DIVISION OF CARDIOVASCULAR and RENAL PRODUCTS

Memorandum

Date: 31 May 2006

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Subject: BB-IND 12504:HBOC-201 (Polymerized Bovine Hemoglobin –B): Amendment SN0006

Consultative reviews of seven trials of HBOC-201 in several porcine models of hemorrhagic shock (HS) to support clinical use in HS per RESUS

CBER's office of blood research and review is taking the Navy's RESUS trial (**R**estore **E**ffective **SU**rvival in **S**hock in hemorrhagic shock) of HBOC-201, currently on hold, to the Blood Products Advisory Committee on July 13, 2006. The office requested a consultative review of several studies of HBOC-201, vs. 6% starch (HEX), lactated Ringers (LR), normal saline (SAL) or no fluid replacement (NON) in USUHS and NMRC porcine models of HS with or without important soft tissue injury. These studies were intended to anticipate use of HBOC-210 to treat life-threatening traumatic HS, in the population at large, prior to hospital arrival and definitive resuscitation. This overview focused on aspects of the models and study design to be considered

in trying to extrapolate veterinary findings to clinical behavior and expectations. This reviewer sought correspondences and deviations vis a vis RESUS with respect to trauma setting; re-infusion criteria ; maximum dosages and rates of administration; extent of monitoring; interpretability and validity of findings (i.e., confounding factors); clin. chem.. and histologic evidence of any re-perfusion injury i.e., excess injury in hex or LR-expanded swine vs. non-reperfused control – and any exacerbation by HBOC-201 (a theoretical concern with HBOCs); and identification of any prognostic endpoints or biomarkers. These opinions are incorporated in responses to the 9 questions asked by the consulting division regarding extent to which each study anticipates safety and efficacy in RESUS.

Content of consult:

- Executive Summary
- Background
- General comments
- Overview of seven swine studies: response to Division questions.
- Appendix: RESUS protocol

Executive Summary/Overview:

The swine HS models: Studies overviewed uniformly involved healthy juvenile swine which, prior to exsanguination to shock levels of hypotension and hypovolemia, were well hydrated, anesthetized, intubated, and mechanically ventilated - usually with oxygen enrichment, and after neuromuscular blockade. In all these regards, they deviate from RESUS. Important soft tissue injury, where protocol-specified, was confined to liver (crush/laceration).
Fluid intervention criteria: In both controlled bleeding low-volume resuscitation scenarios and in scenarios of continuing and uncontrolled hemorrhage , sole target of resuscitation with HBOC-201 was to achieve and maintain a mean art. pressure of 60 mmHg whereas RESUS specifies more (stable ≥90 mmHg SBP / ≤100 BPM HR *and* no signs of occult shock.
Monitoring, and prognosticators: Although swine were monitored for hemodynamic, clinical chemistry, blood gas, and metabolic status, there was no retrospective tracking of survivors and non-survivors to more reliably identify potential prognostic parameters.
Results: In the 5 models of reduced survival, HBOC-201 at 2-12X RESUS dosage , significantly prolonged survival compared to Hex or LR. In these and the 2 non-lethal models, cardiac and urinary outputs were typically depressed; systemic/ pulmonary art BP elevated; recovery of tissue oxygenation accelerated, and, except in "forced'infusion scenarios, resuscitation volumes reduced relative to Hex or LR. Where monitored, hepatic lesions were consistently encountered, and in a 5 day recovery study, renal and myocardial histopathology as well. The safety implications - veterinary or clinical - of the systemic and pulmonary vasoconstrictor activity, oliguria, and histopathology - in the context in which they were encountered (no pre-existing CV disease; anesthesia; resuscitation to 60 mmHg MAP; excessive delay to definitive treatment) – are unclear. What is clear is that when blood pressure and heart rate had stabilized and approached targeted values (approx. 30-50 minutes, MBP ≥60 mmHg, and HR ≈ baseline) lactate, mixed venous oxygen saturation, tissue oxygenation, base deficit and cardiac index and systemic vascular resistance remained abnormal. Accordingly, it is

cardinal to rely on other signs of hypoperfusion - as now specified in RESUS - to guide resuscitation with this vasoactive product. It is further recommended that HBOC-201 be tested in a monkey model of HS ,CV disease, and oxidative stress to perhaps more relevantly predict human response to HBOC-201 oxidative processes based on similarity in reductive status (i.e., neither species produces ascorbate). However, it seems that the available surgical patient safety data - presumably obtained at dosages at least corresponding to the minimal envisioned for RESUS (1-2 units...?), and from a population likely to have had some pre-existing CV disease, should more immediately drive the decision to approve or further modify RESUS protocol.

Background: HBOC-201 is a solution of 13 ± 1 g/dl of ultra-purified glutaraldehyde-polymerized bovine hemoglobin (Biopure Corporation, Cambridge, Ma.) in a modified ringers solution of pH 7.6-7.9. Osmolality (290-310 mOsm/Kg) is comparable to human plasma, but viscosity is appreciably less than that of whole blood (1.3 vs. 3.5 centipoise at 37 ° C.). Oncotic pressure is 17 mmHg. (slightly less than normal: 20-30 mmHg). Oxygen affinity is less than that for native human hemoglobin (P50 of 38mmHg

Phase II and III trials of HBOC-201 (BB-IND 12504), which included use in an elective orthopedic surgery setting, revealed pressor activity and a statistically significant excess of serious cardio-vascular AEs: CVA/TIA/ cerebral ischemia/infarction; MI; and heart failure/pulmonary edema. The context of these adverse CV events is relevant to considering safety of HBOC-201 tested per RESUS. If not known, the relationship, if any of these peri-operative events to the pressor activity; to potential risk factors such as history of diabetes, hypertension, or other CV disease; and/or aspects of anesthesia or surgical setting (depth of anesthesia; use of muscle relaxants, co-medications; any confounding of signs/symptoms of over dosage) must be identified. Reviewer is unaware whether these AEs were acute, delayed, or both and, furthermore, whether they were even dose-related. Perhaps both the recognized pressor and pro-oxidative activities of acellular modified hemoglobins underlie these AEs in pre-disposed patients. It is my expectation that some RESUS patient-subjects will have important pre-existing cardiovascular disease and atherosclerosis, that 34% of them will not survive attempted resuscitation with Lactated Ringer's; and some will undergo very aggressive resuscitation/re-perfusion scenarios via up to 10 units (2500 ml) of HBOC-201 – equivalent to 50% of their blood volume.

A major issue is the adequacy of the swine HS model for informing safety of HBOC-201 given the absence of adverse events in previously healthy swine vs. surgical patients at large (but presumably acceptable surgical risks) . As documented below, adverse clinical CV events cited above were not encountered either during or following resuscitation of anesthetized pigs with HBOC-201, even at appreciable multiples of the clinical dosage and in models of lethal as well as non-lethal HS (although histopathology was observed). Their absence, even at appreciable over dosage in the context of simulated uncontrollable hemorrhage (130 ml/Kg over several hours: see Manning et al, below) calls into question *prima facie*, the utility of swine HS model for predicting peri-resuscitative CV safety. It is evident, from the behavior described below in previously healthy hydrated swine, that this species can compensate for acute decreases in hematocrit down to 5% or less. Such levels would cause overt circulatory collapse in humans. Perhaps this species can also better withstand pressor activity?

At issue also is whether this model is one of detectable re-perfusion injury, as one might expect *a priori*, and any exacerbation by HBOCs. However, I did not detect this when I compared histopathology in non-reperfused vs. Hex or LR - re-perfused animals. *In vivo* models have also

implicated HBOCs as important mediators of injurious reactive oxygen and nitrogen species, especially in an ischemia-reperfusion scenario. The degree of micro vascular injury seems to depend on the pro-oxidative nature of the HBOC, and can be attenuated with anti-oxidants such as sodium selenite (Baldwin et al, 2003/4). Since the swine can produce potentially protective ascorbate, but that the rate of its production is attenuated under stress (Wariss, 1984), I paid attention to whether ischemia-reperfusion injury was present in these swine models, whether it varied with the severity and duration of the hypovolemia and speed/degree to which it was corrected; and whether HBOC-201 could exacerbate such if the model revealed reperfusion injury. The three Nav. Med. Res. Center. studies comprising protocol KO04-02 (see p. 6 below) were most informative in that regard as authors looked at clinical chemistry, histologic, 3-nitrotyrosine (asserted to be an indirect marker for free radical – mediated tissue injury) and inflammation marker evidence histopathology. Tissue 3-nitrotyrosine levels were not elevated after re-perfusion with Hextend or even HBOC-201 vs. non-reperfused./non-resuscitated controls.

General comments pertaining to swine models of HS:

In common with the RESUS population, the severity of HS present in the swine models addressed below ranges from non-lethal insult to uniformly lethal hypoxia and metabolic acidosis absent timely restoration of tissue perfusion . However, swine were uniformly healthy and well hydrated prior to exsanguinations; and were resuscitated under anesthesia, intubation, and mechanical respiration often with 50-100% oxygen;. In those respects (and that they were arterially monitored to assure an accurate blood pressure and blood-gas status) the context in which the trauma was suffered and treated in all the models described below most obviously contrasts from that of RESUS. The young swine in these HS trials presumably have no important pre-existing cardiovascular and metabolic diseases (e.g., local or systemic atherosclerosis, arrhythmias, hypertension, and diabetes). In that regard, they differ importantly from those RESUS patients with compromised baseline cardiovascular reserve because of natural or acquired CV disease, and expected to be at higher risk of sequelae of hypoperfusion of vital organs..

In these swine HS models the only veterinary goal - in the fluid resuscitation cohorts - was, typically, to restore circulating blood volume sufficient to recover, and maintain , MBP of 60 mmHg. This was attempted in the context of either controlled bleeding - where HBOC-201 could be tested at low volume to minimize loss/dilution of native hemoglobin and coagulation factors – or catastrophic uncontrolled bleeding mandating forced titration to inform safety and efficacy at multiples of the maximum envisioned in RESUS. In none of the 7 studies overviewed was any timely attempt made to treat associated lactic acidosis and base deficit abnormality of a severity that , in a trauma center, would be treated with bicarbonate - for example, the pH =7.1 and bicarbonate level = 8 mEq/L scenario in the unsuccessfully resuscitated Lactated Ringers cohort in the Manning, J. *et al* study below. Yes, it simulates resuscitation in a particularly austere environment, but more timely interventions could inform propensity of HBOC-201 to provoke the hepatic, myocardial, and renal lesions The RESUS protocol is silent on pre-hospital use of bicarbonate although such would be envisioned in delayed hospital arrival scenarios.

Swine resuscitation protocols were silent on recognition of, and re-infusion criteria for, signs of any persistent hypoperfusion ("occult " shock) beyond the dogmatic blood pressure/heart rate composite end-point. Regarding occult shock, RESUS recognizes that persistent hypoperfusion may occur in patients despite restoration of stable BP and HR, and need for further fluid reinfusion if signs of HS

and hypoperfusion are still present. These include tachypnea , narrow pulse pressure , cool pale skin, weak and/or thready pulse, decreased oxygen saturation, oximeter failure to obtain reading, decreased capillary filling, and anuria. Accordingly, RESUS defines the scenarios for continuing resuscitation with standard fluids vs. CTM fluids. However the swine HS study protocols are completely silent on the necessity to look for and continue treating were evidence of persisting hypoperfusion to be present despite having arrived at BP and HR-based stopping points. Relying solely on BP and HR , or blood pressure *per se* stopping criteria - especially in view of the pressor (and reflex bradycardic) activity of HBOC-201, and the well-recognized effect of anesthesia on BP and HR – is at variance with the directions given in RESUS.

The clinical, let alone veterinary, safety implications of the positive hepatic (hepatobiliary), renal (multiple infarcts), and myocardial histopathology findings in several of these studies is not at all clear absent trials involving swine with pre-existing CV disease, and trials resuscitating to 90 mmHg SBP - rather than exclusively to 60 mmHg Mean Arterial Pressure as in most of the trials reviewed herein. These lesions were most numerous in the Fitzpatrick et al study (see p.22) where, it should be recognized, they were encountered in the Hex-treated swine as well and at the same incidence. However the four pulmonary edema deaths in the Hex –treated cohort suggests the latter group was over-resuscitated, which confounds comparing the propensity of HBOC-201 vs. HEX for provoking tissue injury in the course of resuscitation - at least to 60 mmHg MAP. The brain is not listed as one of the organs harvested for histopathology; however, the trial reports/publications are also silent on whether macroscopic brain lesions were encountered in any of the trials.

The influence – either salutary or adverse - of isoflurane, the anesthetic state, and the use of mechanical respiration on behavior of HBOC-201 in these trials is indeterminate absent controls for such. In these models where anesthesia-induced apnea necessitated mechanical ventilation, the option of tracheal intubation /ventilatory support or signs of/response to tachypnea does not ,of course, arise. Hyperventilation is the usual compensatory response to acidosis, and that is obviated in the mechanically ventilated intervals in the studies visited below.

There are important differences in monitoring vs. RESUS scenarios. Blood pressure was monitored directly from arterial cannulae in these models, thus obviating the problem, in some HS patients, of accurately measuring BP and, especially, avoiding falsely low values.

These trials of HBOC-201 in HS pigs were, with one exception, silent on incidence, if any, of pulmonary edema encountered in - life in the course of initial or definitive resuscitation. Such would be a contraindication to further fluid administration, or its more judicious use (volume and /or rate of infusion) in RESUS. For example, the Hex-treated cohort in the Fitzpatrick et al trial (see p. 23) Neither central venous pressure (CVP) nor blood volume were monitored to help objectively inform how re-assuring it is to rely exclusively on MBP /HR/digital oximetry profile (or even typical signs of "occult" shock such as thready pulse; impaired capillary refill; etc.) when resuscitating with a "vasoconstrictor " such as HBOC-201. On the other hand, pulmonary artery wedge pressure (which tracks left ventricular diastolic filling pressure) was informatively monitored throughout in some studies; it would not necessarily be in RESUS -even in its ICU phase). ECGs evidently were not always monitored in HS swine models although , per RESUS, such would be ancillary for diagnosing angina, NTSTEMI, SEMI., SEMI, QWMI, and aborted MI)

Overview of Porcine Studies:

[studies overviewed:

A.Protocol KO04-02: [HBOC-201 vs. 6% hetastarch (HEX) vs. no resuscitation fluid in three swine models of shock due to controlled bleeding (moderate and severe) with concurrent skeletal muscle crush injury) and uncontrolled bleeding with concurrent hepatic injury; and incorporating 4 hr. delay to definitive medical treatment].

Performing laboratory: Naval Medical research Center [Protocol KO04-02 is among the most comprehensively monitored of the seven studies overviewed, and involved both controlled and uncontrolled bleeding. Study design is prototypic for that followed, with some variations, in all the porcine HS studies reviewed, and, accordingly will be the only one described in some detail].

B. Katz, LM et al HBOC-201 improves survival in a swine model of hemorrhagic shock and liver injury Resuscitation 54(2002) 77-87)]

Performing laboratory: Carolina Resuscitation Research Group

C. Manning, JE et al Bovine hemoglobin-based oxygen carrier (HBOC-201) for resuscitation of uncontrolled , exsanguinating liver injury in swine,.... Shock 13(no 2) : 152-159,2000]

Performing laboratory: Carolina Resuscitation Research Group

D. York et al. Low volume resuscitation with a polymerized bovine hemoglobin-based oxygen carrying solution .....J Trauma: 2003, 55(5): 873-885.

Performing laboratory: Wilford Hall Medical Center, Lackland AFB.

E. Fitzpatrick et al. Low volume resuscitation with HBOC-201 in a large animal survival model of controlled hemorrhage. J. Trauma. 2005;59:273-283.

Performing laboratory: Wilford Hall Medical Center, Lackland AFB.

A. Protocol KO04-02: Controlled hemorrhagic shock (Moderate and Severe); and uncontrolled hemorrhagic shock

This study compared HBOC-201 vs. hetastarch (HEX) for emergency fluid resuscitation in three anesthetized swine models of lethal HS with concurrent soft tissue damage, each incorporating a 4-hour simulated delay to definitive veterinary care: controlled exsanguinations of either 40 or 55% of estimated normal blood volume; and a third more lethal HS model of severe uncontrolled hemorrhage where mean 1-hr survival is only 50% absent fluid intervention. After 4 hours of BP or HR criteria -dependent fluid intervention, definitive medical care was applied (autologous blood; allogenic packed RBCs for anemia; SAL for hypotension); swine were allowed to regain consciousness; monitored to 3 days post-resuscitation; euthanized; gross autopsy performed; and tissues harvested for histopathology. There is no mention of monitoring for persistent signs of hypoperfusion (e.g., tachypnea) immediately upon recovery from anesthesia.

HS induction: An estimated 40% (moderate controlled hemorrhage; MCHS) and 55% (severe controlled hemorrhage, SCHS) of blood volume was withdrawn, over 15 minutes, to reduce mean blood pressures to approx 30 or 20 mmHg, and cardiac index by 39% and 43%, respectively. For the uncontrolled HS model (UHS; intended to model resuscitation in combat

with severely delayed evacuation to surgery) bleeding from a crushed lacerated liver (25% lobectomy) was unhampered for 4-hours. The MCHS and the SCHS models also incorporated a standardized skeletal muscle crush injury; the UHS model, a liver laceration/25% lobectomy insult. Shed blood was measured and reserved for re-infusion during definitive resuscitation 4 hours later upon "hospital arrival".

Resuscitation: Five minutes after the controlled exsanguination to targeted degrees of estimated blood volume depletion, or after 15 minutes of uncontrolled hemorrhage, fluid intervention commenced - 10 ml/Kg /10 minutes of either HBOC-201 or 6% hetastarch vs. no fluids, and repeated at 5 ml/Kg/10 min at ½, 1, 2, and 3 hours post-injury if SBP remained less than 60 mmHg or HR remained above baseline. Definitive resuscitation was attempted at four hours, and involved, initially, reinfusion of autologous or allogenic RBCs if Hb<7 mg/dL, and 10-20 ml/Kg normal saline for hypotension, and repeated, if re-infusion criteria were met, over the 72 hr recovery period, again for control of anemia or hypotension, respectively.

Monitoring: Survival to 72 hours; and gross/histopathology (heart, lung, liver, kidney, jejunum) were the primary endpoints. A variety of cardiovascular, metabolic , clinical chemistry, and other parameters addressing specific HBOC /HS resuscitation issues were monitored prior to and during both emergency and definitive resuscitation. These included BP; HR; cardiac output index; urine output; systemic vasc. resistance index ; clinical chemistries; hematology; arterial base excess; tissue oxygenation; pre-hospital and hospital phase fluid requirements. Since activation of the coagulation system is often a feature of HS, aPTT, PT, TEG clotting index were monitored. Since acellular Hgb is believed to be pro-inflammatory, and to increase oxidative "stress", 3-nitrotyrosine , cytokines (TNF, INF, IL-2, 6 and 10), and immunocyte apoptosis were monitored as well.

Response to specific CBER questions:

Question1.a.) Model adequately simulates RESUS pre-hospital trauma setting?

Prima facie, and with caveats noted below, HS swine model those RESUS patient who have relatively confined soft tissue injury (e.g., a stab wound), and exsanguination sufficient to severely depress blood pressure, cardiac index, tissue oxygenation, and urine output; markedly elevate HR and serum lactate levels - but of no more than 5-15 minutes duration i.e., when resuscitation of the swine commenced. The consequences of extracellular Hgb-mediated pro-oxidative cascades may be exacerbated in scenarios of more protracted hypoperfusion and greater levels of endogenous oxidants (H_2O_2; $O_2^{\cdot}$; $ONOO^-$) released upon reperfusion]. The natural course of HS in NON pigs was characterized by stable PT, PTT, and fibrinogen during the pre-hospital phase (1st 4 hours). I do not know if this stability is a feature of clinical HS.

The four-hour delay to definitive resuscitation does not anticipate RESUS which envisions a 15-min delay to ER/Hospital where definitive follow-up will be undertaken

Caveats itemized in my general comments apply. Attempted resuscitation was to 60 mmHg MBP as verified by arterial cannula (rather than 90 mmHg SBP by probably less reliable cuff in RESUS) and to baseline HR and pigs were monitored to only 3 days rather than 1 month. Presumably, these swine do not have the pre-existing CV disease. Moreover, the resuscitation protocol did not anticipate. - and provide for adjustment of re-infusion criteria for - occult shock. It is not known whether any pigs restored to stable target BP and HR might have evidenced signs of persistent hypoperfusion if allowed to recover. On the other hand, a more demanding

delay to definitive medical care of 4-hours, rather than the <10-15 minutes expected arrival time to hospital in RES, was imposed.

1 b) Results support use of HBOC-201 in RESUS?

Survival: The MCHS, SCHS, and UHS models all feature reduced survival absent any fluid resuscitation (NON cohort) for 4 hours: NON survival to 4 hours(simulated ER arrival) for MCHS, SCHS, and UHS models was 88%, 25%, and 12%, respectively; and to 72 hr., 63%, 25%, and 12%, respectively. *Prima facie*, the statistically significantly improved 72 hr survival in the two most severe HS models- especially in the very lethal uncontrolled bleeding model where only HBOC-201, but not HEX, improved both 30 minute and 72 hour survival - support its usage in RESUS [In the SCHS model of recovery from 55% blood volume depletion HBOC-201 and HEX cohorts had similarly improved 72 hour survival (100%, and 75%, respect. vs. 25% survival for NON, ($p= 0.007$ overall; $p> 0.05$: HBOC vs. HEX). In the most severe UHS/liver injury model, 72 hours survival was improved by HBOC-201, but not HEX (88%, 13%, and 13%, for HBOC, HEX, and NON, respectively; overall $p=0.002$; HBOC-201 vs. HEX $p=0.004$)] Survival at 72 hours in the least severe MCHS model (i.e., resuscitation from 40% loss of estimated blood volume) also tended to be highest in the HBOC-210 cohort (100%, 87%, and 62%, for HBOC-201, Hex, and NON, resp., $p>0.05$)

Hemodynamics and oxygenation/metabolic parameters : Over the 4-hour course of pre-hospital resuscitation with either HBOC-2 or Hex, blood pressure, cardiac output, and systemic vascular resistance, and HR, tissue oxygenation, and lactate had essentially returned to baseline. However, there were clear differences in the time course of recovery of hemodynamic parameters relative to Hex: Across all models, CO recovered more quickly in the Hex cohorts; conversely, tissue oxygenation and lactate recovered more quickly during resuscitation with HBOC-201 . There were several qualitative differences in the trajectory of resuscitation as well : Hex was not able to restore baseline mean arterial pressure in the severe controlled and uncontrolled bleeding HS models. Conversely, HBOC-201 actually revealed systemic and pulmonary pressor activity.

Severe controlled bleeding HS model: Urine output for HBOC-201 vs. Hex: Reviewer thought it revealing to compare hemodynamic status of HBOC-201 vs. HEX – treated cohorts at the 4-hr. simulated hospital arrival time point since an equivalent volumes of each had been administered at that point [28 ml/Kg - enough to have restored blood volume to near normal since about 32 ml/Kg had been withdrawn to induce HS). At such time mean arterial pressure (both systemic and arterial) were appreciably higher than in the HEX cohort; cardiac index, heart rate, mixed venous oxygen saturation , tissue oxygenation and serum lactate levels were comparable; but mean urine output was only half that of the HEX cohort although both were much improved over that of the NON (no fluid re-infusion) control. It should be pointed out, however, that over the course of this 0-4 hr pre-hospital interval, urine output was only ½ that of the HEX group (2.2 ± 0.8 vs. 5 ± 1.3 ml/Kg) despite both cohorts having required/received virtually identical volume of intravenous fluids (28 ± 2 vs. 29 ± 2 ml/Kg), and MBP having significantly and very appreciably exceeded that of the HEX cohort throughout the pre-hospital interval (SBP never did return to baseline in the HEX cohort). Perhaps the smaller restoration of urine output is related to the reduced cardiac output and greater systemic vascular resistance in the HBOC-201 cohort *vs.* HEX.

Blood loss in uncontrolled bleeding/liver injury model: Furthermore, total unhampered blood loss, normalized for survival, was lower in HBOC-201 (8 ml/Kg/survival hour *vs.* 24 for Hex , $p<0.05$). This might have been expected since fluid and blood transfusion requirements also were less (7 vs. 16 ml/Kg/survival hour for HEX; $p=0.01$). Whether blood loss and resuscitative fluid requirements were least with HBOC-201 mainly because of its vasoactivity , as asserted by authors , is not clear,(and it is not obvious how such was accomplished non-surgically) . Hypercoagulation is evidently not the cause since in neither this nor the 2 other HS models was PT, PTT, fibrinogen, or bleeding time values appreciably affected by HBOC-201 at 4 hours; nor did thromboelastography data, on balance, indicate that the hemostatic effects of HBOC-201 resuscitation in HS were consistent and/or likely to be clinically significant according to the authors.

Histopathology and Clin. Chemistries:

Liver, lung, kidney, cardiac, and intestinal histology; clinical chemistry markers of liver and renal function; and tissue and plasma levels of 3-nitrotyrosine (a marker for free-radical mediated tissue injury) were examined. With a few exceptions, tissue histopathology – whether or not reflecting redox stress and re-perfusion injury – was not obviously exacerbated with HBOC-201. **Cardiac:** There were no excess myocardial lesions in the HBOC cohorts in any of the three HS models. Rather, incidence of LV necrosis , *collapsed across all three models* consistently tended to be lowest in HBOC-201 cohorts (13%, 43%, and 38% for HBOC, HEX, and NON cohorts, respectively; $p> 0.05$); this was *not* supported by any parallel decrease in RV ventricular septal necrosis whose incidence was not consistently treatment-related. At odds with the tendency for LV necrosis incidence to be least with HBOC-201 was the fact that CK-MB was highest in the HBOC-resuscitated cohorts in MCHS ($p=0.03$ overall; $p=0.02$ HBOC-201 vs. HEX.. CK-MB was not monitored (SCHS) or there were too few survivors (UCS) to confirm this effect of HBOC in the more severe HS models. Troponin I levels did *not* support the excess CK-MB levels in the HBOC cohort noted above. Accordingly, there is no really persuasive evidence of excess cardiac injury in the HBOC-201 cohort, at least in this trial.
Lung: Incidence/severity score of lung alveolar edema and inflammation/fibrin was highest in the HBOC-201 cohort in the most severe model (UHS), but its importance is moot because such was not confirmed in the other models, or associated with any excess interstitial edema. The 3-nitrotyrosine levels were not increased by HBOC-201 in either sever HS model.
Kidney: There was excess HBOC-201 - associated incidence and severity of renal papillary necrosis, inflammation (and possible hemorrhage), slightly elevated BUN (but not creatinine) in the MCHS. Neither histopathology nor excess BUN/creatinine relative to NON or Hex were detected in either of the more severe HS models. However, where monitored (the Mod. Controlled and Severe Uncontrolled bleeding HS models) there was a suggestion of elevated renal 3-nitrotyrosine in the HBOC-201 cohorts,
Liver: HBOC-201 promoted hepatobiliary changes, and higher AST, and LDH levels vs. their baseline in all three models. My impression is that increases are higher than in contemporary Hex and NON cohorts. The renal 3-nitrotyrosine levels were not elevated.
Jejunum: Excess macroscopic mucosal or serosal edema was seen in the MCHS model, but *not* confirmed in either of the more severe models. The 3-nitrotyrosine levels were not elevated.

Accordingly, the more conservative, but certainly not the only, interpretation is that HBOC-201 - associated tissue injury is evidently model-dependent except for excess hepato-biliary/LFT pathology in all models. Since background (NON) histopathology did not seem to vary across

the three models, sponsor collapsed data for each organ. Analyzed as such, the overall rates of hepatic sinusoidal ectasia and biliary changes were significantly higher with HBOC-201. Therefor, hepatobiliary changes were the most consistently effected by HBOC-201 in these studies.

Plasma Cytokines and leukocyte adhesion markers: as opposed to HEX, HBOC-201 actually averted a significant rise in PMN β2 and lymphocyte α4 integrin expression in the MCHS model, i.e., it was "immunoprotective"; however in SCHS the leukocyte adhesion markers increased during HBOC-201 resuscitation. Clear differences between treatment groups in IL-2, -6, and –10 expression, or in blood PMN and lymphocyte apoptosis, during resuscitation were not evident to me.

Tissue and plasma 3-nitrotyrosine: HBOC-201's ability to promote tissue oxygenation projects the potential for oxidative stress and increased risk of organ re-perfusion injury. At no injury sites noted above was there any significant increase in 3-nitrotyrosine staining. This includes the severe uncontrolled bleeding HS model, where HBOC-201 resuscitation was associated with borderline signif.(p=0.06) excess right ventricular (and possibly septal), necrosis and fibroplasia; the statist. signif. excess of pulmonary alveolar edema and inflammation/fibrin; and the hepatic and renal findings, . This suggests that the excess pathology was not reperfusion-related. *Nevertheless, plasma nitrotyrosine levels in the HBOC-201 resuscitated uncontrolled- bleeding HS model were unequivocally and persuasively elevated..*

In summary, the adverse effects relative to Hex comprise: transient increases in systemic and pulmonary blood pressure and decreased cardiac output ; reversible oliguria or anuria; and model-dependent reno-papillary., hepatobiliary,+ LFTs, and pulmonary lesions; and, according to authors, mild coagulopathy.

Clinical coagulation parameters: Veterinarily significant HS-or treatment -related coagulopathy (PTT, PT, fibrinogen) was not apparent to me.

Question 2. Is study well designed?

Yes, with caveats below. It was relatively under-powered considering the number of parameters whose mean values varied across cohorts by up to 50% (e.g., urine output between HBOC-201 and HEX cohorts in both severe HS models) but were not statistically significant. Inclusion of both controlled and uncontrolled hemorrhage models varying in mortality absent timely resuscitation was appropriate. It is re-assuring that at least the cardiac histopathology was asserted to be corroborated by a "blinded" 2nd veterinary pathologist.

Following are noted:

No provision was made for recognition/treatment of any evidence of hypoperfusion even if BP/HR stopping criteria were met.

Central venous pressure was not monitored. There is no control for whether the euthanasia solution causes pulmonary and/or liver congestion.

HBOC-201 continued to be given when - per RESUS - it would have been stopped and other standard IV given instead (see next question) .

Question 3. Dosing schedules/rates of administration simulate RESUS?

Yes - except for a maximum use scenario in RESUS where up to 2500 ml is permissible, and given, say, in 20 -30 minutes.

Swine received 10 ml/Kg of HBOC-201 in 10 minutes , which corresponds to that for a RESUS subject weighing 50 Kgs and receiving the anticipated 500ml (2 units) in 10 minutes, and slightly exceeds the 7 ml/Kg initial use in a 70Kg patient receiving 500 ml. It is expected that the typical 70 Kg RESUS subject would receive 2 units (500 ml) of HBOC-201 in the 10 minutes prior to hospital arrival i.e., 7 ml/Kg. This is approx. the same rate as given to the swine in this study who received 15 ml/Kg of HBOC-201 over 30 minutes.

However, dosing does not simulate a maximum RESUS use scenario. The maximum volume of HBOC-201 infused in severe HS swine models was 30 ml/kg (half of estimated blood volume) spread over 4 hours (10 ml/Kg initially as noted above ; 5 ml/Kg additionally at 30, 60, 120, and 180 min). This is less than the maximum total infusion 36 ml/Kg provided to a refractory 60 Kg Kg by the maximum allowed (2500 ml) of HBOC-201 given, say, over 20 minutes. Accordingly, only the first exposure to HBOC-201 is simulated in the HS swine studies. Clinical Cmax after subsequent infusions in a maximum use scenario is expected to appreciably exceed that in the swine if clearance is comparable across HS swine and patients.

[Note: Because of different re-infusion criteria , pigs in the severe HS models received 30 ml/Kg of HBOC-210 - approx 3X as much HBOC- 201 as the typical RESUS patient is expected to receive. Per RESUS directions for fluid re-infusion scenarios, there is to be *no* further CTM (HBOC-201 or LR) to be infused if blood pressure is fully restored (SPB> 100 mmHg) even though signs of HS persisted such as tachycardia; rather standard IV fluids would then be indicated. However in the swine model, HBOC-201 rather than other standard IV fluid , continued to be infused after blood pressure was restored to target since the animal protocol stated that HR > baseline was criteria for continuing either HBOC-201 or LR CTM . Per RESUS, no more HBOC-201 would have been administered after 1.5 hours, at the latest, in either of the severe swine HS models since BP was restored to (or even exceeded) target within that interval; yet pigs - primarily in the 2 severe models - continued to receive HBOC-201 up to the last permissible time point (180 minutes) because of persistently elevated HR]

Question 4. type of pre-hospital monitoring simulates RESUS?

Neither EKG nor signs of persistent hypoperfusion beyond hypotension and tachycardia (e.g., tachypnea and, obviously, mental status,) were monitored to guide continued fluid resuscitation in the HS swine if/when BB and HR stopping criteria were met. Conversely, many useful parameters were monitored in the swine, including cardiac output and pulmonary artery pressure, serum lactate, base excess, urine output, and transcutaneous tissue oxygenation ($tcpO_2$) which although not to be monitored in the pre-hospital phase of RESUS inform the inadequacy of relying exclusively on BP, HR, and $tcpO_2$ as markers of extent of recovery of systemic or local perfusion,

Question 5. Favorably prognostic endpoints/biomarkers identified to guide RESUS?

Serum lactate appears to be one of the better prognosticators, most evident in its trajectory in the severe controlled hemorrhage HS model. It is comparably elevated to three times baseline at 30 minutes in all three cohorts. By 60 minutes it had stabilized in the HEX and HBOC-201 cohorts, but had further increased in the NON cohort. Within the next hour, the majority of fatalities encountered in this model occurred in that NON cohort, and by 180 minutes Lactate had recovered to near baseline levels except in the HEX cohort which was to experience a few more deaths in the next half-hour.

Only the status of survivors at each time point is portrayed. Since terminal swine were not retrospectively segregated from survivors (regardless of whether early fluid resuscitation was applied to either) and their hemodynamic , clinical chemistry (including blood gases, lactate , and base excess) trajectories compared, predictors of early demise – and any salutary effects of fluid therapy thereon, is difficult to identify. This could be performed by combining data from all three studies (since the same parameters were monitored) where the trajectories for the 45 swine who survived to 72 hours could be compared to that of the 27 which did not.

Throughout pre-hospital resuscitation of both of the severe HS models, extent and rate of recovery BP HR and $tcpO_2$ very clearly favored HBOC-201, yet urine output was only ½ that of the HEX cohort., Based on that observation, such parameters, in isolation, do not appear to be reliable markers for extent of recovery of global and local tissue perfusion.

Regarding reliance, in these models, on blood pressure and HR responses *per se* to guide re-infusion of HBOC-201, I note the following: In each of the three model, when blood pressure and heart rate had stabilized and approached targeted values (approx. 30-50 minutes, depending on model, when MBP was at least 60 mmHg, and HR ≈ baseline) lactate, mixed venous oxygen saturation, tissue oxygenation, base deficit and cardiac index and systemic vascular resistance remained abnormal. Accordingly, it is prudent to rely on other signs of hypoperfusion - as now specified in RESUS - to guide resuscitation with a vasoactive product.

Question 6. All animals accounted for?

Not clear. All animals were anesthetized to apnea, and mechanically ventilated, presumably without further adjustment of anesthesia or ventilation to the end of the experiment. However, the statement is made that "Data from animals unable to regain spontaneous breathing prior to resuscitative fluid administration were excluded from analysis". Since the word analysis rather than randomization was used, I do not know if the "data" was excluded retrospectively or prospectively. I strongly suspect that the animals struggle at the end of exsanguination and anesthesia/ventilation is adjusted to overcome that, and that is why there is a range of specifications for isoflurane of 1%-2.5%, for ventilation of 12-15 breaths/minute, and for tidal volume of 5-10 ml/min.

Question 7. Any aspects of the model that confound interpretation and/or relevance to prehospital resuscitation.?

See general comments especially the fact that CV disease-free swine were used; and that anesthesia and mechanical ventilation might affect response (certainly the latter could mask any signs of persistent hypoperfusion e.g., tachypnea . The depth of anesthesia and extent of breathing support was apparently adjusted at the end of exsanguination to fully compensate for dyspnea. Will RESUS subjects be intubated and on mechanical respiration?

There is a protocol violation at the 60 minute time point in the moderate controlled HS model since the mean volume of HEX administered exceeded 5 ml/Kg, indicating that the permitted amount of 5ml/Kg was exceeded in at least one animal. Visiting the raw data revealed that swine # 527 and #785 received more (34 and 36 ml/Kg respectively) than the 30 ml/Kg maximum allowed over the 4-hour pre-hospital interval. It also revealed no infusion volume protocol violations in the severe controlled hemorrhage HS models. I could not verify that the maximum infusion volume of 30 ml/Kg through 180 minutes was not violated in the

uncontrolled hemorrhage/liver injury model severe since infusion volumes at specific time points was not reported; rather only ml/Kg/survival hour.

It is very likely, assuming similar pharmacokinetic behavior in pigs and humans, that HBOC-201 was not tested at up to exposures likely to be achieved in subjects requiring the anticipated 2-3 units of HBOC-201, and even less likely at up to exposures expected in those RESUS subjects requiring up to the maximum of 10 units. However, dosages administered in the Manning et al study below (see p. 17) support maximum dosages envisioned in RESUS.

Question 8. Will absence of any MI, stroke, CHF, hypertension, hypoxemia, or oliguria in swine HS be re-assuring?

No. There absence in these three studies is only re-assuring if re-visiting the clinical adverse CV events compellingly identifies risk factors and those risk factors are absent in these swine model. If CV disease is a clear risk factor, it may be that RESUS should only recruit relatively young trauma patients. Regarding oliguria, urine output was only ½ that in the HEX cohort in both the severe controlled bleeding and uncontrolled bleeding HS models. Regarding risk for MI, stroke, or CHF, it is noted that the swine used in these studies are expected to be virtually devoid of any pre-existing atherosclerosis, or depressed pulmonary, hepatic, cardiac, or renal functional reserve capacity.

Question 9. Pre-clinical results vs. standard therapy justify waiver of informed consent?

Yes, but with two caveats: HBOC-201 was not evaluated at up to maximum dosages and rates of infusion envisioned in RESUS. However in the Katz *et al* study described next, pigs more aggressively resuscitated with HBOC-201 did spontaneously recover from anesthesia and, further, evidently showed no signs of continuing veterinarily important hypoperfusion, or sequelae of such. A 2nd caveat to the waiver is that behavior of HBOC-201 was most likely in the absence of any veterinarily important pre-existing CV disease. It seems that the available surgical patient safety data - presumably obtained at dosages at least corresponding to the minimal envisioned for RESUS (1-2 units…?), and in patients some of whom are likely to have had pre-existing CV disease should inform the decision to approve or further modify RESUS protocol.

An attempt should be made to re-visit case report forms for surgical patients who experienced cardiovascular AEs after use of HBOC-201. If there is persuasive evidence that important pre-existing CV disease is a contra-indication to aggressive emergency use of HBOC-201, such usage should, perhaps, be confined to young trauma patients.

B. Katz ,LM:: HBOC-201 improves survival in a swine model of hemorrhagic shock and liver injury. Resuscitation 54(2002) 77-87); ancillary final study report also submitted containing line listing of data .

As in the previous study, this model simulates resuscitation of HS with concurrent liver injury/bleeding, but simulates a 1 rather than 4 hour delay to definitive resuscitation. Swine were anesthetized with 1-3% isoflurane and balanced air/oxygen,(50% O_2 ?) instrumented and catheterized for periodic or continuous monitoring of a variety of CV parameters including cardiac index, pulmonary artery/ capillary wedge pressure (i.e., LV end diastolic filling pressure); arterial blood gases; clinical chemistries; tongue or ear oximetry, and EKG. Swine (

28-38 Kg were subjected to a standardized liver crush injury contemporary with withdrawal, over 15 min., of 50 ml/kg. of arterial blood. At such time, there were no significant differences in pre-resuscitation MAP (which was depressed down to approx 25-30 mmHg),cardiac index, or HR. Animals were then resuscitated for the next 15 minutes with 6 ml/Kg /min of either HBOC-201 (N=8) or 6% hetastarch (N=8) or no fluid(N=6). Volume of fluid resuscitation was then reduced 3 ml/Kg/min for the next half-hour. Controlled blood loss of 3 ml/kg/min was maintained throughout the 45 min resuscitation period. *[Importantly, FIO_2 was promoted to 100% during resuscitation, and FIO_2 ranged from 21%-100% during recovery].* Minutes 60-90 simulated ER care i.e., autologous blood transfusion (the 50 ml/Kg of shed art. blood was returned over 20 min.); surgical repair of the lacerated liver; ICU care; extubation at approx. 24 hours; and recovery from anesthesia.

Accordingly this is a very aggressive model of lethal acute hemorrhage /emergency resuscitation protocol since approx. 70% of estimated blood volume has been lost at the time of attempted resuscitation, and the 90 ml/Kg infused during the first 15 min of resuscitation would more than compensate for the 45 ml/Kg of controlled depletion from the vascular compartment. In a sense it may even simulate over-resuscitation – although actual blood volume and/or central venous pressure were not monitored to read such.

Question1.a.) model adequately simulates RESUS pre-hospital trauma setting?

Yes, especially those with refractory hemostasis , but with the pig-model caveats noted in my general comments section . It simulates a model of massive rapid blood loss (4 swine died in cardiac arrest during bleed-out prior to randomization) followed by very aggressive resuscitation in the face of appreciable ongoing hemorrhage of 3 ml/Kg/min/45 min..

Question 1.b) results support use of HBOC-201 in RESUS?

Infusion of HBOC-201 at a volume and rate similar to 3% hetastarch prevented acute hypovolemic cardiac arrest in this rapidly lethal model; allowed 96 hr survival; and would support even the maximum envisioned dosage of RESUS *prima facie* . That is, 135 ml/Kg. was administered in the first 30 minutes of resuscitation. This is approx. 4 X more than the 36 ml/Kg provided to the refractory 70 Kg RESUS subject receiving up to the maximum volume of HBOC-201 allowed (2500 ml) given over, say, 30 minutes.

However, such enthusiasm must be tempered by the following: A. Context of the resuscitation: - The forced fixed infusion regimen - independent of BP, HR, and any signs of volume overload ; and under anesthesia, mechanical ventilation, and 50% oxygen – appreciably differs from RESUS. In view of the forced infusion regimen, monitoring of central venous pressure for over-resuscitation would have been desirable (see monitoring below) given the hemodynamic evidence of a hyperdynamic even over-resuscitated state.

B. Mixed salutary and undesirable hemodynamic changes: All control swine had expired by minute 22 whereas by such time MBP had recovered to 70 and 90 mmHg for the HEX and HBOC-201 cohorts, respectively. By min 30 , survival was, however, reduced to 50% in the HEX group, along with a downturn in cardiac index, (1.6 L/min) and marked arterial base excess deficit. This compares to 100% survival in the HBOC-201 cohort in association with variably restored cardiac index and better base deficit. *However at that point, the HBOC-201 cohort is experiencing both systemic mean arterial and pulmonary art hypertension (mean values of 125 and 36 mmHg, respectively now well above baseline pre-hemorrhage values and*

dangerously high in some pigs based on the size of the std. deviation. At that time point , cardiac index in the HBOC-201 is over 6 L/min., having recovered to beyond pre-hemorrhage baseline as well, and very high in some pigs. Arterial average base excess, which had been 5 mmol/L at baseline, and having dropping down to approx 0 just prior to resuscitation, is now -12 for HEX but compromised to only - 6 for HBOC-201. At the end of resuscitation, the re-infusion of the originally shed arterial blood markedly elevated PCWedge Press. (a marker for LV end-diastolic filling pressure) to levels, in some pigs, usually seen in CHF and/or assoc with alveolar or interstitial pulmonary edema. This response is consistent with a volume overload scenario. The autopsy of the one HBOC-201-treated pig that died at 24 hours revealed edematous lungs and necrotic intestines.

Urine output in the first 24 hours of recovery from HBOC-201 averaged approx 2 ml/Kg/hr. I do not know how to determine if that is "normal" absent a comparably hydrated control to compare with (there were no surviving HEX pigs for comparison). However, BUN and creatinine were said to be normal.

Question 2. Is study well designed?

The aggressive forced fluid re-infusion design in anesthetized animals does not match the criteria for re-infusion of either CMT or other standard infusions per RESUS where infusion will be driven by BP, HR, and loss of signs of any occult hypoperfusion. Rather this is a safety study which supports the most aggressive, actually excessive, use of HBOC-201 in the most critical RESUS subject. The absence of monitoring for central venous pressure in such an aggressive bleeding/ resuscitation study design is glaring.

The amount and rates of HBOC-201 infusion and concurrent simulated ongoing hemorrhage of 3 ml/Kg/min were not arbitrary, but rather deliberate and rational. Authors disclose that in pilot studies, an average of 4 ml/min/kg of allogenic blood was required to maintain a 60 mmHg mean arterial BP, and permit survival to 96 hrs. Accordingly, a similar volume of HBOC-201 and 3% hetastarch were chosen for comparison, while the rate of bleeding was kept constant, thus assuring that, at each time point during attempted rescue by HBOC or hetastarch, such was from comparable levels of hematocrit *and* blood volume. This constant infusion and bleeding rate allowed for an accurate comparison of the degree of hemodilution between groups. As entirely expected, the hematocrit 30 min. into the protocol was virtually identical (4 %) for HBOC and hetastarch, and marked the onset of bradycardic cardiac arrest in the starch group. By this thoughtful strategy, rescue was attempted from an identical degree of hemodilution and critically lowered (at least for the pig species) HCT, and unequivocally favored HBOC over 3% hetastarch. The 3 ml/Kg/min rate of simulated ongoing hemorrhage during resuscitation was based on their observation that a similar volume accumulated in the abdomen during fluid resuscitation from uncontrolled hemorrhage, and was kept constant to permit comparison of resuscitation from comparably depleted vascular compartments.

The 96 hour post-resuscitation observation period covers the time-frame for release of inflammatory cytokines associated with post-traumatic multi-organ failure reported clinically .

Necropsy at 96 hours included examinations of external surfaces of peritoneal and thoracic cavities, and surfaces (external only?) of major organs.

Unfortunately, microscopy of tissue was not performed following necropsy.

Question 3. Dosing schedules/rates of administration simulate RESUS?

See responses to question 1 and 2 above. This represents an egregious overdose scenario. The 90 ml/Kg infused during the first 15 min of resuscitation is 3X the dosage afforded by the maximum RESUS volume of 2500 ml given in 15 min by large bore IV push to an 80 Kg victim. In the swine it more than compensated for the 45 ml/Kg of controlled depletion from the vascular compartment in that time frame. The total of 180 ml/ Kg given in ½ hour is approx. 6X the max RESUS dosage of 2500 ml received by an 80Kg patient-subject.

Question 4. Type of pre-hospital monitoring simulates RESUS?

No, in that pigs were anesthetized and on 100% oxygen throughout resuscitation, intubated and mechanically respired under 100% oxygen. Neither BP, HR, nor any of a variety of signs and symptoms of persistent hypotension, if any, (e.g., tachypnea, skin condition, and, obviously, mentation) were used to guide fluid re-infusion

Evidently the high plasma levels of HBOC-201 interfered with lactate and liver transaminase determination, so they were not reported. [It is noted that no such interference with the lactate quantification was disclosed in the Protocol KOO4-02 reviewed above where lactate levels appeared to be a useful prognosticator). One wonders why sponsor even bothered to try to monitor liver transaminases considering the hepatic crush/laceration injury that was a prominent feature of this model.

Question 5. Favorably prognostic endpoints/biomarkers identified to guide RESUS?

Retrospective segregating into survivors and decedents, and comparing their trajectories would facilitate identifying prognostic endpoints/biomarkers. For example, as plotted, the prognostic utility of mean arterial base deficit is not all that evident , and is comparable for HBOC and HEX although their survival would markedly differ. Lactate was not monitored; thus the utility apparent in the previous study could not be confirmed.

Question 6. All animals accounted for?

Yes.

Question 7. Any aspects of the model that confound interpretation and/or relevance to pre-hospital resuscitation.?

See my general comments regarding porcine HS models. It is also noted that resuscitation was attempted under 100% oxygen. I do not know if that is the scenario envisioned for RESUS.

It is difficult to determine the relative contributions, if any, of HBOC-201, catheter obstruction (causing urine to accumulate in the peritoneal cavity, and parasitic infestation to the pulmonary congestion and presumed ARDS in the one HBOC-treated swine which expired at 24 hours.

The controlled bleeding prevented determining whether the obvious pressor effect of HBOC-201 would, in RESUS, increase bleeding in scenarios where hemostasis is not fully controlled.

It is noted that this study was not performed per Good laboratories practice regulations (21 CFR Part 58). However, independent review of data revealed a 1.16% error rate individual data points, but it is asserted that this had no impact on statistically-based conclusions and interpretations..

Question 8 . Will absence of any MI, stroke, CHF, hypertension, hypoxemia, or oliguria in swine HS be re-assuring?

See prior answers to this question. These were not encountered, except for the oliguria. It is noted that one HBOC-201 treated pig did die with pulmonary congestion and signs of ARDS at 24-hours, but co-existing factors render HBOC-mediation moot (see question 7 response).

Question 9. Pre-clinical results vs. standard therapy justify waiver of informed consent?

Yes.

C. Manning, JE et al Bovine hemoglobin-based oxygen carrier (HBOC-201) for resuscitation of uncontrolled , exsanguinating liver injury in swine.

Shock 13(no 2) : 152-159,2000]

This study in anesthetized swine simulated a scenario where, after 9 minutes of uncontrolled bleeding from multiple liver lacerations and loss of 50% of estimated blood volume, the subject is initially infused with 10 ml/Kg /min of either HBOC-201 (N=7) or LR (N=10) to restore MBP to 60 mmHg, and then adjusted to maintain such for two hours with no attempt to control hemorrhage. Minute ventilation and FiO_2 were adjusted to maintain end-tidal CO2 at 40 and pO2 at 90-110 mmHg, respectively, and then maintained at these baseline settings throughout resuscitation. Mean arterial BP, cardiac output, total blood loss, arterial blood gases , and lactate were measured periodically throughout initiation, and the 2-hour resuscitation/ observation, period.

Question1.a.) model adequately simulates RESUS pre-hospital trauma setting?

Yes, it simulates attempted resuscitation from a scenario of severe liver injury and exsanguination to the extent that MBP is at 16 mmHg, Hct is about 12, and cardiac output <0.4 l/min.; and a duration of insult of about 10 minutes. Caveats to utility of swine model are as previously noted; swine also received 0.1 mg/Kg pancuronium, and were ventilated with 100% oxygen. The 2 –hour delay without attempting RBC infusion considerably exceeds the anticipated delay to ER/Hospital in RESUS of ≤ 15 minutes (albeit the latter is unrealistically prompt for some scenarios)

It is noted that these healthy young swine showed an ability to compensate at levels of HCT (2-5) which would be minimally if at all tolerated in humans.

Injury also involved a single organ, vs. a spectrum expected in RESUS.

Question 1.b) results support use of HBOC-201 in RESUS?

Yes.

Safety: Pigs received , and tolerated, a total volume of HBOC-201 (7.2 Liters) equivalent to approx. 4X estimated blood volume (1.6 L for a 23 Kg pig, assuming 70 ml/kg) in the 2 hour course of their resuscitation, and at a normalized rate comparable to the maximum envisioned for RESUS.(approx 2ml/Kg/min, based on a 60 Kg patient receiving 2500 ml HBOC-201 in 20 minutes) and , furthermore for , appreciably longer duration – 120 minutes vs. , say, 15-20 minutes in RESUS. It is noted that the sole criterion for rate and duration of infusion was to maintain MBP at 60 mmHg for two hours with a maximum infusion rate of 10 ml/Kg/min

Efficacy: Study documents ability of HBOC-201 to successfully resuscitate uniformly lethal severe liver injury/hypovolemic shock at a relatively low blood pressure requirement Both LR and HBOC-201 cohorts were initially successfully resuscitated to an MBP of 60mmH g within several minutes (HBOV-201: 1.6 ±0.5 min. vs. 7.4±5.1 min; $p<0.01$), Mean survival times were 120± 0 (i.e., all survived) and 27±10 min, for HBOC-201 and LR, resp., $p<0.05$). Lactate at 30-minute was significantly more elevated in the LR than in the HBOC-201 cohort (19±2 vs. 11±1 nmol/l) , and remained at those levels throughout resuscitation. Despite significant metabolic acidosis, the HBOC-201 treated swine maintained hemodynamic stability, presumably reflecting an adequate degree of tissue oxygenation although the latter was not monitored.

As expected in RESUS, safety and efficacy were assessed absent any attempt to correct metabolic acidosis. It should be noted, that HBOC-201did not correct serum lactate , even when administered at 10 ml/Kg/min for 120 min vs. the maximum of 0.18 ml/Kg/min for 20 minutes (2500 ml given to a 70 Kg patient in, say, 20 minutes) in a refractory RESUS subject.

Question 2. Is study well designed?

Yes. given the limited goals of the study- presumably primarily to determine whether pigs could survive the equivalent of a complete exchange transfusion of their total circulating blood volume, and at a blood pressure expected to limit further loss of native hemoglobin and coagulation factors.

The rate of initial bleeding was adjusted by graded compression of the bleeding liver surfaces to maintain the decline in aortic pressure along a prospectively defined pressure-time curve. This, importantly, standardizes the hemodynamics of the insult over the initial 9-minute trajectory of the injury phase.

On the other hand, long-term survival was not assessed, and direct measurements of tissue oxygenation were not made.

LR was appropriately chosen as the control because isotonic electrolyte solutions are the current standard fluid therapy in hemorrhagic shock. A colloid solution with oncotic properties similar to HBOC-201 could have addressed.

Although resuscitation was intentionally attempted at 60 mmHg, and success suggests adequate oxygen delivery at less than that required for native blood, resuscitation at a more normal MBP would have addressed whether low pressure resuscitation was the reason for the persistent metabolic acidosis.

Question 3. Dosing schedules/rates of administration simulate RESUS?

The initial rate of infusion of 10 ml/Kg/min. is very aggressive ,and about 12X the initial rate in the typical RESUS subject.

The mean infusion rate of 2.6 ml/Kg/min/ 120 minutes tolerated in this study represents a very considerable safety margin over the 0.8 ml/Kg/min/ 20 min expected for the typical RESUS subject - although pigs were not allowed to recover and monitored for any adverse sequelae.

This mean infusion rate is actually comparable to that obtaining at the likely maximum dose/fastest infusion RESUS scenario – i.e., if the maximum permitted volume of 2500 ml is administered over, say, 20 minutes to a refractory RESUS patient it would be providing approx 2

ml/Kg /minute for 20 minutes . Accordingly, the swine in this study received a maximum rate of infusion, and for approx. 6 times the duration I envision for the most refractory RESUS subjects.

Question 4. type of pre-hospital monitoring simulates RESUS?

Yes, with caveats noted previously. Cutaneous oximetry was not performed

Question 5. Favorably prognostic endpoints/biomarkers identified to guide RESUS?

Lactate and acid-base balance parameters. The pH, and base balance tend to be more prognostic than bicarbonate. Mean arterial pO2 at an FiO2 of 100% were similar, and not prognostic, throughout the resuscitation and observation phases. Sponsor asserts that there was no significant difference in pCO2 between the two cohorts during the injury and *early* resuscitation phases.. but that LR treated swine typically showed marked declines in pCO2 that heralded hemodynamic collapse

Question 6. All animals accounted for?

Yes. In comparing the total volumes of LR and HBOC-201 infused, authors excluded 1 pig from each group that showed a substantially lower rate of bleeding , and did not reach a hct of $\leq 1\%$., and further excluded 1 HBOC-201 pig with intractable abdominal wound hemorrhage suggestive of pre-op or induced coagulopathy.

Question 7. Any aspects of the model that confound interpretation and/or relevance to prehospital resuscitation.?

Species difference: swine appear capable of tolerating hematocrits of 5-10 %, considerably below the threshold for collapse in humans as I understand. The use of pancuronium and anesthesia do. The evacuation of the blood from the abdomen during the injury phase may have affected early hemostasis according to the authors.. Only one organ, the liver, was injured (although there was presumably a large abdominal wound as well).

The initial infusion rate of 10 ml/Kg/min is aggressive but can probably be achieved clinically with multiple large-bore IV catheters and applying pressure to the bags.

Question 8 . Will absence of any MI, stroke, CHF, hypertension, hypoxemia, or oliguria in swine HS be re-assuring?

No, for reasons previously given.

Question 9. Pre-clinical results vs. standard therapy justify waiver of informed consent? See my responses to this question for each of the other studies overviewed. HBOC-201 afforded marked survival benefit at, moreover, appreciable multiples of the proposed clinical exposure, and at a blood pressure expected to minimize further loss of native hemoglobin.

D. York et al Low volume resuscitation with a polymerized bovine hemoglobin-based oxygen carrying solution (HBOC-201) provides adequate tissue oxygenation..... ...

J Trauma: 2003, 55(5): 873-885.

This study in anesthetized mechanically-ventilated (40% oxygen;, 1.75% isoflurane) swine simulated a scenario of rapid non-lethal hemorrhage to a mean arterial blood pressure (MAP) of 30 mmHg, and maintained hypotensive for 45 minutes. At that point, pigs were randomized to 4 cohorts where they were resuscitated to maintain a MAP of 60 mmHg for 4 hours with either autologous shed blood (Shed Blood 60 cohort) or HBOC-201 (HBOC-201 60 cohort) ; or resuscitated to baseline MAP with either autologous shed blood (Shed Blood) or lactated Ringers (maximum, 40 ml/Kg) followed by shed blood (LR plus Shed Blood). At the end of hour 4, the two cohorts that had been maintained at 60 mmHg MAP– i.e., Shed Blood 60 and HBOC-201 60 groups- were restored to baseline MAP using remaining shed blood or LR , respectively. All groups were then monitored for an additional hour. Hemodynamic variables, urine output, blood gas analyses, lactate levels, and jejunal oximetry were throughout including the final hour 4 to hour 5 resuscitation period of normotension in all groups. Animals were allowed to survive for three days with no further resuscitation, and then underwent sedation, tissue harvesting, and necropsy.

Question1.a.) model adequately simulates RESUS pre-hospital trauma setting?

See general comments relative to perceived utility use of this model.

It simulates a scenario of exsanguination, from a relatively confined soft tissue injury (e.g., simulating a stab wound), of approx. 40-45 % of estimated blood volume such that , for 45 minutes prior to resuscitation, MBP is at 30 mmHg; cardiac output and tissue oxygenation are only ca. 40% of baseline; and HR and serum lactate levels are markedly elevated. This is a model of non-lethal HS since survival was 100% even after 45 minutes of uncorrected hypotension at the level of 35 mmHg MBP vs. the 35% mortality expected in control RESUS cohorts despite early and aggressive attempts at fluid resuscitation.

Use of HBOC-201 in this study does not anticipate its use in RHESUS where volume and rate will be such as to restore SBP to ≥90mmHg mmHg within 15-20 minutes, or less and, again, resuscitation to a target MAP of only 60 mmHg, and for 4 hours. The 45 minute delay to field resuscitation with HBOPC-201,and the additional 4 hours of treatment with only HBOC-201 and only enough to maintain MAP at 60 mmHg considerably exceeds the expected delay to ER/Hospital in RESUS of ≤ 15 minutes. . On the other hand, this scenario of more delay to field resuscitation i.e., 45 minutes of hypoperfusion vs. 15 min in RESUS provides the scenario for greater levels of endogenous oxidants (H_2O_2; $O_2^{\cdot}$; $ONOO^-$) to be released upon reperfusion with HBOC-201 and for more stringent testing of whether, in such a context, consequences of extracellular Hgb-mediated pro-oxidative cascades may be more severe.

Caveats itemized in my general comments apply re: context of the resuscitation. Unlike the proposed RESUS scenario, the swine were anesthetized,; attempted resuscitation was to 60 mmHg mean BP as verified by arterial cannula (rather than 90 mmHg SBP by probably less reliable cuff in RESUS); and pigs were monitored to only 3 days rather than 1 month as in RESUS. Presumably, these swine do not have pre-existing CV disease. Moreover, the resuscitation protocol did not anticipate. - and provide for adjustment of re-infusion criteria for -

occult shock. It is not known whether any pigs restored to stable target BP and HR might have evidenced signs of persistent hypoperfusion if allowed to recover. On the other hand, a more demanding delay to definitive medical care of 4-hours, rather than the <10-15 minutes expected arrival time to hospital in RES, was imposed.

Question 1.b) results support use of HBOC-201 in RESUS?

Both cardiac and urinary output in pigs resuscitated to 60 mmHg for 4 hours were less than that in pigs resuscitated to the same BP with shed blood. The volume of shed blood proved to be virtually identical to the volume required of HBOC-201. The HBOC-treated swine were also virtually anuric compared to all other treatments. However, both outputs were restored to nearly normal values upon restoration to normal blood pressure at 4 hours following whole blood (Shed Blood cohort) or Lactated ringers (HBOC-201 cohort), indicating a hemodynamic basis for the depressed function. Serum creatinine had also returned to normal by recovery day 1.

Serum hepatic transaminase levels were appreciably elevated by day 1 in all groups, with the highest and most persistent levels observed in the HBOC-201 group. Centrilobular and mid-zonal hepatic necrosis, was observed in 4 of the 6 swine resuscitated with HBOC-201, an unusual and concerning finding indicative of ischemia. Because of the relatively short half-life of the HBOC-201, an expected decrease in hemoglobin levels was seen over time in animals resuscitated with agent. It is not known whether re-dosing with HBOC-201 on days 2 and 3 to maintain higher Hgb levels would have prevented the histologic and ALT evidence of ischemic hepatic injury. Jejunal oximetry revealed hypoxia at the end of the exsanguination period, and restoration to baseline values by HBOC-201 even when the latter was used in amounts intended to restore mean arterial blood pressure only to 60 mmHg.

Accordingly, in this model of non-lethal HS, resuscitation with HBOC-201 to a mean blood pressure of 60 mmHg and maintained for four hours, was associated with reversible oliguria and mild to moderate degeneration and/or necrosis of centrilobular to mid-zonal hepatocytes. The hepatic injury is histologically consistent with ischemic injury according to sponsor,

Question 2. Is study well designed?

Yes, for the purpose for which it was intended – namely for low volume resuscitation to MBP of 60 mmHg as the only target vs. RESUS target of 90-100 mmHg MBP , as well as satisfying HR requirements and further treatment with HBOC-201 or conventional fluids depending on whether signs of persistent hypoperfusion are present despite stable BP and HR.

Question 3. Dosing schedules/rates of administration simulate RESUS?

Rate of administration of HBOC-201 is not specified, only data that a mean value of 2400 ml of HBOC-201 was administered over 4 hours to pigs weighing 55-65 Kg. This more than compensates for the 1700 ml mean volume of blood lost during exsanguination. This would correspond, on average, to a total of 40 ml/Kg having been administered to a 60 Kg pig vs. the 10 ml/Kg anticipated for the average RESUS subject and the 36 ml/Kg maximally allowed for the refractory RESUS subject. The 45-minute delay to resuscitation and 4 hour delay to definitive resuscitation do not anticipate RESUS time courses with its minimal delay to both field and subsequent definitive resuscitation.

Question 4. type of pre-hospital monitoring simulates RESUS?

Neither heart rate nor any other signs of hypovolemia were factors e.g., anuria was ignored, and sponsor is silent on any other signs or symptoms specified in RESUS as indicative of persistent hypoperfusion. Rather maintenance of MBP at 60 mmHg for 4 hours was the only determinant of amount of HBOC-201 infused. Jejunal instead of digital oximetry was used.

Question 5. Favorably prognostic endpoints/biomarkers identified to guide RESUS?

No, but the anuria and the liver injury were associated with elevated serum creatinine and ALT levels, respectively.

Paradoxically, in the HBOC-201 group, serum lactate levels rose significantly during the last hour of the resuscitation period when lactated ringers was used to fully restore cardiac out put, and to restore BP from the 60 mmHg MBP value it had been kept at to the normal baseline value.

Jejunal oximetry is of questionable utility in this model, and may even be misleading. It revealed depressed oxygen levels at the end of exsanguination; indicated restored tissue oxygenation in all cohorts 15 minutes after instituting fluid resuscitation; and thereafter described progressively decreasing intestinal oxygen levels in the LR+ blood cohort, and a progressively increasing oxygenation level in the HBOC-201 cohort ($p<0.05$ for LR + Blood vs. HBOC-201.) However, mild to moderate interstitial edema was detected in the lamina propria mucosa of the small intestines of many animals and was independent of treatment. Furthermore, it was the HBOC-201 cohort (with its highest jejunal oxygen readings) and not the LR+ blood cohort (with its lowest readings) which was to reveal, at three day autopsy, the evidence of hepatic ischemic injury.

Question 6. All animals accounted for?

YES.

Question 7. Any aspects of the model that confound interpretation and/or relevance to prehospital resuscitation.?

All subjects showed signs of pulmonary parenchymal inflammation consistent with sub-clinical infection common in these animals and which precluded evaluation of any subtle treatment-associated pathology.

See also my prefacing General Comments which questions the utility of using not only healthy animals but healthy pigs which evidently have a relatively greater resilience to acute blood loss than humans.

Question 8 . Will absence of any MI, stroke, CHF, hypertension, hypoxemia, or oliguria. in swine HS be re-assuring?

No. In fact there was marked, but reversible, anuria as well as clinical chemistry and histologic evidence of excess hepatic injury vs. pigs resuscitated to a comparable MBP of 60 mmHg with a comparable volume of blood – with both the HBOC-201 and shed-blood resuscitated swine having been recovered from comparable degrees of exsanguination (hemorrhage volume of approx. 1750 ml for either cohort).

Question 9. Pre-clinical results vs. standard therapy justify waiver of informed consent?

No. There is no demonstrable benefit over shed blood or lactated ringers to offset the clinical chemistry and hisologic evidence of hepatic injury. It is not known whether such injury could have been avoided if Hgb was maintained at normal levels in the two day recovery as sponsor did not attempt such. Presumably, RESUS subjects would receive supplemental whole blood or packed RBCs if Hgb fell in the days following resuscitation as it would otherwise since HBOC-201 has a half-life of 19 hours.

E. Fitzpatrick et al Low volume resuscitation with HBOC-201 in a large animal survival model of controlled hemorrhage. J. Trauma. 2005;59;273-283.

This study was selected because it monitored survival and histology five days after resuscitation in a model where all extend and HBOC-201 - treated swine survived an 8-hour resuscitation period, but not to 5 days.

It monitored resuscitation of female pigs (55-65 Kg) exsanguinated to a MAP of 30 mHg and maintained at that level for 45 minutes prior to attempted recovery with either hextend (Hex) or HBOC-201. Over 8 hours, swine received up to 1,000 ml of either fluid aimed at sustaining an MAP of 60 mmHg. At the end of 8 hours, Hex cohort received 2 L of LR followed by shed blood; HBOC –treated swine received 4 L of LR only.(this difference in definitive resuscitation is problematic: see below). Hemodynamic status was monitored during shock and resuscitation. CBC, amylase, lactate, coagulation parameters, and renal and liver function tests were measured throughout the experiment. Animals were killed and necropsied on day 5 for gross and histologic abnormality.

Question1.a.) model adequately simulates RESUS pre-hospital trauma setting?

See responses for other trials above. Use of healthy juveniles, 8-hour anesthesia, a hydrating dose of 2 L of normal saline, and an 8 hour delay to definitive resuscitation all are not as envisioned in RESUS. [Also in comparing results, it is noted that only the Hex-treated animals received shed blood at the end of 8 hours].

Question 1.b) results support use of HBOC-201 in RESUS?

Survival results tend to: whereas all animals survived the initial 8 hours, the 5-day HBOC-201 survival was 88% (7/8) vs. 50% (4/8) for Hex (p. >0.05). However there was also important liver and renal histopathology at autopsy (see below).The one HBOC-201 lethality was believed due to air embolism at time of insertion of an indwelling catheter, and in the Hex group, the 2 dying during, and two after, resuscitation, may have possibly been due to over-resuscitation (see below). Accordingly, the absolute survival supports use of HBOC-201 in RESUS, rather than superiority over Hex since , as noted below, there were important differences in their resuscitative use.

Initial resuscitation volumes favored HBOC-201(i.e., were less). However, the relatively lower total resuscitation volume in the HBOC-201 cohort appears, rather, to reflect a forced over-resuscitation in the Hex cohort due to pre-determined protocol mandates instead of

physiologically-driven fluid "requirements" (see question 3. below.), so relative behavior of HBOC vs. Hex is moot.

Although HBOC-201 had appreciably higher systemic vascular resistance and lower cardiac output , there were no cohort differences in the status and recovery of base excess, pH, lactate, and urine output , mixed venous oxygen saturation, and oxygen extraction ratio during the 8-hour trajectory of resuscitation Accordingly, at least in this time frame, they are not prognostic as subsequent survival during subsequent definitive resuscitation and post-operative recovery periods tended to differ appreciably. Urine output was restored, especially in the HBOC-201 cohort, upon definitive resuscitation at 8 hours.

No clinical or laboratory evidence of organ dysfunction was asserted for the 5 day survivors (urine output was not monitored in the 5-day recovery period). Histopathology on the 5-day survivors (N=4 and 7 for Hex and HBOC-201, respectively) ostensibly revealed a similar incidence of hepatic centrilobular degeneration and necrosis (2/4, and 4/7 for Hex and HBOC) and renal infarctions (2/4 and 1/7, respectively). Regardless of whether the incidences of histopathology are or are not comparable for HBOC-201 vs. Hex(since the resuscitative use of the two fluids differed) there is clear evidence of a high incidence of important liver and kidney histopathology including necrosis and infarction in the HBOC-201 cohort.

Question 2. Is study well designed?

No. Resuscitation was to MAP of 60 mmHg but there were no specific protocol-specified directions on rates and durations of administrations of fluid to achieve such, and evidently the large definitive resuscitation fluid volumes at 8 hours were dictated by protocol rather than in response to physiological end-points.. Accordingly the following relative fluid volume requirements for resuscitation are unreliable until confirmed: HBOC-201 achieved the blood pressure goal (60 vs. 46 mmHg for Hex; $p<0.01$), and required less volume than hex in the 8hour interval in terms of both initial volumes(12.4 vs. 17.3 mL/Kg: for Hex; $p<0.010$) and total resuscitation volume (81 vs. 99 ml/Kg for hex, p,0.01). In fact, one wonders whether both cohorts, but especially the hex, were over-resuscitated since the average volume hemorrhaged in both cohorts was 45 ml/kg vs. total resuscitation volume of 81 ml/Kg of HBOC-201 99 ml/Kg of Hex, and that on top of the 2L hydrating dose of normal saline prior to exsanguination.

Also, absent a control for resuscitation to a more physiologic higher arterial blood pressure, it is indeterminate as to whether the appreciable renal and hepatic histopathology would have been avoided.

Question 3. Dosing schedules/rates of administration simulate RESUS?

Only total dosages, and not rates of administration, are reported; furthermore there is conflicting data on the total dosage administered. The initial resuscitation volume of 12 ml/Kg of HBOC-201 does approx. correspond to the 8 ml/Kg typical RESUS volume (500 ml given to a 60 Kg patient). If total resuscitation volume for HBOC-201 was 81 ml/Kg ,as reported, that represents about 10X the typical RESUS ml/Kg dosage. However authors report that pigs received up to 1000 ml of either fluid with a goal of maintaining MAP of 60 ml/Kg; that represents 17 ml/Kg (since swine weighed 55-65 Kg), or about 2X typical RESUS dosage of 8 ml/Kg.. After the shock period, Hex pigs were resuscitated to a goal of 60 mmHg with an initial 500 ml bolus of Hextend , which was repeated once if goal BP was not achieved. Shed blood was then returned to **only** the Hex-treated swine.

Question 4. type of pre-hospital monitoring simulates RESUS?

No. Blood pressure was exclusively used to guide volume of fluid infusions., and final definitive fluid resuscitation volumes were evidently protocol-driven rather than physiologic – leading to the impression of over-resuscitation in the Hex cohort.

Question 5. Favorably prognostic endpoints/biomarkers identified to guide RESUS?

There were no natural fatalities in the HBOC-201 to inform such (fatality was associated with an air embolism; see 6. next.), and in the Hex cohort, there was no retrospective segregation into survival vs. decedents to more clearly identify prognostic markers.

There were no cohort differences in the status and recovery of base excess, pH, lactate, and urine output , mixed venous oxygen saturation, and oxygen extraction ratio during the 8- hour trajectory of resuscitation. Since subsequent survival tended to differ appreciably, these markers are not obviously prognostic.

Question 6. All animals accounted for?

No. Details of the autopsy, if performed, on the pig in the HBOC-210 cohort which expired at the completion of the low-volume segment of the resuscitation are not presented. .Death was attributed to air embolism during attempted insertion of the Lifeport Port System indwelling catheter

Question 7. Any aspects of the model that confound interpretation and/or relevance to pre-hospital resuscitation.?

Yes, the manner of resuscitation of HBOC-201 and Hex was not equivalent: see response to Question.2 above. Rather than HBOC-201 possessing relative survival benefit vs. Hex, it is as, or even more, likely that the excess pulmonary edema deaths in the Hex cohort may have been due to over-resuscitation (they received 20 ml/Kg more volume than the HBOC group) or a combined insult of over-resuscitation + shed-blood.

An acute bronchi pneumonia observed in a majority of the 5-day survivors in both cohorts was attributed to prolonged anesthesia (8 hours).

See also responses to this question for the other 6 studies overviewed.

Question 8 . Will absence of any MI, stroke, CHF, hypertension, hypoxemia, or oliguria. in swine HS be re-assuring?

No. See previous responses to this question. Histology featured renal (multiple focal infarcts), and degenerative hepatic and myocardial lesions - with and without necrosis - in the HBOC-201 (as well as Hex) cohort at 5 days. It is not known whether this relates to the resuscitation to MAP of only 60 mmHg, or whether the safety implications would be more worrisome in the context of any pre-existing CV disease. While the creatinine levels in both cohorts increased during hemorrhage and low-volume resuscitation, they returned to normal during recovery (by recovery day 1 for HBOC-201; by day 5 for hex). AST, ALT, and amylase levels significantly increased in both cohorts, especially Hex , by morning of recovery day 1. However, only AST had not fully recovered by day 5.

Reviewer does not know whether the swine HS model also can model the delayed Multiple Organ Failure. In any case, the swine were not monitored beyond day 5 for this or any other manifestation of delayed toxicity such as neuronal degeneration.

Question 9. Pre-clinical results vs. standard therapy justify waiver of informed consent?

Not convincingly due to design of the experiment. Behavior relative to Hex is moot because of differences in resuscitative use of HBOC-201 and the standard therapy (Hex).

APPENDIX:

The **RESUS** clinical trial protocol:

Naval Medical Research Center /Biopure Corp. (PI: Daniel Freilach, MD, CDR) propose to evaluate HBOC-201 as a red blood cell substitute vs. lactated Ringer's (LR) for pre-hospital resuscitation of severe traumatic HS absent penetrating brain injury. This single-blind Stage I(phase II) trial involving ca. 6 level I trauma centers, and will be conducted under §50.24. It would compare pre-hospital resuscitation with 10 minute infusions of either HBOC-201 (500 ml/dose) or LR (1000 ml /dose) administered to approx. 50 trauma patients (1:1 ratio) with SBP< 90mmHg, and as necessary to maintain SBP ≥ 90 mmHg; and repeated if SBP remains below 90 mmHg or SBP is 90-99 mmHg and HR remains ≥ 100 BPM. Max. total doses: 5 HBOC-201; LR: 1000 ml for 1st and 2nd infusions; 500 ml for 3rd infusion).It is anticipated that most patients would receive 2 or 3 units (250 ml/unit) i.e.,500-750 ml. of HBOC-201, but that a small minority with prolonged transportation time and refractory hypotension would receive up to the maximum permitted (2500 ml of either HBOC or LR) . Other standard IV fluids will be permitted as clinically indicated. Criterion for stopping CTM is SBP ≥ 150 mmHg. Accordingly, RESUS is designed to compare efficacy of HBOC-201 vs. LR at a resuscitation volume ratio 1:2 – although the most refractory patients would receive a maximum of 2500 ml of either solution. A ten-minute infusion of 500 ml of HBOC-201 (approx. 7 ml/Kg, or 10% of normal blood volume) to a RESUS subject who lost approx. 30 ml/Kg of blood (i.e., when compensatory capacity is exceeded, and hypotension occurs) would replete up to approx. 1/4 of the loss. Presumably that is the basis for sponsor's expectation that, with effective hemostasis, most RESUS patient-subjects would receive several (2-3) 250 ml units, prior to ER arrival, and that such would afford significant restitution of effective blood volume absent significant re-bleeding.

Anticipating occult shock scenarios, CBER has clarified the RESUS protocol to allow additional fluid infusion with either CTM or other std. fluids when criteria for continuing CTM fluids are not met but signs of HS persist despite restoration of stable BP/HR. That is, *further standard fluids indicated* if SBP restored to ≥ 100 mmHg, but signs of HS persist **or** SBP restored to 90-99 mmHg and HR <100 bpm but other signs of HS persist ; *further CTM fluid indicated* if SBP remains < 90 mmHg or recurs, with or without other signs of persistent HS **or** SBP is 90-100 mmHg and HR >100BPM with or without other HS signs. Criteria for no further fluid resuscitation with either CTM or std. IV fluids remain the same: SBP restored to ≥ 100 mmHg absent signs of persistent HS **or** SBP restored to 90-99 mmHg and HR <100 bpm absent signs of HS.

Upon arrival in the ER, additional HBOC-201 would be forbidden and definitive resuscitation would be attempted including use of allogenic blood and other standard medical treatment as needed.

The primary endpoint would be 28 - day RR of mortality, and safety/tolerability. Secondary end-points are multiple, including cardinal hemodynamic parameters, tissue oxygenation, organ and neurocognitive function, blood transfusion requirements, ventilation support requirements, length of stay, and pre-hospital relative risk of mortality. Tertiary end-point is Composite Surrogate Score.