *RESUS*. Some observed AEs were characteristic side effects of HBOCs (e.g., transient elevation of LFTs and lipase, jaundice and skin discoloration, gastrointestinal symptoms such as abdominal discomfort, higher BP responses, mild methemoglobinemia, mild oxygen desaturation [with normal oxygen tension], and oliguria); NMRC predicts that many of these

[59] NMRC contends that SAE differences seen in prior trials (e.g., 7.7% excess of overall SAEs in HEM-0115) may not be *relatively minor* for prediction of benefit:risk in elective indications; however, our use of the phrase *relatively minor* is in the context of expected high mortality in control and subjects and significant survival benefit in HBOC-201 subjects *(i.e., reasonable in relation to what is known about the medical condition…* [21 CFR 50.24]). In fact, early in the pre-IND process, OBRR agreed that *…emergency field trauma and elective orthopedic surgery have different risk-benefit profiles* (04 May 2004).

types of AEs are likely to occur irrespective of the type of trial and comparator (e.g., prior elective surgery/orthopedics and RBC vs. HS and LR in *RESUS*). Thus, it is likely that relatively increased rates of these types of AEs (and rare SAEs) will be seen in *RESUS* as well. However, as aforementioned, because these typically *non-serious AEs* were rarely or minimally clinically important, they do not appreciably affect overall benefit:risk prediction for high mortality indications, such as resuscitation from severe HS in *RESUS*. These AEs are more clearly concerning when considering HBOC-201's benefit:risk as a *blood substitute* intended to replace RBC transfusion and/or for elective indications, but neither of these two conditions is relevant to *RESUS*.

NMRC concurs that potentially more serious adverse safety signals must be considered in prediction of benefit:risk in *RESUS*, especially the observed excess incidences of key adverse safety signals in HEM-0115. But akin to the other AEs mentioned above, some of these adverse safety signals were non-serious (e.g., most hypertension AEs) or not clinically relevant (e.g., isolated troponin elevations) and thus do not appreciably alter predicted benefit:risk in *RESUS*.

Data from the ITT assessment of the Phase 3 HEM-0115 orthopedic trial comparing HBOC-201 to RBC, demonstrated excess incidences of the following key adverse safety signals in HBOC-201 subjects (whether significant or not).

1. **Overall SAEs:** The incidence of overall SAEs was higher with HBOC-201 than RBC (88/350 [25.1%] vs. 59/338 [17.5%], respectively, p = 0.02) → delta 7.7%.
2. **Cardiac system SAEs:** The incidence of cardiac System Oriented Class (SOC) SAEs was higher with HBOC-201 than RBC (22/350 [6.3%] vs. 9/338 [2.7%], respectively, p = 0.03) → delta 4%.
    a. **MI AEs:** The incidence of MI SAEs was similar with HBOC-201 and RBC (4/350 [1.1%] vs. 2/338 [0.6%], respectively, p = 0.7) → delta 0.7%.
    b. **Cardiac troponin elevations:** The incidence of troponin elevations was higher with HBOC-201 than RBC (18/136 [13.2%] vs. 2/122 [1.6%], respectively, p = 0.0003) → delta 11.6%.
    c. **Heart failure/fluid overload AEs (Combination 22):** The incidence of Combination 22 AEs was higher with HBOC-201 than RBC (8/350 [2.3%] vs. 1/338 [0.3%], respectively, p = 0.04), → delta 2%.
    d. **Cardiac arrest AEs (Combination 20):** The incidence of Combination 20 AEs was higher with HBOC-201 than RBC (8/350 [2.3%] vs. 2/338 [0.6%], respectively, p = 0.1) → delta 1.7%.

3. **Cerebral ischemic AEs:** The incidence of cerebral ischemic (CVA/TIA/RIND) AEs was higher with HBOC-201 than RBC (7/350 [2%] vs. 2/338 [0.6%], respectively, p = 0.07) → delta 1.4%.
    a. **CVA AEs:** The incidence of CVA AEs was higher with HBOC-201 than RBC (6/350 [1.7%] vs. 0/338 [0%], respectively, p = 0.03) → delta 1.7%.
4. **Hypertension AEs and SAEs:** The incidence of hypertension AEs was higher with HBOC-201 than RBC (43/350 [12.3%] vs. 18/338 [5.3%], p = 0.002) → delta 7%. Hypertension SAEs were similar in the two groups (2/350 [0.6%] vs. 0/338 [0%], respectively, p = 0.5) → delta 0.6%.
5. **Mortality:** Mortality was similar with HBOC-201 and RBC (10/350 [2.9%] vs. 6/338 [1.8%], p = 0.2) → delta 1.1%.

NMRC appreciates OBRR recommendations that have led to comprehensive analyses of safety data from prior HBOC-201 trials and consequent incorporation of numerous strategies in the *RESUS* protocol to minimize potential risk related to these potential adverse safety signals. Where NMRC and OBRR have disagreed is in the extrapolation of HBOC-201's safety profile from prior surgery/orthopedics clinical trials to prediction of benefit:risk in *RESUS*. OBRR has contended that *a priori* predicted risk is too high or inadequately mitigated in the trial design. In contrast, NMRC has reasoned that *a priori* predicted risk from prior HBOC-201 trials is lower than has been suggested by OBRR (and further reduced by trial design mitigation strategies) and therefore *reasonable* for the following reasons (see Assumption #5, 6, and 7):

1. **Survival affects efficacy and safety:** Accurate assessment of risk includes weighing of known and reasonably likely *risks* against known and reasonably likely *benefits*. As preclinical HS studies have demonstrated beneficial effects on survival, consideration of adverse safety signals from prior HBOC-201 trials (in which the only potential benefit was blood transfusion avoidance) without consideration of predicted survival benefit in *RESUS*, inaccurately predicts benefit:risk in *RESUS*.
2. **Extrapolation of clinical data from prior surgery/orthopedics trials:** Extrapolation of safety data from overall populations in ITT analyses from prior surgery/orthopedics HBOC-201 trials for prediction of *relative* risk in *RESUS* is circumstantial and likely inaccurate based on rationale summarized below. In fact, early in the pre-IND process, OBRR agreed that *...emergency field trauma and elective orthopedic surgery have different risk-benefit profiles* (04 May 2004).

a. **Different indications and potential benefits**: HBOC-201 was evaluated with an indication with minimal potential benefit in the U.S. in prior trials—transfusion avoidance (vs. increased survival in *RESUS*).

b. **Different exposures**: HBOC-201 was evaluated as a blood substitute with prolonged exposure (over days) in prior HBOC-201 trials (vs. minutes in *RESUS*).

c. **Different populations:** HBOC-201 was evaluated in older populations—mean age 61 years old in the prior Phase 3 HEM-0115 trial (vs. a younger population in *RESUS*—expected mean age ~ 35 years old).

d. **Different physiologic states:** HBOC-201 was evaluated in mainly hemodynamically stable populations in prior trials (vs. hypotensive population in *RESUS* in which "higher" BP responses would be unlikely to be clinically adverse).

e. **Different comparators:** HBOC-201 was evaluated against a gold standard of RBC transfusion in prior trials—a *high bar* to pass (vs. non-oxygen carrying LR in *RESUS*—a *low bar*).

3. **Sub-populations:** In sub-populations in prior HBOC-201 trials more closely resembling *RESUS* subjects (especially younger subjects), group differences in safety signals were generally reduced and sometimes nonexistent or reversed.
4. **Protocol design:** Appreciation of these potential risks has led to incorporation of comprehensive strategies to mitigate risk in *RESUS*, including exclusion of the elderly.

NMRC maintains that predicted benefit:risk in *RESUS* is high despite HBOC-201 safety concerns from prior surgery/orthopedics trials because the benefit of HBOC-201 is expected to be high in *RESUS*, *relative* risk of HBOC-201 is expected to be low in *RESUS*[60], and residual known HBOC-201 risk is mitigated in the *RESUS* protocol. These general conclusions were based on the following assumptions (rationale):

1. Predicted mortality is 58.1% with a 95% CI of 51.8-64.3% in the *RESUS* population receiving standard care.
2. Preclinical HS studies with HBOC-201 show improved outcome and support the *potential* to provide *direct benefit* to human subjects enrolled in *RESUS*.
3. Preclinical HBOC-201 data from validated swine HS models likely predict human responses.

[60] Use of the word *relative* is key in describing potential benefit and risk in *RESUS* because HBOC-201's benefit:risk may be *relatively* inferior to RBC transfusions but is still predicted to be *relatively* superior to standard non-oxygen carrying resuscitative fluids (i.e., LR) (i.e., *Risks…are reasonable in relation to what is known about the medical condition...* [21 CFR 50.24]).

4. As efficacy data from preclinical *in vitro* and animal studies and prior clinical trials show that HBOC-201 effectively carries and transports oxygen, similar effects are predicted in *RESUS*.
5. In the HEM-0115 trial, the AE profile of HBOC-201 was inferior to that of RBC in the overall population enrolled in that study.
6. Safety data in overall enrolled populations in prior surgery/orthopedics HBOC-201 trials are unlikely to accurately predict benefit:risk in *RESUS*.
    a. Even if one assumes that prior surgery/orthopedics HBOC-201 trials predict benefit:risk in *RESUS* (as suggested by OBRR), safety data in overall enrolled populations predict *reasonable* risk in *RESUS*.
    b. Even if one assumes that prior surgery/orthopedics HBOC-201 trials predict benefit:risk in *RESUS* (as suggested by OBRR), group differences of key adverse safety signals were narrowed or nonexistent in younger sub-populations more closely resembling the *RESUS* population, further predicting *reasonable* risk in *RESUS*.
7. Interim data from the ongoing HEM-0125 S. Africa trauma trial, comparing HBOC-201 and "high-bar" RBC transfusions for resuscitation from HS in the ER, show equivalent mortality and a favorable safety profile, further predicting *reasonable* risk in *RESUS*.
8. Safety data from prior trauma trials with the first generation HBOC, DCLHb, are unlikely to accurately predict benefit:risk in *RESUS*.
9. Extensive preclinical indication-specific HS data, extensive clinical non-trauma data from HEM-0115, and limited clinical trauma data from HEM-0125 support prospect for benefit and reasonable safety using proposed *RESUS Dosing Guidelines*.
10. Incorporation of multiple strategies to minimize risk in the *RESUS* protocol has further diminished risk in *RESUS*.

Thus, NMRC believes that prior HBOC-201 trials predict an identifiable level of risk in *RESUS*, that this risk has been minimized in the protocol design, and when considered in the context of 58.1% predicted mortality with standard therapy and predicted mortality reduction of at least 15% with HBOC-201, this risk is *reasonable*, and prediction of benefit:risk is favorable in *RESUS*.

Extrapolation Of Data From Prior HBOC-201 Trials For Prediction Of Benefit:Risk In *RESUS*

NMRC maintains that extrapolation of ITT safety data from overall populations in prior HBOC-201 surgery/orthopedics trials for the purpose of predicting *relative* benefit:risk in *RESUS* is indirect because differences in the clinical settings, comparators, exposures, and potential for benefit in prior trials vs. *RESUS* are so great that direct associations are unlikely. NMRC's assumption is based on

the following rationale: in prior trials, HBOC-201 was compared against the relatively safe and effective gold standard treatment of RBC transfusion—a "high bar" (vs. less effective non-oxygen carrying LR in *RESUS*—a "low bar"); in prior trials, HBOC-201 was administered as a *blood substitute* with prolonged exposure over days (vs. a short *oxygen bridge* [minutes]); in prior trials, HBOC-201 was compared in older populations—mean age 61 years old in HEM-0115 (vs. a younger population in *RESUS*—expected mean age ~ 35 years old); in prior trials, HBOC-201 was compared in mainly hemodynamically stable populations (vs. hypotensive population in *RESUS* in which "higher" BP responses would be unlikely to be clinically adverse); and in prior trials, HBOC-201 was compared for an indication with minimal potential benefit in developed countries such as U.S.—transfusion avoidance (vs. increased survival in *RESUS*). Thus, NMRC concludes that data from prior surgery/orthopedics HBOC-201 trials do not accurately reflect expected *relative* risk of clinically relevant SAEs in *RESUS* because predicted physiologic and survival benefits in *RESUS* are not accounted for in prior HBOC-201 trials (i.e., the only potential benefit was transfusion avoidance).

A more detailed quantitative benefit:risk assessment from the overall population in HEM-0115 illustrates the importance of *relative* risk. In HEM-0115, 207/350 (59%) HBOC-201 subjects avoided blood transfusions (benefit) albeit at the expense of incidence excesses (whether significant or not) of 7.7% for SAEs and 1.1% for mortality (risk); this translated to 27 additional subjects (normalized for different group sizes) with SAEs and 4 deaths. It should be recalled that in HEM-0115, no potential survival benefit would offset the adverse safety signals.

In contrast, a predicted 15% mortality reduction with HBOC-201 in *RESUS* (benefit) would translate to potentially 48 fewer deaths[61]. If the same *relative* safety profile were to occur in *RESUS* as occurred in HEM-0115 (7.7% SAE incidence excess) (a worst-case-scenario risk estimate), the 48 fewer deaths would be at the expense of 43 additional subjects with SAEs. Further extrapolation of these HEM-0115 overall population data predicts that for *RESUS*, the number needed to treat (NNT) is 11.5 (to save an additional life), and the number needed to harm (NNH) is 13 (to cause an additional subject to experience an SAE)[62]. These data predict that for every life saved in *RESUS*,

[61] Although it is possible that transfusion avoidance will be similar in *RESUS*, this is unlikely due to expected higher requirements for replenishment of blood oxygen content. If this were to occur, 327 subjects would avoid transfusions.

[62] Risk assessment calculations for *RESUS* based on HEM-0115 safety data in the overall population:

- Potential lives saved: 554 HBOC-201 subjects x 0.581 mortality rate x 0.15 effect size = 48.
- NNT: 554 HBOC-201 subjects / 48 potential lives saved = 12.
- Potential additional SAEs: 554 subjects x 0.077 excess SAE rate = 43.
- NNH: 554 HBOC-201 subjects / 43 potential additional SAEs = 13.

0.92 excess subjects might experience at least one SAE; NMRC believes that the prospect of ~ one additional SAE for every life saved represents *reasonable* risk in a patient population with ~ 1:2 risk of death.

Age Dependence Of HBOC-201's Clinical Safety Profile (Younger Sub-Populations)

Of vital importance for prediction of benefit:risk in *RESUS*, group differences (delta) in all key HEM-0115 ITT adverse safety signals were narrowed, non-existent, and occasionally reversed in younger sub-populations, almost invariably in the following descending order: ≥ 70 years old, overall population, < 70 years old, and < 50 years old. Analysis of safety data from the larger ISS database confirms the same pattern (Appendix B.2) with logistic ORs significantly diminished for the youngest sub-populations for cardiac AEs and SAEs (0.7 and 0.66, respectively), MI AEs (0.61), cardiac arrest AEs (0.72), heart failure/fluid overload AEs (0.18), respiratory AEs and SAEs (0.59 and 0.53, respectively), renal AEs (0.78), CVA and cerebral ischemic AEs (0.11 and 0.29, respectively), and neurologic SAEs 0.6). (Appendix B).

These data show that the relative safety profile of HBOC-201 in HEM-0115 (vs. RBC transfusions) was age-dependent[63]. Simply put, with respect to prediction of relative risk in *RESUS*, within the overall population and in the clinical setting studied in HEM-0115, HBOC-201's *relative* safety profile was suboptimal in elderly (≥ 70 years old) but more equivocal in younger subjects (< 70 and especially < 50 years old).

Review of mortality data from HEM-0115 is illustrative. In the overall population, mortality was statistically similar in HBOC-201 (10/350 [2.9%]) and RBC subjects (6/338 [1.8%]), p = 0.2 (delta 1.1%); thus, there was a statistically insignificant signal of 4 excess deaths in HBOC-201 subjects. But notably, 5/10 (50%) deaths in HBOC-201 vs. 1/6 (16.7%) in RBC subjects occurred in subjects ≥ 80 years old, although they accounted for only 8% of the enrolled population; similarly, 8/10 (80%) HBOC-201 and 6/6 (100%) RBC deaths were in subjects ≥ 70 years old. There were no deaths in either group in < 50 year olds (HBOC-201 0/84 [0%] vs. RBC 0/65 [0%])[64]. Among HBOC-201 subjects, mortality was higher in ≥ 70 year olds (8/111 [7.2%]) than in the overall population (10/350

[63] Age dependence of observed safety profiles in HEM-0115 was greater for HBOC-201 than RBC subjects. Logistic ORs were significantly lower for most key adverse safety signals in the youngest sub-populations (Appendix B).

[64] OBRR's summarization of mortality statistics from prior HBOC-201 Phase 2/3 trials (showing mortality odds ratios [OR] of 1.5 in the *aggregate population*, *3.53* in the > *75 y/o cohort*, *1.11* in the ≤ *75 y/o cohort*, *1.45* in the > *50 y/o cohort*, *and indeterminate* in the ≤ *50 y/o cohort* [06 Mar 2006]), confirm our sub-population analyses which show narrowing and often absence of adverse safety signals or trends in younger populations.

[2.9%], p = 0.049), and especially higher than in < 70 year olds (2/239 [0.8%], p = 0.002) and < 50 year olds (0/84 [0%], p = 0.01).

Similarly, other key adverse safety signals were disproportionately represented in ≥ 70 year old HBOC-201 subjects in HEM-0115 even though they accounted for less than one third of the overall population: 39/88 (44.3%) overall SAEs, 14/22 (63.6%) cardiac SAEs, 4/4 (100%) MI AEs, 5/7 (71.4%) cerebral ischemic (CVA/TIA/RIND) AEs, 4/6 (66.7%) CVA AEs, and 8/18 (44.4%) troponin elevations.

In the HEM-0115 < 70 year old sub-population, HBOC-201 subjects avoided blood transfusions (benefit) albeit at the expense of an incidence excess of 6% for SAEs (risk); this translated to 14 additional subjects with SAEs (normalized) and 2 deaths (risk). As aforementioned, the 15% reduction in mortality predicted in HBOC-201 subjects in *RESUS* (benefit), would translate to 48 fewer deaths. If the same *relative* safety profile occurs in HEM-0115 and *RESUS* < 70 year olds (i.e., 6% excess of SAEs), the 48 fewer deaths would be at the expense of 33 additional subjects with SAEs. Further extrapolation of these data in < 70 year olds shows that for *RESUS*, the NNT is estimated at 11.5 (to save an additional life) and the NNH is estimated at 17 (to cause an additional subject to experience an SAE)[65]. Thus, for every life saved in *RESUS*, 0.71 excess SAEs would be predicted; NMRC concludes that prospect of less than one additional subject experiencing an additional SAE for every life saved represents *reasonable* risk in a patient population with ~ 1:2 risk of death

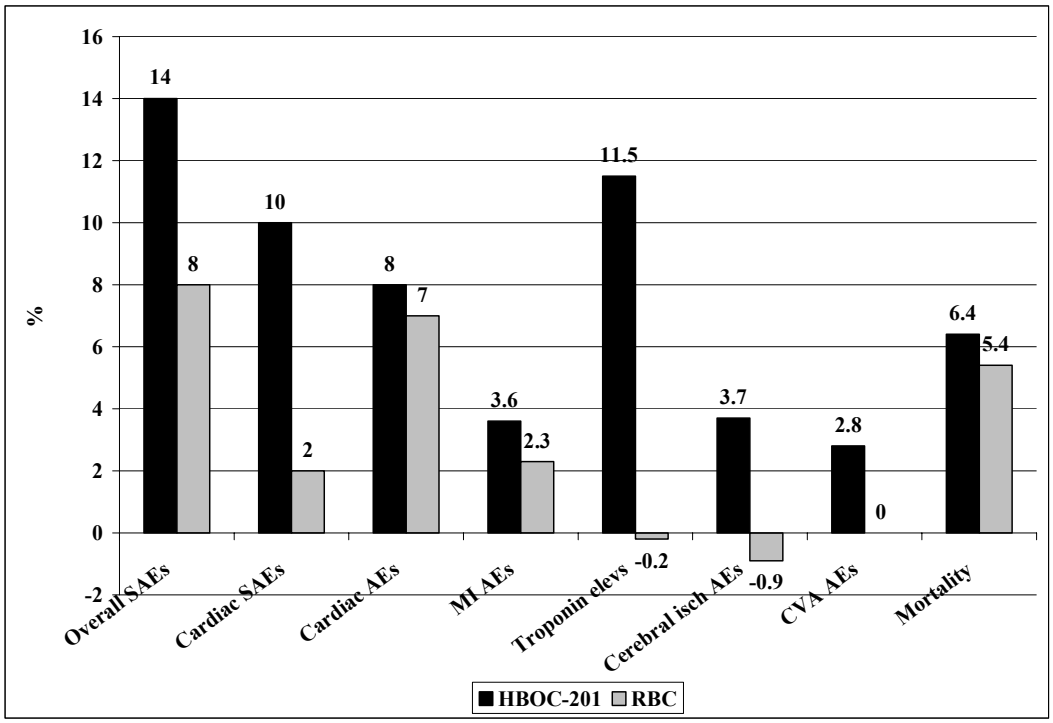
These data suggest that elderly patients may be particularly sensitive to even seemingly relatively mild to moderate vasoactive effects of HBOC-201. Awareness of potential vasoactivity risk in the elderly, exclusion of elderly patients, and comprehensive surveillance for adverse vasoactivity signals should diminish potential risk due to intrinsic vasoactivity in *RESUS*. It is important to note that the relatively inferior AE profile seen in elderly in comparison with younger HBOC-201 subjects was not simply an age phenomenon, but an HBOC-201-related safety profile directly related to vasoactivity (intrinsic toxicity) or indirectly related to *hemoglobin deficit* (practice guideline insufficiency) in HEM-0115. This is exemplified by the observation that differences in incidences (delta) of key safety

[65] Risk assessment calculations for *RESUS* based on HEM-0115 safety data in < 70 year olds:

- Potential lives saved: 554 HBOC-201 subjects x 0.581 mortality rate x 0.15 effect size = 48.
- NNT: 554 HBOC-201 subjects / 48 potential lives saved = 11.5.
- Potential additional SAEs: 554 subjects x 0.06 excess SAE rate = 33.
- NNH: 554 HBOC-201 subjects / 33 potential additional SAEs = 17.

signals in ≥ 70 vs. < 70 years old was consistently higher in HBOC-201 than RBC subjects (Figure 7). This point is an essential element in NMRC's prediction of benefit:risk in *RESUS* because it implies that the younger *RESUS* population will be less prone to HBOC-201 vasoactivity in the first place; and absence of effort to avoid blood transfusions and standardization of transfusion triggers (*in-hospital care guidelines*) in *RESUS* will decrease risk of *hemoglobin deficit*[66].

Figure 7: Differences in incidence of key safety signals in > 70 vs. < 70 years old subjects treated with HBOC-201 vs. RBC in HEM-0115



NMRC believes that these indirect data (from studies with different indications, exposure durations, populations, physiologic states, comparators, and potential benefit) do not necessarily predict risk of *relatively* adverse outcome in elderly subjects in *RESUS* because the elderly may be particularly sensitive to the consequences of tissue hypoxia with standard resuscitation and thus may have significant potential for benefit from the study intervention. Also, as noted above, awareness of and surveillance for potentially "vasoactivity-related" adverse safety signals and optimized practice guidelines in *RESUS* mitigates risk.

[66] The *RESUS in-hospital trauma care guidelines* recommend a Hb target of 8-10 g/dL in unstable subjects prior to control of hemorrhage (early resuscitation); a Hb target of 7-8 g/dL is recommended in hemodynamically stable subjects in whom hemorrhage has been controlled (late resuscitation).

Nevertheless, NMRC's initial trial design (presented at the pre-IND meeting) excluded subjects > 65 years old, but OBRR directed deletion of that exclusion. NMRC subsequently offered to revisit an elderly exclusion criterion on two separate occasions (conditional on Biopure's written assurance to OBRR that it would pursue appropriate trial(s) in vulnerable populations [pediatric and elderly] subsequent to a BLA approval in non-elderly subjects) in an effort to further increase the benefit:risk equation in *RESUS*, but no response was received from OBRR. Therefore, NMRC modified the *RESUS* protocol and IND to exclude subjects > 70 years old (CRL, 14 Aug 2006).

In summary, NMRC contends that preclinical HS studies support the hypothesis that survival will be enhanced with HBOC-201 resuscitation in *RESUS*; and clinical efficacy and safety ITT data from prior HBOC-201 surgery/orthopedics trials cannot be accurately extrapolated for prediction of benefit:risk in *RESUS* because of different trial designs, comparators, exposures, populations, physiologic states, and potential benefits. Even if efficacy and safety data from overall populations in prior trials are used to predict benefit:risk in *RESUS*, a worst-case-scenario because potential survival benefit is not accounted for, one would predict transfusion avoidance and increased rates of frequent AEs but infrequent SAEs. As differences in the AE profile between HBOC-201 and RBC were narrowed in younger sub-populations more closely resembling subjects to be enrolled in *RESUS*, one would predict that the small differences seen in overall populations in prior trials would be narrowed further or be nonexistent or reversed in *RESUS*. Interim data from the ongoing HEM-0125 S. Africa ER trauma trial, showing lower SAE rates in HBOC-201 than RBC subjects, are supportive of this hypothesis.

Prediction Of Risk In Less Severe HS

OBRR has suggested that if predicted mortality in *RESUS* is indeed ~ 50% with standard care, then the other 50% of subjects who will not die (irrespective of treatment) will be exposed to HBOC-201 risks without potential for benefit. As NMRC understands the basis for this argument, OBRR would concede that predicted *benefit:risk* might be high in the ~ 50% of subjects destined to die with standard care because they were going to die in any case; they could benefit from the study intervention but could not have significant additional risk. NMRC's assessment is that this issue does not adversely affect predicted benefit:risk in *RESUS* with the following rationale:

1. NMRC concurs with OBRR's concern as it pertains to the large population, but prospectively, it is unknown who will survive and who will not. Thus, each and every individual subject has a 58.1% chance of dying and benefiting from the predicted 15% decrease in mortality in *RESUS*.

2. As beneficial physiologic effects have been seen with HBOC-201 in comparison with standard fluids in surviving animals in preclinical HS studies, NMRC predicts that similar physiologic benefits will be documented in surviving human subjects with HS in *RESUS*, likely translating to decreased morbidity[67].
3. Safety data from HEM-0115 suggests that in less critical subjects (who might be exposed to HBOC-201 risks without potential survival benefit in *RESUS*), HBOC-201's relative safety profile was improved. Specifically, group differences (delta) in key adverse safety signals were narrowed or absent in the subgroup of subjects treated with HBOC-201 alone in comparison with a matched RBC subgroup vs. comparison of the overall HBOC-201 and RBC populations[68]: overall SAEs (delta 1 vs. 7.7%, respectively), cardiac SAEs (delta 1 vs. 3.6%, respectively), and mortality (delta 0.1 vs. 1.1%, respectively). As expected, deltas for overall AEs (5.1 vs. 4.5%) and cardiac AEs (11 vs. 12%) were similar in the HBOC-201 only subgroup and HBOC-201 overall population[69] (this is expected because characteristic typically non-serious HBOC-201 side effects can be expected to occur irrespective of the clinical situation [e.g., jaundice]). However, group deltas in the key adverse signals, more relevant to prediction of risk in *RESUS*, including overall SAEs, cardiac SAEs, and mortality, were all reduced to insignificant levels[70]. Similar to the pattern in the overall ITT HBOC-201 population in HEM-0115, incidence of key adverse signals was less in younger than older subjects (e.g., cardiac SAEs: in subjects $\geq$ 70 years old were 16.9%, where as the overall population had a rate of 12.3%, and < 70 and < 50 years old had a rate of 10.3%). These data should be reassuring in the context of the high mortality *RESUS* trial, because even in subjects who might survive irrespective of treatment, risk of harm from HBOC-201 is *relatively* low and *reasonable*.

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[67] Beneficial physiologic effects in preclinical HS studies have included stabilized hemodynamics, improved cutaneous and brain tissue oxygenation, decreased blood LA and BD, increased SSO_2sat, decreased SSLA and SSBD, decreased blood transfusion requirements, and improved CPP, ICP, and intracranial injury volume and hemorrhage. Many of these physiologic parameters are known to independently correlate with morbidity (e.g., multi organ failure, SIRS/sepsis, ICU length of stay, transfusion requirements, neurocognitive outcome (with concomitant TBI). [12, 18, 20, 29, 53, 55, 126, 127]

[68] For the purpose of this analysis, a cutoff of $\leq$ 3 RBC units was chosen as the cutoff for the RBC group because this resulted in close matching of hemorrhage (i.e., similar pre-treatment physiologic derangement). This cutoff resulted in pre-treatment EBL of 536.8 ml and 524.6 ml in the HBOC-201-only and RBC ($\leq$ 3 units) subgroups.

[69] Other AE SOC group deltas were also higher in the HBOC-201 only than the RBC ($\leq$ 3 units) subgroup. These included GI, hepatobiliary, investigations, skin, and dermatologic disorders. Tachycardia accounted for nearly 70% of cardiac AEs. Constipation, nausea, and vomiting accounted for the majority of GI AEs. Jaundice accounted for 95% of hepatobiliary AEs. BP, temperature, and lipase elevations accounted for the majority of investigations AEs. Hypertension was the major contributor to vascular AEs.

[70] There were no statistically significant group deltas in any SAE SOC class, comparing the HBOC-201 only and RBC ($\leq$ 3 units) subgroups.

Potential Benefit

There is no disagreement that HS is associated with significant mortality. Based on queries of the NTDB and *RESUS* trauma center trauma registries, and a critical review of the literature, NMRC estimates that 58.1% of subjects meeting *RESUS* inclusion/exclusion criteria will die, assuming current standard of care. Even if these numbers are not precise, the population at risk is substantial risk of death (95% CI is 51.8-64.3%), and as required by 21 CFR 50.24 (a)(1), *the human subjects are in a life-threatening situation* and *available treatments are…unsatisfactory*.[71]

There is every reason to believe that administering HBOC-201 is likely to improve survival and other clinical outcome. There is no debate that HBOC-201 carries and unloads oxygen adequately. [35, 36] In comparison to standard resuscitative fluids, HBOC-201 significantly improved clinical outcome in almost all preclinical swine HS studies, especially in severe HS. [12, 17, 20, 23, 24, 31, 55]. In addition to improved survival, HBOC-201 benefits have included stabilization of hemodynamics, improved tissue oxygenation, reversal of anaerobic metabolism, blood transfusion avoidance, and improved myocardial histopathology. In HS with concomitant TBI in swine and rat models, similar systemic physiologic benefits were seen. As well, neurophysiologic benefits were documented, including improved CPP, $brPO_2$ and SSO_2sat, and SSLA and SSBD, maintained cerebral and renal blood flow and CNS autoreactivity (mild HS: [20, 24, 27, 29-31, 33, 52, 54] (severe HS: [17, 18, 23, 24, 53]. As these HS studies ($\pm$ TBI) predict an overall decrease in mortality of 74.6% (82% in severe HS), our estimate of a 15% decrease in mortality in *RESUS* is conservative.

OBRR has noted one preclinical study in which one of four cohorts (HS/TBI with a short delay [30 minutes]) did not show a significant HBOC-201 survival advantage. The cohort showed a trend toward survival advantage but did not reach significance. Given that the study was not powered to reach significance in each cohort, and that the short delay cohort is not representative of conditions in the field, this finding does not undermine the benefit calculus. Indeed, with a trend toward survival in favor of HBOC-201, and significantly improved physiological parameters (MAP, CPP, $tcPO_2$, $brPO_2$, and CBF) with HBOC-201, it strengthens it.

Although there are limited clinical data on HBOC-201 for a HS indication, clinical data from prior HBOC-201 surgery/orthopedics trials showed high transfusion avoidance (efficacy) in the first 24

[71] We believe OBRR's mortality estimates have not taken into account the RTS inclusion criterion in the study. Inclusion of the RTS inclusion criterion results in a study population at greater risk for death than the overall traumatic HS population.

hours (≥ 95%). Only Baxter's DCLHb trauma trials, where clinical outcome was unfavorable in the in-hospital Phase 3 trial [40]and equivocal in the prehospital Phase 3 trial [41], have raised an efficacy issue. These studies, however, were conducted on a different drug and are simply inapplicable to HBOC-201[72]. In light of OBRR's concerns, however, and to further maximize potential benefit, NMRC previously amended the protocol to more systematically eliminate most patients with short (10-15 minutes) transportation delays. Our preclinical data suggest that those facing longer transit times, and therefore, less immediate access to transfusion, stand to benefit most from HBOC-201 (e.g.,[18]); focusing the trial on this group should therefore maximize potential benefit.

Potential Benefit—Focus On Blood Transfusion Avoidance

Extensive preclinical and clinical data robustly predict that blood transfusion requirements (incidence and dose) will be decreased and delayed in subjects treated with HBOC-201 in *RESUS*. In preclinical HS studies, transfusion avoidance was high (NMRC combined analysis: 36%, $p < 0.0001$) and time to first transfusion was increased (Table 53). In HEM-0115, > 95% of HBOC-201 subjects avoided transfusions in the first 24 hours, and 59% avoided transfusions by 42 days. Interim data from the HEM-0125 S. Africa ER trial reveals a trend to decreased transfusion requirements as well (RBC units/subject: HBOC-201 group 5.4 ± 1.2 vs. RBC 16.8 ± 5.7, $p = 0.08$).

[72] OBRR has referred to the prior adverse outcome in the DCLHb in-hospital study as a component of its basis for the Clinical Hold [15, 40], citing HBOC-201 and DCLHb as *vasoactive HBOCs* and implying essentially similar vasoactivity potency. However, preclinical studies strongly suggest that HBOC-201 is significantly less vasoactive than DCLHb (e.g., [13]). As well, in the in-hospital trial, DCLHb was administered as an adjunct to standard care (including blood transfusions); in contrast, in *RESUS*, HBOC-201 is compared with non-oxygen carrying IV fluids where blood transfusions are unavailable. Thus, the two HBOCs have different intrinsic vasoactive properties, and the comparators and potential for benefit differs in the in-hospital DCLHb and *RESUS* trials. Hence, NMRC believes results of the in-hospital DCLHb trial are largely irrelevant to prediction of benefit:risk in *RESUS*.

Table 53: Blood transfusion avoidance in NMRC preclinical HS studies

		HBOC-201	HEX/LR	p-value
Blood Transfusion Avoidance (%)	**Overall**	36% (16/45)	0% (0/31)	$p < 0.0001$
Dose (avg # transfusions)	**4 Hour Models**	0.4 ± 0.7	1.2 ± 0.4	$p = 0.0002$
	Uncon HS/TBI SD	1.9 ± 0.3	2.0 ± 0.4	$p = 0.02$
	UnconHS/TBI LD	1.83 ± 0.3	2.5 ± 0.5	$p < 0.0001$
Time to 1st Transfusion (hours)	**4 Hour Models**	27.5 ± 5.1	6.6 ± 2.8	$p < 0.0001$
	Uncon HS/TBI SD	1.8 ± 0.3	0.6 ± 0.1	$p < 0.001$
	UnconHS/TBI LD	2.3 ± 0.2	1.25 ± 0.0	$p < 0.0001$

Data from the medical literature predict that lower blood transfusion requirements (incidence and dose) and delay in need for blood transfusions in *RESUS* would contribute to improved clinical outcome. Specifically, the literature (cited below) suggests that blood transfusion is an independent predictor of adverse outcome in trauma with regard to mortality; MOF, SIRS, and infection; and ICU admission and length of stay (LOS):

- Sauaia A [128]: blood transfusion is an independent risk factor for MOF.
- Moore F [129]: blood transfusion is an independent risk factor for MOF.
- Dunne J [130]: blood transfusion is an independent risk factor for SIRS, ICU admission and LOS, and mortality.
- Malone D [131]: blood transfusion is an independent and time-dependent (24 hours) risk factor for ICU admission, ICU and hospital LOS, and mortality.
- Hill G [132]: blood transfusion is an independent risk factor for post-operative bacterial infection (meta analysis).
- Claridge J [126]: blood transfusion is an independent and dose-dependent risk factor for post-operative infection.

Potential Risk

NMRC and OBRR agree that HBOC-201 demonstrates some vasoactivity, as shown in both preclinical and clinical studies. While there may be some disagreement between OBRR and Biopure about the precise incidences of vasoactive responses[73], the decisions about *RESUS* do not depend on

[73] There has been some question about the validity of Biopure's AE and laboratory databases. To the best of our knowledge, these databases are valid and accurate as per accepted standards. The following passages describe validation of the AE database and recent Biopure restructuring of the laboratory database:

Validity of the AE database: The AE database was created following monitoring and complete remonitoring of source documentation and CRFs at HEM-0115 study sites to address GCP noncompliance issues identified

resolving this question. That is because it is possible to estimate the risks to subjects in *RESUS* with sufficient precision to see that they are outweighed by the estimated benefits, and are not, therefore, *unreasonable*.

Two potential risks have been hypothesized: first, that BP increases will lead to clinically important AEs in some subjects, and second, that BP increases will affect treatment decisions, leading to under-resuscitation, and therefore, hypoperfusion. NMRC believes that these risks, although real, are low and have been further mitigated in the *RESUS* protocol design.

Potential Risks By Organ System And Minimization Of Risks

Neurologic

In the HEM-0115 overall population, CVA AE incidence was higher with HBOC-201 (6/350 [1.7%]) than RBC (0/338 (0%) (p = 0.03); but TIA/RIND (reversible ischemic neurological deficit) AE incidences (1/350 [0.3%] vs. 2/338 [0.6%], p = 0.6) and all (combined) cerebral ischemic AE (CVA/TIA/RIND) incidences (7/350 [2%] vs. 2/338 [0.6%], p = 0.07) were not significantly different between groups. For combined CVA/TIA/RIND incidence, mean (± SEM) age was 75.6 ± 3.3 years (range 62-91 years) for HBOC-201 vs. 58.5 ± 7.2 for RBC subjects (range 48-69 years).

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during site audits. The remonitoring was performed to specifically look for AEs potentially missed during the initial monitoring process. A comprehensive report describing the details of the clinical laboratory database restructuring process was submitted by Biopure to FDA 27 January 2006 (Amendment to BB-IND 2935, SER375).

Laboratory database restructuring: A laboratory restructuring process was completed aiming to provide a laboratory database where all inconsistencies associated with the BLA laboratory database were resolved. The BLA database had several issues and inconsistencies such as lack of reliability in reported units and laboratory norms for the collected data; inability to trace reported data to source documents; significant number of contradictions in the analysis database submitted in the BLA; and an absence of a clear system for referencing multiple datasets in the original locked database. To resolve these issues, Biopure reconstructed the laboratory database such that:

1. The laboratory database is based on source-verified laboratory data generated from the central laboratories and local laboratories in this study.
2. Uniform units of measurements are used across the study designated laboratories,
3. The laboratory normal reference ranges are based upon values originally reported by respective laboratories.
4. Complete traceability of all data and all edits to original locked databases and source documents is possible.

The restructuring of the lab database was achieved in a three phase process:
Phase 1: Creation of "Intermediate Database" with all available local and central laboratory data.
Phase 2: Creation of the "Verified Database" without duplicate measurements and all laboratory data verified by source documentation.
Phase 3: Laboratory normal reference ranges were applied from source documents for all measurements with uniform units of measure across laboratories comprising the "Reporting Database." Restructuring of the reporting database to permit data analysis resulted in creation of the "Analysis Database."

Similar but insignificant trends were seen for SAEs: CVA SAE incidence was 5/350 (1.4%) vs. 0/338 (0%) (p = 0.06); TIA/RIND incidence was 0/350 (0%) vs. 2/338 (0.6%) (p = 0.2); and CVA/TIA/RIND incidence was 5/350 (1.4%) vs. 2/338 (0.6%) (p = 0.3). In the < 50 year old sub-population, incidence was the same in HBOC-201 (0/84 [0%]) and RBC subjects (1/65 [1.5%]) (p = 0.4). Among HBOC-201 subjects, there was a trend to lower incidence in < 50 year olds (0/84 [0%]) vs. the overall population (7/350 [2%]) (p = 0.4) or vs. ≥ 50 year olds (7/266 [2.6%]) (p = 0.6). Thus, although potentially ischemic cerebral AEs (especially CVAs) were more common with HBOC-201 than RBC in the overall population, they were uncommon, the increase in incidence was small (< 1.7%), and all occurred in older subjects.

In a combined analysis of CVA/TIA/RIND AEs in all HBOC-201 surgical Phase 2/3 surgical trials, NMRC found a similar pattern. CVA AE incidence was higher with HBOC-201 (9/557 [1.6%]) than RBC (1/512 (0.2%) (p = 0.02); but TIA/RIND AE incidences (1/557 [0.2%] vs. 2/512 [0.4%], p = 0.6) and CVA/TIA/RIND incidences (10/557 [1.8%] vs. 3/512 [0.6%], p = 0.09) were not significantly different. For CVA/TIA/RIND incidence, mean age (± SEM) was 74.2 ± 3.4 years (range 53-91 years) for HBOC-201 and 64.3 ± 6.9 for RBC subjects (range 48-76 years). In the < 50 year old sub-population, incidence was the same in HBOC-201 (0/111 [0%]) and RBC subjects (1/90 [1.1%]) (p = 0.4). Among HBOC-201 subjects, there were trends to lower incidence in < 50 year olds (0/111 [0%]) vs. the overall population (10/557 [1.8%]) (p = 0.4) or vs. ≥ 50 year olds (10/446 [2.2%]) (p = 0.2).

In summary, these data show slightly increased risk of CVA AEs in HBOC-201-treated subjects in comparison with gold standard RBC treatment, but exclusively in older subjects, most of whom were hemodynamically stable. As mean age of HBOC-201 subjects with CVA/TIA/RIND AEs was ~ 75 years old, none occurred in < 50 years olds, incidence and group differences were low, and stroke risk is low in trauma patients in the first place[74], risk is predicted to be low in the younger *RESUS*

[74] *Post-traumatic cerebral infarction* (*PTCI*) is a recognized complication of neurotrauma, but even in this sub-population of the overall trauma population, its occurrence is rare. In the largest series in the literature, among neurotrauma patients requiring CT at UMD Shock-Trauma Center, incidence was only 1.9% (25/1,332).[133] As TBI is expected in ~ 1/3 of *RESUS* subjects, these data would predict an overall 0.6% risk of PTCI in control subjects in *RESUS*. Furthermore, as noted by the authors, *CT findings in 24* (of 25) *patients suggested that cerebral infarction was a result of focal mass effects and/or gross mechanical displacement of the brain producing transfalcine and/or transtentorial herniation.* Similar observations were reported by Server et al.[134] Unless HBOC-201 increased hematoma size and/or brain edema, it would be unlikely to have any effect on such pathophysiology. In comparison with standard fluids, preclinical studies evaluating HBOC-201 in HS/TBI, models have shown decreased contusion volume and equivalent brain edema [33] and decreased ICH volume.[18] Cerebral vasospasm is an additional possible pathophysiologic basis for PTCI, but preclinical data has shown equivalent cerebral blood flow (CBF) with HBOC-201 and standard fluids.[18] In conclusion,

population, especially considering comparison with suboptimal LR rather than gold standard RBC. Thus, NMRC believes low risk of stroke does not significantly affect overall predicted benefit:risk in *RESUS* and should not be a basis for the Clinical Hold. Our rationale is detailed as follows:

In order to further diminish stroke risk, he *RESUS* protocol includes numerous risk mitigation strategies for rapid detection and reporting of potentially adverse signals (allowing further action should they occur):

- Inclusion/exclusion criteria targeting a population with high mortality and unavailability of blood transfusions
- Comparison with suboptimal (but standard) asanguinous fluids
- Potential vasoactivity risk mitigation
    - Comprehensive *CTM Dosing Guidelines*
    - Access to standard fluids, as needed
    - *In-hospital trauma care guidlienes (blood transfusion and fluid guidelines)*
    - CTM stopping criterion (for high SBP response)
    - *Hypoperfusion markers reports*
    - Expedited AE/SAE reporting to DMC, IRB, and FDA
    - *ESRs* starting at 50 subjects (only 25 HBOC-201 subjects)

Cardiovascular

Cardiovascular risk has been addressed in detail above. HBOC-201 has vasoconstrictive properties that can manifest as elevated BP, potentially resulting in accelerated hypertension or misleading of EMS personnel (and/or physicians) regarding adequacy of fluid resuscitation and secondary under-resuscitation and consequent hypoperfusion. All of the following have been reported with HBOC-201: rare hypertensive responses classified as SAEs (0.3% HBOC-201 vs. 0% controls, $p = 0.50$ [prior Phase 1-3 trials), common hypertensive responses classified as AEs (15% HBOC-201 vs. 7% controls, $p < 0.0001$ [prior Phase 1-3 trials]), mild to moderate hypertensive response, mild to moderate increased SVR, mildly increased PVR, and mildly decreased CO. In addition, incidences of cardiac SAEs, especially MI and CHF/fluid overload, have been higher with HBOC-201 than with

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baseline *PTCI* risk is expected to be low in the overall *RESUS* population, its mechanism is unlikely to be affected by HBOC-201, and preclinical HBOC-201 data in HS/TBI models suggest physiologic benefit rather than harm in this context. These observations support NMRC's assessment that risk of stroke is low in *RESUS* and does not sufficiently affect overall benefit:risk to be a basis for the Clinical Hold.

control fluids. However, the populations previously studied concentrated on mainly older adults (mean age 60.8 years old in HEM-0115) and not surprisingly, the increased incidence of cardiovascular AEs/SAEs occurred mostly in elderly patients and in those with pre-existing cardiovascular diseases. Hence, group differences were minimal in younger subjects, more akin to the general population to be enrolled in *RESUS*. Importantly, cardiovascular co-morbidity is expected to be significantly lower in *RESUS* subjects than those studied in prior surgery/orthopedics HBOC-201 trials. A recent NTDB review found that only 6.5% (4,716/72,517) of admitted trauma patients had a history of cardiovascular disease.[92] In contrast, 70.7% of HEM-0115 subjects had history of cardiovascular disease. Moreover, there are no preclinical or clinical data showing that cardiovascular events will be higher in subjects who receive HBOC-201 who are in HS, especially younger subjects. Troponin levels and histopathologic evidence of myocardial damage were equivocal or better in HBOC-201- vs. standard fluid-resuscitated animals in preclinical HS studies conducted at NMRC. ([18, 32]

NMRC concludes that cardiovascular risks are low in *RESUS* because the potency of HBOC-201's vasoactivity is only mild to moderate; preclinical HS studies show high efficacy and only mild to moderate vasoactive responses to HBOC-201 infusion; clinical HBOC-201 data from prior surgery/orthopedics trials show frequent mild but rare severe BP responses and uncommon cardiovascular SAEs; clinical HBOC-201 data from prior surgery/orthopedics trials show lower and minimal group differences in BP and cardiovascular adverse signals in younger subjects; preclinical and clinical data with other HBOCs suggests reasonable risk in the indication sought in *RESUS* (e.g., prehospital DCLHb HS trial [41]; baseline co-morbid cardiovascular risk is expected to be low in the younger *RESUS* population *a priori*; and extensive risk mitigation strategies have been incorporated into the *RESUS* protocol. That the SAE rate is equivalent in HBOC-201 and RBC treated subjects in an interim analysis of the ongoing HEM-0125 S. Africa trauma trial in a younger population, is supportive of these risk predictions.

Minimization of risk: See above (neurologic summary of risk mitigation strategies).

Gastrointestinal

Dysphagia, abdominal pain, chemical pancreatitis, elevated liver transaminases, hyperbilirubinemia. Elevations in LFTs and amylase/lipase are expected and are almost always clinically insignificant.

Minimization of risks: Treat dysphagia and/or abdominal pain symptomatically. Follow LFTs and lipase.

Genitourinary

Free Hb is toxic to the kidneys. In contrast, polymerized Hb has reduced renal effects, thought due to decreased glomerular filtration and renal vascular vasoactivity. In the prior Phase 3 HEM-0115 trial, oliguria AEs occurred more commonly in HBOC-201 (39/350 [11%]) than RBC (16/338 [5%]) subjects (p = 0.002), but more clinically important acute renal failure (ARF) AEs (5/350 [1.4%] vs. 4/338 [1.2%], p = 1.0) and overall renal SAEs (6/350 [2%] vs. 4/338 [1%], p = 0.8) were similar in the two treatment groups. In some preclinical HS studies, slightly increased creatinine and BUN blood levels have been seen, as well as mild papillary necrosis, especially in models in which fluid infusion volumes were decreased.[32] Thus, the preclinical and clinical database with HBOC-201 suggest possibly increased risk of oliguria, but extrapolation of oliguria risk to *RESUS* is circumstantial due to different indications, patient populations, physiologic states, comparators, and potential benefits; in addition, no increased risk of acute renal failure and renal SAEs are predicted. As a significant survival benefit is expected with HBOC-201 resuscitation in *RESUS*, the small increased risk of oliguria does not significantly alter the overall benefit:risk prediction.

Minimization of risks: The overall strategy to minimize renal risk in *RESUS* is to reduce risk in via training (to reduce primary risk in the first place) and close surveillance (to reduce secondary risk). Specifically, renal risk is minimized by maximizing predicted benefit:risk by rigorous attention to inclusion/exclusion criteria (targeting patients with severe HS who are most likely to potentially benefit from HBOC-201), and attention to *RESUS in-hospital care guidelines* to ensure adequate fluid resuscitation. *RESUS* Trauma Center training includes detailed education of physicians about potential oliguria risk and reinforcement about utilizing a number of clinical parameters to assess fluid status, especially in HBOC-201-treated subjects. Fluid/HS status is not determined using any single clinical parameter such as urine output; trauma center physicians will use multiple parameters to assess fluid and HS status, including renal perfusion indirectly. For example, decreased urine output in the setting of additional abnormalities of other relevant clinical parameters (e.g., LA) would suggest renal hypoperfusion due to inadequate fluid resuscitation; decreased urine output in the setting of normal other relevant clinical parameters might suggest other etiologies (e.g., HBOC-201 side effect). In addition, to ensure close surveillance and rapid detection of potentially adverse signals, renal AEs will be reported as *always expedited* (to the DMC and FDA), and infusion volumes

will be closely monitored and regularly and frequently reported in *Hypoperfusion Reports* (to the DMC and FDA).

Methemoglobinemia

HBOC-201 specifications allow up to 10% metHb. Also, when HBOC-201 is exposed to oxygen, such as *in vivo* after IV infusion, Hb in HBOC-201 can oxidize and form metHb. Clinically significant methemoglobinemia ordinarily occurs at $\geq$ 20%; lower metHb levels (such as 10-20%) can be significant with concomitant cardiovascular alterations (e.g., shock). In cardiac surgery subjects, by days 1 and 2, 15% and 40%, respectively, of remaining *in vivo* HBOC-201 was oxidized to metHb (Levy, 2002). But actual metHb levels were much lower due to the short half-life of HBOC-201. Mean peak metHb levels in subjects in surgical Phase 2 [135], cardiac surgery Phase 2 [136], and HEM-0115, were 3.7, 4.6, and 4.5%. In the surgical trial, among subjects infused high dose HBOC-201 (2-6 g/kg), mean peak metHb reached 7.1%. [136] These data show that clinically significant oxidation of HBOC-201 to metHb occurs over 1-3 days; by then, most subjects should be adequately volume resuscitated. Thus, even at the highest allowed dose of HBOC-201 in *RESUS* (i.e., 6 units) (~ 2.8 g/kg), metHb levels are unlikely to surpass 10%. Similar metHb levels have been seen in preclinical HS studies. Thus, methemoglobinemia is unlikely to be clinically significant in the overwhelming majority of subjects infused HBOC-201 in *RESUS*. However, because at least theoretically, clinically significant methemoglobinemia could occur in subjects infused large volumes of HBOC-201 (e.g., 6 units) (especially with reduction in intravascular volume due to hemorrhage), clinicians will need to take this into consideration when calculating effective blood oxygen content.

Minimization of risks: To minimize risk of methemoglobinemia, metHb levels will be assessed serially, metHb will be considered in evaluation of oxygen content for blood transfusion decisions, and clinicians will be comprehensively educated about diagnosis and treatment as part of the Trauma Center training program.

Integument

Transient yellowish discoloration of skin occurs commonly after HBOC-201 administration. Application of $tcPO_2$ monitoring electrodes for > 4 hours can cause small skin burns under the electrodes.

Minimization of risks: No action planned regarding skin discoloration—this side effect is transient. The maximum time for application of $tcPO_2$ monitoring electrodes in one skin site will be 3–4 hours; the site will be changed within 4 hours.

Allergic

Rare allergic reaction to bovine protein may induce an allergic delayed or immediate hypersensitivity reaction. No immediate hypersensitivity reactions occurred in Phase 1-3 clinical trials to date.
Minimization of risks: Exclude patients with known allergy to HBOC-201.

Pregnancy and lactation (category X): There are no human data regarding the safety of HBOCs in pregnancy and in lactation.

Reproductive Toxicology Studies Summary

Reproductive toxicology studies were initially conducted in rats, and irrespective of the dosing regimen (IV route of administration) during gestation, excessive embryo-fetal toxicity and mortality were reported. Serious maternal side effects were seen only with clinically-irrelevant suprapharmacological repeat-dosing regimens, and included petechiae (skin microhemorrhage), tissue histiocyte pigmentation (pigmentation of histiocytes), hepatic sinusoidal ectasia (swelling of liver sinuses), renal pigmentation and vacuolation (kidney toxicity), and increased mortality; generally, histopathologic changes were less severe than in hestastarch-treated control animals. However, in multiple studies with clinically-relevant doses, none of these serious side effects were seen, and mortality was not increased. Decreased food intake, maternal weight gain, and uterine gravid weight were seen, and were probably related to NO binding with consequent GI smooth muscle contraction, causing anorexia. In pigs treated with HBOC-201, NMRC also observed temporary decreased food intake. In rat studies, fetal side effects were severe, occurred irrespective of dose, and included yolk sac abnormalties, severe developmental malformations, spontaneous abortions, and fetal death. Importantly, no maternal or fetal side effects were seen in dog studies.

Overall Studies: Reproductive toxicology studies were carried out in rats and dogs to examine effects of HBOC-201 and BPH on embryo-fetal development (teratology studies). Three studies in the rat included administration of HBOC-201 by continuous IV administration [gestation day (6 through 18], daily continuous IV administration (gestation day 6 to 7, 7 to 8, 8 to 9, 9 to 10, 10 to 11, 11 to 12 and 12 to 13) or hemodilution followed by IV infusion on gestation day 9. An additional study was carried out in rats in which hemodiluted pregnant animals were administered BPH IV on gestation

day 9. In dogs, HBOC-201 was administered IV on either gestation days 21, 25, 29 or 33. Finally, an HBOC-201 repeat-dosing (during gestation) dog study was completed. A study was carried out in pregnant sheep to examine the ability of HBOC-301, fresh whole blood and hetastarch to resuscitate hypovolemic animals (maternal hemodynamics and cardiopulmonary parameters) and restore fetal oxygen content. A mechanistic study was conducted using rat embryos to examine HBOC-201-related embryotoxicity in the rat.

***Conclusions**:* Reproductive toxicology study data demonstrated significant embryo-fetal toxicity with IV administration of HBOC-201 to pregnant rats whether administered continuously or by daily IV infusion or by IV infusion on a single critical day (gestation day 9). However, IV infusion of HBOC-201 to pregnant dogs at critical gestation times (gestation days 21, 25, 29 or 30), at doses 2-fold greater than the MHD on a weight-to-weight basis, did not cause any embryo-fetal toxicity. Upon repeat dosing of HBOC-201 to pregnant dogs during organogenesis, no teratological effects were reported. Mechanistic study results indicated that HBOC-201 related embryo-fetal toxic effects in rats were likely due to effects on the inverted yolk sac, a developmental system fairly unique to the rat. Again, since dogs and humans do not utilize such a developmental system during pregnancy and there were no embryo-fetal effects of HBOC-201 in pregnant dogs, it is likely that these teratogenic effects of HBOC-201 are unique to rats and not relevant to humans. Finally, the results of the study in pregnant sheep indicated that HBOC-301 was equivalent to whole blood at improving fetal oxygen content and was not transported to the fetus in this model.

Minimization of risks: Female patients known or suspected to be pregnant or lactating will be excluded from enrollment. A β–HCG will be checked upon admission to the hospital. However, due to the emergency nature of this prehospital study, it is possible that a few subjects will be enrolled who will turn out to be pregnant. Pregnancy outcome will be followed to term. Potential benefits/risks related to pregnancy are summarized in *RESUS CCD* materials; although routine self-exclusion (e.g., medical jewelry) of all pregnant women in communities where *RESUS* is being conducted is not specifically advised, it is recommended that pregnant women weigh the benefits/risks and consider this option individually.

Other Potential General Risks

General (Laboratory Interactions)

As HBOCs interfere with the accuracy and cause expected abnormalities of some laboratory assays, there is potential risk for failure to provide standard of care at the trauma centers.

Minimization of risks: This risk is mitigated in two fashions: comprehensive laboratory interference evaluation/challenge system and EMS/Trauma Center training programs.

General (Blood Drawing)

~ 50% (161-191 ml) of the blood volume required in this study (322-382 ml) would be ordered as part of standard care, 25% for maximizing safety because of HBOC administration, and ~ 25% for other research purposes.

Minimization of risks: To decrease the likelihood of venipuncture related complications (i.e., brief discomfort, and rare hematoma and infection), only trained medical personnel will draw blood. In order to minimize phlebotomy-induced anemia, only 38-86 ml will be drawn solely for research purposes.

General (Delay In Care)

A brief delay in care due to study-related interventions (screening, *IC* or *Pre-ED*, and enrollment) is likely. However, *RESUS* study investigators believe that the potential benefits of HBOC-201 outweigh this risk.

Minimization of risks: *RESUS* study-specific research procedures have been minimized to avoid diversion of attention of EMS providers and delay in treatment. The screening review of inclusion/exclusion criteria is absolutely necessary but no undue delay is expected for that process. The review of inclusion criteria has to be complete but it is not expected to delay medical care significantly. EMS providers will be trained to complete *IC* and/or *Pre-ED* processes only when feasible and thus not to delay necessary care. Enrollment includes only the opening of the randomization study box to identify the patient's grouping and then to administer the appropriate CTM. EMS providers will complete the EMS CRF after the run, so paperwork will not hinder or delay medical care.

Transmission Of BSE And Other Infections

HBOC-201 treated subjects: As HBOC-201 is cow-derived, there are theoretical risks for transmission of bovine bacterial, viral, and prion infections (i.e., variant Creutzfeldt-Jakob disease

[vCJD]), the human disease caused by prions implicated in BSE (mad cow disease). Bovine (BSE) and human vCJD infections require exposure to infected animal brain/spine and other tissue, but probably not blood. This potential risk is especially relevant after BSE was reported in Washington State in 2003 [137] and in Texas in 2004. Human vCJD is fatal and has no known treatment, but to date has not been reported in the U.S. [138]

LR control subjects: There is a higher risk of transmission of human transfusion-related (blood-borne) infectious agents (e.g., HIV and hepatitis B and C) in LR than HBOC-201 patients, as they are likely to receive more allogeneic blood transfusions.

Minimization of risks: HBOC-201 is derived from isolated cow herds (in Pennsylvania), which are not fed animal-source feed. Veterinary and animal husbandry processes include thorough avoidance of cross contamination as well. Thus, the risk of BSE transmission among cows (and therefore, human vCJD) is practically eliminated. In addition, HBOC-201 is sterilized by heat-treatment and undergoes a prion-elimination process. For the purposes of the *RESUS* study, OBRR had accepted Biopure's manufacturing process. The risk of human transfusion-related infections is the same in LR subjects as in non-enrolled patients; the overall risk in study subjects is lower than in non-enrolled patients because the transfusion avoidance rate for HBOC-201-treated subjects was high in prior surgical clinical trials.

Mortality Database From Prior HBOC-201 Trials

At a recent *RESUS* Type A Meeting, OBRR stated that the trend (not statistically significant) to higher mortality in HBOC-201 than RBC subjects in prior HBOC-201 trials (25/797 [3.1%] vs. 14/661 [2.1%], $p = 0.26$), was also a consideration in OBRR's Clinical Hold decision. NMRC reiterates that in those trials, no beneficial effect on mortality was expected and the comparator was gold standard RBC; in contrast, in *RESUS*, a 15% reduction in survival is predicted and the comparator is suboptimal non-oxygen carrying LR; hence, extrapolation of a negative safety signal for prediction of risk in *RESUS* cannot be done with any confidence of accuracy.

Furthermore, stratification of mortality in prior HBOC-201 trials based on age demonstrated that mortality appeared related to age. As shown in Table 54, overall mortality rates were statistically equivalent in the two groups (25/797 [3.1%] in HBOC-201 vs. 14/661 [2.1%] in RBC subjects, $p > 0.05$); but in subjects > 75 years old, mortality rates trended to be higher in HBOC-201 than RBC

subjects (13/95 [13.7%] vs. 5/89 [5.6%], respectively, p = 0.08). Within treatment groups, it is apparent that mortality differences between ≤ 75 and > 75 year olds are greater for HBOC-201 than RBC subjects (13% vs. 5%). Additionally, among HBOC-201 subjects, mortality in ≤ 50 year old subjects (1.2%) trended to be lower than in the overall population (3.1%, p > 0.05) and significantly lower than in > 75 year olds (13.7%, p < 0.001). Looking at the data slightly differently, NMRC observed that the mortality Odds Ratio (OR) was 1.5 for the overall population, but almost equivalent (1.06) for < 75 years olds. Thus, these mortality differences are not simply an overall age phenomenon, but a particular safety profile observation in HBOC-201 subjects, suggesting that when compared with RBC in stable surgery/orthopedics patients, the elderly do not tolerate side effects of HBOC-201 as well as younger patients. These mortality data support NMRC's observations that SBP responses, AEs, SAEs, and troponin elevation rates were also lower in younger sub-populations, and predict that benefit:risk will be more favorable in the younger population to be enrolled in *RESUS* (especially since addition of the elderly exclusion).

Table 54: Mortality in prior HBOC-201 surgery/orthopedics trials

Overall population **HBOC-201** 25/797 (3.1%) **P = 0.26**		**RBC Control** 14/661 (2.1%)	
HBOC-201 only		**RBC only**	
≤ 50 years old 3/245 (1.2%) **P < 0.001**	**> 75 years old** 13/95 (13.7%)	**≤ 50 years old** 0/158 (0%) **P = 0.01**	**> 75 years old** 5/89 (5.6%)
≤ 50 years old 3/245 (1.2%) **P = 0.07**	**> 50 years old** 22/552 (4%)	**≤ 50 years old** 0/158 (0%) **P = 0.08**	**> 50 years old** 14/503 (2.8%)
≤ 75 years old 12/690 (1.7%) **P < 0.001**	**> 75 years old** 13/95 (13.7%)	**≤ 75 years old** 9/572 (1.6%) **P = 0.05**	**> 75 years old** 5/89 (5.6%)

Semi-Quantitative Analysis Of Benefit:Risk In RESUS Using SOC Mortality Equivalents (Analysis #3)

Introduction

Benefit:risk analysis is generally assumed to involve estimation of a *benefit:risk ratio* (*BRR*) when, in fact, this statistic is hardly ever calculated. This may be due, in large part, to the difficulty often encountered when attempting to assess relative value or weight associated with the observed benefit vs. risk. An extreme case is that of *RESUS* where lives saved vs. risk of SAEs is being compared. Clearly, for SAEs with no permanent incapacitating sequelae, benefit of saving a life is unequivocal. However, because the *RESUS* trial will assess efficacy and safety of a potentially life-saving therapy, and will be conducted with *EIC*, objective and quantitative benefit:risk assessment is imperative.

In the Executive Summary section, risk:benefit Analyses #1-3 were described. In Analysis #1, a simple quantification of benefit:risk was performed utilizing overall SAE data from the overall population and < 70 year old sub-population in HEM-0115. This analysis showed that the *benefit:risk ratio* (*BRR*) exceeded 1.0 (i.e., *favorable* benefit:risk) but because it overly conservatively equates death and mortality as having equivalent clinical significance, it does not accurately predict benefit:risk in *RESUS*. Analysis #2 partially accounted for disparity in the clinical significance of death and SAE occurrence by calculating an *Excess SAE Score* (*ESS*) which rates benefit:risk favorability based on an estimate of the number of excess SAEs that would be expected to be tolerable to patients and physicians in order to save a life. The analysis showed that the *ESS* is < 1 for *RESUS*, predicting < 1 excess SAEs for each life saved even using a conservative rating scale and conservatively basing the analysis on overall SAE data from HEM-0115. These analyses showed that prediction of *favorable* benefit:risk is robust, being confirmed even with a wide range of mortality reduction and effect size variations. However, a comprehensive approach, including a comprehensive comparison of prior SAE databases vs. mortality for prediction of benefit:risk in *RESUS*, was not included in Analyses #1 and #2.

Accordingly, a systematic and quantitative method for benefit:risk assessment is detailed below utilizing a scoring system for assessment of the relative severity of SOC SAEs compared to mortality (Analysis #3). This system allowed translation of SAEs into *mortality equivalents*, permitting a more meaningful quantitative estimate of benefit:risk based upon evaluation of the ratio of the number needed to benefit (NNB) to the number needed to harm (NNH).

Methods

The assessment of benefit:risk described herein is similar to that described by Holden, incorporating quantitative assessment (scoring) of the relative severity of AEs and SAEs as compared to mortality

(determined by an experienced trauma surgeon). The *BRR* was calculated based upon estimation of the NNT to show benefit vs. the NNH, as follows:

$$NNB = 1/(p_1-p_2)$$

Where p_1 and p_2 are probabilities of mortality in control (LR) and treatment (HBOC-201) groups, respectively.

The benefit:risk analysis for HBOC-201 was completed using *RESUS* inclusion criteria. Queries of the NTDB and UAB/UMD databases show that this population has a mortality of ~ 58 ± 3%. More than half of the subjects in this population will die as a result of their injuries. The projected benefit of HBOC-201 has been conservatively estimated to be a 15% reduction in mortality (or 0.15 X 58% = 8.7%). That is, it is hypothesized that mortality will be reduced from 58 to 49%. The details of how the effect size was determined, is presented in 2.0 Executive Summary. On this basis,

$$NNT = 1/(0.58-0.49) = 11.5$$

indicating that ~ 11 subjects will need to be treated to show beneficial effect measured in terms of a life saved.

The risk associated with HBOC-201 is derived from the risk of AEs. Accordingly, the

$$NNH = 1/(q_1-q_2)$$

where q_1 and q_2 are the risk of AEs in control and treatment groups, respectively.

As death and SAEs have different clinical significance, stressing AE incidence alone in benefit:risk analysis for *RESUS* has limitations. This simple approach results in calculation of the ratio (i.e., the *BRR*) of the number of individuals that would need to be treated to save a life vs. number of individuals needed to see an AE. Evaluation of the *BRR* becomes a subjective determination based on the reviewer's individual assessment of the relative value of saving a life compared to SAEs.

The major idea of the proposed benefit:risk analysis enhancement is to replace assessment of probability of excess SAEs [q1-q2] by expected *net risk*, calculated in terms of mortality. If every subject in the studied population is assigned an outcome score (0-1 scale) where 0 means "no reported/unresolved problems" and 1 means "patient is dead", then the mean value of such a score will give a statistical expectation for outcome in terms of mortality. Actually, mortality itself is an extreme version of such a score, where every survivor gets a score of 0 and every dead subject is assigned a score of 1. The opposite extreme is the SAE(AE) incidence score, when any reported SAE guarantees a of score 1 and a score of 0 means "nothing happened at all". These two approaches are well known as *morbidity and mortality evaluations.* The biggest problem of standard benefit:risk calculations is a desperate try "to marry" those extremes: NNT for benefit is expressed in mortality terms while NNH is expressed in morbidity terms. The morbidity/mortality score that was developed (see below) permits an assessment of the entire (evidence-based) *net risk* as a difference between average scores for compared arms in mortality terms. Calculations of two standard auxiliary indicators (NNH and NTT) stay unchanged. It should be noted that the *BRR* is a ratio of expected benefit divided by expected risk. In the situation when benefit and risk are expressed in the same terms, NNH and NTT, while illustrating the same concepts as before, become unnecessary for accurate assessment.

Assessing AE Severity

To more rigorously approach an objective and quantitative evaluation of *BRR*, a scoring system was developed to quantify the relative severity of AEs by SOC, compared to mortality. This was a two-step process, the first step involved tabulation of the scoring and critique by three critical care physicians or trauma surgeons with significant experience conducting clinical trials. Table 55 lists the average severity score (0-100) for SOC SAEs relative to mortality. The score for the presence of any non-serious AE was set to 0.01. This score can be referred to as a *mortality equivalent*.

Table 55: Scoring of SOC SAEs relative to mortality

SYSTEM ORGAN CLASS*	Mortality		
	30%	60%	90%
	Score	Score	Score
BLOOD AND LYMPHATIC SYSTEM DISORDERS	5	5	5
CARDIAC DISORDERS	15	10	10
GASTROINTESTINAL DISORDERS	10	5	5
GENERAL DISORDERS AND ADMINISTRATION SITE	5	5	5

SYSTEM ORGAN CLASS*	Mortality		
	30%	60%	90%
	Score	Score	Score
CONDITIONS			
HEPATO-BILIARY DISORDERS	5	5	5
INFECTIONS AND INFESTATIONS	10	5	5
INJURY AND POISONING	10	5	5
INVESTIGATIONS	5	10	15
METABOLISM AND NUTRITION DISORDERS	5	5	5
MUSCULOSKELETAL, CONNECTIVE TISSUE AND BONE DISORDERS	5	5	5
NERVOUS SYSTEM DISORDERS	10	5	5
PSYCHIATRIC DISORDERS	5	5	5
RENAL AND URINARY DISORDERS	10	5	5
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	10	5	5
SURGICAL AND MEDICAL PROCEDURES	15	5	5
VASCULAR DISORDERS	10	5	5

* SOC are based upon the MedDRA medical dictionary used for the classification of AEs.

The method for calculating the total score for each subject utilized the following recursive equation:

$$R = r_1 + r_2/(1 + r_1 * r_2)$$

Note: this equation is symmetric in that the result will not depend on the order of operation. In addition, this equation is asymptotic—the sum approaches but never reaches 1.0 when all summed scores are <1.0. If any reported score equals 1.0, then the total score for that subject will be 1.0. For scores < 0.3, the formula essentially yields the summation of scores.

This was used for the calculation of morbidity/mortality score for subjects who died or experienced any SAE. For the remaining subjects, a score of 0.01 was assigned to subjects that experienced any AE and a score = 0.0 was assigned to those who did not experience an AE.

Analysis of Benefit:Risk

The analysis of benefit:risk is divided into three parts: the first is assessment of *BRR* based on matching group comparisons in HEM-0115; the second involves ITT and worse-case assessments of *BRR* in HEM-0115; and the third utilizes interim results from the HEM-0125 S. Africa ER traumatic HS trial.

Risk Assessment Using Matching Subgroups Analysis (from HEM-0115)

In FDA's critique of the RESUS protocol, the risk to subjects who may not benefit from HBOC-201, on the basis of low need, was assumed to be high. That is, the risk to subjects who are at the lowest risk of mortality, and thus the lowest need for HBOC-201 or RBC, was considered unreasonable. The first analysis herein will estimate the BRR for subjects with the lowest needs, based on HEM-0115 results. Table 56 summarizes the risk observed for the *HH* and *R*- subgroups, expressed in mortality equivalents specifically in the trauma setting. As noted above, AEs were scored relative to mortality on a 1 to 100 scale with death receiving a score of 100. This score is referred to as a mortality equivalent. The data show that the net risk = 0.001, suggesting that there is no net risk because the group difference is within the margin of rounding error.

Table 56: Risk Scores (mortality equivalents) in HEM-0115 matching groups

Treatment	N	Mean (q)	SD	Min	Max
H Group					
HH	211	0.028	0.118	0	1
R Group					
R-	231	0.027	0.113	0	1
Net risk **(q_{1-2})**					
HH vs. *R-*	0.028 - 0.027 = 0.001				

These data suggest that for subjects with relatively low need for treatment with HBOC-201 and RBC, the level of risk relative to mortality is not measurable. Furthermore, these data suggest that subjects in *RESUS* who will not benefit from treatment with HBOC-201 in terms of reduced mortality because of low need for therapy, are at no additional risk.

Risk Assessment Using ITT And Worse-Case Analysis (From HEM-0115)

The extrapolated risk of AEs associated with exposure to HBOC-201 was estimated from data in the largest trial conducted with HBOC-201, HEM-0115. Table 57 summarizes the risk, expressed in *mortality equivalents*, for the overall population (ITT) and < 70 year old sub-population.

Table 57: Risk Scores (mortality equivalents) in HEM-0115 overall population and < 70 year old sub-population

Treatment	N	Mean (q)	SD	Min	Max
Overall population					
H Group	350	0.050	0.165	0	1
R Group	338	0.035	0.132	0	1
Net risk **(q_{1-2})**	0.050 - 0.035 = 0.015				
< 70 year old sub-population					
H Group	239	0.029	0.093	0	1
R Group	227	0.017	0.021	0	0.149
Net risk **(q_{1-2})**	0.029 - 0.017 = 0.012				

Calculating the *BRR*, the NNT = 11.5 defined above and the estimate of NNH of 1.0/0.015 = 67, gives,

BRR = 67/11.5 = 5.8

suggesting that expectation for benefit is 6 times greater than that of harm. In subjects < 70 years old, the estimate of NNH of 1.0/0.012 = 83.3 gives, the BRR is as follows:

BRR = 83.3/11.5 = 7.2

This estimation of risk using *mortality equivalents* to account for the asymmetry in the clinical significance of death vs. SAE occurrence, showing *BRR* values of 5.8-7.2 despite absence of compensation for the asymmetry of study design in HEM-0115, predicts *highly favorable* benefit:risk for enrolled subjects in *RESUS*. It is apparent that reduction in the age of subjects improves the *BRR* by ~ 24%.

Worse Case Analysis

Table 58summarizes risk, expressed in *mortality equivalents*, observed in the *HR* and *R+* matching subgroups, providing a worse-case estimation of relative risk because these subgroups represent the sickest subjects with the greatest need for treatment with HBOC-201 and RBC. These subjects had

the highest baseline level and group difference in AEs, reflected in the larger risk difference, or *net risk* (0.030), which is twice that *net risk* derived from the ITT analysis described above.

Table 58: Risk Scores (mortality equivalents) in HEM-0115 in HR and R+ Subgroups

Treatment	N	Mean	S.D.	Min	Max
H Group					
HR	139	0.083	0.119	0	1
R Group					
R+	107	0.053	0.164	0	1
Net risk					
HR vs. *R+*	0.083 - 0.053 = 0.030				

The *BRR*, based on benefit:risk prediction comparing the HEM-0115 HR and R+ groups is:

BRR = 33.3/11.5 = 2.9

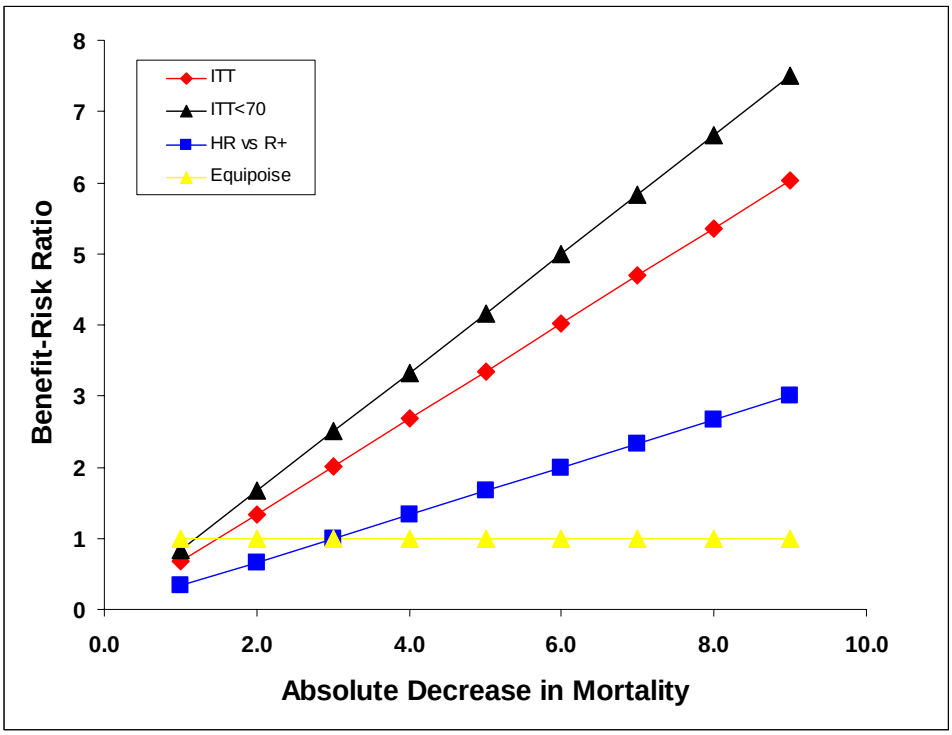
The BRR in the < 70 year sub-population was more favorable (*BRR* = 3.4 vs. 2.9) compared to the entire population and is not shown. In any case, these assessments of the *BRR* for worse-case scenarios show that *BRR* remains *highly favorable* as it exceeds equipoise (*BRR* = 1.0) by a factor of ~ 3. Nevertheless, a number of assumptions in this worse-case analysis should be noted. First, there is the assumption that direct extrapolation of safety data from HEM-0115 to prediction of benefit:risk in *RESUS* is accurate; this remains questionable. Similarly, root cause analysis has identified key factors that likely contributed to the increased risk in HEM-0115. The assumption that these same risk factors will apply equally in *RESUS* may not be justified. In HEM-0115, potential to replete blood oxygen content was significantly lower in HBOC-201 than RBC subjects; specifically, HBOC-201's Hb concentration was 13 g/dl lower than in RBC, contributing to under-treatment and volume overload. In *RESUS*, the converse is true, with the concentration of Hb in HBOC-201 being 13 g/dL higher than in the comparator group, LR. Similarly, in HEM-0115, delayed effective treatment with an oxygen carrying solution occurred while attempting to substitute for blood transfusion requirements of anemic subjects over six days; in contrast, in *RESUS*, there will be no added delay in administration of RBC. In fact, risk associated with delayed effective treatment with an oxygen carrying solution will occur exclusively in control subjects (LR).

This *RESUS* benefit:risk prediction analysis, using data comparisons from HEM-0115 HR and R+ groups, represents a scenario unlikely to occur in *RESUS*; yet, by using worse-case scenarios (regarding safety data), it exaggerates risk, defines the lowest *BRR*, and hence is useful for discussion of robustness of the benefit:risk assessment (examined in detail below).

Impact Of Parameter Estimates On *BRR*

To illustrate the robustness of a *favorable* benefit:risk prediction for *RESUS* (i.e., *BRR* $\geq$ 1.0), the impact of variance in key parameter estimates are shown in Figure 8 & Figure 9. Figure 8 shows the relationship between the *BRR* and effect size, plotted in terms of absolute reduction in mortality. It should be recalled the *RESUS* assumptions predict a 15% relative reduction in mortality (i.e., 8.7% absolute reduction). The three sloped lines represent results from three of the aforementioned *net risk* estimates. The horizontal line represents *equipoise*, *BRR* = 1.0.

Figure 8: Dependence of BRR on Treatment Effect Size (based on HEM-0115 data)



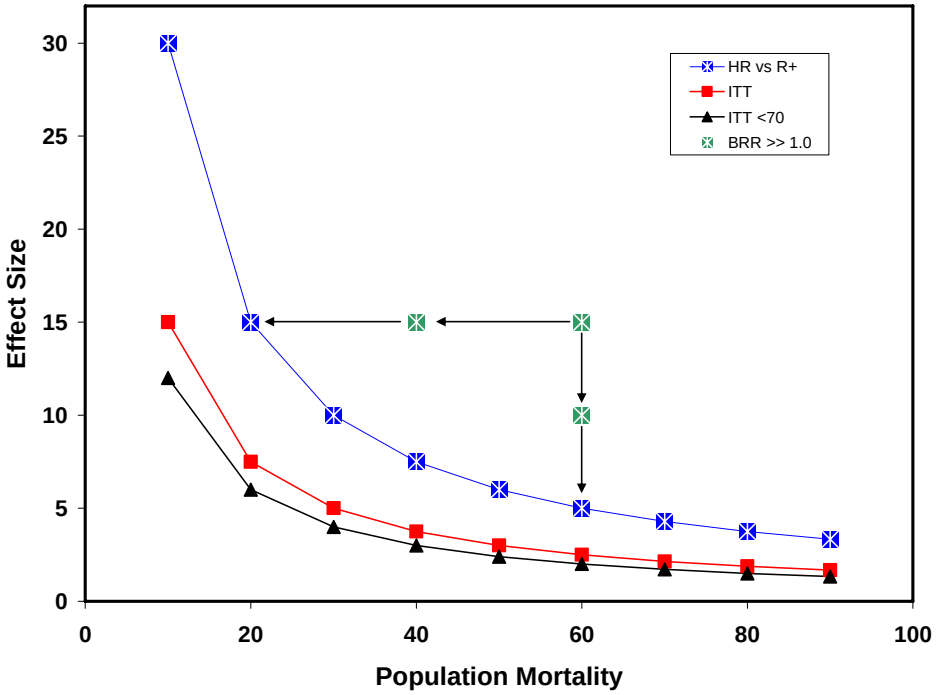
A critical factor in the assessment of *BRR* is related to the possible range of the key parameters used in this assessment. That is, what level of variance in these key parameters is permitted for the benefit:risk assessment to remain *favorable* (i.e. *BRR* $\geq$ 1.0). In this particular situation, *BRR* = f (control population mortality, effect size, risk), that is, *BRR* is a function of the three parameters,

control population mortality, effect size, and risk. *BRR* is inversely proportional to risk; that is, the higher the risk the lower the *BRR*. The higher risk requires a larger absolute decrease in control mortality (effect size) in order for the *BRR* to remain ≥ 1.0 (*equipoise*). This is illustrated in Figure 8 which shows that *equipoise* is predicted for *RESUS* even with absolute decreases in mortality as low as 3, 1.5, and 1.2% based HEM-0115 HR vs. R+ groups comparisons (worse-case scenario), ITT overall population, and ITT < 70 year old sub-population, respectively. These benefit:risk estimates predict a 3-7 fold cushion margin of error as the absolute reduction in mortality expected in *RESUS* is > 8.7%.

To further illustrate the relationship between variance in effect size, control population mortality, and estimates of risk, three *equipoise* curves for each of the three levels of risk analyzed above are shown in Figure 9. Any point above the individual *equipoise* curves predicts a *favorable BRR* relative to the associated estimate of risk. Any point at or above the top curve (HR vs. R+) unequivocally predicts a *favorable BRR* because the top curve (HR vs. R+) represents a worse-case scenario (in terms of safety data input) prediction.

The *RESUS* protocol estimates that the study population will have an average mortality of ~ 60% and the treatment effect size will be 15%. This is illustrated in Figure 9 by the square with coordinates 60 and 15. Using the worse-case scenario *equipoise* curve, the arrows show that even if a 3-fold reduction in either control population mortality (60 → 20%) or effect size (15 → 5%) occurs, *equipoise* is maintained.

Figure 9: Equipoise Lines as a Function of Risk in Terms of Control Population Mortality and Effect Size (based on HEM-0115 data)



The robustness of the *RESUS* study design for showing a beneficial effect is further illustrated by examination of the relationships summarized in Table 59, which shows the ranges of projected control mortality rates and effect sizes that will guarantee *favorable* outcome. These examples show that *BRR* is likely to remain *favorable* even outside the range of expected changes in control population mortality, effect size, and estimated risk.

Table 59: Effect of Error in Estimate of Mortality or Treatment Effect Size

Estimated Mortality (%)	Effect Size (%)	Absolute Change (%)
20	15	3
30	10	3
40	7.5	3
60	5	3

Benefit:Risk Analysis Based On Interim Data From The HEM-0125 S. Africa ER Trauma Trial

The benefit:risk analyses described above are based upon results from the largest study conducted with HBOC-201 in the setting of orthopedic surgery (HEM-0115). Extrapolation of risk from HEM-

0115 is recognized as an approximation from a very different clinical setting that could over- or under-estimate actual risk in the trauma setting (i.e., in *RESUS*). A study of HBOC-201 is underway in S. Africa where the product is being evaluated for safety in a population of trauma patients with severe HS. The study has enrolled 22 subjects of the intended 1:1 randomization of 50 subjects into this trial.

The purpose of the HEM-0125 trial was to assess the safety of HBOC-201 when added to standard therapy compared to standard therapy alone, when administered in doses up to a maximum cumulative dose of 10 units in a 4 hour period following randomization. The intent is to determine if there is an increase in AEs or signals associated with adding HBOC-201 on top of standard therapy (e.g. HBOC-201 + RBC vs. RBC) in subjects with unstable traumatic HS. In this regard, this study provides the best assessment of risk associated with HBOC-201 above standard therapy in a setting mimicking *RESUS*. In contrast, extrapolation of benefit:risk from HEM-0125 to *RESUS* is limited by the fact that HBOC-201 was not administered in the pre-hospital setting where the primary benefit is expected.

The final DSMB meeting was conducted 10 Oct 2006 with the Committee recommending that the study proceed. In light of the fact that *RESUS* will be conducted under provisions of *EIC*, it was determined that unblinding of results prior to completion of this study was necessary to provide an initial estimate of the risk in a clinical setting closer to that of the proposed *RESUS* trial. This decision was also prompted by necessity to address FDA's concern that there could be a greater risk of excess SAEs associated with HBOC-201 treatment, particularly in the sicker subjects anticipated in the trauma setting compared to subjects in the HEM-0115. Thus, the benefit:risk scoring system described above was applied to interim safety data from HEM-0125 Table 60).

Table 60: Risk Scores (mortality equivalents) in HEM-0125

Treatment	N	Mean	Min	Max
H Group	10	0.406	0.01	1
R Group	10	0.410	0.01	1
Net risk				
H vs. R	0.406 - 0.41 = - 0.004			

The results show that the difference in risk is within the rounding error for this analysis. Thus, in the setting of severe HS with a mortality of 40% in the in-hospital setting, there is no evidence of increased risk with HBOC-201. Although these interim results do not completely rule out small

differences (increased or decreased) in risk, they strongly suggest that treatment with HBOC-201 does not result in a large increase in risk in unstable trauma patients with severe HS[75].

Summary

Quantification of the relationship between benefit and risk derived from any new treatment is important in determining whether to go forward with a clinical trial evaluating any proposed intervention. This can prove difficult when the units of measure of benefit and risk are not equivalent. In the *RESUS* trial, the issue is one of determining how to weigh the potential benefit of saving a life against the potential risk of assuming the occurrence of intervention-associated SAEs. To address this issue, a *benefit:risk ratio* (*BRR*) was derived with the aid of quantitative scoring of the relative severity of AEs compared to the baseline mortality expected in the *RESUS* trial. The risk associated with SAEs was expressed in terms of *mortality equivalents*. Using these scores, it was possible to accurately predict the *BRR*, the number of subjects needed to treat (NNT) vs. the number of patients needed to harm (NNH).

The primary estimates of *net risk* were obtained from safety data from the largest homogeneous trial with HBOC-201, HEM-0115. Specifically, these estimates included the low needs sub-population (matched *HH* vs. *R*- groups), the ITT overall population, the < 70 year old sub-population, and a high needs sub-population (*HR* vs. *R*+) which was considered a worse-case scenario (in terms of safety data input). Additionally, assessment of *net risk* was performed using interim safety data from the ongoing HEM-0125 S. Africa ER trauma trial, which more closely resembles the *RESUS* trial (exception HEM-0125 assessed only expected risks and not benefits). The absence of a measurable *net risk* following analysis of the low needs population ruled out any "extrapolatable" risk in a hypothetical low needs/low risk population in *RESUS*. The estimates of *BRR* ranged from 3 to 7 for the other HEM-0115-based models, whereas, there was an absence of measurable risk based on trauma safety data from HEM-0125 ($BRR \rightarrow \infty$). Analysis of the robustness of a *favorable* outcome ($BRR \geq 1.0$), indicated that benefit outweighed risk over a large variation in parameter estimates even for the worse-case scenario estimate of *net risk* based on safety data in the high needs sub-population in HEM-0115.

[75] Note: This analysis included one patient in the control group that survived but was discontinued from the study and AEs for this patient were not collected. Thus, this represents a worse-case analysis appropriately biased in favor of control.

In summary, this benefit:risk analysis showed that the *BRR* is highly *favorable* based on all extrapolated risk estimates and is not sensitive to even reasonably large variations in control population mortality and effect size estimates and *net risk*. This robust relationship overwhelmingly supports lifting the *RESUS* IND Clinical Hold.

7.d *Exception from Informed Consent (EIC)*

This *RESUS* trial will enroll subjects who, by virtue of the inclusion criteria, will be unable to provide *IC* prior to enrollment. *IC* will not be feasible in the majority of subjects due to the acuity of their medical condition and a therapeutic window of minutes before treatment must be initiated. Thus, the conduct of the *RESUS* trial requires a provision for *EIC*. Recognizing high ethical standards necessary for *EIC* trials, NMRC researchers went to extraordinary lengths to ensure a scientifically- and ethically-optimal trial design. Specifically, a comprehensive *CCD* process was developed to maximize informed decision making by affected communities; inclusion/exclusion criteria were selected to target subjects with severe HS, a high rate of mortality, and for whom blood transfusions were unavailable (i.e., those subjects predicted to benefit most from the proposed intervention); provisions were included to prevent any delay in providing standard care (especially blood transfusions); *IC* will be obtained or *Pre-ED* will be provided when feasible to maximize individual subject autonomy; multiple risk mitigation strategies were incorporated to minimize risk to subjects (especially exclusion of the elderly because safety data from prior trials showed that they appeared most sensitive to potentially adverse vasoactive effects of HBOC-201); and independent government direction, funding, and sponsorship of the trial were insisted upon to minimize potential bias. Moreover, despite OBRR's concern that uncontrolled hemorrhage and concomitant TBI might not be adequately treatable with HBOC-201, in a complex OBRR-directed *RESUS* IND-enabling swine study, NMRC showed significantly improved outcome in that simulated clinical setting.

The requirements for research involving subjects enrolled under an *EIC* are set forth in 21 CFR 50.24. These requirements are listed below along with bulleted statements demonstrating how the *RESUS* study protocol has met and in many cases exceeded them. Details regarding *EIC, CCD*, and *RESUS* protocol details are provided in Appendix C.

Sec. 50.24 EIC 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 IC 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.

- As noted previously, using redundant sources (i.e., NTDB query, UAB/UMD trauma center prehospital data registries [Appendix A]), NMRC estimates a mortality rate of ~ 58.1% in the targeted population of subjects receiving standard care in *RESUS*. Thus, each subject is *facing a life threatening situation.*
- Each subject enrolled in the *RESUS* study will be at substantial risk of death (58.1%) despite the current standard of care. Thus, *available treatments are unsatisfactory.*
- To validate the efficacy and safety of HBOC-201 for use in traumatic HS, HBOC-201 must be studied in a trial such as *RESUS.*

(2) Obtaining IC 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.

- Victims of profound HS are not competent to provide adequate *IC*.
- Prehospital interventions must be immediate to optimize opportunity for success.
- It would not be feasible to identify and enroll subjects suffering traumatic HS prior to the event leading to their injuries, given the random epidemiology of trauma.

(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.

- Traumatic HS *carries a significant risk of death and necessitates immediate intervention.*
- An extensive preclinical database of 23 indication-specific HS studies in a wide variety of animal models (mainly swine models simulating younger adults with HS without co-morbid diseases) shows improved survival with HBOC-201 in comparison with standard

therapy (with combined analyses effect sizes of 74.6-82% with p values < 0.0001). Thus, *preclinical studies support the potential for direct benefit to RESUS subjects*[76].

- Given that 1) the relative AE profile of HBOC-201 was only mildly adversely shifted (in the context of *RESUS*) in prior HBOC-201 surgery/orthopedics trials despite comparison with gold standard RBC transfusion, 2) HBOC-201's relative AE profile was significantly better in younger subjects, and elderly subjects are now excluded from enrollment in *RESUS*, 3) mortality is estimated to be 58.1% in the *RESUS* population receiving standard care, and 4) preclinical studies support an estimated mortality reduction in significant excess of 15%, NMRC concludes that *risks associated with the investigation are reasonable in relation to what is known about the medical condition…risks and benefits of standard therapy*.

  NMRC agrees with OBRR that the overall relative AE profile of HBOC-201 might preclude product approval for a general orthopedic *blood substitute* indication in the U.S. because standard care includes efficacious alternatives (i.e., blood transfusions). In other words, NMRC agrees that for elective orthopedic surgery in the U.S., risks may not be *reasonable in relation to what is known about the medical condition of the potential class of subjects, the risks and benefits of standard therapy…* (i.e., availability of blood transfusions) (in an unrestricted population). But for a prehospital severe HS indication, the same safety profile must be considered in the context of a different standard of care that does not include efficacious alternatives (i.e., absence of blood transfusions). Thus, the *relative* benefit:risk equation is favorable.

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

- Enrolling subjects with appropriately severe HS can only be accomplished with *EIC*.

(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.

[76] One limitation of the preclinical HS swine studies is that they were in juvenile swine. Hence, their ability to predict cardiac and cerebral ischemic AE occurrence in older human subjects may be limited. However, they support prediction of efficacy and safety in analogous younger adult humans with HS—similar to the *RESUS* target population since addition of proposed exclusion of the elderly.

- As described above, prehospital interventions must be initiated immediately to ensure the best opportunity for success; thus, the therapeutic window is very short in time and would preclude the general application of *IC*.
- When considered feasible by the EMS provider, attempts to obtain *IC* will be made.
- When a legally authorized representative (LAR) or family member is present, *Pre-ED* will be conducted with a predefined script to be read by the EMS provider.
- An extensive program of ongoing post-enrollment disclosure and continuing *IC* will be conducted with subjects, their LARs, and family members.
- All attempts have been made to maximize subject autonomy.

(6) The IRB has reviewed and approved informed consent procedures and an informed consent document consistent with Sec. 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.

- The procedure and documentation for *IC* will be vetted by the NMRC IRB as well as each individual participating trauma center IRB.
- Reasonable/feasible effort will be made to obtain *IC* or provide *Pre-ED* prior to exercising *EIC*.
- Following enrollment, investigators will actively pursue ongoing consent from subjects, their legally authorized representative, or family members.

(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.

- An extensive program of *CCD* has been developed.

- An independent DMC will oversee trial conduct and subject safety.
- Reasonable/feasible effort will be made to obtain the highest level of consent feasible at the time of enrollment as well as in a continual consent process.

(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.

- These provisions have been included in detail in the *RESUS* protocol.

(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 Sec. 56.115(b) of this chapter.

- These provisions have been included in detail in the *RESUS* protocol.

(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 Secs. 312.30 or 812.35 of this chapter.

- The *RESUS* protocol is clearly identified in the IND as requiring an *EIC*.

(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 IRBs that have been, or are, asked to review this or a substantially equivalent investigation by that sponsor.

- All appropriate correspondence between Navy and Trauma Center IRBs will be documented and provided as necessary to FDA.

8.0 CONCLUSION

NMRC concludes that *in vitro* and *in vivo* studies show that HBOC-201 efficiently transports and unloads oxygen [35, 36]; indication-specific preclinical HS studies support prediction of survival benefit in *RESUS* (efficacy and safety) with mortality reduction effect sizes of 74.4% overall and 82% in severe HS; clinical data from overall populations in surgery/orthopedics blood substitute trials for other indications show transfusion avoidance (efficacy) and an HBOC-201 safety profile only mildy worse than the gold standard RBC safety profile. These observations must be taken in the context of the high mortality *RESUS* trial where the comparator is LR and not RBC. In populations closely resembling subjects to be enrolled in *RESUS*, current standard therapy (e.g., LR) is associated with high mortality (~ 1 in 2). Even if the same *relative* safety profile observed in HBOC-201-treated subjects in prior surgery/orthopedics trials was seen in HBOC-201-resuscitated subjects in *RESUS*, the potential benefit of increased survival outweighs circumstantial evidence suggesting potential for higher incidence of common non-serious AEs and uncommon SAEs in comparison with RBC. In fact, the incidence of SAEs is expected to be lower in HBOC-201 than in LR subjects in *RESUS* because the comparator (LR) is known to be suboptimal in comparison with RBC. That SAE incidence is lower in HBOC-201 than RBC subjects in an interim analysis of the ongoing HEM-0125 ER trauma trial is S. Africa is highly supportive of this hypothesis. NMRC believes that that NMRC has met and in many cases exceeded provisions of 21 CFR 50.24 (including predicted benefit); specifically, *Risks associated with the intervention are reasonable in relation to what is known about the medical condition of the potential class of subjects* (21 CFR 50.24 (a) (3) (iii)).

Balancing the great potential benefit to be derived from HBOC-201 against potential risks, which appear *relatively* small in non-elderly adults, NMRC believes the *RESUS* trial should be allowed to proceed. OBRR's allowance of the *RESUS* trial would enable a fair assessment of HBOC-201's potential use as a resuscitative fluid for treatment of trauma victims with severe HS without access to blood transfusions. NMRC agrees with OBRR that HBOC-201 should be tested under conditions that pose the least risk to subjects, and NMRC has tried to design a trial that will do so. Therefore, NMRC believes that it is ethically, scientifically, and medically appropriate for OBRR to lift the *RESUS* Clinical Hold and to allow research predicted to diminish mortality to proceed, particularly because HBOC-201 does not pose unreasonable risk to individual subjects under the conditions of *RESUS*. Clearly, HBOC-201 has potential to have a transformational impact on trauma medicine.

The Way Forward

NMRC hopes that the BPAC will consider recommending lifting of the *RESUS* Clinical Hold. If the BPAC does not agree with NMRC's position, NMRC hopes that the BPAC will make specific recommendations about necessary IND modifications that should allow lifting of the Clinical Hold. Finally, NMRC suggests that after the BPAC meeting, NMRC and OBRR convene for a day-long conference to deliberate about the BPAC recommendations with the aim of ironing out remaining differences in order to lift the Clinical Hold and allow the *RESUS* trial to proceed.

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10.0 APPENDICES

List of Appendices

Appendix A: National Trauma Data Bank and UAB and UMD trauma registry mortality data

Table A.1. Mortality and N in hypotensive non-elderly adults admitted to U.S. trauma centers stratified by RTS and TBI (NTDB [hospital arrival data])

Table A.2. Mortality and N in hypotensive non-elderly adults at the prehospital scene after trauma stratified by RTS and TBI (UAB/UMD [prehospital data])

Figure A.3. Mortality in hypotensive non-elderly adults admitted to U.S. trauma centers stratified by RTS and TBI (NTDB [hospital arrival data])

Figure A.4. Mortality in hypotensive non-elderly adults at the prehospital scene after trauma stratified by RTS and TBI (UAB/UMD [prehospital data])

Figure A.5. N in hypotensive non-elderly adults admitted to U.S. trauma centers stratified by RTS and TBI (NTDB [hospital arrival data])

Figure A.6. N in hypotensive non-elderly adults at the prehospital scene after trauma stratified by RTS and TBI (UAB/UMD [prehospital data])

Table A.7. Mortality and N in all hypotensive adults (> 18 years old) admitted to U.S. trauma centers stratified by RTS (NTDB [hospital arrival data])

Table A.8. Mortality and N in hypotensive elderly adults (> 70 years old) admitted to U.S. trauma centers stratified by RTS (NTDB [hospital arrival data])

Appendix B: Key Adverse Safety Data (HEM-0115, ISS)

Table B.1. HEM-0115 key adverse safety signals stratified by age
Table B.2. ISS key adverse safety signals stratified by age
Table B.3. Logistic odds ratios (OR) for ISS key adverse safety signals stratified by age

Appendix C: *RESUS* protocol details

Appendix D: *RESUS* in-hospital trauma care guidelines

Appendix E: HEM-0125 S. Africa ER Trauma Trial - Biopure Provided Documents

E.1. Preliminary data HEM-0125 – Biopure Corporation
E.2. Protocol HEM-0125 DSMB Recommendation and DSMB meeting minutes – Biopure Corp.

Appendix F: Document prepared by Fred Apple, PhD re: codification of cardiac biomarker (troponin) data in diagnosis of MI

Table A.1. Mortality and N in <u>hypotensive non-elderly adults</u> admitted to U.S. trauma centers <u>stratified by RTS and TBI</u> (NTDB [hospital arrival data])

		Dead N	**Total N**			**Dead %**	
1 to < 2	379	789	1168	**1 to < 2**	32.4	67.6	**1 to < 2**
2 to < 3	223	293	516	**2 to < 3**	43.2	56.8	**2 to < 3**
3 to < 4	218	172	390	**3 to < 4**	55.9	44.1	**3 to < 4**
4 to < 5	258	109	367	**4 to < 5**	70.3	29.7	**4 to < 5**
5 to < 6	481	96	577	**5 to < 6**	83.4	16.6	**5 to < 6**
6 to 6.5	1391	159	1550	**6 to 6.5**	89.7	10.3	**6 to 6.5**
Total	**2950**	**1618**	**4568**		**64.6**	**35.4**	
1 to 6	**1559**	**1459**	**3018**	**1 to 6**	**51.7**	**48.3**	
1 to < 5	**1078**	**1363**	**2441**		**44.2**	**55.8**	
2 to < 5	**699**	**574**	**1273**		**54.9**	**45.1**	
NTDB RTS stratified, SBP < 90 mm Hg, no GCS exclusion, 18-69 years old, w/o TBI							
	Alive N	**Dead N**	**Total N**		**Alive %**	**Dead %**	
1 to < 2	371	729	1100	**1 to < 2**	33.7	66.3	**1 to < 2**
2 to < 3	213	252	465	**2 to < 3**	45.8	54.2	**2 to < 3**
3 to < 4	207	158	365	**3 to < 4**	56.7	43.3	**3 to < 4**
4 to < 5	246	99	345	**4 to < 5**	71.3	28.7	**4 to < 5**
5 to < 6	451	90	541	**5 to < 6**	83.4	16.6	**5 to < 6**
6 to 6.5	1337	157	1494	**6 to 6.5**	89.5	10.5	**6 to 6.5**
Total	**2825**	**1485**	**4310**		**65.5**	**34.5**	
1 to 6	**1488**	**1328**	**2816**	**1 to 6**	**52.8**	**47.2**	
1 to < 5	**1037**	**1238**	**2275**		**45.6**	**54.4**	
2 to < 5	**666**	**509**	**1175**		**56.7**	**43.3**	
NTDB RTS stratified, SBP < 90 mm Hg, no GCS exclusion, 18-69 years old, w/ TBI							
	Alive N	**Dead N**	**Total N**		**Alive %**	**Dead %**	
1 to < 2	8	60	68	**1 to < 2**	11.8	88.2	**1 to < 2**
2 to < 3	10	41	51	**2 to < 3**	19.6	80.4	**2 to < 3**
3 to < 4	11	14	25	**3 to < 4**	44.0	56.0	**3 to < 4**
4 to < 5	12	10	22	**4 to < 5**	54.5	45.5	**4 to < 5**
5 to < 6	30	6	36	**5 to < 6**	83.3	16.7	**5 to < 6**
6 to 6.5	54	2	56	**6 to 6.5**	96.4	3.6	**6 to 6.5**
Total	**125**	**133**	**258**		**48.4**	**51.6**	
1 to 6	**71**	**131**	**202**	**1 to 6**	**35.1**	**64.9**	
1 to < 5	**41**	**125**	**166**		**24.7**	**75.3**	
2 to < 5	**33**	**65**	**98**		**33.7**	**66.3**	

RTS range	**% W/O TBI**	**% W/ TBI**
1 to 6.5	94.4	5.6
1 to < 6	93.3	6.7
1 to < 5	93.2	6.8
2 to < 5	92.3	7.7

Table A.2. Mortality and N in hypotensive non-elderly adults at the prehospital scene after trauma stratified by RTS and TBI (UAB/UMD [prehospital data])

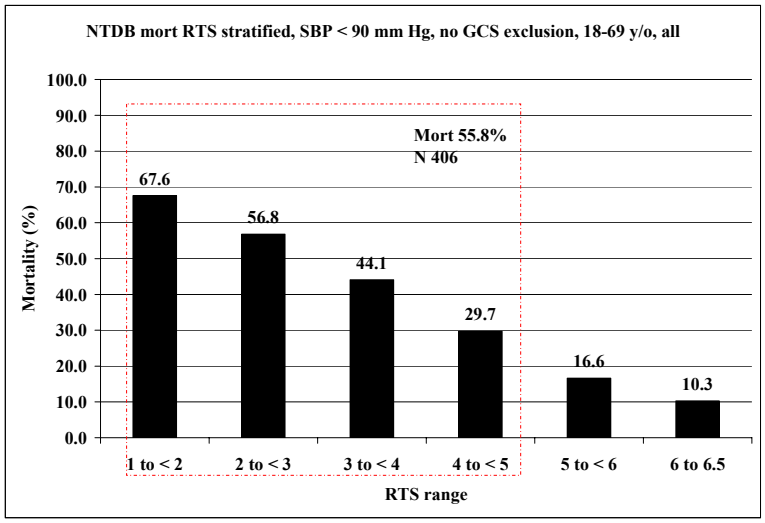
UAB/UMD COMBINED RTS stratified, no GCS exclusion, 18 to 69 years old, all							
	Alive N	Dead N	Total N		Alive %	Dead %	
1.1 to 2	5	39	44	1.1 to 2	11.4	88.6	1.1 to 2
2.1 to 3	40	55	95	2.1 to 3	42.1	57.9	2.1 to 3
3.1 to 4	30	25	55	3.1 to 4	54.5	45.5	3.1 to 4
4.1 to 5	23	17	40	4.1 to 5	57.5	42.5	4.1 to 5
5.1 to 6	61	10	71	5.1 to 6	85.9	14.1	5.1 to 6
6.1 to 6.5	181	11	192	6.1 to 6.5	94.3	5.7	6.1 to 6.5
1.1 to 6.5	**340**	**157**	**497**	**1.1 to 6.5**	**68.4**	**31.6**	
1.1 to 6	**159**	**146**	**305**	**1.1 to 6**	**52.1**	**47.9**	
1 .1 to 5	**98**	**136**	**234**	**1 .1 to 5**	**41.9**	**58.1**	
2.1 to 5	**93**	**97**	**190**	**2.1 to 5**	**48.9**	**51.1**	

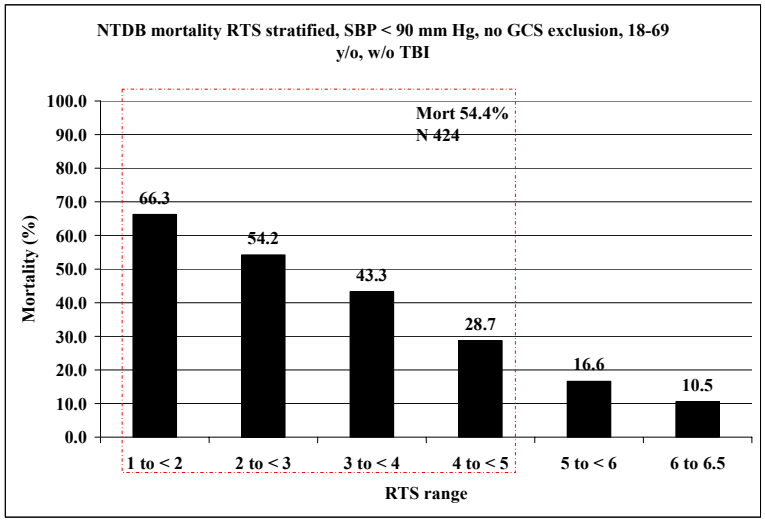
UAB/UMD COMBINED RTS stratified, 18 to 69 years old, w/o TBI							
	Alive N	Dead N	Total N		Alive %	Dead %	
1.1 to 2	2	26	28	1.1 to 2	7.1	92.9	1.1 to 2
2.1 to 3	25	38	63	2.1 to 3	39.7	60.3	2.1 to 3
3.1 to 4	23	15	38	3.1 to 4	60.5	39.5	3.1 to 4
4.1 to 5	18	11	29	4.1 to 5	62.1	37.9	4.1 to 5
5.1 to 6	48	6	54	5.1 to 6	88.9	11.1	5.1 to 6
6.1 to 6.5	167	9	176	6.1 to 6.5	94.9	5.1	6.1 to 6.5
1.1 to 6.5	**283**	**105**	**388**	**1.1 to 6.5**	**72.9**	**27.1**	
1.1 to 6	**116**	**96**	**212**	**1.1 to 6**	**54.7**	**45.3**	
1 .1 to 5	**68**	**90**	**158**	**1 .1 to 5**	**43.0**	**57.0**	
2.1 to 5	**66**	**64**	**130**	**2.1 to 5**	**50.8**	**49.2**	

UAB/UMD COMBINED RTS stratified, 18 to 69 years old, w/ TBI							
	Alive N	Dead N	Total N		Alive %	Dead %	
1.1 to 2	3	13	16	1.1 to 2	18.8	81.3	1.1 to 2
2.1 to 3	15	17	32	2.1 to 3	46.9	53.1	2.1 to 3
3.1 to 4	7	10	17	3.1 to 4	41.2	58.8	3.1 to 4
4.1 to 5	5	6	11	4.1 to 5	45.5	54.5	4.1 to 5
5.1 to 6	13	4	17	5.1 to 6	76.5	23.5	5.1 to 6
6.1 to 6.5	14	1	15	6.1 to 6.5	93.3	6.7	6.1 to 6.5
1.1 to 6.5	**57**	**51**	**108**	**1.1 to 6.5**	**52.8**	**47.2**	
1.1 to 6	**43**	**50**	**93**	**1.1 to 6**	**46.2**	**53.8**	
1 .1 to 5	**30**	**46**	**76**	**1 .1 to 5**	**39.5**	**60.5**	
2.1 to 5	**27**	**33**	**60**	**2.1 to 5**	**45.0**	**55.0**	

RTS range	% W/O TBI	% W/ TBI
1 to 6.5	78.1	21.7
1 to < 6	69.5	30.5
1 to < 5	67.5	32.5
2 to < 5	68.4	31.6

Figure A.3. Mortality in <u>hypotensive non-elderly adults</u> admitted to U.S. trauma centers <u>stratified by RTS and TBI</u> (NTDB [hospital arrival data])





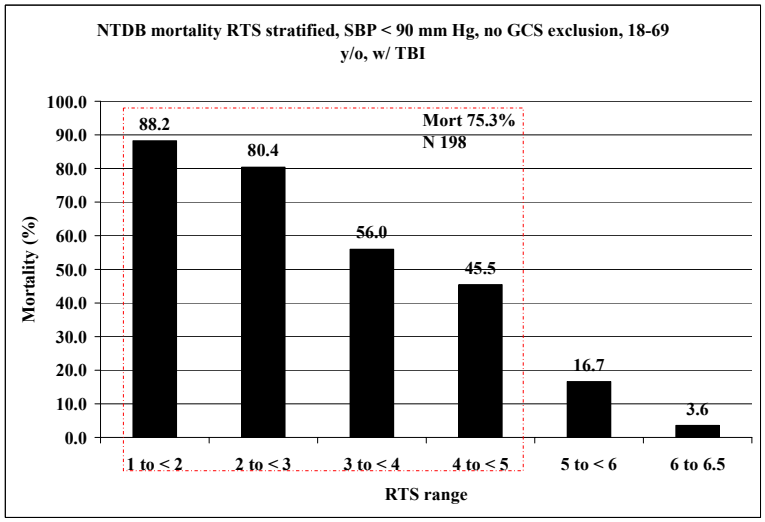
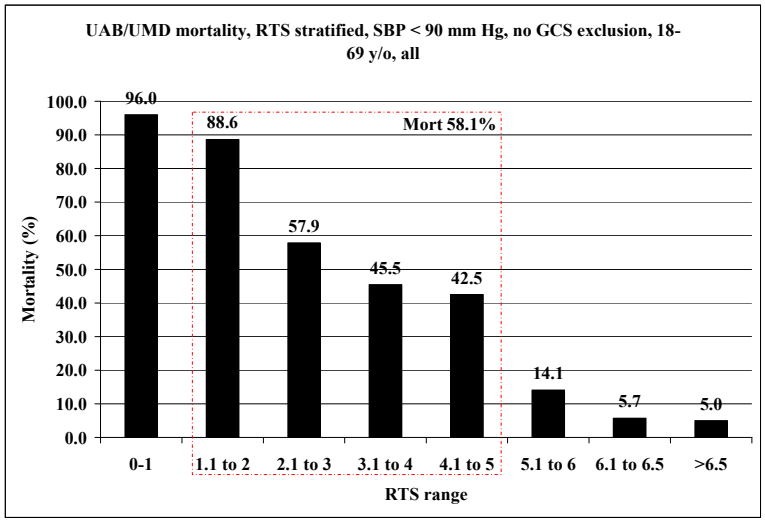
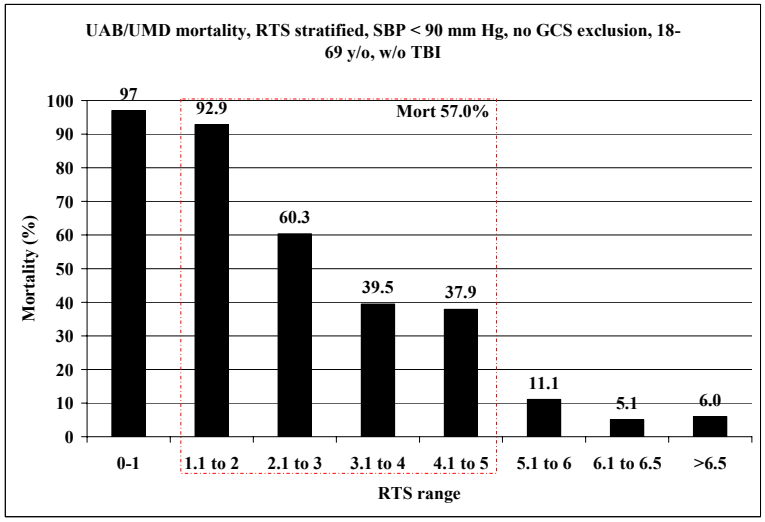


Figure A.4. Mortality in <u>hypotensive non-elderly adults</u> at the prehospital scene after trauma <u>stratified by RTS and TBI</u> (UAB/UMD [prehospital data])





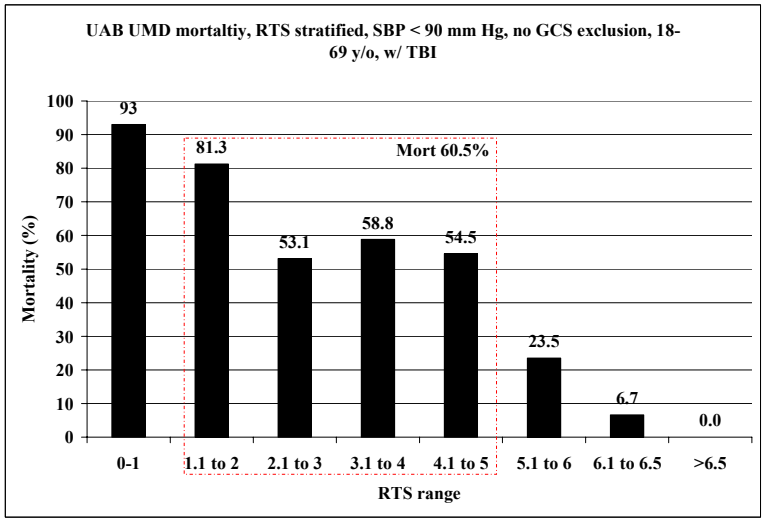
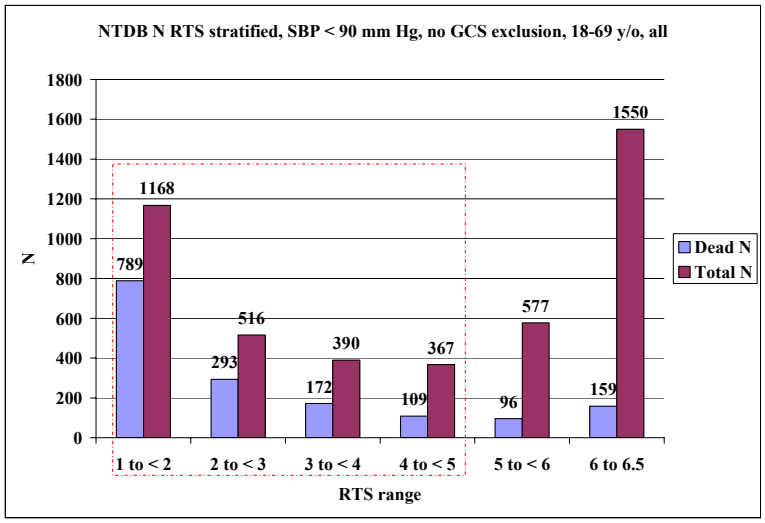
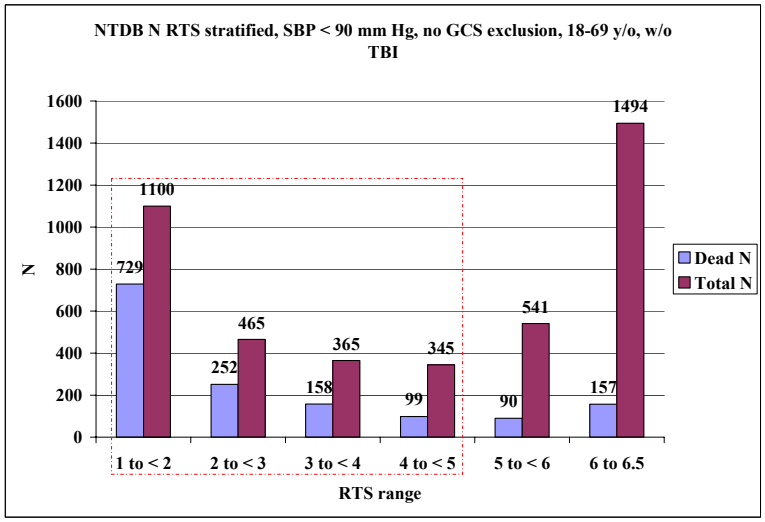


Figure A.5. N in <u>hypotensive non-elderly adults</u> admitted to U.S. trauma centers <u>stratified by RTS and TBI</u> (NTDB [hospital arrival data])





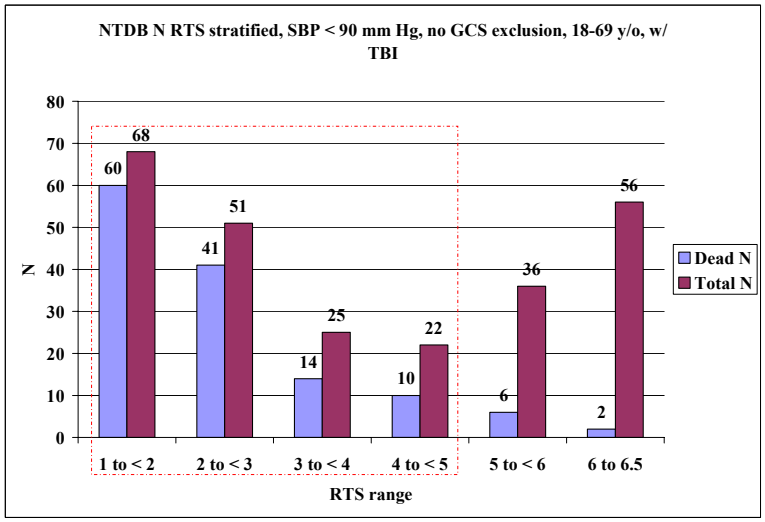
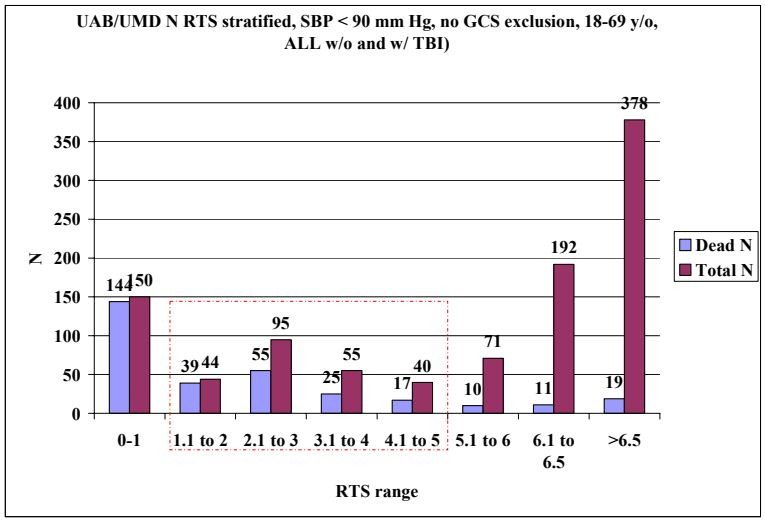
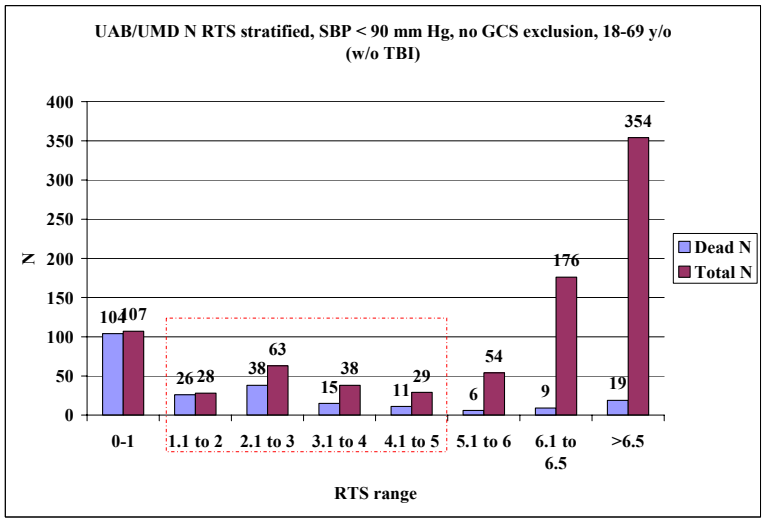


Figure A.6. N in <u>hypotensive non-elderly adults</u> at the prehospital scene after trauma <u>stratified by RTS and TBI</u> (UAB/UMD [prehospital data])





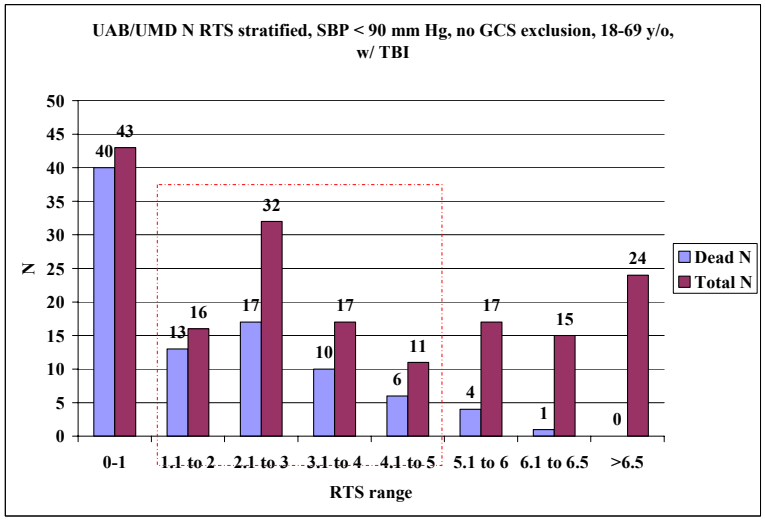


Table A.7. Mortality and N in <u>all hypotensive adults</u> (≥ 18 years old) admitted to U.S. trauma centers <u>stratified by RTS</u> (NTDB [hospital arrival data])

NTDB RTS stratified, SBP < 90 mm Hg, no GCS exclusion, > 18 years old							
	Alive N	Dead N	Total N		Alive %	Dead %	
1 to < 2	412	899	1311	1 to < 2	31.4	68.6	1 to < 2
2 to < 3	237	338	575	2 to < 3	41.2	58.8	2 to < 3
3 to < 4	233	205	438	3 to < 4	53.2	46.8	3 to < 4
4 to < 5	276	128	404	4 to < 5	68.3	31.7	4 to < 5
5 to < 6	535	116	651	5 to < 6	82.2	17.8	5 to < 6
6 to 6.5	1578	215	1793	6 to 6.5	88.0	12.0	6 to 6.5
Total	**3271**	**1901**	**5172**		**63.2**	**36.8**	
1 to < 5	**1158**	**1570**	**2728**		**42.4**	**57.6**	
2 to < 5	**746**	**671**	**1417**		**52.6**	**47.4**	

Table A.8. Mortality and N in <u>hypotensive elderly adults</u> (≥ 70 years old) admitted to U.S. trauma centers <u>stratified by RTS</u> (NTDB [hospital arrival data])

NTDB RTS stratified, SBP < 90 mm Hg, no GCS exclusion, ≥ 70 years old							
	Alive N	Dead N	Total N		Alive %	Dead %	
1 to < 2	33	110	143	1 to < 2	23.1	76.9	1 to < 2
2 to < 3	14	45	59	2 to < 3	23.7	76.3	2 to < 3
3 to < 4	15	33	48	3 to < 4	31.3	68.8	3 to < 4
4 to < 5	18	19	37	4 to < 5	48.6	51.4	4 to < 5
5 to < 6	54	20	74	5 to < 6	73.0	27.0	5 to < 6
6 to 6.5	187	56	243	6 to 6.5	77.0	23.0	6 to 6.5
Total	**321**	**283**	604		**53.1**	**46.9**	
1 to < 5	**80**	**207**	**287**		**27.9**	**72.1**	
2 to < 5	**47**	**97**	**218**		**21.6**	**44.5**	

Appendix B.1 HEM-0115 key adverse safety signals stratified by age

Incidence (%)

	N		Overall AEs (%)			Overall SAEs (%)			Card AEs (%)			Card SAEs (%)			MI AEs^(%)		
	HBOC	Control	HBOC	Control	Diff	HBOC	Control	Diff	HBOC	Control	Diff	HBOC	Control	Diff	HBOC	Control	Diff
> 70 y/o	111	111	95.5	91	4.5	35.1	23.4	11.7	37.8	22.5	15.3	12.6	4.5	8.1	3.6	1.8	1.8
Overall	350	338	95.4	91.1	4.3	25.1	17.5	7.7	29.1	17.2	12	6.3	2.7	3.6	1.1	0.6	0.6
< 70 y/o	239	227	95.4	91.2	4.2	20.5	14.5	6	25.1	14.5	10.6	3.3	1.8	1.6	0	0	0
≤ 50 y/o	97	69	96.9	91.3	5.6	20.6	14.5	6.1	22.7	15.9	6.7	2.1	0	2.1	0	0	0

	N		Cardiac arrest AEs*			HTN AEs			HTN SAEs			Heart failure AEs**			Mortality		
	HBOC	Control	HBOC	Control	Diff	HBOC	Control	Diff	HBOC	Control	Diff	HBOC	Control	Diff	HBOC	Control	Diff
> 70 y/o	111	111	5.4	1.8	3.6	11.7	4.5	7.2			0	5.4	0.9	4.5	7.2	5.4	1.8
Overall	350	338	2.3	0.6	1.7	12.3	5.3	7	0.6	0	0.6	2.3	0.3	2	2.9	1.8	1.1
< 70 y/o	239	227	0.8	0	0.8	12.6	5.7	6.8	0.8	0	0.8	0.8	0	0.8	0.8	0	0.8
≤ 50 y/o	97	69	1	0	1	8.2	5.8	2.5	1	0	1	0	0	0	0	0	0

	N		CVA AEs			Cereb isch AEs		
	HBOC	Control	HBOC	Control	Diff	HBOC	Control	Diff
> 70 y/o	111	111	3.6	0	3.6	4.5	0	4.5
Overall	350	338	1.7	0	1.7	2	0.6	1.4
< 70 y/o	239	227	0.8	0	0.8	0.8	0.9	-0.1
≤ 50 y/o	97	69	0	0	0	0	1.4	-0.4

* Combination 20 (OBRR-Biopure June 2006)

** Combination 22 (OBRR-Biopure June 2006)

^ Data include 3 HBOC-201 MI AEs classified as "non-serious" in the < 70 year old sub-pop

AEs	N		Card			Vasc			Resp			Neuro			Renal/urinary		
	HBOC	Control	HBOC	Control	Diff	HBOC	Control	Diff	HBOC	Control	Diff	HBOC	Control	Diff	HBOC	Control	Diff
> 70 y/o	111	111	37.8	22.5	15.3	34.2	14.4	19.8	37.8	26.1	11.7	38.7	32.4	6.3	33.3	19.8	13.5
Overall	350	338	29.1	17.2	12	28.9	15.1	13.8	27.7	20.7	7	43.7	36.1	7.6	24.9	18.9	5.9
< 70 y/o	239	227	25.1	14.5	10.6	26.4	15.4	10.9	23	18.1	5	46	37.9	8.1	20.9	18.5	2.4
≤ 50 y/o	97	69	22.7	15.9	6.7	18.6	11.6	7	17.5	20.3	-2.8	50.5	36.2	14.3	16.5	17.4	-0.9

SAEs	N		Card			Vasc			Resp			Neuro			Renal/urinary		
	HBOC	Control	HBOC	Control	Diff	HBOC	Control	Diff	HBOC	Control	Diff	HBOC	Control	Diff	HBOC	Control	Diff
> 70 y/o	111	111	12.6	4.5	8.1	3.6	3.6	0	1.8	0	1.8	2.7	0	2.7	1.8	1.8	0
Overall	350	338	6.3	2.7	3.6	4	3.3	0.7	2	0.9	1.1	1.4	0.6	0.8	2	1.2	0.8
< 70 y/o	239	227	3.3	1.8	1.6	4.2	3.1	1.1	2.1	1.3	0.8	0.8	0.9	-0.1	2.1	0.9	1.2
≤ 50 y/o	97	69	2.1	0	2.1	4.1	2.9	1.2	3.1	1.4	1.6	0	1.4	-1.4	3.1	1.4	1.6

Appendix B.2 ISS key adverse safety signals stratified by age

Incidence %

	N		Overall AEs (%)			Overall SAEs (%)			Card AEs (%)			Card SAEs (%)			MI AEs^(%)			CVA AEs (%)		
	HBOC	Control	HBOC	Control	Diff	HBOC	Control	Diff	HBOC	Control	Diff	HBOC	Control	Diff	HBOC	Control	Diff	HBOC	Control	Diff
> 70 y/o	233	196	93.13	91.84	1.30	37.77	28.57	9.20	38.63	29.08	9.54	11.59	7.14	4.45	3.00	1.02	1.98	2.58	0.51	2.06
Overall	797	661	92.85	87.90	4.95	23.46	18.15	5.31	27.10	21.63	5.47	5.77	3.93	1.84	1.63	0.61	1.03	1.13	0.15	0.98
< 70 y/o	564	465	92.73	86.24	6.49	17.55	13.76	3.79	22.34	18.49	3.85	3.37	2.58	0.79	1.06	0.43	0.63	0.53	0.00	0.53
< 50 y/o	245	158	93.88	85.44	8.43	14.69	12.03	2.67	13.88	15.82	-1.95	1.22	1.27	-0.04	0.82	0.00	0.82	0.00	0.63	-0.63

	N		Cereb isch AEs (%)			Cardiac arrest AEs* (%)			HTN AEs (%)			HTN SAEs (%)			Heart failure AEs**			Mortality (%)		
	HBOC	Control	HBOC	Control	Diff	HBOC	Control	Diff	HBOC	Control	Diff	HBOC	Control	Diff	HBOC	Control	Diff	HBOC	Control	Diff
> 70 y/o	233	196	3.00	0.51	2.49	3.86	2.04	1.82	21.89	8.16	13.7	0.00	0.00	0.00	3.86	1.02	2.84	6.87	5.61	1.25
Overall	797	661	1.25	0.45	0.80	1.63	0.91	0.72	15.81	7.87	7.94	0.25	0.00	0.25	1.63	0.45	1.18	3.14	2.12	1.02
< 70 y/o	564	465	0.53	0.43	0.10	0.71	0.43	0.28	13.30	7.74	5.56	0.35	0.00	0.35	0.71	0.22	0.49	1.60	0.65	0.95
< 50 y/o	245	158	0.00	0.63	-0.63	0.82	0.63	0.18	8.57	7.59	0.98	0.41	0.00	0.41	0.41	0.63	-0.22	1.22	0.00	1.22

* Combination 20 (OBRR-Biopure June 2006)
** Combination 22 (OBRR-Biopure June 2006)
^ Data include 3 HBOC-201 MI AEs classified as "non-serious" in the < 70 year old sub-pop

AEs by SOC

	N		Card (%)			Vasc (%)			Resp (%)			Neuro (%)			Renal/urinary (%)		
	HBOC	Control	HBOC	Control	Diff	HBOC	Control	Diff	HBOC	Control	Diff	HBOC	Control	Diff	HBOC	Control	Diff
> 70 y/o	233	196	38.63	29.08	9.54	41.20	23.47	17.7	36.05	30.10	5.95	33.91	27.55	6.35	36.05	22.45	13.6
Overall	797	661	27.10	21.63	5.47	30.49	21.03	9.46	26.60	21.79	4.81	33.88	30.11	3.77	25.47	17.40	8.07
< 70 y/o	564	465	22.34	18.49	3.85	26.06	20.00	6.06	22.70	18.28	4.42	33.87	31.18	2.68	21.10	15.27	5.83
< 50 y/o	245	158	13.88	15.82	-1.95	17.96	13.92	4.04	15.10	20.89	-5.78	39.18	32.91	6.27	13.06	11.39	1.67

SAEs by SOC

	N		Card (%)			Vasc (%)			Resp (%)			Neuro (%)			Renal/urinary (%)		
	HBOC	Control	HBOC	Control	Diff	HBOC	Control	Diff	HBOC	Control	Diff	HBOC	Control	Diff	HBOC	Control	Diff
> 70 y/o	233	196	11.59	7.14	4.45	5.58	3.57	2.01	2.58	2.04	0.53	2.15	1.02	1.13	1.29	2.04	-0.8
Overall	797	661	5.77	3.93	1.84	3.76	2.87	0.89	2.51	1.66	0.85	1.00	0.76	0.25	1.25	1.21	0.04
< 70 y/o	564	465	3.37	2.58	0.79	3.01	2.58	0.43	2.48	1.51	0.98	0.53	0.65	-0.11	1.24	0.86	0.38
< 50 y/o	245	158	1.22	1.27	-0.04	3.27	2.53	0.73	2.04	2.53	-0.49	0.00	0.63	-0.63	1.22	0.63	0.59

Appendix B.3 Logistics odds ratio (OR) for ISS key safety signals stratified by age

Key adverse signals	N		Logistic OR											
	HBOC	Control	Overall AEs	Overall SAEs	Card AEs	Card SAEs	MI AEs	CVA AEs	Cereb isch AEs	Card arrest AEs*	HTN AEs	HTN SAEs	Heart failure/fluid overload**	Mortality
Overall vs. > 70 y/o	233	196	1.04	0.98	0.94	0.90	0.92	1.48	0.47	0.95	0.75	0.50	0.95	1.21
<70 vs. > 70 y/o	797	661	1.06	0.96	0.91	0.80	0.84	0.21	0.21	0.87	0.64	0.71	0.87	2.02
< 70 vs. > overall	564	465	1.02	0.99	0.96	0.89	0.92	0.14	0.45	0.92	0.85	1.41	0.92	1.67
< 50 y/o vs. overall	245	158	1.04	0.95	0.70	0.66	0.61	0.11	0.29	0.72	0.56	1.63	0.18	1.65

AEs	N		Logistic OR				
	HBOC	Control	Card	Vasc	Resp	Neuro	Renal/urinary
Overall vs. > 70 y/o	233	196	0.94	0.83	1.02	0.91	0.91
<70 vs. > 70 y/o	797	661	0.91	0.74	1.04	0.88	0.86
< 70 vs. > overall	564	465	0.96	0.90	1.02	0.97	0.94
< 50 y/o vs. overall	245	158	0.70	0.89	0.59	1.06	0.78

SAEs	N		Logistic OR				
	HBOC	Control	Card	Vasc	Resp	Neuro	Renal/urinary
Overall vs. > 70 y/o	233	196	0.90	0.84	1.20	0.63	1.64
<70 vs. > 70 y/o	797	661	0.80	0.75	1.31	0.39	2.29
< 70 vs. > overall	564	465	0.89	0.89	1.09	0.62	1.39
< 50 y/o vs. overall	245	158	0.66	0.98	0.53	0.60	1.87

Appendix C: *RESUS* Protocol Details

Setting and population

The study population will be comprised of prehospital civilian patients with HS due to blunt and penetrating injuries (~ 10-15 and ~ 40 trauma centers for Stages I and II, respectively). International sites may be recruited (e.g., S. Africa and Australia). A recent cohort study of HS trauma patients from Harborview Medical Center in Seattle, WA, predicts the expected population (Heckbert, 1998). The population will be predominantly male, young, frequently under the influence of alcohol, severely traumatized, with high Injury Severity Score, requiring frequent prehospital life-saving procedures, and having high mortality. Minorities accounted for 44% of subjects enrolled in Baxter's in-hospital DCLHb study in HS.

Subject enrollment

A NTDB query demonstrated that ~ 4.8% (range: 0-9.5%) of level I trauma center patients will have the key *RESUS* inclusion criterion of SBP < 90 mm Hg. Adding the RTS inclusion/exclusion criteria enumerated in the protocol (to exclude subjects with penetrating TBI, subjects with non-salvageable injuries who are likely to die irrespective of treatment, and subjects with less severe injuries who are highly likely to survive irrespective of treatment), reduces this number considerably. As *IC* and *Pre-ED* may only be feasible in a very small minority of subjects in *RESUS*, necessitating reliance mainly on *EIC*, recruitment and consenting are not expected to affect enrollment; it is estimated that almost all eligible patients will be enrolled.

Summary of study interventions

EMS interventions will include brief screening of patients with HS requiring fluid resuscitation by review of inclusion/exclusion criteria; obtaining *IC* or alternatively providing brief *Pre-ED* (*right of refusal*) when feasible, or enrollment with *EIC*; opening a *randomization* box (containing CRFs, and HBOC-201 or LR, depending on randomization assignment); infusion of Clinical Test Material (CTM)—HBOC-201 (500 ml) or LR (1,000 ml); re-infusion of CTM for persistent severe hypotension (SBP < 90 mm Hg) or moderate hypotension (SBP 90-99 mm Hg) with associated tachycardia (HR > 100 bpm), and standard fluids for signs of hypoperfusion without hypotension (SBP ≥ 100 mm Hg); and completion of *Screening* and *Resuscitation CRF*s. Other prehospital care will follow standard EMS protocols (e.g., PHTLS).

After hospital arrival, no additional CTM infusions will be initiated (although incomplete infusions will be finished) as blood transfusions (usually O- PRBC) will generally be available. Subjects will receive standard care, including immediate blood transfusions as needed and available. In order to try to improve standardization of in-hospital care across trauma centers, *RESUS in-hospital trauma care guidelines* (for fluids, blood transfusions, and inotropes) have been developed and are included in the protocol. Where local evidence-based trauma center guidelines do not exist, *RESUS guidelines* will be used, which include fluid resuscitation to a target SBP of 90 mm Hg during active bleeding for most patients without known hypertension or microvascular disease; fluid resuscitation to achieve euvolemia in most patients post-hemorrhage control, including targeting maximum sustainable CO, and normalization of lactic acidosis and mixed venous oxygenation; blood transfusion to a target Hb of 8-10 g/dL in most patients during *early Resuscitation* (hemodynamically unstable and pre-hemorrhage control); and blood transfusion to a target Hb of 7-8 g/dL (minimum 6 g/dL) in most patients during *late Resuscitation* (hemodynamically stable and post-hemorrhage control).

Unless subjects or their legally authorized representative/family member opt to withdraw from the study, subjects will be followed intensely through 24 hours, then at 48 hours, 72 hours, 1 week, day of discharge, and 28 days. Subsequent follow-up at 60 days will include survival and resolution of clinically significant AEs and, if necessary, additional follow-up at 90 days for unresolved clinically significant AEs, and through delivery in subjects determined to be pregnant after enrollment.

Inclusion/exclusion criteria

The main objective of selected *RESUS* inclusion/exclusion criteria is to maximize benefit:risk by targeting a population of subjects with severe HS with potentially salvageable injuries but without access to blood transfusions. The majority of such subjects will have ATLS Class III and IV shock, almost invariably requiring blood transfusions for optimal care.

Enrollment Inclusion criteria (at time of enrollment)	[1] Age 18 to less than 70 years old [2] Injury with obvious/suspected massive bleeding [3] SBP less than 90 mm Hg [4] RTS 1 to less than 5 [5] Expected transport to participating hospital [6] IV access secured
Enrollment exclusion criteria	[1] Penetrating traumatic brain injury [2] Paralysis [3] Pregnancy—known/suspected [4] Cardiac arrest (absence of spontaneous circulation) [5] Known allergy to HBOC-201 [6] Known opposition to HBOC-201 [7] Burn > 20% BSA (partial or full thickness) [8] Blood transfusion available (guideline: expected < 10-15 minutes to hospital arrival)

Inclusion/exclusion criteria justification

- **Age:** Regarding exclusion of patients < 18 years old, there are insufficient data regarding the safety and efficacy of HBOC-201 in the pediatric population. Also, children are frequently resuscitated using intraosseous infusion, and the efficacy and safety of HBOC administered by the intraosseous route has not been established (other resuscitative fluids have shown toxicity by this route e.g., hypertonic saline [Alam, 2002]). Finally, physiological compensation after trauma is different in children (Sloan EP, Moront ML). These factors are especially pertinent to a trial requiring *EIC*. Regarding exclusion of patients > 70 years old, safety data from prior HBOC-201 Phase II/III surgical trials reveal that the risk of key AEs/SAEs is highest in the older population. Specifically, group differences in key adverse safety signals were higher in older than younger subjects (especially cardiac and cerebral ischemic SAEs). Almost all group differences were decreased and sometimes absent or even reversed in the younger < 70 and < 50 year old sub-populations. Additionally, although preclinical acute coronary occlusion models have shown decreased MI size and improved myocardial viability with HBOC-201 infusion (George I), all preclinical HS studies have been done in young healthy animals without comorbid illnesses common in elderly subjects. Although it is possible that elderly subjects (who have diminished physiologic reserve) will particularly benefit from increasing tissue oxygenation with HBOC-201 in the setting of HS, there are no objective data available to support the hypothesis. Thus, due to potential for higher safety risk in elderly subjects and absence of preclinical data to directly support potential for benefit in elderly subjects, and to minimize risk in an *EIC* trial, patients > 70 years old will be excluded from enrollment. On the other hand, exact age cannot always be determined at the scene of an accident, and thus, as has occurred in previous emergency trauma trials, it is expected that some subjects will be enrolled who will be outside this age range. Hypotension (SBP < 90 mm Hg): Although BP is an insensitive indicator of HS, the presence of hypotension is a specific indicator of severe blood loss (usually > ATLS class III hemorrhage with loss of > 30% of EBV [in absence of less common and usually obvious causes of hypotension]). Despite the above caveat, BP is easy to assess in the field, is reproducible, and is included as a routine evaluation parameter in PHTLS training. Almost all such patients require blood transfusions.
- **Penetrating TBI:** There are no preclinical or clinical data to predict the potential therapeutic benefit and safety of HBOCs in this population. Furthermore, the impact of severe TBI on mortality is significant and may be independent of the therapeutic intervention being studied in this protocol (i.e., increasing oxygen content).

- **Revised Trauma Score (RTS):** The *RESUS* trial design targets enrollment of an intermediate severity traumatic HS population by: (a) SBP < 90 mm Hg (targets patients with moderate to severe HS); (b) cardiac arrest exclusion criterion (targets patients with reasonable potential to be salvaged); and (c) RTS 1 to < 5. The RTS, consisting of weighted scores based on SBP, RR, and GCS, has been shown to predict survival in trauma patients (Riou B). Our detailed stratification of RTS databases (NTDB, UAB, and UMD trauma registries) revealed that trauma patients with an RTS range of 1 to < 5 have an intermediate mortality risk (mean 55.8-58.1%) and relatively homogeneous RTS stratification ranges (42.5-88.6% in the UAB/UMD prehospital database) (normalized—bell-shaped). As hypotensive patients with RTS < 1 have mortality 96% and those with RTS ≥ 5 have mortality 6% (UMD data), these will be excluded.
- **Paralysis:** Paralyzed patients may have severe TBI or spinal cord injury. Paralysis in the spinal cord injured patient is usually due to neurogenic shock, a condition unlikely to be benefited by an HBOC.
- **Pregnancy—known/suspected:** There are no human data to document or suggest that HBOCs are safe or unsafe in pregnancy (pregnancy category X).
- **Cardiac arrest:** For severe injuries that are highly unlikely to be survivable, standard medical care focuses on comfort care; administering an investigational agent to patients with injuries for whom survival is very unlikely would be unethical and would adversely affect the outcome of the study possibly negating a true potential benefit in patients with potentially survivable injuries. Survival after traumatic cardiac arrest is highly unlikely. Falcone reported that survival among 320 patients with traumatic cardiac arrest in the field was only 1.9%—the mechanism of injury (blunt vs. penetrating trauma) did not affect these results. Dull showed that only 103 of 633 (16%) patients who had trauma or nontrauma prehospital cardiac arrests, survived to hospital discharge. Per PHTLS/ATLS, absence of palpable central pulse equates with cardiac arrest, requiring CPR (unless declared dead). The cardiac arrest exclusion criterion directly targets a non-futile population with potentially salvageable injuries, but also because central pulse cannot usually be palpated below SBP ~ 40 mm Hg, the exclusion criterion further focuses the target population indirectly by limiting the SBP component of the RTS score to a minimum of 0.73 (SBP 1-49 mm Hg).
- **Allergy to HBOC-201:** An immediate type hypersensitivity reaction related to HBOC-201 may worsen HS morbidity and increase mortality. Although there are no data to document that pre-existing allergy to HBOC-201 increases risk of an allergic reaction to HBOC-201, this contention is scientifically plausible.

- **Known opposition to bovine blood products:** Although most Jehovah's Witnesses will accept bovine Hb transfusions, a minority may object based on stricter interpretation of the religion's prohibitions.
- **Severe burn (partial- or full-thickness**): Burns covering > 20% of BSA require significant augmentation of circulatory support due to very high fluid requirements. Thus, significant confounding of data in this HS resuscitation trial would be expected.
- **Blood transfusion availability (guideline: expected < 10-15 minutes to hospital arrival):** This study's power and benefit:risk ratio assumptions are based on comparison with traditional IV fluids; a larger study would be needed to show superiority in comparison with blood transfusion (rarely available in the prehospital arena). Thus, the intent of the *blood transfusion available* exclusion criterion is to exclude enrollment of subjects who will have access to blood transfusions in a reasonable amount of time (depending on the patient's clinical urgency), and who will have insufficient prehospital time in order to gain potential benefits of HBOC-201.
  The 10-15 minute expected delay guideline will optimize the efficacy:toxicity equation by minimizing enrollment of subjects who are unlikely to have sufficient prehospital time for potential benefits of HBOC-201 infusion; but maintenance of EMS personnel judgment for the "final call" regarding the definition of "*available*" will ensure that critical patients who are likely to expire prior to or soon after hospital arrival, and thus may desperately need an oxygen bridge, will not be excluded and will have an opportunity to potentially benefit from participation in *RESUS*. In effect, this guideline will shift the "transportation delay" curve to the right, eliminating enrollment of the majority of patients with short transportation delay.

Assessment of exclusions in the field

Known opposition to HBOC-201 and *HBOC-201 allergy* exclusions are statistically unlikely but are included for thoroughness. In most cases, these exclusions will be assessed by EMS providers looking for relevant medical jewelry or reporting by an accompanying family member or friend. *Pregnancy* may not be obvious early in gestation and exclusion will rely on history (similar to standard emergency medicine clinical practice in females of childbearing age). In more stable patients in whom a screening history can be obtained, EMS providers will ask the patient or an accompanying family member or friend.

The *blood transfusions available* exclusion has the following practical applications. First, EMS systems that carry blood on board will not be used in *RESUS*. Second, patients with expected very short transportation delays (to hospital arrival) will generally be excluded. Analogous to *RESUS Dosing Guidelines*, which call for a default infusion duration of 10 minutes (but allow for clinical

judgment dictating faster infusion rates for more critical subjects), we defined a default guideline for defining "*available*" as an expected delay of < 10-15 minutes from the time of screening to hospital arrival (i.e., insufficient time to be infuse ≥ 1 standard rate dose of CTM). In more critical subjects, with severe vital sign abnormalities and at high risk of death prior to hospital arrival (per EMS personnel judgment), we recognize that even shorter delays may not equate with "*available*" as subjects may expire prior to blood transfusions becoming "*available*"; thus, EMS personnel may enroll critically ill subjects with expected < 10-15 minute delays (e.g., extremely unstable vital signs or low RTS scores) deemed at high risk of death prior to hospital arrival.

EMS personnel will assess the *blood transfusion available* exclusion criterion as follows:

- **Guideline (applies to majority of patients)**
    - Expected < 10-15 minutes to hospital arrival → "blood transfusion available" → do not enroll
    - Expected ≥ 10-15 minutes to hospital arrival → "blood transfusion unavailable" → enroll
- **Exception in critical patients (applies to minority of subjects)**
    - May enroll critical patients with expected < 10-15 minutes to hospital arrival, deemed at high risk of death prior to hospital arrival (e.g., extremely unstable VS and low RTS scores)

Screening procedures

Medical care and monitoring throughout the screening and enrollment phases of the study will follow standard local EMS procedures (usually PHTLS, ATLS, or similar guidelines), including primary evaluation and securing of adequate airway and breathing, and then addressing circulation concerns including hemostasis and treatment of HS (ABCDEs). The priority of transportation for definitive medical care (i.e., surgery) will remain paramount, and transportation and standard medical care will not be significantly delayed for purposes or interventions related to this trial. After airway and breathing concerns are addressed (with spinal inline immobilization, as indicated) and hemostasis is achieved or attempted (if possible), further completion of the circulation ("C" in ATLS) evaluation/treatment phase will specifically address HS diagnosis and treatment options. Screening for enrollment will occur at this point in the ABCDEs sequence, only for patients diagnosed as having severe HS requiring fluid resuscitation. This will minimize unnecessary distraction and potential risk of delay in care. ATLS class I and II HS patients will usually not meet inclusion criteria, will not be enrolled, and will receive "usual care." Some ATLS class II and almost all class III and IV HS casualties will meet inclusion criteria for enrollment. ATLS class II, III, and IV HS patients are

predicted to have hemorrhaged 15-30%, 30-40%, and > 40% of blood volume, respectively. Thus, screening criteria will include:

(1) Diagnosis of traumatic HS

(2) SBP < 90 mm Hg

(3) IV fluid resuscitation indicated

Informed consent (IC)* and *Pre-enrollment Disclosure (Pre-ED)

After screening, if inclusion/exclusion criteria are satisfied, enrollment will be considered. If the patient is conscious or an accompanying legally authorized representative is present, and it is *feasible*, *IC* will be obtained prior to enrollment (small minority of cases). If full *IC* is not feasible, but a brief scripted disclosure is feasible, *Pre-ED* will be provided (minority of cases). If *IC* and *Pre-ED* are not feasible, subjects will be enrolled utilizing *EIC* (majority of cases). Feasibility of obtaining *IC* or providing *Pre-ED* will be based on the judgment of the EMS provider using *RESUS feasibility guidelines*. If *IC* or *Pre-ED* is feasible and participation is agreed to, then the patient will be enrolled; if participation is not agreed to, the patient will not be enrolled.

Trauma center block and individual subject randomization

EMS providers will open a sealed study envelope (Sayre, 2002), which will contain: (1) *Prehospital (EMS) CRF* which will identify group assignment by random allocation (i.e., HBOC-201 or LR), a unique subject PIN, and study labels. The theoretical advantages of smaller blocks (diminished potential for bias) are offset by logistical complexities of tracking numerous small blocks in a multi-center, -EMS system, and -ambulance prehospital trial. Thus, a compromise of random blocks of 20-40 was reached for *RESUS*. An enrollment census including all trauma centers will be frequently updated by the *RESUS* Advisory Board to ensure that the target sample sizes of 50 and 1,130, for Stages I and II, respectively, are not significantly surpassed. For ITT analyses, opening of the study box will be considered enrollment. In order to ensure that randomization is not biased, EMS providers will only open study boxes after completion of screening, the *IC* process (*IC*, *Pre-ED*, or *EIC*), and enrollment has been determined.

CTM infusion

CTM (HBOC-201 or LR) will be administered through 1-2 “large bore” IV lines. An HBOC-201 dose will consist of two 250 ml units, each containing 32.5 g Hb (total of 500 ml/65 g Hb); the 1st dose of LR will consist of two 500 ml units (total of 1,000 ml) and the 2nd dose will consist of one 500 ml unit (500 ml). For a maximum total CTM volume of 1,500 ml, up to 3 doses of HBOC-201 (6 units) and 2 doses of LR may be administered if indicated.

EMS providers will be advised generally to infuse an entire dose of CTM over ~ 10 minutes (for HBOC-201, 0.71 ml/kg/min for a 70 kg subject), as for standard resuscitative fluids. However, individual subject infusion rates will be determined by judgment of the EMS providers, based on the clinical urgency of the subject's condition and on practical logistic constraints (e.g., gravity, manual pressure or pressure bag, and angiocatheter size availability).

EMS providers will be educated about HBOC-201's vasoactive properties, specifically, that prior clinical and preclinical studies suggest that potentially adverse BP responses (and possibly secondary complications such as fluid overload) appear to be dose- and infusion-rate dependent, and maximal shortly after the 1st infusion and much less after subsequent infusions. EMS providers will be instructed to take these factors into account in determining individual subject infusion rates, most notably, that BP responses are likely to be less with slower infusion rates. These clinical judgment calls are not novel to EMS providers who are well aware that risks associated with standard IV fluids (e.g., LR) are also dose- and infusion-rate dependent (e.g., fluid overload).

Initial infusion criteria for CTM are the *RESUS* inclusion (including SBP < 90 mm Hg) and exclusion criteria. Per PHTLS/ATLS guidelines, EMS providers will repeatedly reassess ABCDEs, VS, and other clinical parameters of HS. Specific attention will be directed towards assessment of re-infusion criteria, as persistent or recurrent severe hypotension (SBP < 90 mm Hg) alone, or moderate hypotension (SBP 90-99 mm Hg) with tachycardia (HR ≥ 100 bpm), would dictate that re-infusion of HBOC-201 or LR is indicated. For subjects with SBP > 90 mm Hg and HR < 100 bpm, and for all subjects with SBP ≥ 100 mm Hg, HBOC-201 will not be administered, and standard care will be substituted.

Although this risk is expected to be significantly lower in hypotensive subjects enrolled in *RESUS* (and not seen in preclinical HS animal studies), idiosyncratic severe BP responses are a theoretical possibility. Thus, EMS providers will be instructed to discontinue and not restart (during the same episode of HS) CTM infusion if SBP increases to ≥ 120 mm Hg in order to decrease risk of a serious BP response, and then, to crossover to standard care—including reassessment for need for further IV fluids (usually with assistance from medical control) and infusion of standard IV fluids if indicated.

LR control

LR was chosen as the control fluid because it is commonly used for both prehospital and hospital resuscitation of trauma patients. LR is manufactured and distributed in the U.S. as either pure levo L-

isomer (l-LR, Baxter Healthcare Corp.) or as a 50:50 racemic mixture of levo and dextro isomers (dl-LR, Abbott Laboratories and B. Braun Medical Inc.). There are theoretical reasons (i.e., toxicity, LA accumulation, and immune activation) to choose l-LR over racemic dl-LR. However, the current standard of care in the U.S. includes use of either dl-LR or l-LR. Moreover, dl-LR is used in HBOC-201. Thus, as directed by OBRR, dl-LR (Abbott Laboratories or B. Braun Medical Inc.) will be used as control prehospital resuscitative fluid CTM in this protocol. Whether or not LR is warmed prior to infusions will be determined by local EMS protocols.

CTM re-infusion

Repeat administration of CTM (HBOC-201 [500 ml] or LR [500 ml]) will occur similarly to general PHTLS/ATLS guidelines—i.e., persistent signs of class III or IV (and sometimes class II) HS. As summarized above, for consistency in the study, persistent severe hypotension (SBP < 90 mm Hg) will suffice as a sole criterion for re-infusion of CTM. In addition, moderate hypotension (SBP 90-99 mm Hg) in combination with tachycardia (HR ≥ 100 bpm) will be a 2nd CTM re-infusion criterion. A maximum (total) of 6 units of HBOC-201 may be administered (HBOC-201: 250 ml = 32.5 g Hb/unit x 6 units =195 g Hb in 1,500 ml). A maximum of 1,500 ml of LR (three 500 ml units in two doses) will be considered CTM. Because urban transportation times are usually short, it is expected that most subjects will receive only 2 units of HBOC-201 (and 1,000 ml of LR), a minority will receive 4 units of HBOC-201, and a smaller minority with prolonged transportation time and persistent hypotension, will receive 6 units of HBOC-201.

Other/standard IV fluids

All other medical care and monitoring will follow standard procedures. This includes administration of standard IV resuscitative fluids (crystalloid and/or colloid solutions) if deemed clinically indicated where SBP and/or tachycardia criteria for re-administration of CTM are not met. Occult shock may occur in patients despite restoration of stable BP and HR. For example, if other medical conditions that can also cause shock are ruled out in a patient with SBP restored to ≥ 100 mm Hg but with persistent tachycardia, tachypnea, narrow pulse pressure, cool pale skin, and/or mental status changes, he/she is likely to have persistent HS and further IV fluid administration will be indicated. Similarly, if SBP is restored to > 90 mm Hg even without tachycardia, if other such signs of shock are present, he/she is likely to have persistent HS and further IV fluid administration will be indicated. EMS providers will be educated that further IV fluid resuscitation will be indicated in such cases (per standard of care). EMS providers will be reminded about potential for paradoxical bradycardia in HS patients.

Some of the other signs of occult hypoperfusion are listed below (EMS personnel should recall that these signs are nonspecific and may reflect other physiological derangements [e.g., compromised airway, hypoxemia and hypoventilation due to pulmonary injuries, and TBI]):

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

Non-CTM infusions will include all fluid infusions after 1,500 ml of CTM and for indications not meeting CTM administration criteria.

Fluid re-infusion scenarios

The following scenarios will occur after initial infusion of CTM (HBOC-201 or LR):

A. No further fluid resuscitation indicated

a. SBP is restored to ≥ 100 mm Hg without other signs of persistent HS. Neither CTM nor standard IV fluids indicated.

b. SBP is restored to 90-99 mm Hg and HR is < 100 bpm, without other signs of persistent HS. Neither CTM nor standard IV fluids indicated.

B. Further standard fluid resuscitation indicated

a. SBP is restored to ≥ 100 mm Hg, but with other signs of persistent HS. CTM not indicated, but standard IV fluids indicated.

b. SBP is restored to 90-99 mm Hg and HR is < 100 bpm, but with other signs persistent HS. CTM not indicated, but standard IV fluids indicated.

C. Further CTM fluid resuscitation indicated

a. SBP remains < 90 mm Hg or recurs, with or without other signs of persistent HS. CTM (HBOC-201 or LR) indicated.

b. SBP is 90-99 mm Hg and HR is ≥ 100 bpm, with or without other signs of persistent HS. CTM (HBOC-201 or LR) indicated.

CTM/standard fluids dosing guidelines summary

- **CTM dose**
    - **HBOC-201:** Two 250 ml units (500 ml).
    - **LR**: Two 500 ml units (1,000 ml) for 1st infusion, one 500 ml unit (500 ml) for 2nd infusion.
- **Maximum total CTM dose (1,500 ml, if indicated)**
    - **HBOC-201:** Up to 3 doses (6 units).
    - **LR:** Up to 2 doses (3 units).
- **CTM route of administration**: Intravenously.
- **CTM infusion duration**: Bolus infusion of entire dose generally over ~ 10 minutes.
    - Clinical judgment should determine infusion rates in individual subjects.
        - More rapid rates usually required in severely unstable subjects.
        - Slower rates recommended in less unstable subjects.
- **CTM initial infusion criteria**
    - Meeting inclusion (including SBP < 90 mm Hg) and exclusion criteria.
- **CTM re-infusion criteria**
    - SBP < 90 mm Hg

      or-
    - SBP 90-99 mm Hg and HR ≥ 100 bpm
- **CTM re-infusion criteria**
    - **Standard care**
        - For subjects with SBP ≥ 90 mm Hg and HR < 100 bpm, and for all subjects with SBP ≥ 100 mm Hg, CTM will not be administered and standard care will be substituted.
    - **Standard IV fluids** will usually be indicated if there are other persistent signs of HS (some are listed below):
        - 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 traumatic brain injury)
    - EMS personnel should recall that these signs are nonspecific and may reflect other physiological derangements (e.g., compromised airway, hypoxemia and hypoventilation due to pulmonary injuries, and traumatic brain injury).
- **Stopping**: CTM infusion should be discontinued for SBP $\geq$ 120 mm Hg, and not restarted.
    - Standard care should be substituted.
- **Comments**: Potentially adverse HBOC-201 related BP effects appear to be dose- and infusion rate-dependent. This effect is highest after the 1st dose and less substantial after subsequent doses.

Rationale for fluids re-infusion criteria

Hypotension will remain the only purely hemodynamic physiological criterion (RTS is also physiological) for initial CTM infusion because of its relative simplicity, association with severe decompensated HS (ATLS Class III/IV hemorrhage).

Inclusion of tachycardia as a re-infusion criterion in *RESUS* is likely to increase HBOC-201 infusion volume, especially in subjects with long transportation times who may receive at least 2-3 infusions. Inclusion of tachycardia is likely to decrease risk of BP roller coaster effect and of under-resuscitation with consequent tissue hypoperfusion. In the general trauma population, tachycardia alone has low sensitivity and specificity as an indicator of HS requiring fluid administration. Tachycardia may be a consequence of pain, fear, or other injury patterns requiring another therapy (e.g., tension pneumothorax) (lowering specificity). However, the combination of hypotension and tachycardia select patients at high risk of adverse outcome (Victorino, 2003). Thus, to increase sensitivity of tachycardia, HR $\geq$ 100 bpm was selected (as per ATLS criteria for Class II shock) as an additional fluid re-infusion criterion only when combined with concomitant hypotension (SBP 90-99 mm Hg).

Training to minimize risk related to HBOC-201 vasoactivity

There is risk of under-resuscitation in *RESUS* due to "*potential elevations in BP*", but study design strategies and training program make the risk low. As EMS personnel will be treating subjects in *RESUS* for relatively short periods of time, the consequences of potential under-resuscitation will not be apparent in most subjects until hospital arrival, at which point sophisticated technology (including multiple diagnostic modalities), will be available (standard care) to trauma center physicians to evaluate fluid status.

Thus, intensive training of EMS personnel and trauma center physicians will occur in order to minimize risk of under-resuscitation. The *EMS and Trauma Center Training Plan* and *Modules* (and IB) will be used to educate EMS personnel and trauma center physicians about these risks, and provide guidance regarding fluid administration for HS subjects enrolled in *RESUS*. Both training modules will emphasize risk and mitigation guidelines regarding HBOC-201 vasoactivity.

Outcome measurements

The primary objectives of the study are to compare the relative effects of prehospital resuscitation with HBOC-201 and LR on 28-day mortality (efficacy) and safety and tolerability. Secondary measurements will include key and other surrogates of morbidity and mortality. Prospectively defined key surrogates include SBP, LA and BD at hospital arrival, and survival to hospital arrival.

Sample size

The sample size of 50 subjects for Stage I was chosen arbitrarily as a pilot study. To decrease the 28-day RR of mortality from 58.1% in LR to 49.4% in HBOC-201 subjects ($\Delta = 15\%$, $\alpha = 0.045$, power = 0.80, drop out 2%): Stage II sample size = 1,130 subjects (1,108 evaluable subjects).

Stopping criteria

The *absolute efficacy stopping-criterion* requires a significantly decreased 28-day relative rate (RR) of mortality with O'Brien-Fleming boundary adjustment and *intent-to-treat (ITT)* analysis. *Safety* stopping-criteria include absolute and relative criteria, requiring significance levels of $p < 0.05$ and $p < 0.05$-0.1, respectively. The *relative stopping-criteria* include SAEs identified by OBRR as always requiring expedited reporting in the following organ systems: cardiovascular, pulmonary, neurologic, and renal.

ESRs and data management

ESRs will occur after accrual of 50 (~5%), 222 (20%), 554 (50%), 1,108-1,158 (100%) evaluable subjects. The data management plan is to capture, clean, validate, submit, and store all clinical data associated with the trial.

AE reporting and DMC

Trauma centers will submit SAE reports to the *RESUS* Drug Safety Officer (unblinded data). Trauma center staff will have only access to unblinded data generated at their trauma center. The *RESUS* Drug Safety Officer will have access to individual subject unblinded data only as required for SAE reporting to the DMC and FDA. *RESUS Advisory Board members* will have access only to trial

conduct data and aggregate efficacy/safety data. Three DMC reports (i.e., open session, closed session, and code) will be prepared for ESR time points and submitted to the DMC for efficacy and safety review. An independent statistician will analyze and collate interim data from the database and prepare DMC closed session and code reports and provide input for DMC open session reports.

Exception from Informed Consent (EIC)

In accordance with 21 CFR 50.24 *(EIC)*, *IC* will be obtained (or alternatively *Pre-ED* will be provided) when feasible. However, in most cases, due to the subject's condition and short therapeutic window, neither *IC* nor *Pre-ED* will be feasible. Therefore, most subjects will be enrolled per *EIC*. Comprehensive *CCD* and *Post-Enrollment Disclosure/Option to withdraw (Post-ED)* programs are included in the trial design. Implementation of the *EIC* program will include a detailed program of *CCD* (e.g., media public service announcements, house mailing brochures, town hall discussions, church meetings, www.RESUS.com website [in development], and health fairs). A summary and analysis of the adequacy of the *CCD* process will be submitted for review and approval by each trauma center's IRB and NMRC's IRB prior to subject enrollment.

EMS and trauma center training

Detailed EMS and trauma center training modules are included in the protocol, focusing on the specifics of the protocol, HBOC-201 physiologic effects, toxicities, laboratory interference, and appropriate follow-up. NMRC researchers believe that potential benefit can be maximized and risk minimized by comprehensively training ambulance personnel and physicians about optimal care of patients resuscitated with an oxygen-carrying resuscitative fluid with intrinsic mild to moderate vasoactivity. Significant time is devoted to training about risk of elevated BP responses, hypoperfusion due to inadequate resuscitation, possible secondary AEs, and EMS- and trauma center-focused risk mitigation strategies (EMS *fluid reinfusion guidelines* and *in-hospital trauma care guidelines*). In addition, training for EMS and trauma center research personnel, regarding protection of human research subjects, is included in the protocol design.

Trial startup

After adequate *CCD* and EMS and trauma center training, ~ 2 dry runs will be performed, during which the protocol will be followed through the 1st 24 hours. Any identified deficiencies in the protocol will be corrected, changes will be submitted to respective IRBs and *RESUS Advisory Board*, and approval secured prior to proceeding to Stage I of the trial. CTM (HBOC-201 and LR) will be supplied to trauma center pharmacies which will maintain study supplies (and logs) and dispense CTM to trained EMS units.

Study interventions summary

Field

1. ABCDEs (routine PHTLS/ATLS)
2. Diagnose traumatic HS requiring fluid resuscitation
3. Screen for enrollment
    - Assess inclusion/exclusion criteria
    - *Informed consent (IC)*
        - If feasible, obtain *IC* from a conscious patient or accompanying LAR
        - If *IC* is not feasible, but *Pre-ED* is feasible, provide *Pre-ED* to a conscious patient or accompanying legally authorized representative/family member
        - If neither *IC* nor *Pre-ED* are feasible, enroll with *EIC*
4. Open study box
    - Assigns study group intervention and PIN
    - Contains *Prehospital (EMS) CRF*
5. Administer CTM
    - HBOC-201—2 bags (250 ml each) (total of 500 ml = 65g Hb)
    - LR—2 bags (500 ml each) (total of 1,000 ml)
6. Reassess patient's fluid status
    - Assess CTM re-infusion criteria
    - Assess standard fluid resuscitation indication (standard care)
    - If CTM re-infusion criteria met → administer CTM
        - HBOC-201 2 units, LR 1unit for 2nd infusion
            - Maximum volume: 1,500 ml (HBOC-201 3 doses [6 units]); (LR 2 doses [3 units])
            - If CTM re-infusion criteria not met but there are signs of persistent HS → administer standard IV fluids
7. Follow up time points—collect data
8. Document on "*Prehospital EMS screening"* and "*Prehospital EMS" CRFs*

Hospital

1. Finish incomplete CTM infusions
2. Routine trauma care
3. *In-hospital trauma care guidelines* (proposed)

4. Post-HBOC specific care issues (e.g., assay interference)
5. *Post-ED*
6. Follow up time points—collect data
7. Document and forward CRFs
8. Report AEs

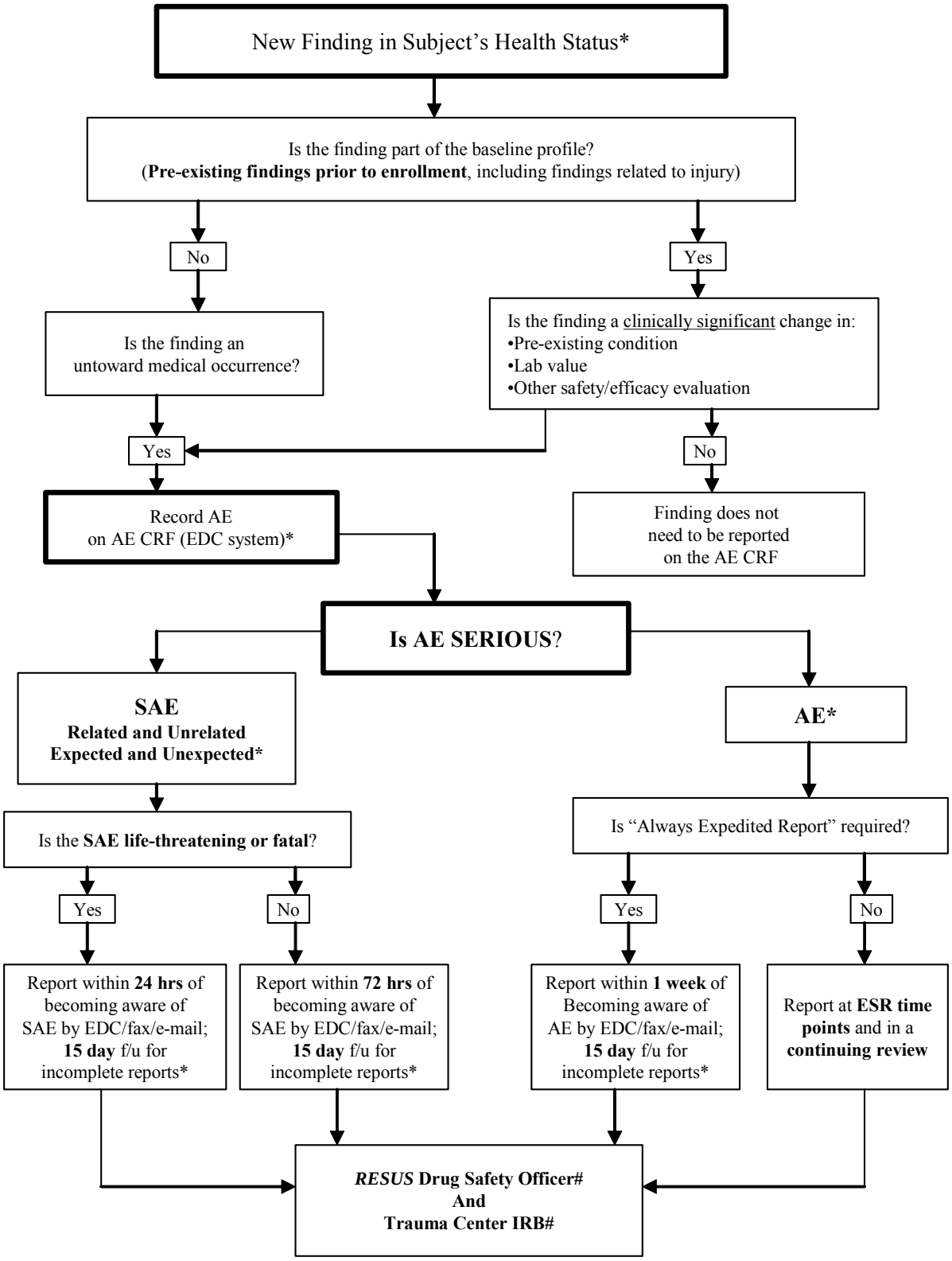
Adverse Events

Documenting AEs

Most CTM related AEs are expected to occur during the first 1-3 days after infusion. At each of the Trauma Center sites, subjects will be monitored carefully for clinical signs and symptoms of any untoward medical occurrences (including clinically significant laboratory findings and any pre-existing medical conditions, which increase in severity or frequency) regardless of whether these findings are considered related to test product. The baseline profile will be based on any pre-existing findings prior to enrollment, considered part of the subject's health status (including injury to the subject). Untoward occurrences will be considered AEs if they occur in a subject from the time of enrollment through the day-28 follow-up assessment. Should the subject expire prior to day-28 or prematurely withdraw from the study, an untoward occurrence will be considered an AE through that time point. Should the subject withdraw from the study, the study team will try to obtain permission to make a follow-up telephone call to review any unresolved SAEs that were considered clinically significant at the time that the subject withdrew from the study. AEs and SAEs from previous studies with HBOC-201 are referenced in the current IB.

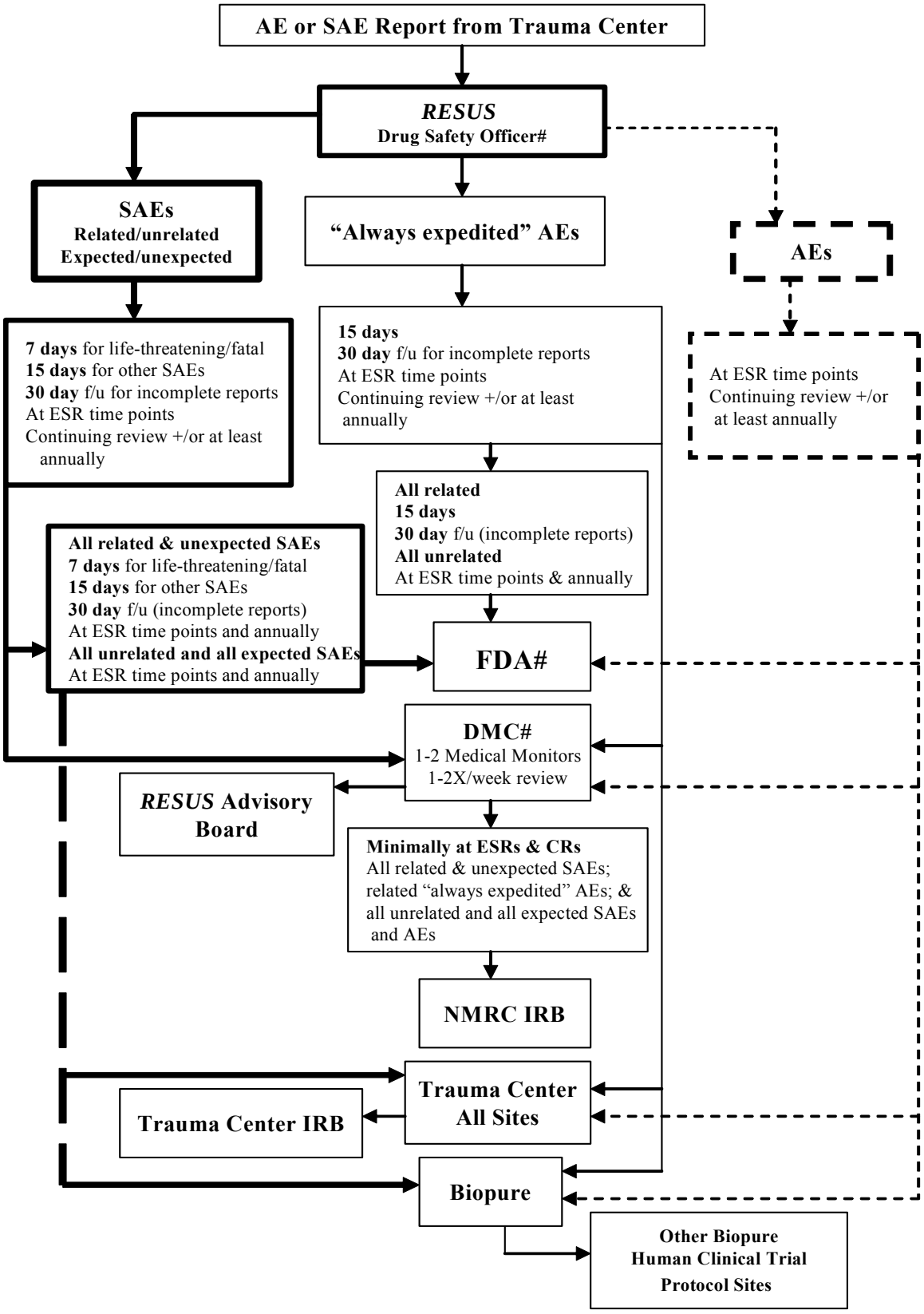
For data collection purposes, the outcome of all AEs will be designated and recorded on the AE CRF when resolved or at the day-28 follow-up assessment. SAEs that are clinically significant and unresolved at the day-28 follow-up assessment will be followed until they are resolved or to 90 days post-enrollment. If a pregnant subject receives HBOC-201, she will be followed-up monthly until delivery.

Figure 13.1: Trauma Center AE Reporting



* Denotes unblinded data. # Denotes who receives unblinded data.

Figure 13.2: *RESUS* Drug Safety Officer AE Reporting



# Denotes who receives unblinded data.

Hypoperfusion markers reporting

In order to minimize risk of potential occult HBOC-201 vasoactivity-related under resuscitation and secondary hypoperfusion, serial follow-up and reporting of hypoperfusion-related clinical markers (i.e., LA, BD, and prehospital fluid infusion volume) will be conducted within ~ 1 month of enrollment of every ~ 10-12 HBOC-201 subjects to the DMC and FDA (until the 2nd *ESR* [222 subjects]).

HBOC-201 interference with clinical laboratory measurements

Serum and plasma collected from those subjects treated with HBOC-201 will contain Hb at various concentrations from the day of treatment for up to 3-4 days (HBOC-201 $T_{½}$ ~ 19 hours) following the last treatment. Certain laboratory tests may not be performed and/or reported by the hospital's clinical laboratory because of the interference with colorimetric methods when specimens are above a certain concentration of HBOC-201. The participating hospital's laboratory equipment will be assessed, followed by provision of an *assay limitation sheet* (listing the parameters being assessed in this study) to the hospital laboratory. Any parameter that cannot be measured by the laboratory while HBOC-201 is in the plasma (measured as the plasma Hb level) will be "suppressed" (i.e., not recorded on the CRF or used for subject management).

Potential risks by organ system and minimization of risks

Neurologic: In the HEM-0115 general population (Appendix N2), CVA AE incidence was higher with HBOC-201 (6/350 [1.7%]) than RBC (0/338 (0%) (p = 0.03); but TIA/RIND AE incidences (1/350 [0.3%] vs. 2/338 [0.6%], p = 0.6) and all (combined) cerebral ischemic AE (CVA/TIA/RIND) incidences (7/350 [2%] vs. 2/338 [0.6%], p = 0.07) were not significantly different between groups. For combined CVA/TIA/RIND incidence, mean (+ SEM) age was 75.6 + 3.3 years (range 62-91 years) for HBOC-201 vs. 58.5 + 7.2 for RBC subjects (range 48-69 years). Similar but insignificant trends were seen for SAEs: CVA SAE incidence was 5/350 (1.4%) vs. 0/338 (0%) (p = 0.06); TIA/RIND incidence was 0/350 (0%) vs. 2/338 (0.6%) (p = 0.2); and CVA/TIA/RIND incidence was 5/350 (1.4%) vs. 2/338 (0.6%) (p = 0.3). In the < 70 year old sub-population, incidence was the same in HBOC-201 (2/239 [0.8%]) and RBC subjects (2/227 [0.9%]) (p = 1.0). Additionally, incidence was significantly greater in > 70 vs. <7 0 year olds (5/111 [4.5%] vs. 2/239 [0.8%], p = 0.04). In the < 50 year old sub-population, incidence was the same in HBOC-201 (0/84 [0%]) and RBC subjects (1/65 [1.5%]) (p = 0.4). Thus, although potentially ischemic cerebral AEs (especially CVAs) were more common with HBOC-201 than RBC in the general population, they were still uncommon, the increase in incidence was small (< 1.7%), and all occurred in older subjects.

In a combined analysis of CVA/TIA/RIND AEs in all HBOC-201 surgical Phase II/III trials, we found a similar pattern. As shown in Table 17.1, CVA AE incidence was higher with HBOC-201 (9/557 [1.6%]) than RBC (1/512 (0.2%) (p = 0.02); but TIA/RIND AE incidences (1/557 [0.2%] vs. 2/512 [0.4%], p = 0.6) and CVA/TIA/RIND incidences (10/557 [1.8%] vs. 3/512 [0.6%], p = 0.09) were not significantly different between groups. For CVA/TIA/RIND incidence, mean age (+ SEM) was 74.2 + 3.4 years (range 53-91 years) for HBOC-201 and 64.3 + 6.9 for RBC subjects (range 48-76 years). In the < 50 year old sub-population, incidence was the same in HBOC-201 (0/111 [0%]) and RBC subjects (1/90 [1.1%]) (p = 0.4). Among HBOC-201 subjects, there were trends to lower incidence in <50 year olds (0/111 [0%]) vs. the general population (10/557 [1.8%]) (p = 0.4) or vs. > 50 year olds (10/446 [2.2%]) (p = 0.2).

In summary, these data show slightly increased risk of CVA AEs in HBOC-201-treated subjects in comparison with gold standard RBC treatment, but exclusively in older subjects, most of whom were hemodynamically stable. But because mean age of HBOC-201 subjects with CVA/TIA/RIND AEs was ~ 75 years old, none occurred in < 50 years olds, and incidence and group differences were low in any case, risk is predicted to be low in the younger population to be enrolled in *RESUS*, especially considering that HBOC-201 will be compared with suboptimal LR rather than gold standard RBC transfusions. Our rationale is detailed as follows:

- Although CVA AE incidence was higher with HBOC-201 than RBC, incidence in HBOC-201 subjects (< 2%) and group differences (< 1.7%) were low.
- CVA/TIA/RIND AE risk was equivalent in < 50 year old sub-populations, which more closely resemble the younger population to be enrolled in *RESUS*.
    - In HBOC-201 subjects, all CVA/TIA/RIND AEs occurred in older subjects
    - Mean age was ~ 75 years old.
    - None occurred in < 50 year olds
- Rationale for extrapolation of risk of CVA/TIA/RIND AEs to *RESUS* from the general populations in HEM-0115 and prior HBOC-201 Phase II/III trials is circumstantial and indirect due to:
    - Different indications (blood substitute vs. resuscitative fluid)
    - Different populations (mainly older adults vs. mainly younger adults)
    - Different comparators (RBC vs. LR)
    - Different physiologic states (mainly hemodynamically stable vs. unstable)

    - Different predicted benefits (blood transfusion avoidance vs. survival)
- The protocol includes numerous risk mitigation strategies to diminish risk in the 1st place and for rapid detection and reporting of potentially adverse signals (allowing further action should they occur):
    - Inclusion/exclusion criteria targeting a population with high mortality and unavailability of blood transfusions
    - Comparison with suboptimal (but standard) asanguinous fluids
    - Potential vasoactivity risk mitigation
        - Comprehensive CTM Dosing Guidelines
        - Access to standard fluids, as needed
        - *RESUS in-hospital trauma care guidelines*
        - CTM stopping criterion (for high SBP response)
        - Hypoperfusion markers reports
        - Expedited AE/SAE reporting to DMC, IRB, and FDA
        - ESRs (interim analyses) starting at 50 subjects (25 HBOC-201 subjects)

CVA/TIA/RIND AE/SAE incidence in HEM-0115 and HBOC-201 Phase II/III surgical trials			
	HBOC-201	RBC	**P (Fisher's Exact test, two-tailed)**
HEM-0115 general population			
AEs			
CVA	6/350 (1.7%)	0/338 (0%)	0.03
TIA/RIND	1/350 (0.3%)	2/338 (0.6%)	0.6
Total	7/350 (2%)	2/338 (0.6%)	0.07
SAEs			
CVA	5/350 (1.4%)	0 (0%)	0.06
TIA/RIND	0/350 (0%)	2/338 (0.6%)	0.2
Total	5/350 (1.4%)	2/338 (0.6%)	0.3
HEM-0115 < 50 year old sub-population			
Total	0/84 (0%)	1/65 (1.5%)	0.4
Phase II/III surgical trials general population			
AEs			
CVA	9/557 (1.6%)	1/512 (0.2%)	0.02
TIA/RIND	1/557 (0.2%)	2/512 (0.4%)	0.6
Total	10/557 (1.8%)	3/512 (0.6%)	0.09
Phase II/III surgical trials < 50 year old sub-population			
Total	0/111 (0%)	1/90 (1.1%)	0.4

Minimization of risk: Maximize risk/benefit ratio by rigorous attention to inclusion/exclusion criteria (targeting patients with severe HS who are most likely to potentially benefit from HBOC-201),

exclusion of elderly subjects who had higher group differences in cerebral ischemic AEs in prior trials, and training to optimize care and minimize risk of potentially vasoactivity-related hypoperfusion and secondary cerebral ischemic AEs. All AEs reported as "always expedited" AEs for close surveillance in *RESUS*.

Cardiovascular: All of the following have been reported with HBOCs, including HBOC-201: rare hypertensive responses classified as SAEs (0.3% HBOC-201 vs. 0% controls, $p = 0.50$ [prior Phase I-III trials), common hypertensive responses classified as AEs (15% HBOC-201 vs. 7% controls, $p < 0.0001$ [prior Phase I-III trials]), mild to moderate hypertensive response, mild to moderate increased SVR, mildly increased PVR, and mildly decreased CO. These AEs were reported mostly in elderly patients and in those with preexisting cardiovascular diseases (Phase III trial). However, there are no preclinical or clinical data to suggest that cardiovascular events will be higher in subjects who receive HBOC-201 who are in HS, especially younger adults. The relative benefit:risk equation is expected to differ significantly. Troponin levels and histopathologic evidence of myocardial damage were equivocal or better in HBOC-201- vs. standard fluid-resuscitated animals in preclinical HS studies conducted at NMRC (Johnson J, Crit Care Med 2006; Stern S, submitted to *Crit Care Med*).
Minimization of risk: Maximize risk/benefit ratio by rigorous attention to inclusion/exclusion criteria (targeting patients with severe HS who are most likely to potentially benefit from HBOC-201), exclusion of elderly subjects who had higher group differences in cerebral ischemic AEs in prior trials, and training to optimize care and minimize risk of potentially vasoactivity-related hypoperfusion and secondary cerebral ischemic AEs. All AEs reported as *"always expedited"* AEs for close surveillance in *RESUS*. Please see summary of risk mitigation strategies in neurologic section above.

Gastrointestinal: Dysphagia, abdominal pain, chemical pancreatitis, elevated liver transaminases, hyperbilirubinemia. Elevations in LFTs and amylase/lipase are expected and are almost always clinically insignificant.
Minimization of risks: Treat dysphagia and/or abdominal pain symptomatically. Follow LFTs and lipase.

Genitourinary: Free Hb is toxic to the kidneys. In contrast, polymerized Hb has reduced renal effects, thought due to decreased glomerular filtration and renal vascular vasoactivity. In the prior Phase 3 HEM-0115 trial, oliguria AEs occurred more commonly in HBOC-201 (39/350 [11%]) than RBC (16/338 [5%]) subjects ($p = 0.002$), but more clinically important acute renal failure (ARF) AEs

(5/350 [1.4%] vs. 4/338 [1.2%], p = 1.0) and overall renal SAEs (6/350 [2%] vs. 4/338 [1%], p = 0.8) were similar in the two treatment groups. In some preclinical HS studies, slightly increased creatinine and BUN blood levels have been seen, as well as mild papillary necrosis, especially in models in which fluid infusion volumes were decreased (Johnson, 2006). Thus, the preclinical and clinical database with HBOC-201 suggest possibly increased risk of oliguria, but extrapolation of oliguria risk to *RESUS* is circumstantial due to different indications, patient populations, physiologic states, comparators, and potential benefits; in addition, no increased risk of acute renal failure and renal SAEs are predicted. As a significant survival benefit is expected with HBOC-201 resuscitation in *RESUS*, the small increased risk of oliguria does not significantly alter the overall benefit:risk prediction.

Minimization of risks: The overall strategy to minimize renal risk in *RESUS* is to reduce risk in via training (to reduce primary risk in the 1st place) and close surveillance (to reduce secondary risk). Specifically, renal risk is minimized by maximizing predicted benefit:risk by rigorous attention to inclusion/exclusion criteria (targeting patients with severe HS who are most likely to potentially benefit from HBOC-201), and attention to *RESUS in-hospital care guidelines* to ensure adequate fluid resuscitation. *RESUS* Trauma Center training includes detailed education of physicians about potential oliguria risk and reinforcement about utilizing a number of clinical parameters to assess fluid status, especially in HBOC-201-treated subjects. Fluid/HS status is not determined using any single clinical parameter such as urine output; trauma center physicians will use multiple parameters to assess fluid and HS status, including renal perfusion indirectly. For example, decreased urine output in the setting of additional abnormalities of other relevant clinical parameters (e.g., LA) would suggest renal hypoperfusion due to inadequate fluid resuscitation; decreased urine output in the setting of normal other relevant clinical parameters might suggest other etiologies (e.g., HBOC-201 side effect). In addition, to ensure close surveillance and rapid detection of potentially adverse signals, renal AEs will be reported as *always expedited* (to the DMC and FDA), and infusion volumes will be closely monitored and regularly and frequently reported in *Hypoperfusion Reports* (to the DMC and FDA).

Methemoglobinemia

HBOC-201 specifications allow up to 10% metHb. Also, when HBOC-201 is exposed to oxygen, such as *in vivo* after IV infusion, Hb in HBOC-201 can oxidize and form metHb. Clinically significant methemoglobinemia ordinarily occurs at $\geq$ 20%; lower metHb levels (such as 10-20%) can

be significant with concomitant cardiovascular alterations (e.g., shock). In cardiac surgery subjects, by days 1 and 2, 15% and 40%, respectively, of remaining *in vivo* HBOC-201 was oxidized to metHb (Levy, 2002). But actual metHb levels were much lower due to the short half-life of HBOC-201. Mean peak metHb levels in subjects in surgical Phase 2 (Sprung, 2002), cardiac surgery Phase 2 (Levy, 2002), and HEM-0115, were 3.7, 4.6, and 4.5%. In the surgical trial, among subjects infused high dose HBOC-201 (2-6 g/kg), mean peak metHb reached 7.1% (Sprung, 2002). These data show that clinically significant oxidation of HBOC-201 to metHb occurs over 1-3 days; by then, most subjects should be adequately volume resuscitated. Thus, even at the highest allowed dose of HBOC-201 in *RESUS* (i.e., 6 units) (~ 2.8 g/kg), metHb levels are unlikely to surpass 10%. Similar metHb levels have been seen in preclinical HS studies. Thus, methemoglobinemia is unlikely to be clinically significant in the overwhelming majority of subjects infused HBOC-201 in *RESUS*. However, because at least theoretically, clinically significant methemoglobinemia could occur in subjects infused large volumes of HBOC-201 (e.g., 6 units) (especially with reduction in intravascular volume due to hemorrhage), clinicians will need to take this into consideration when calculating effective blood oxygen content.
Minimization of risks: To minimize risk of methemoglobinemia, metHb levels will be assessed serially, metHb will be considered in evaluation of oxygen content for blood transfusion decisions, and clinicians will be comprehensively educated about diagnosis and treatment as part of the Trauma Center training program.

Integument: Transient yellowish discoloration of skin occurs commonly after HBOC-201 administration. Application of transcutaneous oxygen monitoring electrodes for > 4 hours can cause small skin burns under the electrodes.
Minimization of risks: No action planned regarding skin discoloration—this side effect is transient. The maximum time for application of transcutaneous oxygen monitoring electrodes in one skin site will be 3–4 hours; the site will be changed within 4 hours.

Allergic: Rare allergic reaction to bovine protein may induce an allergic delayed or immediate hypersensitivity reaction. No immediate hypersensitivity reactions occurred in Phase 1-3 clinical trials to date.
Minimization of risks: Exclude patients with known allergy to HBOC-201.

Pregnancy and lactation (category X): There are no human data regarding the safety of HBOCs in pregnancy and in lactation.

Reproductive Toxicology Studies:

Summary: Reproductive toxicology studies were initially conducted in rats, and irrespective of the dosing regimen (IV route of administration) during gestation, excessive embryo-fetal toxicity and mortality were reported. Serious maternal side effects were seen only with clinically-irrelevant suprapharmacological repeat-dosing regimens, and included petechiae (skin microhemorrhage), tissue histiocyte pigmentation (pigmentation of histiocytes), hepatic sinusoidal ectasia (swelling of liver sinuses), renal pigmentation and vacuolation (kidney toxicity), and increased mortality; generally, histopathologic changes were less severe than in hestastarch-treated control animals. However, in multiple studies with clinically-relevant doses, none of these serious side effects were seen, and mortality was not increased. Decreased food intake, maternal weight gain, and uterine gravid weight were seen, and were probably related to NO binding with consequent GI smooth muscle contraction, causing anorexia. In pigs treated with HBOC-201, we also observed temporary decreased food intake. In rat studies, fetal side effects were severe, occurred irrespective of dose, and included yolk sac abnormalities, severe developmental malformations, spontaneous abortions, and fetal death. Importantly, no maternal or fetal side effects were seen in dog studies.

Overall Studies: Reproductive toxicology studies were carried out in rats and dogs to examine effects of HBOC-201 and BPH on embryo-fetal development (teratology studies). Three studies in the rat included administration of HBOC-201 by continuous IV administration [gestation day (6 through 18], daily continuous IV administration (gestation day 6 to 7, 7 to 8, 8 to 9, 9 to 10, 10 to 11, 11 to 12 and 12 to 13) or hemodilution followed by IV infusion on gestation day 9. An additional study was carried out in rats in which hemodiluted pregnant animals were administered BPH IV on gestation day 9. In dogs, HBOC-201 was administered IV on either gestation days 21, 25, 29 or 33. Finally, an HBOC-201 repeat-dosing (during gestation) dog study was completed. A study was carried out in pregnant sheep to examine the ability of HBOC-301, fresh whole blood and hetastarch to resuscitate hypovolemic animals (maternal hemodynamics and cardiopulmonary parameters) and restore fetal oxygen content. A mechanistic study was conducted using rat embryos to examine HBOC-201-related embryotoxicity in the rat.

Conclusions*:* Reproductive toxicology study data demonstrated significant embryo-fetal toxicity with IV administration of HBOC-201 to pregnant rats whether administered continuously or by daily IV infusion or by IV infusion on a single critical day (gestation day 9). However, IV infusion of HBOC-201 to pregnant dogs at critical gestation times (gestation days 21, 25, 29 or 30), at doses 2-fold greater than the MHD on a weight-to-weight basis, did not cause any embryo-fetal toxicity. Upon

repeat dosing of HBOC-201 to pregnant dogs during organogenesis, no teratological effects were reported. Mechanistic study results indicated that HBOC-201 related embryo-fetal toxic effects in rats were likely due to effects on the inverted yolk sac, a developmental system fairly unique to the rat. Again, since dogs and humans do not utilize such a developmental system during pregnancy and there were no embryo-fetal effects of HBOC-201 in pregnant dogs, it is likely that these teratogenic effects of HBOC-201 are unique to rats and not relevant to humans. Finally, the results of the study in pregnant sheep indicated that HBOC-301 was equivalent to whole blood at improving fetal oxygen content and was not transported to the fetus in this model.

Minimization of risks: Female patients known or suspected to be pregnant or lactating will be excluded from enrollment. A β–HCG will be checked upon admission to the hospital. However, due to the emergency nature of this prehospital study, it is possible that a few subjects will be enrolled who will turn out to be pregnant. Pregnancy outcome will be followed to term. Potential benefits/risks related to pregnancy are summarized in *RESUS CCD* materials; although routine self-exclusion (e.g., medical jewelry) of all pregnant women in communities where *RESUS* is being conducted is not specifically advised, it is recommended that pregnant women weigh the benefits/risks and consider this option individually.

Potential general risks

General (laboratory interactions): As HBOCs interfere with the accuracy and cause expected abnormalities of some laboratory assays, there is potential risk for failure to provide standard of care at the trauma centers.

Minimization of risks: This risk is mitigated in two fashions: comprehensive laboratory interference evaluation/challenge system and EMS/Trauma Center training programs.

General (blood drawing): ~ 50% (161-191 ml) of the blood volume required in this study (322-382 ml) would be ordered as part of standard care, 25% for maximizing safety because of HBOC administration, and ~ 25% for other research purposes.

Minimization of risks: To decrease the likelihood of venipuncture related complications (i.e., brief discomfort, and rare hematoma and infection), only trained medical personnel will draw blood. In order to minimize phlebotomy-induced anemia, only 38-86 ml will be drawn solely for research purposes.

General (delay in care): A brief delay in care due to study-related interventions (screening, *IC* or *Pre-ED*, and enrollment) is likely. However, *RESUS* study investigators believe that the potential benefits of HBOC-201 outweigh this risk.

Minimization of risks: *RESUS* study-specific research procedures have been minimized to avoid diversion of attention of EMS providers and delay in treatment. The screening review of inclusion/exclusion criteria is absolutely necessary but no undue delay is expected for that process. The review of inclusion criteria has to be complete but it is not expected to delay medical care significantly. EMS providers will be trained to complete *IC* and/or *Pre-ED* processes only when feasible and thus not to delay necessary care. Enrollment includes only the opening of the randomization study box to identify the patient's grouping and then to administer the appropriate CTM. EMS providers will complete the EMS CRF after the run, so paperwork will not hinder or delay medical care.

General (transmission of BSE and other infections):

HBOC-201 treated subjects: As HBOC-201 is cow-derived, there are theoretical risks for transmission of bovine bacterial, viral, and prion infections (i.e., variant Creutzfeldt-Jakob disease), the human disease caused by prions implicated in BSE (mad cow disease). Bovine (BSE) and human variant Creutzfeldt-Jakob disease infections require exposure to infected animal brain/spine and other tissue, but probably not blood. This potential risk is especially relevant after BSE was reported in Washington State in 2003 (CDC, 2003) and in Texas in 2004. Human variant Creutzfeldt-Jakob disease is fatal and has no known treatment, but to date has not been reported in the U.S. (CDC).

LR control subjects: There is a higher risk of transmission of human transfusion-related (blood-borne) infectious agents (e.g., HIV and hepatitis B and C) in LR than HBOC-201 patients, as they are likely to receive more allogeneic blood transfusions.

Minimization of risks: HBOC-201 is derived from isolated cow herds (in Pennsylvania), which are not fed animal-source feed. Veterinary and animal husbandry processes include thorough avoidance of cross contamination as well. Thus, the risk of BSE transmission among cows (and therefore, human variant Creutzfeldt-Jakob disease) is practically eliminated. In addition, HBOC-201 is sterilized by heat-treatment and undergoes a prion-elimination process. For the purposes of the *RESUS* study, OBRR had accepted Biopure's manufacturing process. The risk of human transfusion-related infections is the same in LR subjects as in non-enrolled patients; the overall risk in study subjects is lower than in non-enrolled patients because the transfusion avoidance rate for HBOC-201-treated subjects was high in prior surgical clinical trials.

Risk of hypertensive responses: HBOC-201 has vasoconstrictive properties that can manifest as elevated BP, potentially resulting in accelerated hypertension or misleading of EMS personnel (and/or physicians) regarding adequacy of fluid resuscitation and secondary under-resuscitation and consequent hypoperfusion. But these risks are considered low in *RESUS* because the potency of HBOC-201's vasoactivity is only mild to moderate, preclinical HS studies show high efficacy and only mild to moderate vasoactive responses to HBOC-201 infusion, clinical HBOC-201 data from prior trials show frequent mild but exceedingly rare severe BP responses, preclinical and clinical data with other HBOCs suggests reasonable risk in the indication sought in *RESUS* (e.g., prehospital DCLHb HS trial (Kerner, 2003) and extensive risk mitigation strategies have been incorporated into the *RESUS* protocol.

Efficacy and Safety Reviews (ESRs)

4 ESRs will occur (3 interim and 1 final analyses), after accrual of 50, 222, 554, and 1,108-1,158 evaluable subjects (trial completion). If the sample size is revised after completion of Stage I of the trial, although the n will differ at ESRs #2-4, the proportion of total subjects enrolled at these ESRs will remain the same (i.e., 20%, 50%, and 100%). The ESRs will be conducted by an independent DMC. ESR time points were selected based on the following power estimates (evaluable subjects):

(#1)	n = 50	Stage I 100% enrollment (arbitrary sample size) (5% of Stage II if data integrated)
(#2)	n = 222	20% enrollment
(#3)	n = 554	50% enrollment
(#4)	n = 1,108-1,158	Stage II 100% enrollment (LR = 58.1%, HBOC-201 = 49.4%→ Δ = 15%)

DMC

21 CFR 50.24 (a)(7)(iv) requires an independent DMC for *EIC* trials, in order to exercise oversight of the clinical investigation. The DMC will approve the study design and plan prior to initiation of enrollment. Based on its safety, tolerability, and efficacy review of the data and the prospectively defined stopping criteria, the DMC will be responsible for making study disposition recommendations regarding appropriateness of continuing the investigation on both safety and scientific grounds (whether due to generation of data showing greater benefit or greater risk than predicted). In order to avoid pitfalls of prior emergent therapy trials (i.e., providing aggregative data to DMCs), group as well as aggregative data will be reviewed. The *RESUS Advisory Board* will summarize and address DMC recommendations/issues. This summary, as well as a copy of the

written DMC recommendations, will be forwarded to the NMRC IRB, Biopure Corp., trauma center PIs (to be forwarded to local IRB), and FDA within ~ 2 weeks (~ 8 weeks post-target accrual).

Program review and DMC ESR schedule

ESR time points (based on subject accrual) as well as semi-annual programmatic reviews will occur as outlined below. ESRs will focus on efficacy and safety data. Program reviews will focus on milestones accomplishment—especially the *EIC* process and subject accrual.

DMC ESR reviews		**Program reviews (from initiation of subject enrollment in Stage I)**		
			Subject accrual target (N = 1,108-1,158)	#
#	Subject accrual			
1	50	2 month	~ 25	1
		4 months	~ 50	2
2	222	12 months	~ 200	3
3	544	16 months	~ 350	4
4 (final)	1,108-1,158 (total)	18 months	~ 500	5
		24 months	~ 750	6
		36 months	1,108-1,158	7 (final)

Appendix D. *RESUS in-hospital trauma care guidelines*

1. Introduction

These are protocol guidelines applicable to resuscitation of most subjects (referred to as patients in these guidelines) in *RESUS*. These are not instructions. Clinical care of individual patients must always be dictated by judgment of responsible physicians in accordance with local standards and the patient's unique needs.

It is expected that precision in adherence to these guidelines will increase over the duration of resuscitation of individual patients. Initial management of hemorrhaging trauma patients is dictated by vital signs, clinical assessment, and experience; adjustments for laboratory values, invasive monitoring data, and advanced diagnostic studies (especially CT, angiography, and ultrasound) are incorporated, as possible, later in resuscitation.

The goal of resuscitation from HS is the restoration and preservation of tissue oxygen delivery. This is achieved by two mechanisms: hemostasis and effective circulating volume expansion using plasma volume expanders (summarized in Figures A and B).

2. Surgical hemostasis

Hemostasis (cessation of bleeding) is the primary diagnostic and therapeutic goal. No other intervention or study procedure should interfere with an indicated diagnostic study to define ongoing bleeding or indicated procedure to arrest hemorrhage. The standard of care for achieving hemostasis is established by the ATLS curriculum, and is largely the realm of the trauma surgeon[77], but may involve the interventional radiologist or hematologist as appropriate.

3. Fluid resuscitation

Fluid resuscitation begins in the prehospital arena and has two important components: quantity and composition[78]. These will be addressed separately.

The quantity of fluid administered should ideally be the least possible amount that will restore adequate tissue perfusion without exacerbating ongoing hemorrhage. During early resuscitation, while the patient is still actively bleeding, the quantity of resuscitated fluid should be carefully titrated to avoid increasing the patient's BP and thus encouraging disruption of clots and inappropriate dilution of RBC mass and clotting factors. A target SBP of 90 mm Hg is recommended in the patient without known hypertension or microvascular disease. In the typical trauma patient, this value has been shown to be as safe as the traditionally identified normal SBP of 120 mm Hg and possibly better.

Once hemorrhage is definitively controlled and the patient becomes hemodynamically stable, enough fluid should be administered to achieve euvolemia. Whether or not advanced monitoring is available (see below), fluids should be administered until vital signs, serum lactic acid (LA), and urine output

[77] There are preliminary data supporting use of recombinant factor VIIa (rfVIIa) in trauma patients with hemorrhage uncontrollable by surgical means. Currently, there are insufficient data to include a recommendation in these guidelines.

[78] Currently, there are insufficient data to recommend whether crystalloid (e.g., LR, NS) vs. colloid fluids (e.g., Hextend®, Hespan®, HSA) vs. both should be used for fluid resuscitation. Therefore, local practice should be followed.

have normalized. If advanced monitoring is available, fluids should be administered until cardiac output (CO) has reached its maximum value or until mixed venous oxygenation is normal or high.

4. Inotrope/vasoconstrictor drugs

Inotropic and vasoconstrictive medications (dopamine, dobutamine, norepinephrine, epinephrine, and phenylephrine) should only be administered when fluid therapy alone has failed to achieve desired hemodynamic goals. Bolus pressor therapy (typically epinephrine 0.1-1 mg) may be used to transiently support cerebral and coronary perfusion during early resuscitation, with the understanding that a requirement for repeated pressor therapy or a continued vasopressor infusion prior to definitive control of bleeding, usually indicates exhaustion of the compensatory mechanisms of the cardiovascular system and a state of fatal HS. Continuous vasoactive infusion during late resuscitation should always be guided by advanced monitoring, and titrated to maintenance (with fluid therapy) of the maximum sustainable CO.

5. Advanced hemodynamic monitoring

Advanced monitoring of cardiovascular function (PA catheter, continuous venous oximetry, transesophageal echocardiography, etc.) is indicated when multiple etiologies might be contributing to cardiovascular dysfunction (e.g., the patient with both myocardial ischemia and ongoing hemorrhage). Diagnosis and control of ongoing hemorrhage should never be delayed for placement of advanced cardiovascular monitoring.

6. Laboratory assessment

Laboratory assessments of CBC, ABG, blood chemistry, electrolytes, LA, coagulation function, and metHb are indicated in *RESUS* on a frequent basis during resuscitation, especially in patients with active, ongoing hemorrhage. Frequent laboratory and urine output assessment should be used to guide the composition of administered fluids.

7. Maintenance of effective blood composition

Preservation of an effective blood composition is essential for successful resuscitation from HS. This requires the clinician to anticipate expected derangements, often in the absence of definitive diagnostic data. Therefore, the data elements presented below requires continuous assessment and adjustment in therapeutic interventions. The following targets are recommended:

7a. Blood oxygen carrying capacity

Balancing a sufficiency of RBC mass against the pro-inflammatory consequences of transfused blood products remains controversial in the trauma community. We recommend a target Hb concentration of 8-10 g/dL during early resuscitation (active bleeding), and 7-8 g/dL in the hemodynamically stable patient (a minimum of 6 g/dL). Higher values are acceptable in patients with a history of microvascular disease or evidence of end-organ distress, and in the elderly.

7ai. HBOC-201 short half-life

As HBOC-201 has a short half-life (~ 19 hrs), there is potential for rapid fall in blood Hb levels if RBC are not transfused. Thus, to maintain adequate blood oxygen content, in the 1st 48 hrs, Hb should be followed frequently (minimum q 8 hrs in the 1st 24 hrs, q 12 hrs in the 2nd 24 hr period) and PRBC transfused as needed using the above transfusion triggers.

7aii. Methemoglobinemia

HBOC-201 may oxidize to methemoglobin (metHb) *in vivo*. Although metHb levels have remained < 4-5% in prior clinical trials, indicating probably clinically insignificant metHb loads, higher levels are possible in trauma patients. Thus, in the 1st 48 hrs, metHb levels should also be followed frequently (minimum q 8 hrs in the 1st 24 hrs, q 12 hrs in the 2nd 24 hr period), metHb should be deducted from total Hb to assess blood oxygen carrying capacity. Furthermore, metHb should be treated if indicated based on recommendations for initiating and providing therapy in the *RESUS* metHb training module.

7b. Blood hemostasis capacity

7bi. RBC mass

Maintenance of red cell mass is an important consideration in optimizing hemostasis in transfused trauma patients with potential ongoing hemorrhage. HBOC-201 replenishes blood Hb but not red cell mass. Hence, in patients with known or suspected uncontrolled hemorrhage and coagulopathy, hematocrit (a rapidly available measure of red cell mass) should also be taken into account in order to optimize hemostasis (i.e., hematocrit goal of 24-30% if actively bleeding, and 21-24% if hemodynamically stable).

7bii. Coagulation factors

Coagulation factor replacement should begin early in resuscitation, and should be titrated to maintain an INR $\leq$ 1.5 in the actively bleeding patient. In late resuscitation, thawed fresh frozen or fresh plasma or cryoprecipitate should be administered on the basis of abnormal laboratory values or clinical evidence coagulopathy.

7biii. Platelets

Platelet replacement should begin early in the patient with massive hemorrhage, and should be titrated to maintain a value of at least 50,000 per hpf during early resuscitation. As with clotting factor replacement, platelets should not usually be given in the absence of ongoing hemorrhage.

8. Electrolytes

Electrolyte values should be maintained in the normal range. Hypocalcemia (citrate intoxication) is treated with the intravenous administration of calcium. Hyperkalemia is treated with improved perfusion (further fluid administration), improved ventilation, and, rarely, with insulin and glucose therapy.

9. Body temperature

Maintenance of normothermia (core temperature > 35.5°C) is recommended throughout resuscitation.

10. Elevated BP responses

Elevated BP responses in trauma patients usually reflect increased sympathetic activity, often due to inadequate analgesia or anesthesia as well as stimulation from direct laryngoscopy during endotracheal intubation. However, in *RESUS*, HBOC-201's vasoactivity also carries the potential for elevated BP, especially during the 1st hrs post-infusion. In HBOC-201-treated subjects with elevated SBP responses in prior trials, 94% were mild to moderate and 0.6% severe. In most trauma patients, elevated BP does not require treatment; similarly, most elevated BP responses in *RESUS* are unlikely to require treatment. However, elevated BP in the setting of uncontrolled (especially arterial) hemorrhage may require treatment. Occasionally, elevated BP may require treatment for other reasons (e.g., to decrease afterload in the setting of LV failure, to decrease myocardial work in the setting of cardiac ischemia, or malignant hypertension). Thus, in HBOC-201-treated patients with elevated BP responses: (1st) common causes (unrelated to HBOC-201) should be sought and treated as indicated; (2nd) clinicians should ensure that other parameters of fluid status are adequate in order to ensure that the patient is not hypoperfused despite the elevated BP response (e.g., LA [and CO, SVO_2, PCWP, and CVP, if available); and (3rd) elevated BP responses (related to HBOC-201) should be treated if deemed clinically indicated (nitroglycerine or nitroprusside infusions should be considered because of rapid onset of action, ability to titrate dose with precision, and mechanistic logic [HBOC-201's vasoactivity is mainly related to nitric oxide binding]).

11. HBOC-201 colloidal properties/fluid overload

HBOC-201's colloidal properties result in potential for fluid shifts in hemodiluted patients, and although not common, heart failure/fluid overload AEs occurred more frequently in HBOC-201 than control subjects in prior trials (principally in elderly subjects). Consequently, clinicians should frequently and comprehensively assess fluid status, considering symptoms and signs of both fluid overload as well as hypoperfusion. In awake patients, symptoms of heart failure/fluid overload such as congestion, shortness of breath, and orthopnea should be sought. In all patients, signs of heart failure/fluid overload such as increased oxygen requirements, decreased lung compliance, dyspnea, tachycardia, tachypnea, rales and decreased breath sounds, elevated jugular venous pressure, cardiac gallop, edema, CXR evidence of pulmonary edema and pleural effusions, and elevated CVP and PCWP (if available) should be sought. It should be recalled that in trauma patients, pulmonary edema is more likely to be non-cardiogenic (ALI/ARDS) than cardiogenic (hydrostatic). Heart failure/fluid overload should be treated as indicated using routine methods (e.g., furosemide).

12. Traumatic brain injury (TBI)

12a. Introduction

The following guidelines are relevant to *RESUS* patients with severe HS and TBI; about 1/3 of *RESUS* subjects are expected to have severe HS and severe TBI (stratified in *RESUS* by Head AIS 4-6 and clinically with GCS 3-8). These guidelines are based on expert recommendations of the Brain Trauma Foundation:[79]

- *Management and Prognosis of Severe Traumatic Brain Injury, 2000.* http://www2.braintrauma.org/guidelines/index.php

[79] Brain Trauma Foundation, 708 3rd Avenue, New York, NY 10017 (Tel 212-772-0608).

- *Update to guidelines for the management and prognosis of severe traumatic brain injury, 2003.* http://www2.braintrauma.org/guidelines/downloads/btf_guidelines_cpp_u1.pdf

It should be recalled that these are general guidelines are based on expert consensus but evidence-based consensus is lacking in some aspects of resuscitation of patients with HS and TBI. The guidelines should not hinder appropriate medical judgment and clinicians should adjust care based on local standards and specific pathophysiologic conditions in individual patients.

The main neurologic objectives in the management of patients with HS and concomitant TBI are to minimize primary and secondary brain injury via maintenance of adequate brain tissue oxygenation. As even brief episodes of hypotension and/or hypoxia correlate with worse neurologic outcome, the first treatment priority is optimization of systemic resuscitation using ATLS guidelines—ABCDEs—with emphasis on ventilation, oxygenation, intravascular volume, and BP.

There is significant Class II evidence that even a single episode of hypotension (defined as SBP < 90 mm Hg) or hypoxia (defined as apnea or cyanosis in the field or O_2 saturation < 90% or PaO_2 < 60 mm Hg), are associated with increased morbidity and mortality. However, in the presence of signs of transtentorial herniation, the primary treatment priority should be decreasing intracranial pressure (ICP). Because hypotension can result in increased ICP, correction of hypotension (and hypoxemia) is important early in the management of intracranial hypertension. Two Class I and one Class II studies support use of mannitol for the purpose of reducing ICP after adequate volume repletion. Hyperventilation reduces CO_2, CBF, and thus ICP; hyperventilation should be used when there are clinical signs of herniation (unilateral or bilateral pupillary dilatation, asymmetric papillary reactivity, motor posturing, or other deterioration of the neurologic examination) prior to the availability of ICP monitoring, or for ICP $\geq$ 20-25 mm Hg in conjunction with a cerebral perfusion pressure (CPP) < 70 mm Hg (CPP = MAP – ICP), after ICP monitoring is available. Excessively decreased CO_2 via aggressive hyperventilation can decrease CBF to critically low levels. A balance of adequate fluid resuscitation and modalities aimed at minimizing ICP should be individualized for each patient.

12b. ICP monitoring

ICP monitoring is recommended in patients with severe TBI with an abnormal head CT (i.e., hematoma, contusion, brain edema, or compressed basal cistern) on admission. In the absence of an abnormal CT, ICP monitoring should be considered if $\geq$ 2 of the following criteria are present: age > 40 years old, SBP < 90 mm Hg, or motor posturing.

12c. Oxygenation and resuscitation of BP and CPP

The highest priority is maintenance of an adequate airway. Patients with a GCS $\leq$ 8 require administration of a definitive airway, usually by endotracheal intubation. All patients should initially be administered 100% oxygen, with subsequent adjustment to maintain a minimum blood PaO_2 > 60 mm Hg (and O_2 saturation > 90%) but a target PaO_2 > 100 mm Hg is ideal during the 1st 24 hours after injury. Prior to availability of ICP monitoring, fluid resuscitation[80] should target a minimum SBP of 90 mm Hg (Class II evidence) especially prior to definitive control of hemorrhage. Targeting a minimum MAP of 90 mm Hg may be preferable, especially after control of hemorrhage. Aggressive fluid resuscitation has potential to increase ICP and brain edema but the association has not been

[80] Some (but not all) clinical studies suggest that hypertonic saline (HTS) and hypertonic saline-dextran solution (HSD) resuscitation result in higher SBP and survival than with crystalloid fluid. Some clinical data show lower ICP with HTS resuscitation in the setting of TBI and intracranial hypertension. There are insufficient data currently for a formal recommendation.

consistent in the literature. Prior to control of hemorrhage, a balance between minimization of uncontrolled bleeding (dislodging of clot) and optimization of cerebral perfusion should be individualized in each patient. After availability of ICP monitoring, CPP management should be instituted, titrating fluid resuscitation to optimize cardiac performance and to manipulate CPP for a minimum target $\geq$ 60 mm Hg. CPP < 50 mm Hg is associated with decreased brain tissue oxygenation and increased morbidity and mortality. Aggressive CPP management (targeting CPP $\geq$ 70 mm Hg) should be avoided due to risk of ALI/ARDS.

12d. ICP treatment

Brain edema and severely elevated ICP are a common cause of death and disability in TBI patients. Thus, reversal of intracranial hypertension is an essential component of optimal treatment of patients with TBI. General supportive care includes control of body temperature, seizure prophylaxis, elevation of the head of the bed, avoidance of jugular venous flow obstruction, sedation, paralysis, maintenance of adequate arterial oxygenation, volume resuscitation, and cardiac performance optimization for a minimum CPP $\geq$ 60 mm Hg. Specific ICP treatment should be instituted for clinical signs of herniation (unilateral or bilateral pupillary dilatation, asymmetric pupillary reactivity, motor posturing, or other deterioration of the neurologic examination) prior to availability of ICP monitoring, or for ICP $\geq$ 20-25 mm Hg after ICP monitoring is available. If a ventricular catheter is inserted, CSF drainage should be titrated to effect. Ventilation should be adjusted to maintain a low normal $PaCO_2$ of ~ 35 mm Hg. A cause amenable to surgical correction/drainage should repeatedly be sought, typically using CT scanning.

If ICP remains high despite these interventions (or they are unavailable), hyperventilation should be adjusted to a target PCO_2 30-35 mm Hg. If this is unsuccessful in lowering ICP to an acceptable level (< 20 mm Hg), mannitol (0.25-1.0 g/kg) should be administered—preferably by intermittent bolus rather than infusion and preferably after and concomitant to adequate fluid resuscitation; serum osmolarity should be < 320 mOsm to reduce renal failure risk. It should be recalled that mannitol can deplete intravascular volume, cause hypotension, and thus result in increased secondary brain injury. Mannitol should be used to achieve isovolemic dehydration and must be titrated in conjunction with appropriate fluid management to prevent hemoconcentration and diminished effective circulating volume. Thus, intravascular volume, including urine output, should be followed and optimized. A balance of adequate fluid resuscitation and modalities aimed at decreasing ICP should be individualized in each patient. A cause amenable to surgical correction/drainage should repeatedly be sought. High-dose barbiturate therapy may be indicated in the setting of intracranial hypertension refractory to surgical and medical therapy.

Figure A
RESUS—In-hospital Trauma Care guidelines
Assumes resuscitation is ongoing

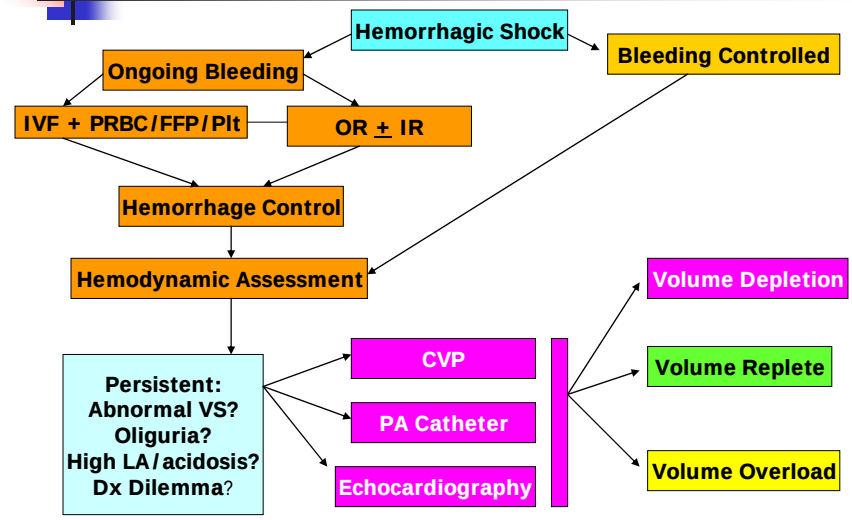
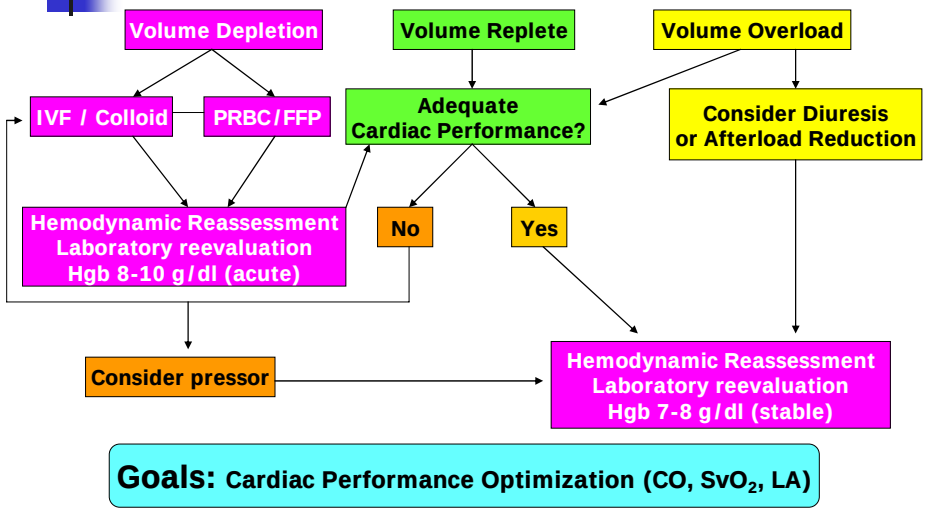


Figure B
RESUS—In-hospital Trauma Care guidelines
PVE and Component Therapy



A Single-Center, Study to Evaluate the Safety and Tolerability of Hemoglobin-Based Oxygen Carrier-201 (HBOC-201) in Trauma Subjects

HEM-0125
Preliminary Data

Study Designation:

HEM-0125

Principal Investigator:

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Study Sites:

Johannesburg General Hospital
South Africa

Trial Registration:

Identifier: NCT00301483
www.clinicaltrials.gov

Purpose of Preliminary Data Summary:

This preliminary summary of the results of study HEM-0125 was prepared in response to a request from FDA. The results from the first DMC meeting is scheduled for the midway point in this study. The recommendations from the DMC, the minutes prepared by the DMC Chairman, and the data submitted to the DMC for review will be provided to FDA when these data are made available by the DMC Chairman. The final study report and all line listings will be submitted to FDA upon completion of this study.

HEM-0125 STUDY DESCRIPTION

This is Phase II study a single-center, randomized, single-blind, parallel-group, standard therapy-controlled, variable dose study of HBOC-201 administered to trauma subjects with bleeding or potential for bleeding who require standard fluid therapy for treatment of hypoperfusion. Subjects are considered for enrollment into the study if at randomization there is evidence of hypoperfusion as defined by a Base Deficit (BD) of > 5.0 and one of either the following two: a Systolic Blood Pressure (SBP) of ≤ 90 mm Hg or a sustained (≥ 10 minutes) heart rate (HR) of ≥ 120 beats per minute (bpm). Subjects with a known head injury are not enrolled into the study and those with suspected head injury and Glasgow Coma Score (GCS)of ≥ 13 require head CT to confirm lack of head injury. All trauma subjects who meet the inclusion criteria are eligible for enrollment. ~ fifty (50) eligible trauma subjects will be randomized (1:1) to receive either HBOC-201 plus standard therapy or Standard Therapy alone.

Study Objectives:

- **Primary:** Assess the safety and tolerability of HBOC-201 for the treatment of hypoperfusion in trauma subjects by the type and incidence of adverse events attributed to the study drug.

- **Secondary:** Assess efficacy parameters and other information that will aid in the design of subsequent studies to evaluate HBOC-201 treatment in trauma subjects in a pre-hospital setting.

Eligible trauma subjects are randomized (1:1) to receive either HBOC-201 plus standard therapy or Standard Therapy for a total of 25(twenty-five) evaluable subjects in each treatment group. Subjects randomized to the HBOC-201 group receive intravenous infusion of HBOC-201 (250 mL [1 Unit]). At the end of the initial infusion, the subject is evaluated as *Unstable* (BD > 5 and either SBP ≤ 100 [for 2 consecutive readings at standard of care intervals], or HR ≥ 110 bpm) or *Stable*. At the end of the initial and each successive infusion, the BD, SBP, and HR for the subject is assessed in order to receive additional treatment, except where rapid successive (back-to-back) infusions are required based on clinical judgment. When back-to-back infusions are required, SBP and HR must be recorded between infusions. BD should be obtained for data collection, however, therapy should not be withheld pending the result.

Measurements of BD, SBP, and HR are performed immediately following the last infusion in a series of successive infusions.

Subjects that are deemed *Unstable* after the first infusion receive a second infusion of HBOC-201. Subjects who continue to be deemed *Unstable*, or who become unstable again, may receive additional treatment with HBOC-201 until one of the following has occurred:

A maximum dose of 10 Units of HBOC-201 has been administered, or
HCT ≤ 18%, or
4 hours from baseline (initiation) of treatment has been reached, or
Blood has been given after randomization

Subjects randomized to receive Standard Therapy receive treatment according to standard treatment protocols adopted by treatment facility.

- Target enrollment of 50 subjects
- Duration of participation will be 28 Days
- A maximum HBOC-201 dose of 10 units
- Subjects randomized to the Control group receive Standard of Care (fluids, RBCs)
- Subjects randomized to the HBOC-201 group receive Standard of Care fluids, HBOC-201, and RBCs (if needed)

Study Entry Criteria

1. Inclusion Criteria

- Male or Female with negative pregnancy test
- 18 years ≤ 65 years
- In-hospital admission, directly from scene of injury (blunt/penetrating) and randomized within 2 hours of admission
- Bleeding or potential for bleeding
- Signs of hypoperfusion with :
- BD > 5
- SBP ≤ 90 mmHg OR Sustained (≥ 10 minutes) HR of ≥ 120 bpm
- GCS ≥ 9 , with the exception of documented drug-induced lowered GCS, Possible head trauma & GCS ≥ 13 may be enrolled
- Must receive head CT scan within 2 hours of ER arrival
- Informed Consent / Independent Physician Authorization

2. Exclusion Criteria

- Known Head Injury
- Head injury confirmed on CT scan
- Head injury with GCS < 13
- Non survivable injury (Falcone criteria)
- Traumatic cardiac arrest

- Known prior cardiac arrest (preceding trauma episode)
- Known / suspected pregnancy
- Known allergy to bovine products
- Transferred from other treatment facilities
- No consent / physician authorization
- Unable to meet protocol or follow-up criteria

Assessments

When a subject in the HBOC-201 treatment group or in the Standard Treatment group is determined to be Stable, assessment for continued stability (BD, SBP, and HR) is performed as follows: once every 30 minutes for that first hour after initial treatment, once every 1 hour for the next 3 hours (assess at hours 2, 3, and 4), and subsequently every 4 hours up to 24 hours from the initial treatment. At the end of the treatment phase, all subjects have follow-up assessments for the primary safety endpoint and the secondary efficacy endpoints on Day 1 (24-hour time point from initial CTM infusion), Day 2, Day 3, Day 7 or day of discharge, and Day 28.

PRELIMINARY DATA SUMMARY

The following data for the study subjects are summarized consistent with the extent and nature of data to be supplied to the Chairman of the DMC for the first DMC meeting prospectively scheduled to occur when 50% (25 subjects) of subjects have been enrolled in the study. These data have undergone QC sufficient to support assessment of safety by the DMC but not equivalent to the level of data cleaning required for locking the database. Thus, the results are subject to change based upon the results of the data checking and cleaning required prior to locking the database. Accordingly, the data summarized in this report represent "preliminary data".

Randomized Population:

Table 1 summarizes the randomization of 21 subjects enrolled in this study. Of these 21 subjects, one (1) subject (0122) was found to be ineligible based upon a positive CT scan demonstrating TBI. In accordance with the study protocol, if "subjects receive study material but are subsequently determined to be ineligible for the study due to a head injury confirmed by a positive CT Scan", they will not be included in the "evaluable analysis set". Accordingly, the evaluable analysis set comprises the data from those subjects who receive CTM, do not have confirmed head injuries, and have evaluable data available after initiation of treatment.

Subject 0117 is not included in the evaluable dataset but was incorrectly withdrawn 2 hours after enrollment because of the failure of the blood gas machine; no follow-up data was collected for this subject. This was a protocol violation as the protocol does not permit withdrawal of a patient from the study for equipment failure or inability to perform certain assessments. The site has been queried regarding whether or not the subject survived to the 28

day follow-up point and once this information is known, this subject will be added to the evaluable study population. In addition, the medical records will be reviewed for suspected adverse and serious adverse events which will be reviewed by the principle investigator and those events confirmed by the investigator will be included in the database. Subject 0121 does not exist because the randomization envelope with this number was inadvertently opened and the treatment assignment for this number was revealed.

Table 1. Summary of Patients Randomized Versus Evaluable

	Total N (%)	Std. Care **N (%)**	HBOC + Std. Care **N (%)**
Patients Randomized	21	10	11
Patients in Evaluable Dataset*	19 (91)	9 (90)	10 (91)

* Does not include subjects 0117 and 0122.

Blinding:

Blinding for safety (Groups 1 vs. 2) and efficacy (Groups A vs. B) was done independently. Sample sizes for efficacy tables were removed to maintain blinding.

Patient Demographics:

Table 2 summarizes the demographics for the 19 subjects comprising the evaluable dataset. These data show that the majority of subjects randomized to this study were male and this trend was seen in both treatment groups. The p-values for between group differences are also provided. The revised trauma score (RTS) is included to provide an estimate of overall patient trauma status in each study group near the time of randomization.

Table 2. Study Demographics*

	All	**Std. Care**	**HBOC + Std. Care**	**p-value**
	n=19	**n=9**	**n=10**	
Male (%)	14 (74%)	7 (78%)	7 (70%)	
Female (%)	5 (26%)	2 (22%)	3 (30%)	
Mean Age	38±3.3	39±4.8	37±4.7	0.82
20-29years of age (%)	6 (32%)	3 33%)	3 (30%)	-
30-39 years of age (%)	7 (37%)	3 (33%)	4 (40%)	-
40-49 years of age (%)	2 (11%)	1 (11%)	1 (10%)	-
50-59 years of age (%)	2 (11%)	1 (11%)	1 (10%)	-

60-69 years of age (%)	2 (11%)	1 (11%)	1 (10%)	-
Revised Trauma Score**	6.59±0.24	6.66±0.41	6.51±0.27	0.76

* Does not include subjects------ and -------

** The RTS was obtained following arrival in the emergency room.

Study Mortality:

Overall mortality and mortality in each treatment group is summarized in Table 3.

Table 3. Summary of Study Mortality*

	Total N (%)	Std. Care **N (%)**	HBOC + Std. Care **N (%)**
N	19	9	10
Deaths	8 (42)	4 (44)	4 (40)

* Based on evaluable study population.

Summary of Adverse Events Data:

The primary safety endpoint for this study is comparison of serious adverse events (SAE) between treatment groups within evaluable set. A complete listing of serious adverse events by study subjects is provided in Table A1 in the Appendix. The summary statistics for SAEs and mortality are presented in Table 4.

Table 4. **Serious Adverse Events***

	All	**Std. Care**	**HBOC + Std. Care**	**p-value**
	19	**9**	**10**	
Number of SAEs	23	13	10	-
Patients with ≥1 SAEs	9 (44%)	5 (55%)	4 (40%)	1.0
SAE/patient	-	1.4	1.0	0.58

* Based on evaluable study population.

The descriptive statistics for the non-serious adverse events (AE) are summarized in Table 5. A complete listing of adverse events by study subject is provided in Table A1 in the Appendix.

Table 5. **Adverse Events***

	All	Std. Care	HBOC + Std. Care	p-value
Number of AEs	244	153	91	-
Patients with ≥1 AE	19 (95%)	9 (100%)	10 (100%)	1.0
AE/patient	-	17	9.1	0.12

* Based on evaluable study population.

Table 6 summarizes the data for all resuscitation fluids administered to subjects in this study with the exception of HBOC-201.

Table 6. **Comparison of Resuscitation Fluid* Administration**

	HBOC+Std. Care	Std. Care	p-value
Total Units RBC/subject	5.4±1.2	16.8±5.7	0.08
Volume Colloid/subject	3,552±630	5,224±783	0.12
Volume Crystalloid/subject	11,525±2571	19,264±4,979	0.19
Total Volume All** Fluids/subject	15716±2570	30242±8088	0.12

*The HBOC-201 infusions were not included
**Includes Colloids, Crystalloids and Nutrition solutions

.

Table 7 lists the infusion rates for all HBOC-201 infusions, the average rate was 73 ml/min and ranged from 3 to 286 ml/min. Infusion 9 represents back-to-back infusions of 8 units of HBOC-201.

Table 7. Rate of HBOC-201 Administration

Infusion #	Volume (ml)	Duration (min)	Rate (ml/min)	Patient ------- - D
1	250	7	36	------
2	250	4	63	------
3	250	8	31	------
4	250	45	6	------
5	250	5	50	------

6	250	5	50	------
7	250	25	10	------
8	250	15	17	------
9	2,000	7	286	------
10	250	6	42	------
11	250	20	13	------
12	250	55	5	------
13	250	35	7	------
14	250	24	10	------
15	250	49	5	------
16	250	92	3	------
17	250	76	3	------
18	250	2	125	------
19	250	1	250	------
20	250	1	250	------
21	250	2	125	------
22	250	3	83	------
23	250	1	250	------
24	250	2	125	------
25	250	28	9	------
26	250	27	9	------
27	250	2	125	-------
28	250	4	63	-------
29	250	2	125	-------
30	250	4	63	-------
31	250	5	50	-------
32	250	5	50	-------
Average	**305**	**18**	**73**	**N/A**

*This patient was excluded from the evaluable set.

Clinical Summary:

The following provides a brief summary for each subject in the evaluable dataset describing the nature of the trauma and the outcome.

Subject ID/ Treatment Group	Clinical Summary
------ / HBOC + Standard Care	**INJURY** Gunshot wound anterior right chest at third intercostal, exiting left posterior above seventh intercostal space. Lung laceration of right upper lobe T4 fracture with paraplegia. **OUTCOME** Cardiac arrest during the surgical procedure, leading to death
------ / HBOC + ----- dard Care	**INJURY** Bleeding from multiple stab wounds to neck, forearm and abdomen. The bleeding mesenteric vessels were ligated, and small bowel perforations repaired. Multiple

	stab wounds sutured. **OUTCOME** Patient survived, was discharged from the hospital and completed Day 28 follow-up visit.
------ / Standard ------	**INJURY** Patient presented with reduced breath sounds – right chest, right haemopneumothorax of 2000mL. Gunshot wounds to the following: right axilla 5cm below apex in midaxillary line, right shoulder left aspect, and right bicep medial aspect proximal third. There was no detectable radial pulse. **OUTCOME** Patient died despite all efforts.
------ / Standard ------	**INJURY** Female patient presented with stab wound – midclavicular line, central margin. Patient underwent laparotomy, splenectomy, and liver repair of grade II laceration in the third segment. Also present was a severed spleen and haemopneumothorax. **OUTCOME** Patient recovered well but was discharged from hospital without study personnel first being notified and is lost to follow-up.
------ / HBOC + Standard Care	**INJURY** Multiple gunshot wounds to left flank, arm and thigh. Fracture of right clavicle and right proximal humerus (open). Large laceration right antoid axillar fold. Fracture right tib-fib midshaft open 15cm with partial amputation. **OUTCOME** Patient survived, was discharged and completed Day 28 follow-up visit

------ / HBOC + ------- ard Care	**INJURY** Admitted after vehicular accident with multiple injuries to chest, abdomen and limbs. During surgery it was determined that patient had a ruptured right hemi-diaphragm, and multiple grade 5 liver lacerations. **OUTCOME** Patient died post surgery, suffering a cardiac arrest while in the ICU.
------ / Standard ------	**INJURY** Gunshot wound below umbilicus with evisceration. Patient underwent surgery; Haemoperitoneum ±2500 ML. 2 holes located in jejunum: 10 cm resection, 2 holes in ileoceal junction: 10 cm resection. 2 hole in mesenterium: resected.

	Retroperitoneal hematoma explored. **OUTCOME** Patient survived but lost to follow-up, thus Day 7 and Day 28 assessments not done.
------ / Standard ------	**INJURY** Female admitted with a gunshot wound to the posterior T9 paravertebral area. Victim of a drive-by shooting and admitted in a severely shocked state. **OUTCOME** The patient developed ventricular fibrillation while undergoing surgery and died.
------ / Standard ------	**INJURY** Admitted following gunshot wound to right sub costal margin, midclavicular line, and gunshot wounds (x2) to the right thigh. A right thoracotomy was performed. Four holes in the lung were found with ± 500mL blood in right pleural cavity. **OUTCOME** Patient recovered well and returned for Day 28 follow-up visit.
------ / HBOC ----- ndard Care	**INJURY** Gunshot wounds to upper left and right quadrants. During surgery, it was discovered that patient had Grade IV through and through lacerations to transverse colon, grade II lacerations to kidney, and laceration to right hemi diaphragm. Blood pressure at 22h00 was too low to be detected by standard equipment. First recordable blood pressure taken at 22:05, which was 66/35mmHg; heart rate 88. Subject then randomized at 22:40.Internal hemorrhaging requiring a second surgery. **OUTCOME** Patient suffered cardiac arrest on the table and died during second surgical procedure
------ / Standard Care	**INJURY** Multiple gunshot wounds. Extremities: Gunshot wounds X2 left buttock, Gunshot wound right biceps medially, Gunshot wound right biceps laterally, and decreased anal tone – prostate normal **OUTCOME** Patient survived and completed Day 28 follow-up assessments.
------ / HBOC + Standard Care	**INJURY** Stab wound to chest, 3rd intercostal space lateral to sternum. Reduced air entry on right side. Two chest drains on right. Swinging tachypnea **OUTCOME**

	Patient survived, was discharged and lost to follow-up
------ / ------ C+Standard Care	**INJURY** Gunshot wound to neck, scalp laceration right front to parietal region. Anterior mandible fracture and reduced entry on the right side. Loose teeth, laceration right lower lip, laceration tongue, missing teeth. Head and neck extremely swollen. Patient hypertensive at baseline. **OUTCOME** Patient survived, was discharged from the hospital and completed the Day 28 follow-up visit.
------ / Standard Care	**INJURY** Two bullet holes in left and right upper quadrants, abdomen distended during resuscitation, voluntary quarding. **OUTCOME** Patient survived and completed the study follow-up.
------ / HBOC + Standard Care	**INJURY** Stab wound to chest. Poor air entry right base. Drained 900ml of blood **OUTCOME** Patient survived, was discharged from the hospital and completed the Day 28 follow-up visit.
------ / HBOC + Standard Care	**INJURY** Patient admitted with severe hypothermia and progressive hypovolemic shock, after being stabbed 24 times in the following areas sternomastoid area, right ear lower chest, 9th intercostal space, mid clavicular line, anterior axillary line, back and spine (14 lacerations)A thoracotamy and laparotamy were both performed. Blood loss estimated at least 4000 ml **OUTCOME** Patient remained unstable and continued to deteriorate rapidly, and then died.

------ / Standard Care	**INJURY** Patient admitted with stab wound to left 4th intercostal anterior mid clavicular line. Decreased entry left. Left hemapneumothorax. Patient distressed. **OUTCOME** Patient was discontinued from study after blood gas machine malfunctioned and site personnel were unable to record study specified measurements. Sponsor only notified afterwards.

------ / Standard Care	**INJURY** Patient in hypovolemic shock at admission, from bullet wound to chest which entered abdomen. There was evidence of early coagulopathy and metabolic acidosis. A laparotomy was performed and the injuries stapled, including part of the duodenum. The left ureter was stented. This patient had multiple surgeries including a splenectomy, small bowel resection and decompression, and colon decompression. **OUTCOME** The patient was on a ventilator and never recovered from his renal failure He progressed into multi-organ failure and died.
------ / HBOC + Standard Care	**INJURY** A 60 year old male victim of pedestrian vehicular accident. His injuries included a traumatic amputation of his left leg, fractures of the pelvis, scalp and hip, ruptured urethra/bladder, and bowel lacerations. A midline laparotomy was performed, repairing small bowel injuries and extra peritoneal bladder rupture. A left above-knee amputation was also done. Post surgery, the patient was admitted to the ICU fully ventilated and on adrenalin. The first ECG post-surgery showed clinically significant cardiac ischemia, cause of which was under perfusion due to hemorrhagic shock. Premorbid ischaemic heart disease could not be ruled out. **OUTCOME** Patient survived, was discharged from the hospital, and returned for Day 28 follow-up.
------ / Standard ------	**INJURY** A 45 year old male victim of pedestrian vehicular accident, admitted in severe hemorrhagic shock with evidence of coagulopathy. Injuries included a crushed pelvis and thighs, fracture of left and right femur, crushed calf muscles, left ankle dislocation with extensive tissue loss, and a ruptured popliteal vein. Patient underwent surgery on left leg and ankle, as well as fixation of the pelvis and the left and right femur. The patient developed acute renal failure and remained on a ventilator. **OUTCOME** The patient's condition continued to worsen and he suffered a cardiac arrest and died.

------	**Not Applicable**
------ / HBOC + Standard Care	Patient fell from a significant height. Presented with lower extremity, and pelvic fractures, and femoral bone protruding distally from right thigh. Scalp laceration to occipital regions with exposed skull. This patient did not meet the entry

	criteria and was randomized without consulting the Sponsor **OUTCOME**: The patient suffered a cardiac arrest on the way to surgery, and died.

Appendix

Table A1. Adverse Event and Serious Adverse Event Listing (The results are obtained from the evaluable study population of n=19 patients).

Subject Number	Adverse Event: Verbatim Terms	Serious = 1
- ---- ---------	**BLEEDING (WORSENING)**	**1**
----------	**HYPOTENSION (WORSENING)**	**1**
---- - ---------	**CARDIAC ARREST**	**1**
- ----	WORSENING OF RESPIRATORY ACIDOSIS	2
- ----	WORSENING OF LACTIC ACIDOSIS	2
- ----	HYPOXIA (WORSENING)	2
- ----	CREPITATIONS	2
- ----	HYPOKALEMIA (K=2.7)	2
- ----	REDUCED BREATH SOUNDS	2
- ----	INCREASED WITE SECRETIONS	2
- ----	ANEMIA	2
- ----	ELEVATED AMYLASE	2
- ----	ELEVATED CK	2
- ----	REDUCED BASE EXCESS (WORSENING)	2
- ----	HYPERTENSION	2
- ----	LOW URINE OUTPUT	2
- ---- ---------	**MASSIVE BLEEDING**	**1**
---- - ---------	**CARDIAC ARREST**	**1**
- ----	HYPERNATREMIA (148)	2
- ----	HYPONATREMIA (117)	2
- ----	ABSENT BOWEL SOUNDS	2
- ----	ANEMIA	2
- ----	HYPOGLYCAEMIA (GLUC 2.1)	2
- ----	HYPOKALEMIA	2
- ----	METABOLIC ALCALOSIS	2
- ----	METABOLIC ACIDOSIS	2
- ----	REDUCED BICARBONATE	2
- ----	INCREASED BICARBONATE	2
- ----	REDUCED CO2	2
- ----	INCREASED CO2	2
- ----	REDUCED BASE EXCESS	2
- ----	CHEST INFECTION	2
- ----	HYPOKALEMIA DURING SURGERY	2
- ----	INCREASED LIPASE	2
- ----	INCREASED ALKALINE PHOSPHATASE	2
- ----	INCREASED AST	2
- ----	INCREASED AMYLASE	2
- ----	INCREASED GAMMA GT	2
- ----	INCREASED ALT	2
- ----	POST-OPERATIVE PARALYTIC ILEUS	2

Subject Number	Adverse Event: Verbatim Terms	Serious = 1
- ----	CHEST INFECTION	2
- ----	METABOLIC ACIDOSIS (LACTIC)	2
- ----	REDUCED CALCIUM WORSENING	2
- ----	HYPOKALEMIA	2
- ----	THROMBOPHLEBITIS RIGHT ARM	2
- ----	LEG WOUND INFECTION (LEFT)	2
- ----	HYPOTENSION (BP 90/60)	2
- ----	REDUCED HCT AND HB. (ANEMIA)	2
- ----	RAISED CK (WORSENING)	2
- ----	INCREASED TOTAL BILIRUBIN	2
- ----	WORSENING OF LOW PROTEIN COUNT	2
- ----	INCREASED CONJUGATED BILIRUBIN	2
---- - ---------	**SEVERE HAEMORRHAGE**	**1**
---- - ---------	**CARDIAC ARREST**	**1**
- ----	ATRIAL FIBRILLATION	2
- ----	EARLY REPOLARISATION IN ST VARIANT	2
- ----	TACHYCARDIA	2
- ----	ELEVATED AST	2
- ----	ELEVATED CK	2
- ----	ELEVATED AMYLASE	2
- ----	ECG CHANGES: INCREASED R-WAWE VOLTAGE	2
- ----	DISTENDED, TENDER ABDOMEN	2
- ----	WORSENING ANEMIA	2
- ----	SCANTY BOWEL SOUNDS	2
- ----	ECG CHANGES: NON-SPECIFIC CHANGES ST-T WAVE	2
- ----	METABOLIC ACIDOSIS	2
- ----	HYPERGLYCAEMIA	2
- ----	HYPOTENSION	2
- ----	INCREASED ALT	2
- ----	INCREASED LDH	2
- ----	HYPOXIA	2
- ----	LOW PACO2	2
----------	**HEMORRHAGE**	**1**
---- - ---------	**VENTICULAR FIBRILLATION**	**1**
- ----	ELEVATED ALT	2
- ----	ELEVATED AST	2
- ----	ELEVATED AMYLASE	2
- ----	ELEVATED LDH	2
- ----	ELEVATED CK	2
- ----	TACHYCARDIA	2
- ----	PNEUMOTHORAX (NEW)	2
- ----	INCREASED CKMB	2
- ----	METABOLIC ACIDOSIS	2
- ----	HYPOTENSION	2
- ----	WORSENING DRAINAGE FROM CHEST DRAIN	2
- ----	BLOODY, WATERY SECRETIONS FROM	2

Subject Number	Adverse Event: Verbatim Terms	Serious = 1
	ENDOTRACHEAL TUBE	
- ----	REDUCED AIR ENTRY ON RIGHT LUNG	2
- ---- ---------	**OLIGURIA**	**1**
----------	**HEMORRHAGE**	**1**
---- - ---------	**ACIDOSIS**	**1**
---- - ---------	**DEATH/CARDIAC ARREST**	**1**
- ----	HYPERKALAEMIA	2
- ----	TACHYCARDIA	2
- ----	POOR CAUGHING EFFORTS	2
- ----	HYPERTENSION	2
- ----	HYPOTENSION	2
- ----	TACHYCARDIA	2
- ----	HYPOXIA	2
- ----	HAEMORROIDS	2
- ----	WOUND INFECTION	2
- ----	INCREASED ALKALINE PHOSPHATE	2
- ----	INCREASED GAMMA GLUTANYL TRANSFERASE	2
- ----	INCREASED ALT (ALANINE TRANSAMNASE)	2
- ----	INCREASED AST (ASPARTCAT TRANSOMINASE)	2
- ----	INCREASED AMYLASE	2
- ----	CHEST INFECTION	2
- ----	MEDIASTINITIS	2
- ----	CARDIAC ISCHEMIA	2
- ----	WHITE CELLS INCREASED	2
- ----	HYPERVENTILATION	2
- ----	MILD HEADACHE	2
- ----	DECREASED PHOSPHORUS	2
- ----	INCREASED LIPASE	2
- ----	PRODUCTIVE COUGH	2
- ----	ABNORMAL BREATH SOUNDS	2
- ----	FEVER	2
- ----	TACHYCARDIA	2
- ----	DRY MOUTH	2
- ----	ANEMIA	2
- ----	RIGHT LOWER LOBE COLLAPSE/CONSOLATION	2
- ----	OLIGURIA	2
- ----	***HYPOTHERMIA***	***??***
- ----	TACHYCARDIA	2
- ----	ENDOTRACHEAL SECRETIONS	2
- ----	PYREXIA	2
- ----	HYPERKALEMIA	2
- ----	TACHYCARDIA	2
- ----	HYPOKALEMIA	2
- ----	HYPERTENSION	2
- ----	HYPOTHERMIA	2
- ----	ONGOING BLEEDING WITH HYPOVOLEMIC SHOCK	2

Subject Number	Adverse Event: Verbatim Terms	Serious = 1
- ----	HYPERGLYCAEMIA	2
- ----	SWELLING RIGHT HAND	2
- ----	RESPIRATORY ALKALOSIS	2
- ----	OOZING ABDOMINAL WALL	2
- ----	ADVENTITIOUS BREATH SOUNDS	2
- ----	HYPOVOLEMIA	2
- ----	DEHYDRATION	2
- ----	PEDAL EDEMA GRADE 1	2
- ----	TACHYPNEA	2
- ----	GASTRIC OUTLET OBSTRUCTION	2
---- - ---------	**SEPTICAEMIA**	**1**
- ----	INCREASED WHITE CELL COUNT	2
- ----	DECREASED RED CELL COUNT	2
- ----	DECREASED PLATELET COUNT	2
- ----	DECREASED PHOSPHATE	2
- ----	LACTIC METABOLIC ACIDOSIS	2
- ----	HYPOCAPNOEA	2
- ----	LOW PARTIAL PRESSURE OF OXYGEN	2
- ----	PRESSURE ULCER BUTTOCKS	2
- ----	PRESSURE ULCER LEFT NOSTRIL	2
- ----	ABRASION LEFT KNEE	2
- ----	VOMITING	2
- ----	NAUSEA	2
- ----	NEURALGIA	2
- ----	WOUND SEPSIS	2
- ----	PYREXIA	2
- ----	PAIN	2
- ----	DIARRHEA	2
- ----	DIZZINESS	2
- ----	DECREASED URINE OUTPUT	2
- ----	RESPIRATORY DISTRESS	2
- ----	HYPERTENTION	2
- ----	HEMATURIA	2
- ---- ---------	**PROGRESSIVE HYPOVOLEMIC SHOCK**	**1**
- ---- ---------	**HYPOTHERMIA**	**1**
- ----	ACUTE LUNG INJURY	2
- ----	COAGULOPATHY	2
- ----	HYPERKALEMIA	2
- ----	HYPOGLYCEMIA	2
- ----	PYREXIA	2
- ----	POOR PERFUSION ILEOSTEMY COLOSTOMY	2
- ----	DUODENAL FISTULA	2
- ----	GENERALISED LYMPHODENOPATHY	2
- ----	DUODENAL RUPTURE	2
- ----	DIAPHRAGMATIC RUPTURE	2
- ----	SMALL PRESSURE SORE ON BACK	2

Subject Number	Adverse Event: Verbatim Terms	Serious = 1
- ---- ---------	**SECONDARY PERITONITIS**	**1**
- ----	HYPOTENSION	2
- ----	SYSTOLIC HYPERTENSION	2
- ----	ENDOTRACHEAL SECRETIONS	2
- ---- ---------	**ABDOMINAL COMPARTMENT SYNDROME**	**1**
- ----	ECTOPIC HEARTBEATS	2
- ----	INCREASED WHITE CELL COUNT	2
- ----	DECREASED RED CELL COUNT	2
- ----	WORSENING ANEMIA	2
- ----	**LOW PLATELET COUNT**	**1**
- ----	INCREASED RED CELL DISTRIBUTION WIDTH	2
- ----	DECREASED METABOLIC CO2	2
- ----	INCREASED ANION GAP	2
- ----	DECREASED CALCIUM	2
- ----	DECREASED TOTAL PROTEIN	2
- ----	DECREASED ALBUMIN	2
- ----	TOTAL BILIRUBIN INCREASED	2
- ----	INCREASED CONJUGATED BILIRUBIN	2
- ----	INCREASED ALANINE TRANSAMINASE	2
- ----	INCREASED ASPARTATE TRANSAMINASE	2
- ----	INCREASED LACTATE DEHYDROGENASE	2
- ----	DECREASED METHEMOGLOBIN	2
- ----	DECREASED HEMOGLOBIN	2
- ----	DECREASED HEMATOCRIT	2
- ----	LACTIC METABOLIC ACIDOSIS	2
- ----	HYPERCAPNIA	2
---- - ---------	**ACUTE RENAL FAILURE**	**1**
- ----	HYPOKALEMIA	2
- ----	SINUSTACHYCARDIA	2
- ----	HYPOTHERMIA	2
- ----	METABOLIC ACIDOSIS	2
---- - ---------	**MULTI-ORGAN FAILURE**	**1**
- ---- ---------	**SEPSIS**	**1**
- ----	SINUS TACHYCARDIA	2
- ----	ALCALOSIS (METABOLIC	2
- ----	HYPOTHERMIA	2
- ----	HYPOTENSIA	2
- ----	OLIGURIA	2
- ----	NO BOWEL SOUNDS	2
- ----	DIARRHOEA	2
- ----	NEUROLOGICAL LOSS OF CONSCIASNESS	2
- ----	OEDEMA	2
- ----	BLISTER ON SACRUM	2
- ----	BLOOD STAINED SECRETIONS	2
- ----	ABNORMAL BREATH SOUNDS	2
- ----	PUPILS UNEQUAL	2

Subject Number	Adverse Event: Verbatim Terms	Serious = 1
- ----	SELF EXTUBATION	2
- ----	SELF EXTUBATION	2
- ----	OOZING WOUNDS	2
- ----	HYPOKALEMIA	2
- ----	HYPOCALCEMIA	2
- ----	HYPOGLYCEMIA	2
- ----	BLISTERS ON FOOT AND ANKLE	2
- ----	SWOLLEN FACE	2
- ----	ILEUS	2
- ----	HYPERGLYCEMIA	2
- ----	SINUS TACHYCARDIA	2
- ----	HYPERTENSION	2
- ----	HYPOTHERMIA	2
---- - ---------	**ACUTE RENAL FAILURE**	**1**
- ----	COAGULOPATHY	2
- ----	ACUTE LUNG INJURY	2
- ----	ELIVATED LIPASE	2
- ----	PYREXIA	2
- ----	INCREASED ALT	2
- ----	INCREASED AMYLASE	2
- ----	INCREASED AST	2
---- - ---------	**CARDIAC ARREST**	**1**

EXHIBIT A

DSMB RECOMMENDATION FORM

DATE OF MEETING: OCTOBER 13, 2006

ATTENDEES:
DRS CIOFFI, CONN, HEYMAN & SLOAN +
BIOPURE REPS

FINAL DECISION:

1. **Name of DSMB member:** ALASDAIR CONN MD FACS FRCS(C)

2. **Decision of DSMB member:**
   - A. Continue study as planned ☑
   - B. Make changes to the investigational plan ☐
   - C. Terminate the study ☐

Date: Nov 2 '06 **Signature:**

Each DSMB member should forward their completed *DSMB Recommendation Form* to the DSMB Chairman (Dr. Eugene Heyman) using the enclosed pre-paid envelopes.

The DSMB Chairman (or designee) will compile the *DSMB Recommendation Form* for all DSMB members, send a written summary of the minutes, including a list of attendees, and any specific recommendations (if any), to the Sponsor.

EXHIBIT A

DSMB RECOMMENDATION FORM

DATE OF MEETING: 10-13-2006

ATTENDEES: DRS. CIOFFI, CONN, HEYMAN & SLOAN

FINAL DECISION:

1. Name of DSMB member: EUGENE HEYMAN

2. Decision of DSMB member:
   - A. Continue study as planned ☒
   - B. Make changes to the investigational plan ☐
   - C. Terminate the study ☐

Date: 11/6/06 Signature: Eugene R. Heyman

Each DSMB member should forward their completed *DSMB Recommendation Form* to the DSMB Chairman (Dr. Eugene Heyman) using the enclosed pre-paid envelopes.

The DSMB Chairman (or designee) will compile the *DSMB Recommendation Form* for all DSMB members, send a written summary of the minutes, including a list of attendees, and any specific recommendations (if any), to the Sponsor.

EXHIBIT A

DSMB RECOMMENDATION FORM

DATE OF MEETING: 10/13/06

ATTENDEES:

FINAL DECISION:

1. Name of DSMB member: William G. Cioffi, MD

2. Decision of DSMB member:

A. Continue study as planned ☒

B. Make changes to the investigational plan ☐

C. Terminate the study ☐

Date: 11/2/06 Signature: [signature]

no changes on the minutes

Each DSMB member should forward their completed *DSMB Recommendation Form* to the DSMB Chairman (Dr. Eugene Heyman) using the enclosed pre-paid envelopes.

The DSMB Chairman (or designee) will compile the *DSMB Recommendation Form* for all DSMB members, send a written summary of the minutes, including a list of attendees, and any specific recommendations (if any), to the Sponsor.

To HAZEL Kropme
3 pages

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EXHIBIT A

DSMB RECOMMENDATION FORM

DATE OF MEETING: Oct 13 2006

ATTENDEES:

FINAL DECISION:

1. **Name of DSMB member:** Edward Sloan
2. **Decision of DSMB member:**
   - A. Continue study as planned ☒
   - B. Make changes to the investigational plan ☐
   - C. Terminate the study ☐

Date: 11/06/06 **Signature:** [signature]

Each DSMB member should forward their completed *DSMB Recommendation Form* to the DSMB Chairman (Dr. Eugene Heyman) using the enclosed pre-paid envelopes.

The DSMB Chairman (or designee) will compile the *DSMB Recommendation Form* for all DSMB members, send a written summary of the minutes, including a list of attendees, and any specific recommendations (if any), to the Sponsor.

Protocol HEM-0125 DSMB Meeting Minutes

The meeting was held on October 13, 2006 by teleconference and was opened with Dr. Greenburg, VP Medical Affairs, and DSMB members Drs. Cioffi, Conn, and Sloan. The first portion of the meeting was an open discussion of the protocol and of the roles of the DSMB members. This was carried out by Dr. Greenburg and a representative from South Africa. The protocol was discussed in detail in terms of inclusion and exclusion criteria, endpoints, etc. All members of the Board had the opportunity to ask any questions concerning the protocol. Questions concerning the protocol dealt primarily with inclusion and exclusion criteria. Dr. Heyman had the opportunity to go through the protocol and the roles of the DSMB with the company separately on Oct 12.

Further questions were raised to Dr. Greenburg regarding all of the current data on HBOC-201. The questions dealt specifically with cardiac risk. The Board asked specifically of the risk of cardiac SAE/AEs. Dr. Greenburg noted that it was confined to elderly patients over 85, and that the current protocol enrolled patients only up to the age of 65. In addition, Dr. Greenburg noted that he had recently discussed this issue with the FDA and the consensus was that there was not a statistical increase in cardiac risk.

There were no further questions in the open discussion, and therefore Dr. Greenburg and all representatives of Biopure signed off the conference call. Dr. Heyman then joined the teleconference and the closed portion of the meeting commenced. The DSMB unanimously agreed that there was no safety concern and that the study should continue as planned.

The DSMB expressed concern about the slow patient enrolment and would like to propose that consideration be given to relaxing the entry criterion of SBP $\leq$ 90 mmHg to increase enrolment.

The DSMB then discussed the data that were provided and would like to request the following additions and/or changes for future meetings.

- Information about patient enrolment, i.e., number of patients enrolled by month
- All tables presented by Group A and B to allow us to link the baseline data with the safety data
- The number of patients enrolled in each treatment group A and B
- Greater detail for medical history. If the investigator terms are coded then provide the numbers of patients in each coded category within each body system. If not coded then we would like the investigator terms in the table beneath their corresponding body system.
- The numbers of patients in each baseline Injury Severity Score (ISS) grouping, using the following categories:
    - 0 – 12
    - 13 – 16
    - 17 – 20
    - 21 – 24

- 25 – 28
- 29 – 32
- 33 – 36
- 37 – 40
- 41 – 44
- ≥ 45

- The numbers of patients in each treatment group with blunt vs. penetrating injuries
- Provide confirmation that all inclusion criteria were met in addition to the numbers of patients in each treatment group with
  - SBP ≤ 90 mmHg
  - Heart rate ≥ 120 bpm for ≥ 10 min
- Summaries of all adverse events (AEs) and of serious AEs by coded terms. We understand that all AEs may not be coded, but an AE summary is necessary to understand the overall safety profile.
- The AE and the SAE patient listings should be presented by treatment group, sorted by patient within treatment group. It would also be helpful if a line is skipped between patients (i.e. in SAS print; by patient; id patient;)
- Replace onset date and stop date by study day (Day 1 = day of randomization).
- On the SAE listing add the date of death for patients who died
- The onset and stop times are not necessary.
- Add a summary of the numbers of patients in each treatment group with 0, 1, 2, etc. AEs

Eugene R. Heyman 11-6-06

Eugene R. Heyman, PhD date
DSMB Chairman

Draft Prepared by Fred Apple PhD, 062206

A 2000 ESC/ACC consensus publication has codified the role of biomarkers, specifically cardiac troponin, by advocating that the diagnosis of myocardial infarction be evidence of myocardial injury based on biomarkers of cardiac damage in the appropriate clinical situation.[1] Either one of the following criteria satisfies the diagnosis for an acute, evolving, or recent MI.

1. Typical rise or gradual fall (cardiac troponin) or more rapid rise or fall (CK-MB) of biochemical markers of myocardial necrosis with at least one of the following:
    a. Ischemic symptoms
    b. Development of pathological Q waves on the ECG
    c. ECG changes indicative of ischemia (ST segment elevation or depression)
    d. Coronary artery intervention (e.g., coronary angioplasty)
2. Pathological findings of an acute MI

The guidelines thus recognized the reality that neither the clinical presentation nor the ECG had adequate sensitivity and specificity. The guideline does not suggest that all increases of these biomarkers, i.e. cardiac troponin, should elicit a diagnosis of AMI; only those associated with the appropriate clinical and ECG findings. When cardiac troponin increases that are not caused by acute ischemia occur, the clinician is obligated to search for another etiology for the elevation.[2,3] The criteria for use of these biomarkers suggested by the Biochemistry Panel of the ESC/ACC Committee for use of cardiac biomarkers for detection of myocardial injury and myocardial infarction were as follows.[1,4]

• Increases in biomarkers of cardiac injury are indicative of injury to the myocardium, but not an ischemic mechanism of injury
• Cardiac troponins (I or T) are preferred markers for diagnosis of myocardial injury

• Increases in cardiac marker proteins reflect irreversible injury
• Myocardial infarction is present when there is cardiac damage, as detected by marker proteins (an increase above the 99th percentile of the normal range) in a clinical setting consistent with myocardial ischemia.
• If an ischemic mechanism is unlikely, other etiologies for cardiac injury should be pursued.
• Samples must be obtained at least 6 to 9 hr after the symptoms begin.
• After PCI and CABG, the significance of marker elevations and patient care should be individualized.

References

1. Alpert JS, Thygesen K, Antman E, et al. Myocardial infarction redefined—a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. J Am Coll Cardiol 2000;36:959-9.
2. Apple FS. Tissue specificity of cardiac troponin I, cardiac troponin T, and creatine kinase MB. Clin Chim Acta 1999;284:151-9.
3. Jaffe AS. Elevations in cardiac troponin measurements: False false-positives. Cardiovas Tox 2001;1:87-92.
4. Jaffe AS, Ravkilde J, Roberts R, et al. It's time for a change to a troponin standard. Circulation 2000;102:1216-20.