Review #2

Executive summary BPAC Meeting on RESUS, Use of HBOC-201.

[redacted]

Major technical concerns:

1) All/many of the studies in pigs (excluding the 2 in rats) used anesthesia, ventilation, paralysis, splenectomy, laparotomy, ventilation with oxygen at different times and to different end-points and especially used atropine (I suspect to block tracheal secretions from the anesthesia) which has marked effects on heart rate (tachycardia) and circulating catecholamines.
2) The hemorrhage was almost always sterile unlike RESUS.
3) The fluid administered was often warmed and the pig was warmed.
4) Measurements of cardiac output and heart rate were variable prior to hemorrhage due to anesthesia, atropine and variability in use of thermodilution (even though it is used longitudinally in each animal, see range of cardiac outputs and CI in studies).
5) It is obvious that all hemoglobin solutions are not the same, This includes recombinant HB, Diasprin hemoglobin, 70% poly hemoglobin etc. Those studies indicate caution but the outcomes can not be directly extrapolated to HBOC-201. In fact the one study using HBOC-301 should also be viewed separately.
6) The nature of the invasive measurements (mostly to examine mechanisms) during the preclinical phase (from hemorrhage to blood, simulated in hospital, support) not only confounds the conclusions but will not be used in RESUS.
7) The removal of blood from the abdomen in experimental studies to measure total hemorrhage volume is unlike RESUS (may build up and suppress bleeding).
8) In many instances (most) HBOC-201 is administered to a fixed volume and certainly not to a high systolic pressure.

Major conclusions with HBOC-201

1) In general the use of HBOC-201 increased survival to simulated hospital arrival and for longer periods
2) The results are uniform across many models including hemorrhage and injury to a) the brain,b) the liver, 3) the lung, 4) the abdomen (iliac bleeding) and 5) rectus crush (skeletal muscle).
3) The results are applicable to varying times to treatment and times to simulated hospital arrival.
4) Generally the use of HBOC to support pressure to various levels (50 mmHg, 60 mmHg and above) after hemorrhage is beneficial.
5) Generally less fluid is needed for resuscitation when giving HBOC-201.
6) Generally, there appears to be vasoconstriction after HBOC-201, either pressure rises more or calculated resistance rises more than with LR (or other fluids)
7) There may be some utility in measuring lactate or tissue oxygen levels (non-invasively) if the time to hospital is long (and technically feasible).
8) The histology seems to indicate minimal damage and the immunologic response is small and not organized (selective for IL-10 for instance).

Henderson et al ECMO Study 1 section 2.

Summary: The overall goal of this study was to determine whether HBOC-201 could replace blood during prolonged ECMO in a model of ARDS caused by oleic acid infusion in neonatal swine. Oleic acid was administered prior to placement on ECMO w/without HBOC-201,, or blood. Arterial pressure rose in all animals on ECMO and was higher in both HBOC and RBC. All animals (n=24, 3 groups) survived 8 hours.

Questions to be addressed.

1a) Does the animal model adequately simulate RESUS prehospital trauma setting?
There is no similarity between RESUS and the current study. Specifically, all RESUS participants will be 18 years old. ECMO is not to be given prehospital. Animals were paralyzed, ventilated and anesthetized. They were given oxygen and heparin.

1b) To what extent do the preclinical data support use in humans in the RESUS trial? Except for the safety of HBOC-201 compared to blood, these data do not address a significant question in RESUS. Blood is not an option in the preclinical setting.

2) Is the study well designed? Yes the study is well designed.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? The dosing does apply to RESUS, blood pressure was titrated to 100mmHg whereas RESUS will titrate systolic blood pressure between 90 and 150 mmHg.

4) Does the type of monitoring simulate the RESUS prehospital truma setting? The monitoring is invasive and more addresses mechanisms than prehopsital setting.

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? The outcome is survival of neonatal pigs for up to 8 hours comparing HBOC to blood. Because there was no mortality in 3 groups survival was not an endpoint. Blood and HBOC were equivalent to no treatment.

6) Can all animals entered into the study be accounted for? Yes all the animals are accounted for.

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation? This is a technical study which related to in hospital treatment not prehospital.

8) Is the absence of similar adverse events reported in preclinical studies with HBOC-201 reassuring in terms of the RESUS prehospital trauma setting? There was no adverse effects of HBOC-201, it was equivalent to no treatment and to blood.

9) Have the preclinical requirements for exception from informed consent been met? 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. There is no evidence of a direct benefit, HBOC-201 performed in a manner similar to blood or to no treatment.

McNeil et al study 2 section 2.
The purpose of this study was to determine whether resuscitation from severe hemorrhage with HBOC-201 was different from LR or LR plus blood. Another objective was to determine whether resuscitation to 60 mmHg with HBOC was different from 50 mmHg. There was no difference in the mortality in the 5 groups tested (4 hours observation). Animals were hemorrhaged to 40 mmHg and held there for 45 minutes. HBOC resuscitated to 50 mmHg died (not powered for small differences). Cardiac output reduced in HBOC groups, Figure 1, perhaps due to vasoconstriction . Lactate production reduced in groups except LR.

Questions to be addressed.
1a) Does the animal model adequately simulate RESUS prehospital trauma setting? The model does simulate the RESUS protocol with respect to the lower blood pressure due to blood withdrawal. The blood pressure end points are low. HBOC are titrate to 50 or 60 mmHg not SBP greater than 90 as in RESUS.

1b) To what extent do the preclinical data support use in humans in the RESUS trial? Supports use of HBOC in patients due to lack of mortality. Animals are anesthetized and paralyzed.

2) Is the study well designed? The studies are well designed to generate potential markers for in hospital setting and to investigate mechanisms.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? The dosing schedule and rates of infusion are designed to a low blood pressure, not to SBP greater than 90mmHg. However data indicate that resuscitation to 60 mmHg with HBOC is better than 50 mmHg.

4) Does the type of monitoring simulate the RESUS prehospital trauma setting? The monitoring is more invasive than prehospital setting.

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? The only marker besides blood pressure is blood lactate which fell with HBOC.

6) Can all animals entered into the study be accounted for? Yes, all of the animals are accounted for.

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation? The use of anesthesia, ventilation, paralyzed pigs is not relevant to RESUS protocol. All pigs were female.

8) Is the absence of similar adverse events reported in preclinical studies with HBPC-201 reassuring in terms of the RESUS prehospital trauma setting? The only possible concern

is the HBOC to 50 mmHg resulted in one death (ns). There was no change in plasma free hemoglobin w/HBOC, table 5?

9) Have the preclinical requirements for exception from informed consent been met? 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. In terms of the 5 hour experiment, HBOC was neutral compared to the other protocols.

Fitzpatrick et al paper 3 section 2.

This project investigated the comparison between hetastarch and HBOC-201 in pigs hemorrhaged to a pressure of 30 mmHg for 45 minutes. Either Hetastarch or HBOC were given to increase MAP to 60 mmHg. All animals survived the initial 8 hours but 4/8 survived with hetastarch while 7/8 survived with HBOC for 5 days. Less volume was given with HBOC and MAP of 60 was achieved. At 8 hours, hetastarch animals given LR plus blood but HBOC animals given only LR (no blood). HBOC required less total fluid. Cardiac output was lower in both groups but slightly higher in HBOC. Resistance maintained higher in HBOC.

Questions to be addressed.

1a) Does the animal model adequately simulate RESUS prehospital trauma setting? The model does approximate RESUS protocol since HBOC will be given at 45 minutes of hemorrhagic shock. Blood pressure was only titrated to 60 mmHg not SP greater than 90 mmHg as in RESUS. In addition only received LR after 8 hours (not RBC as in hospital). Mortality was 4/8 with hetastarch and 1/8 with HBOC (and might be catheter induced not HBOC induced).

1b) To what extent do the preclinical data support use in humans in the RESUS trial? Support the use of HBOC since mortality was low at 8 hours.

2) Is the study well designed? Except for the use of anesthetized animals the study is well designed.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? The dosing schedule dose not simulate RESUS since MAP was titrated to 60 mmHg not SP greater than 90 mmHg.

4) Does the type of monitoring simulate the RESUS prehospital trauma setting? The monitoring prehospital is not simulated by this experiment. The experiment is more technical and invasive.

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? Both 8 hours survival (prehospital time long) and 5 day survival correlate with favorable outcome.

6) Can all animals entered into the study be accounted for? Yes, 16 animlas aare accounted for.

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation? The technical nature of the studies using invasive instrumentation and anesthesia may confound interpretation of the data.

8) Is the absence of similar adverse events reported in preclinical studies with HBPC-201 reassuring in terms of the RESUS prehospital trauma setting? Yes, the outcomes are reassuring since 100% survived for 8 hours and 1/8 died at 5 days (perhaps unrelated to HBOC).

9) Have the preclinical requirements for exception from informed consent been met? 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. Yes, these studies support a potential benefit, only 1/8 died before 5 days in the HBOC group, while 4/8 died in the hetastarch group.

York et al paper 4 section 2
This study compared resuscitation with blood versus HBOC-201 to different pressure end-points. Hemorrhage to MAP of 30 mmHg was followed by holding for 45 minutes. 4 Groups were studied: 1) returned blood to original pressure; 2)shed blood to MAP of 60 mmHg; 3) shed blood and LR to control MAP; and 4) HBOC-201 to MAP of 60 mmHg, all for 4 hours. Animals kept for 3 days. Only one (blood ± LR) died before 3 days. 4/6 HBOC had liver enzyme elevation. Laparotomy to insert catheter.

Questions to be addressed.
1a) Does the animal model adequately simulate RESUS prehospital trauma setting? The initial animal protocol does simulate RESUS protocol. Severe hemorrhage followed by 45 minutes sustained hypotension. Blood given at 45 minutes is appropriate but blood not given to HBOC is not per RESUS..

1b) To what extent do the preclinical data support use in humans in the RESUS trial? Except for some indication of mild liver dysfunction in 4/6 HBOC-201, results support use of HBOC. No mortality with HBOC.

2) Is the study well designed? The study is well designed and the end-points are survival for 4 hours and 3 days.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? The dosing schedules do not represent RESUS since HBOC given to only MAP of 60 mmHg not systolic pressure greater than 90 mmHg as in RESUS..

4) Does the type of monitoring simulate the RESUS prehospital trauma setting? The type of monitoring is much more invasive and designed to examine mechanisms.

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? The potential biomarkers are limited by the relatively untechnical nature of the prehospital environment. Blood pressure and heart rate can be measured.

6) Can all animals entered into the study be accounted for? All of the animals are accounted for.

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation? The use of anesthetized, ventilated, paralyzed animals is a confounding influence (including PEEP). Unlike other studies the amount of HBOC was not less than other groups (Table 2).

8) Is the absence of similar adverse events reported in preclinical studies with HBOC-201 reassuring in terms of the RESUS prehospital trauma setting? Yes, there was no additional mortality with HBOC.

9) Have the preclinical requirements for exception from informed consent been met? 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. The study performed is appropriate and the fact that all lived 3 days is reassuring.

Fitzpatrick et al study 5 section 2

This study examined vascular reactivity especially the role of NO, with hemorrhage to 30 mmHg for 45 minutes followed by resuscitation with shed blood, LR or HBOC-201. Iliac artery diameter changes to acetylcholine were measured at baseline, 1 and 4 hours after resuscitation. All fluids supported function. Ach response reduced at 4 hours. Nitric oxide levels in plasma were not different. The amount of HBOC-201 needed for resuscitation was lowest. No indication of resuscitation endpoint in HBOC group. NO measurements are useless.

Questions to be addressed.
1a) Does the animal model adequately simulate RESUS prehospital trauma setting? Yes simulates the RESUS protocol except for invasive nature of the experiments. Animals were anesthetized etc.

1b) To what extent do the preclinical data support use in humans in the RESUS trial? There is no difference in survival for 4 hours of resuscitation thus neutral in respect to LR or blood.

2) Is the study well designed? The study is well designed to determine the role of NO in response to HBOC. Many technical problems.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? The rate of dosing of HBOC not actually given

4) Does the type of monitoring simulate the RESUS prehospital trauma setting? The type of monitoring is much more invasive than RESUS.

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? The markers except pressure and heart rate are too technical for RESUS.

6) Can all animals entered into the study be accounted for? No, there is no indication of mortality.

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation? The use of ventilated, anesthetized and instrumented animals is a problem.

8) Is the absence of similar adverse events reported in preclinical studies with HBPC-201 reassuring in terms of the RESUS prehospital trauma setting? HBOC-201 was at least as effective as LR or blood. Smaller volumes of HBOC needed.

9) Have the preclinical requirements for exception from informed consent been met? 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. The lack of presentation of mortality data is a problem.

Sampson et al paper 6 section 2
This study was designed to compare survival and hemodynamics following hemorrhage to 45 mmHg MAP and resuscitation with 6 different solutions including HBOC-201. Resuscitation was designed to keep MAP at 60 mmHg (or above). The control group and hypertonic saline group had high mortality rates whereas the pentastarch, hexastarch, LR and HBOC-201 had no mortality at 4 hours.. Resuscitation volumes were lowest with HBOC-201 group. Cardiac output lowest in HBOC-201 as was urine volume.

Questions to be addressed.
1a) Does the animal model adequately simulate RESUS prehospital trauma setting? Yes, the initial model of hemorrhage and resuscitation for 4 hours simulates the RESUS protocol. Comparison with LR also resembles RESUS.

1b) To what extent do the preclinical data support use in humans in the RESUS trial? These was no mortality up to 4 hours in the LR and HBC groups indicating that HBOC had no disadvantage.

2) Is the study well designed? Yes, the study is technically well designed. The cardiac ouputs are variable across all the studies.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? Yes, the dosing to a prescribed MAP is important in principle, but the end-point for RESUS is systolic pressure greater than 90 mmHg.

4) Does the type of monitoring simulate the RESUS prehospital trauma setting? The monitoring is much more technical.

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? No the endpoints are technical.

6) Can all animals entered into the study be accounted for? No, it seems there are animals missing (page 749).

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation? The use of anesthetics, paralytics, ventilation etc are confounding influences.

8) Is the absence of similar adverse events reported in preclinical studies with HBPC-201 reassuring in terms of the RESUS prehospital trauma setting? Yes, there is no difference between LR and HBOC.

9) Have the preclinical requirements for exception from informed consent been met? 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. There is no increase in mortality.

King et al Paper 1 section 3.
Swine underwent brain injury and hemorrhage of 30 ml/kg. After 30 minutes LR or HBOC-301 or both were titrated to MAP greater than 100 mmHg and HR less than 100 b/min. After 60 minutes other fluids were given to maintain MAP greater than 70 mmHg alone or with mannitol if at 90 minutes intracranial pressure was greater than 20 mmHg. RBC were also given. In second part, animals underwent hemorrhage and resuscitation with LR ± Mannitol ± RBC or HBOC-301. Animals were weaned from the pump and 100% of HBOC survived 72 hours whereas none of the LR ± Mannitol ± RBC survived. Suggest that HBOC is best but MAP and HR are not good endpoints. Authors talk about anesthesia limiting applicabtility.

Questions to be addressed.
1a) Does the animal model adequately simulate RESUS prehospital trauma setting? Yes, this model does simulate RESUS in that pressure was restored to 100 mmHg and HR less than 100 b/min. This model also deals with patients who potentially have brain injury.

1b) To what extent do the preclinical data support use in humans in the RESUS trial? Preclinical data indicate that HBOC-301 prolongs survival to 72 hours with no mortality (a reasonable behavioral score) and little need for additional LR or mannitol.

2) Is the study well designed? The study is well designed.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? The dosing schedule does simulate RESUS since the endpoints are pressure greater than 100 mmHg and HR less than 100 b/min.

4) Does the type of monitoring simulate the RESUS prehospital trauma setting? The type of monitoring is more sophisticated compared to RESUS prehospital.

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? No new endpoints were developed and in fact the authors question the use of MAP and HR as endpoints.

6) Can all animals entered into the study be accounted for? Yes all the animals are accounted for.

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation? The use of anesthesia and invasive nature of the hemodynamic studies may confound interpretation (as pointed out by the authors). However, survival at 72 hours with HBOC-301 is the best endpoint.

8) Is the absence of similar adverse events reported in preclinical studies with HBPC-201 reassuring in terms of the RESUS prehospital trauma setting? Yes, the survival at 72 hours is important.

9) Have the preclinical requirements for exception from informed consent been met? 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. The lack of ability to recover the Mannitol ± LR group after 6 hours is in marked contrast to survival of 100% of HBOC-301 animals at 72 hours.

Malhotra paper 2 section 3.
This study compared 4 groups of swine randomized after hemorrhage to a systolic pressure of 35 mmHg. Animals received LR, Blood, rHB (recombinant hemoglobin) or DCLHB (diasparin cross linked hemoglobin) based on volume for 15 minutes. After 2 hours animals returned to cage and redosed with resuscitation fluid and killed after 5 days (for 6 days total). Blood pressure after resuscitation was 128 and 108 mmHg in rHB and blood groups. Lower in DCLHB and LR group. Death includes: 0/6 in blood; 1/6 in rHB; 4/6 in LR and 6/6 in DCLHB (figure 8). Conclusion is that rHB is safe. Splenectomy, anesthesia and FiO2 of 50% are all complications.

Questions to be addressed.
1a) Does the animal model adequately simulate RESUS prehospital trauma setting? The model does simulate the hemorrhage phase but the resuscitation is based on volume of fluid not pressure as in RESUS. Followed for total of 6 days. The 2 HBOC used recombinant and aspirin cross linked will not be used in RESUS.

1b) To what extent do the preclinical data support use in humans in the RESUS trial? The use of rHB was beneficial whereas the use of DCLHB was not. Since DCLHB is no longer being tested and rHB is another HBOC, these data support the use of HBOC in principle.

2) Is the study well designed? The study is well designed.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? Dosing schedule is to fixed volume not pressure or HR.

4) Does the type of monitoring simulate the RESUS prehospital trauma setting? The type of monitoring is very invasive.

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? The only new endpoint would be lactate and SVO2 via hemoglobin saturation.

6) Can all animals entered into the study be accounted for? Yes, all animals are accounted for.

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation? Use of anesthesia is confounding.

8) Is the absence of similar adverse events reported in preclinical studies with HBOC-201 reassuring in terms of the RESUS prehospital trauma setting? There is no use of HBOC-201 in these studies.

9) Have the preclinical requirements for exception from informed consent been met? 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. Unless the toxicity of DCLHB can be explained (related to cardiac dysfunction) then the data are equivocal since rHB was beneficial and DCLHB was not.

Malhotra et al paper 3 section 3

In this study animals received head trauma followed by 45% blood volume hemorrhage. After 60 minutes animals were randomized to 3 groups: saline, or 250 or 500 mls DCLHB (DCLBH 1or 2). Animals were observed for 210 minutes. 5/20 with saline had brain death and one dead of shock.0/13 of DCLHB had brain death, one died of heart failure. DCLHB had higher cerebral perfusion pressure and cerebral vasodilation (to pCO2) was maintained in high DCLHB group. Cardiac output was lower and pulmonary pressures higher with DCLHB.

Questions to be addressed.

1a) Does the animal model adequately simulate RESUS prehospital trauma setting? Head trauma followed by hemorrhage for 60 minutes does simulate RESUS, resuscitation with saline versus DCLHB roughly follows RESUS but products different. Observed for 210 minutes. DCLHB given to fixed volume not to MAP of 100 initially only after initial infusion.

1b) To what extent do the preclinical data support use in humans in the RESUS trial? Does simulate RESUS in duration of hemorrhage (although much shorter in RESUS), Does not in combined use of saline and DCLHB. HBOCs are different. DCLHB has been withdrawn from testing.

2) Is the study well designed? The study is well designed.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? The initial use of DCLHB to fixed volume does not simulate RESUS however, later titration of fluid to MAP=100 and HR less than 100 does.

4) Does the type of monitoring simulate the RESUS prehospital trauma setting? Monitoring is more invasive in pursuit of mechanisms.

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? No new endpoints are found.

6) Can all animals entered into the study be accounted for? Yes, all are acconted for. Some confusion in how many DCLHB died (either 1/8 or 3/8).

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation? Yes, anesthesia, surgery etc confound interpretation (fentanyl, ketamine, xylazine ...).

8) Is the absence of similar adverse events reported in preclinical studies with HBOC-201 reassuring in terms of the RESUS prehospital trauma setting? These studies did not use HBOC-201.

9) Have the preclinical requirements for exception from informed consent been met? 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. Did not use HBOC-201.

Maxwell et al Paper 4 section 3.

These studies examined a model of lung trauma and 25% blood volume hemorrhage. Treatments were with 4 hemoglobins 60 minutes after the hemorrhage followed by 6 hours observation, Swine were given 250 mls: 1) polynitroxlated (PnaaHB); 2) aaHB; 3) Polynitroxylated (70%) hemoglobin; 4) Polymerized HB; or 5) saline. All hemoglobins reduced volume requirements; all hemoglobins were pressors, mortality less with polymerized hemoglobins. Saline given to maintain pressure at 100 mmHg and heart rate less than 100 b/min. All animals with polymerized HB (2 groups) survived 480 minutes (see table 1). Fluid needed lowest for HB compared to saline.

Questions to be addressed.

1a) Does the animal model adequately simulate RESUS prehospital trauma setting? The initial insult and resuscitation do simulate RESUS except the HBs given are different from HBOC-201. In general polymerized HB (70%) reduced mortality. Keeping MAP = 100 mmHg and heart rate less than 100 b\min with saline does not resemble RESUS.

1b) To what extent do the preclinical data support use in humans in the RESUS trial? The preclinical data with polymerized HB dies simulate RESUS. The duration of the low pressure is within guidelines. No long term recovery studied (480 minutes).

2) Is the study well designed? The study is well designed.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? The dosing schedules are initially only 250 mls of polymerized HB. Multiple doses are not given rather support is with saline.

4) Does the type of monitoring simulate the RESUS prehospital trauma setting? The monitoring is more invasive than RESUS prehospital.

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? There are no new endpoints.

6) Can all animals entered into the study be accounted for? Yes, all animals are accounted for.

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation? The use of anesthesia etc are confounding influences.

8) Is the absence of similar adverse events reported in preclinical studies with HBOC-201 reassuring in terms of the RESUS prehospital trauma setting? HBOC-201 was not used in this protocol. Use of polymerized HB supports use of HBOC-201. However, for

whatever reason, use of aaHB does not support HBOC-201 use in people. There does not appear to be a general action but rather a product specific action (see also studies with DCLHB).

9) Have the preclinical requirements for exception from informed consent been met? 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. The lack of death in the 2 polymerized hemoglobin groups at 480 minutes support the use of HBOC-201.

Gurney Paper 1 section 4

The goal of the study was to determine the relative effects of HBOC-201, Hex or no resuscitation in 24 swine for 4 hours followed by iv fluids and blood transfusions (to maintain pressure above 60 mmHg and heart rate near normal). HBOC-201 had 7/8 survivors to 240 minutes and to 72 hours after severe liver injury whereas only 1/8 with Hex or 1/8 with no resusitation survived. MAP was higher and cardiac output maintained lower with HBOC. HBOC had lower fluid requirements, fluids given to keep pressure greater than 60 mmHg and reduce the tachycardia.

Questions to be addressed.

1a) Does the animal model adequately simulate RESUS prehospital trauma setting? RESUS prehospital criterea are like the liver injury with uncontrolled bleeding in these studies. Hemorrhage was held for 30 minutes and then resuscitation fluids given, studies were performed at 240 minutes and 72 hours.

1b) To what extent do the preclinical data support use in humans in the RESUS trial? The HBOC-201 reduced mortality at 240 minutes and at 72 hours. Also reduced bleeding and amount of fluids needed. HBOC given to maintain pressure greater than 60 mmHg and HR near normal.

2) Is the study well designed? The study is well designed.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? Dosing is to lower endpoint than RESUS since MAP is only back to 60 mmHg and heart rate near normal.

4) Does the type of monitoring simulate the RESUS prehospital trauma setting? The type of monitoring is very invasive compared to RESUS.

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? The most useful endpoint may be lactate but this is difficult to measure in RESUS. MAP and HR are useful.

6) Can all animals entered into the study be accounted for? All the animals are accounted for.

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation? The anesthesia etc are confounding. The use of atropine eliminates the significance of heart rate control in the study entirely.

8) Is the absence of similar adverse events reported in preclinical studies with HBPC-201 reassuring in terms of the RESUS prehospital trauma setting? Since only 1/8 died at 4 hours or 72 hours with HBOC-201, this study supports the use of HBOC-201 in humans.

9) Have the preclinical requirements for exception from informed consent been met? 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. 1/8 survived in Hex or non resuscitated groups compared to 7/8 for HBOC-201.

Arnaud et al paper 2 section 4.

This study compared HBOC-201, Hex and non resuscitation over 4 hours prehospital phase and 72 hour recovery with a 40% blood loss (BP=26 mmHg) following crushing of the rectus abdominus muscle.. Hemorrhage was held for 20 minutes and then fluid given at 10 ml/kg and an additional 5 ml/kg every 30 minutes to keep MAP greater than 60 mmHg and no tachycardia. At 4 hours hospital care was begun (animals kept for a total of 72 hours). Survival was 8/8 in HBOC-201, 7/8 in Hex and 5/8 in not treated. (not splenectomized so that HCT rose with hemorrhage).

Questions to be addressed,
1a) Does the animal model adequately simulate RESUS prehospital trauma setting? The model does simulate early RESUS protocols with severe hemorrhage and soft tissue damage. Fluids were infused after 20 minutes at 10 ml/kg, not to an initial pressure endpoint. Later the goal was to maintain pressure at 60 mmHg and eliminate tachycardia.

1b) To what extent do the preclinical data support use in humans in the RESUS trial? Since there was no mortality at 72 hours with HBOC-201 these data support the use of HBOC-201 in humans.

2) Is the study well designed? The study is well designed.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? The dosing does not support the RESUS protocol since a fixed volume of HBOC was given and not titrated to restore arterial pressure and heart rate initially.

4) Does the type of monitoring simulate the RESUS prehospital trauma setting? The monitoring is invasive and technical.

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? No new endpoints or biomarkers are evidenced.

6) Can all animals entered into the study be accounted for? All of the animals are accounted for.

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation? Anesthesia is a confounding influence as is acute surgery.

8) Is the absence of similar adverse events reported in preclinical studies with HBPC-201 reassuring in terms of the RESUS prehospital trauma setting? The 100% survival at 72 hours in the HBOC-201 group is important.

9) Have the preclinical requirements for exception from informed consent been met? 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. Since HBOC was comparable to Hex for death, these studies support the use of HBOC in the clinical trial.

Dong et al Paper 3 section 4

This is a project to determine the potential immune repsonse to HBOC-201 during and after hemorrhage. Pigs were anesthetized, ventilated and hemorrhaged 40% of blood volume after rectus abdominus crushing. Animals were monitored 4 hours and then resuscitated , observation continuted for 72 hours. Animals were divided into 3 groups. HBOC-201 , Hex or no resuscitation. Hemorrhaged over 15 minutes then given fixed volume of Hex or HBOC-201. Additional fluids were given to keep MAP greater than 60 mmHg and eliminate the tachycardia. After 4 hours blood was given and the animals kept for 72 hours. No mortality or hemodynamics were presented. IL-10 was elevated in the HBOC group but TNFa was not.

Questions to be addressed.

1a) Does the animal model adequately simulate RESUS prehospital trauma setting? The endpoint for infusion was 10 ml/kg not to a fixed pressure initially. Afterwards, fluids given to keep pressure greater than 60 mmHg and eliminate tachycardia. Resuscitation kept for 4 hours and then blood given. This is an extended peroid of time compared to RESUS.

1b) To what extent do the preclinical data support use in humans in the RESUS trial? The preclinical data indicate a lack of specific immune stimulation by HBOC. Hemodynamics or survival not reported.

2) Is the study well designed? The focus on white cell activation and immune stimulation and is well designed.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? The dosing schedule is to a fixed volume not to maintain MAP 100 and eliminate tachycardia.

4) Does the type of monitoring simulate the RESUS prehospital trauma setting? There is no monitoring of the hemodynamics only blood and tissue samples.

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? No new biomarkers are defined in this study.

6) Can all animals entered into the study be accounted for? No, mortality is not discussed.

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation? Anesthesia, ventilation etc do not fit the RESUS protocol.

8) Is the absence of similar adverse events reported in preclinical studies with HBPC-201 reassuring in terms of the RESUS prehospital trauma setting? The study is largely irrelevant to RESUS prehospital.

9) Have the preclinical requirements for exception from informed consent been met? 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. There does not appear to be a major difference between Hex and HBOC in this model. Only that there were fewer transfusion with HBOC in the discussion, no data presented.

Philbin et al paper 4 section 4.

This study used 24 pigs to determine the relative effects of HEX, HBOC-201 or no resuscitation on hemodynamics and survival. Hemorrhage was performed by 40% of blood volume along with rectus abdominus crushing. Resuscitation was begun 40 minutes and animals kept for 4 hours. Finally, additional fluids and blood were given and animals maintained for 72 hours. 8/8 HBOC-201, 7/8 Hex and 5/8 not resuscitated animals survived for 72 hours. Fluids were initially given at 10 ml/kg and again if pressure was less than 60 mmHg or heart rate higher than control. Transcutaneous tissue monitoring showed that TcO2 was higher with HBOC-201 and could be measured prehospital. Lower volume of HBOC needed.

Questions to be addressed.

1a) Does the animal model adequately simulate RESUS prehospital trauma setting? This mod4l does resemble RESUS except for the 4 hour resuscitation time, should be shorter for RESUS. Invasive monitoring is unlike RESUS. Rectus crush and hemorrhage by 40% reasonable with soft tissue injury included. Short time, 5 minutes until Hex or HbOC administered is also reasonable.

1b) To what extent do the preclinical data support use in humans in the RESUS trial? Given that there was no mortality with HBOC-201 at 72 hours these data do support the use of HBOC-201 in humans.

2) Is the study well designed? The study is well designed.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? Initially the HBOC was given at 10 ml/kg, fixed volume, and additional fluids given to keep MAP greater than 60 mmHg and eliminate the tachycardia.

4) Does the type of monitoring simulate the RESUS prehospital trauma setting? The monitoring is more invasive and technical than RESUS. The use of oxygenation measurements is non-invasive these may be important as an end-point.

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? The only new measurement is tissue O2 measurements.

6) Can all animals entered into the study be accounted for? All of the animals are accounted for.

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation? Use of atropine greatly confounds HR and Cardiac Index data. Use of anesthetics may alter results.

8) Is the absence of similar adverse events reported in preclinical studies with HBPC-201 reassuring in terms of the RESUS prehospital trauma setting? The high survival, 100%,in the HBOC-201 group gives confidence to the studies. The study was not powered for survival.

9) Have the preclinical requirements for exception from informed consent been met? 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. Lack of mortality in HBOC-201 group is an important end-point.

Johnson et al paper 5 section 4

This is a study to determine survival and the difference in 3 models of hemorrhagic shock: 40, 55% and uncontrolled in swine. Swine were treated with HBOC-201, Hex or nothing. Cardiovascular function, histology and survival to 72hours was monitored. (Rectus crushing was also used). Hemorrhage occurred over 15 minutes and then at 20 minutes HBC-201, Hex or nothing were given. Additional fluids were given for hypotension or tachycardia. At 4 hours animals were given blood and hospital like treatment initiated. Survival (Table 1) was 7/8 to 8/8 for HBOC-201, 1/8 to 7/8 with hex, and 1/8 to 5/8 with nothing. HBOC-201 received less fluid, MAP better maintained with HBOC in all models (figure 2). Some renal papillary and hepatic changes noted as mild.

Questions to be addressed.

1a) Does the animal model adequately simulate RESUS prehospital trauma setting? Three models are described and all are relevant to RESUS. Hemorrhage was studied for 4 hours.

1b) To what extent do the preclinical data support use in humans in the RESUS trial? Survival data support use of HBOC-201 in RESUS.

2) Is the study well designed? The study is well designed.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? The doses are initially a fixed volume and then titrated to prevent hypotension and tachycardia.

4) Does the type of monitoring simulate the RESUS prehospital trauma setting? Monitoring is more invasive and technical.

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? No new biomarkers or endpoints are evident. Figure 2 (MAP) should be made into a nomogram.

6) Can all animals entered into the study be accounted for? All animals are accounted for.

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation? Anesthesia and especially atropine confound the results. The small N, even though survival was statistically different, is potentially a problem.

8) Is the absence of similar adverse events reported in preclinical studies with HBOC-201 reassuring in terms of the RESUS prehospital trauma setting? The high survival in the HBOC-201 group is important.

9) Have the preclinical requirements for exception from informed consent been met? 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. The fact that pressures are higher and survival better are both important.

Knudson MM Paper 1 section 5.

This is perhaps the best RESUS model, the study was entirely blinded. Animals were splenectomized and hemorrhaged to a MAP=40mmHg over 20 minutes, kept for 20 minutes and then randomized to HBOC-201, LR or HSD to fixed small volume. Animals were monitored to 2 hours. HSD maintained CO better. MAP returned best with HBOC-201, then HSD and then LR. 9/10 HBOC animals had systolic pressure greater than 90 mmHg compared to 1/10 LR and 4/10 HSD.

Questions to be addressed.
1a) Does the animal model adequately simulate RESUS prehospital trauma setting? This is probably the best for current RESUS prehospital protocol since kept for 20 minutes and then 2 hours to hospital.

1b) To what extent do the preclinical data support use in humans in the RESUS trial? There was no mortality with HBOC-201 thus data do support the use in humans.

2) Is the study well designed? The study is well designed.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? The design is to test a small volume of resuscitation fluid not to a MAP end point. Thus somewhat unlike RESUS.

4) Does the type of monitoring simulate the RESUS prehospital trauma setting? The monitoring is more invasive and technical for prehospital.

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? No new endpoints are presented.

6) Can all animals entered into the study be accounted for? Yes, all animals are accounted for.

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation? Anesthetics, paralytics and oxygen 100% may complicate interpretation. Splenectomy is also a confounding influence.

8) Is the absence of similar adverse events reported in preclinical studies with HBPC-201 reassuring in terms of the RESUS prehospital trauma setting? There was no mortality for 2 hours with HBOC-201.

9) Have the preclinical requirements for exception from informed consent been met? 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. The use of less fluid, and no mortality in 2 hours supports use of HBOC-102 in patients.

Lee paper 2 section 5.

A single group of 7 pigs was hemorrhaged to 40 mmHg and then given a small dose of HBOC-201 (6ml/kg). Observations were continued for 2 hours. Cardiac ouput and MAP returned to near control and brain O2 increased. Hemorrhaged to 40 mmHg for 20 minutes. 100% O2 gieven.

Questions to be addressed.

1a) Does the animal model adequately simulate RESUS prehospital trauma setting? This is a close simulation of REUS except that HBOC-201 is given as a fixed small volume.

1b) To what extent do the preclinical data support use in humans in the RESUS trial? There is no mortality at 2 hours, no comparison made to other solutions.

2) Is the study well designed? The study is a single group and as such well designed.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? The single dose of HBOC-201 does not simulate RESUS.

4) Does the type of monitoring simulate the RESUS prehospital trauma setting? The type of monitoring is more invasive.

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? No new end points are obvious, MAP appears to be useful.

6) Can all animals entered into the study be accounted for? Yes all the animals are accounted for.

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation? Anesthesia, paralytics and splenectomy are confounding, use of 100% O2 also potentially confounding.

8) Is the absence of similar adverse events reported in preclinical studies with HBPC-201 reassuring in terms of the RESUS prehospital trauma setting? No adverse effect for 2 hours is encouraging,

9) Have the preclinical requirements for exception from informed consent been met? 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. Study indicates that MAP and CO are near normal, heart rate is lower and there is no mortality over 2 hours.

Manley et al. Paper 3 section 5.

Six pigs were hemorrhaged to 40 mmHg over 20 minutes, held for 20 minutes and then given 4 ml/kg HBOC-201 with recording for 2 hours. Cardiac output increased as did MAP. Mortality is not reported. MAP not held to any goal. Given 100% O2. There was some restoration of brain oxygen with 100% O2 and HBOC-201 resuscitation.

Questions to be addressed.
1a) Does the animal model adequately simulate RESUS prehospital trauma setting? The loss of blood to MAP of 40 mmHg and holding for 20 minutes represents RESUS. The administration of HBOC-201 to a fixed volume (4ml/kg) does not. No pressure end points.

1b) To what extent do the preclinical data support use in humans in the RESUS trial? The preclinical data presented suggests that HBOC-201 maintains arterial pressure and cardiac output during resuscitation for 2 hours and supports brain oxygenation.

2) Is the study well designed? The study is limited, no mortality discussed.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? The dosing schedule is not like RESUS no pressure or heart rate endpoint only a fixed volume.

4) Does the type of monitoring simulate the RESUS prehospital trauma setting? The monitoring is very invasive.

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? No new endpoints are evident, MAP is useful.

6) Can all animals entered into the study be accounted for?
All of the animals can be accounted for.

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation? Anesthetized and paralyzed pigs were used, these are confounding influences.

8) Is the absence of similar adverse events reported in preclinical studies with HBPC-201 reassuring in terms of the RESUS prehospital trauma setting? Recordings of hemodynamics and maintenance of arterial pressure and heart rate indicate favorable effects of HBOC-201 in the preclinical setting.

9) Have the preclinical requirements for exception from informed consent been met? 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. The only direct benefit of low volume HBOC-201 is maintenance of arterial pressure and heart rate.

Katz et al Paper 1 section 6.

This was a study of the effect of hemorrhage (50 ml/kg) followed by liver bleeding via laceration of the liver in 22 swine randomized to no fluid, hetastarch or HBOC-201. All HBOC-201 lived 60 minutes whereas non of the other groups survived. Importantly 7/8 of the HBOC-201 swine survived 96 hours. Resuscitation held for 60 minutes (figure 1). HBOC-201 maintained MAP and cardiac index during 90 minutes. Infusions at 6 ml/kg/hr and then reduced to 3 ml/kg/hr not to a constant pressure. Hematocrit increased after 60 minutes perhaps due to contraction of the spleen.

Questions to be addressed.

1a) Does the animal model adequately simulate RESUS prehospital trauma setting? Hemorrhage and liver damage for 60 minutes does resemble RESUS prehospital. HBOC-201 given to fixed volume not to pressure endpoint.

1b) To what extent do the preclinical data support use in humans in the RESUS trial? 8/8 HBOC-201 survived 60 minutes, 0/8 with no fluid and 0/8 with hetastarch.

2) Is the study well designed? The study is well designed.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? No the dosing schedule is to a fixed volume or rate (6ml/kg/hr) and then 3 ml/kg/hr.

4) Does the type of monitoring simulate the RESUS prehospital trauma setting? The type of monitoring is more invasinve.

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? Only MAP can be used prehospital.

6) Can all animals entered into the study be accounted for? Yes all animals are accounted for. Interestingly, at 96 hours the one pig in the HBOC-201 group that died had ascaris antigen.

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation? Initial anesthesia, warming, change in anesthesia are all complicating factors.

8) Is the absence of similar adverse events reported in preclinical studies with HBPC-201 reassuring in terms of the RESUS prehospital trauma setting? The 100% survival at 1 hour is the best endpoint, there is some statement that the groups are not big enough for statistical comparisons.

9) Have the preclinical requirements for exception from informed consent been met? 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. There were 0/8 deaths in the HBOC-201 group and only 1/8 (ascaris antigen?) at 96 hours.

Manning et al Paper 2 section 6.

This study used a model of liver injury and 2 hours bleeding to study the relative effects of HBOC-201 or LR in resuscitation, 2 hours survival was 1/10 with LR and 7/8 with HBOC-201. 9/10 with LR died at 36±10 minutes. Blood pressure was held at 60 mmHg during resuscitation instead of 100 mmHg as in RESUS. Fluids were given after 9 minutes. 1 HBOC-201 died during hemorrhage and thus really 7/7 survived. Time to increase pressure back to 60 mmHg for HBOC-201 and 7.4 minutes for LR. Blood loss measured as 40 ml/kg. 100% oxygen used during resuscitation phase. 1/10 survived with LR in comparison to 7/8 with HBOC-201.

Questions to be addressed.
1a) Does the animal model adequately simulate RESUS prehospital trauma setting? The initial hemorrhage and resuscitation phase do. The 2 hour resuscitation is probably too long.

1b) To what extent do the preclinical data support use in humans in the RESUS trial? There was 100% survival during the resuscitation phase with HBOC-201 despite keeping MAP at 60 mmHg. There was 1/10 survival with LR.

2) Is the study well designed? The study was well designed.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? The dosing schedule is to a MAP of 60 mmHg so not fixed volume but fixed low pressure compared to RESUS.

4) Does the type of monitoring simulate the RESUS prehospital trauma setting? The type of monitoring is much more invasive and technical then RESUS prehospital.

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? The best correlate would be pressure.

6) Can all animals entered into the study be accounted for? Yes, all animals are accounted for.

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation? Anesthesia, warming fluid and 100% oxygen are all confounding influences.

8) Is the absence of similar adverse events reported in preclinical studies with HBPC-201 reassuring in terms of the RESUS prehospital trauma setting? Yes, survival after

hemorrhage (during resuscitation) was really 100% if we leave out the 1 that died during the hemorrhage.

9) Have the preclinical requirements for exception from informed consent been met? 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. Survival at 129 minutes was conservatively 7/8 with HBOC-201 and 1/10 with FR.

Manning et al paper 3 section 6

This study as designed to incorporate both the use of an aortic balloon catheter and either HBOC-201 or LR in hemorrhage induced cardiac standstill. 13 pigs underwent hemorrhage induced cardiac standstill, catheter placement and resuscitation. HBOC had 6/6 survive with MAP greater than 60 mmHg for 60 minutes whereas 0/6 survived with LR.

Questions to be addressed.
1a) Does the animal model adequately simulate RESUS prehospital trauma setting? No this is very invasive, highly technical and of very limited scope.

1b) To what extent do the preclinical data support use in humans in the RESUS trial? The survival of 6/6 in this extreme hemorrhage example and technically sophisticated approach is encouraging for RESUS.

2) Is the study well designed? The study is well designed to answer a specific question.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? The dosing to establish a fixed pressure, 60 mmHg, is like RESUS but the endpoints are very different.

4) Does the type of monitoring simulate the RESUS prehospital trauma setting? The whole approach is very technical.

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? MAP is the only prehospital endpoint to be identified.

6) Can all animals entered into the study be accounted for? Yes, all of the animals are accounted for.

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation? This is a very technical protocol and way beyond the RESUS prehospital setting.

8) Is the absence of similar adverse events reported in preclinical studies with HBOC-201 reassuring in terms of the RESUS prehospital trauma setting? The 100% survival is important.

9) Have the preclinical requirements for exception from informed consent been met? 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 The high level of survival is encouraging but this is a ver technical, almost unrelated study.

Handrigan et al Paper 1 section 7.

This study examined the effects of hypotensive resuscitation in awake rats following hemorrhage to MAP of 40 mmHg for 4 hours. Six groups were compared including control hemorrhage; no hemorrhage; HBOC- polyhem; hex; LR and regular pressure resuscitation with LR. LR or Hex with hypotensive resuscitation performed better than control and as well as LR with normal pressure. HBOC performed only as well as control. (There was no hospital arm). The total infusion to keep volume was greatest with LR-normal and least with HBOC. However, HBOC survival no greater than non-resuscitated.

Questions to be addressed.

1a) Does the animal model adequately simulate RESUS prehospital trauma setting? Yes, in that the hemorrhaged occurred in the conscious state and mobile. In addition the endpoint was to infuse fluid to keep MAP at 60 mmHg and then at 80 mmHg (still hypotensive). End point was set pressure not volume.

1b) To what extent do the preclinical data support use in humans in the RESUS trial? The data do not support HBOC-201 in that polyhem did not increase survival although there does not seem to be much mortality early (figure 3) with any treatment.

2) Is the study well designed? The study is well designed with clear blood pressure goals and survival over 24 hours.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? The dosing is to a blood pressure endpoint not volume.

4) Does the type of monitoring simulate the RESUS prehospital trauma setting? Blood pressure and heart rate are monitored as with RESUS.

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? Blood pressure LR plus standard pressure seem to be best. Therefore MAP is important. No new endpoints.

6) Can all animals entered into the study be accounted for? Yes all of the animals are accounted for.

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation? The use of recent surgery and lidocaine may be confounding influences however, the use of conscious animals is very important.

8) Is the absence of similar adverse events reported in preclinical studies with HBPC-201 reassuring in terms of the RESUS prehospital trauma setting? HBOC-201 was not used in these studies only a polyhem from Northfield. Thus the comparisons are different.

9) Have the preclinical requirements for exception from informed consent been met? 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. There do not appear to be any differences in survival early (see figure) and thus neutral. However, mortality was high in polyhem group over 24 hours. Thus not encouraging.

Hayward and Lefer. Paper 2 section 7.

The purpose of this study was to examine the effects of varying amounts of HBOC-201 in a model of traumatic shock in pentobarbital anesthetized rats. Rats were placed in a drum for 500 revolutions and quickly the carotid artery and jugular vein were cannulated. Rats were given 10% HBOC alone (sham), or after trauma 5. 10. 15% HBOC (with comparable withdrawl of blood). Rats were monitored for 5 hours. Control time to 45 mmHg or death was 102+20 minutes. A second group with no hemorrhage was was designed to determine the role of NO (no comment). Administration of HBOC-201 at 5, 10 and 15% of BV enhanced survival although only the 10% was statistically significant (figure 2). 10% HBOC-201 increased survival time to 228±31 minutes. All the HBOCs increased arterial pressure (figure 3).

Questions to be addressed.

1a) Does the animal model adequately simulate RESUS prehospital trauma setting? The animal model represents non-hemorrhagic shock with unknown and unmeasured bleeding. The use of fixed volumes of HBOC is unlike RESUS.

1b) To what extent do the preclinical data support use in humans in the RESUS trial? There was an increase in survival from approximately 100-200 minutes with HBOC.

2) Is the study well designed? The study is well designed to determine the role of No in this model. The trauma studies are straightforward.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? The dosing schedules are to a fixed volume with removal of blood to keep the animal normovolemic.

4) Does the type of monitoring simulate the RESUS prehospital trauma setting? The hemodynamic monitoring is simple MAP and HR, like RESUS.

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? NO, in all cases blood pressure increased but there was only one significant (10% HBOC) in time to death, due to small sample size.

6) Can all animals entered into the study be accounted for? Yes, all animals are accounted for.

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation? Pentobarbital anesthesia and recent surgery are confounding influences.

8) Is the absence of similar adverse events reported in preclinical studies with HBPC-201 reassuring in terms of the RESUS prehospital trauma setting? HBOC-201 to replace fluid which was removed, prolonged survival.

9) Have the preclinical requirements for exception from informed consent been met? 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. The time to death (or 45 mmHg) was prolonged.

Protocol K004-02

Using anesthetized and ventilated pigs, three hemorrhage models were studied: 1) 40% hemorrhage with rectus crush; 2) 55% hemorrhage and rectus crush; and 3) uncontrolled hemorrhage with liver injury. HBOC-201, Hex or no fluid were administered and the animals kept prehospital of 4 hours. Animals were monitored for 72 hours total. All animals were given atropine, but no splenectomies performed. HBOC-201 and Hex were given as 10 ml/kg over 10 minutes and more if MAP< 60mmHg or there was tachycardia. In all animals heart rate is high (140s) and MAP is low (67 mmHg) before hemorrhage.

In model 1, 40% hemorrhage, Less HBOC was required to maintain pressure. 0/8 with HBOC-201 got fluid in the hospital. 8/8 in hex and HBOC-201 survived 4 hours. 8/8 in HBOC, 7/8 in Hex and 5/8 in non survived 72 hours. MVO2 may be non invasive measure if technically simple.

In 55% hemorrhage model, HR was high but MAP 78 before hemorrhage. HBOC required less fluid to restore MAP to guideline, less so with hex and MAP very low with NON (19 mmHg). 72 hours survival 8/8 with HBOC, 6/8 with hex and 2/8 with non. Average survival time is 72, 55 and 19 hours respectively. Four hour survival best with HBOC, less with hex and worst with non (see figure).

In liver injury model, HR high before hemorrhage (atropine). Less fluid used in HBOC group. Blood loss lower in HBOC group. MAP restored by HBOC but not hex or non. Survival to 4 hours was 7/8 in HBOC, 3/8 in hex and 1/8 in non. Survival at 72 hours was 7/8 with HBOC, 1/8 with Hex and 1/8 with non.

Compiled survival across all three protocols was highest for HBOC (96%), 58% for hex and 33% for Non (page 2689).

Questions to be addressed.

1a) Does the animal model adequately simulate RESUS prehospital trauma setting? The animal model with anesthesia, atropine, ventilation does not simulate RESUS. The time course does.

1b) To what extent do the preclinical data support use in humans in the RESUS trial? Survival in each group and across groups for 4 hours with HBOC was equal or better than Hex. Survival to 72 hours was superior with HBOC-201.

2) Is the study well designed? The study is well designed but to look at mechanisms.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? The MAP endpoint above 60 mmHg and reduced tachycardia is less than the 100 mmHg for RESUS.

4) Does the type of monitoring simulate the RESUS prehospital trauma setting? The type of monitoring is more invasive as is the use of anesthesia.

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? Measurement of MVO2 non-invasively may be a useful end-point.

6) Can all animals entered into the study be accounted for? Yes, all animals are accounted for.

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation?The use of anesthetics, ventilator, and especially atropine confound interpretation of the data. The withdrawing of blood from the abdomen in the severe hemorrhage study may confound interpretation of the data.

8) Is the absence of similar adverse events reported in preclinical studies with HBPC-201 reassuring in terms of the RESUS prehospital trauma setting? The survival in the preclinical studies with HBOC both to hospital and for 72 hours is encouraging.

9) Have the preclinical requirements for exception from informed consent been met? 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. The reduced intergroup mortality is encouraging.

Section 8.

This is the most confusing and inconsistent of all the studies. Three groups of studies were performed in anesthetized, ventilated (if needed) with O2 pigs. Uncontrolled abdominal bleeding was created using the iliac artery and vein and hemorrhage controlled to 50% of estimated blood volume. Hemorrhage occurred over 30 minutes, resuscitation to 4 hours and hospital like treatment for 2 more hours. Hemodynamics were recorded and the animals terminated at 6 hours after hemorrhage. In one group no resuscitation was used. In the second group resuscitation to 50 mmHg was used (hypotensive) and in the third resuscitation to normotension performed. Three fluids were administered: a) saline, b) hex, c) HBOC-201 initially at fixed volumes (10 or 5 ml/kg) and then to pressure endpoint. The total blood loss was highest in the normotensive HBOC and Hex groups. Total volume infused was lowest in the hypotensive hem and normotensive hem for HBOC-201 (page 3187).

Survival in the hypotensive hemorrhage group was:
50% for HBOC; 71% in the sal and 87% in the hex groups.
Survival times were 5.1 (sal), 4.6 (HBOC) and 5.9 (Hex) Hours.
Survival in the normotensive hemorrhage groups was:
50% in HBOC, 37.5% in Hex, and 75% in sal
Survival times in norm hemorrhage was:
4 hours in HBOC, 3.3 hours in Hex and 5.2 hours in sal

The authors believe that there are lots of technical problems.

Questions to be addressed.

1a) Does the animal model adequately simulate RESUS prehospital trauma setting? This is a model of uncontrolled abdominal bleeding over 30 minutes and does simulate RESUS. The technical nature of the study (anesthesia) does not simulate RESUS.

1b) To what extent do the preclinical data support use in humans in the RESUS trial? These data do not indicate increased or even (except for small N) the same survival as with Hex or Saline comparted to HBOC.

2) Is the study well designed? The study is well designed.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? The normotensive resuscitation does simulate RESUS whereas the other 2 groups do not.

4) Does the type of monitoring simulate the RESUS prehospital trauma setting? The monitoring is more invasive and the presence of anesthesia is problematic (although necessary).

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? No new

biomarkers are seen. Pressure is slightly better but the survival with HBPC is not better than Saline.

6) Can all animals entered into the study be accounted for? Yes all 59 animals are accounted for.

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation? Anesthesia, some variability in assessing hemorrhage volume as indicated by the authors, the use of ventilation.

8) Is the absence of similar adverse events reported in preclinical studies with HBOC-201 reassuring in terms of the RESUS prehospital trauma setting? These studies do not support the use of HBOC, it is not as good as saline regardless of the potential mechanisms.

9) Have the preclinical requirements for exception from informed consent been met? 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. The lack of equal or enhanced survival with HBOC does not add confidence.

Protocol 9:

This seems to be an update of previous data using severe hemorrhage with liver crush and bleeding to 50 ml/kg . 22 animals were anesthetized and ventilated and instrumented for the measurement of pressures and cardiac output. Blood was removed for 15 minutes and then resuscitation was performed using no fluid, hex or HBOC-201 to 60 minutes, transferred to a hospital like treatment and then for total of 96 hours. The most important data indicate that survival was (page 11 of 289) 8/8 at 24 hours and 7/8 for 96 hours in the HBOC group. On page 33 of 289 mean survival time (minutes) was 21±4.7 in no fluid; 32±2.2 in HES; and 5249±1444 in HBOC-201. Arterial pressure rose above 100 mmHg in the HBOC group but not in the HES group 71 mmHg.

Questions to be addressed.

1a) Does the animal model adequately simulate RESUS prehospital trauma setting? Acute hemorrhage Yes the acute hemorrhage in 15 minutes, 60 minutes to hospital and survival does simulate RESUS. The technical nature of these studies is beyond that of RESUS prehospital.

1b) To what extent do the preclinical data support use in humans in the RESUS trial? Survival at both 24 hours and 96 hours as well as the average survival support the use of HBOC-201.

2) Is the study well designed? The study is well designed although there was some discussion of error in the data sets.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? The dosing schedule is to a fixed volume (6ml/kg/min or 3ml/kg/min) but did increase MAP to 109 mmHg with HBOC as is the goal of RESUS.

4) Does the type of monitoring simulate the RESUS prehospital trauma setting? The monitoring is more invasive.

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? No new endpoints are identified.

6) Can all animals entered into the study be accounted for? Yes, although some difficulty due to early death.

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation? The anesthesia, ventilation and use of O2 does confound interpretation of data. The presence of Ascaris in pigs is a problem in determining the cause of death.

8) Is the absence of similar adverse events reported in preclinical studies with HBOC-201 reassuring in terms of the RESUS prehospital trauma setting? The good survival with HBOC does add confidence to the use of HBOC.

9) Have the preclinical requirements for exception from informed consent been met? 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. The low mortality with HBOC is important.

Protocol 10.

This is a project using 17 pigs to examine the relative effects of lactated rings or HBPC-201 in resuscitation of pigs after severe hemorrhage caused by laceration of the liver. Hemorrhage occurred over 9 minutes and resuscitation was continued for another 120 minutes. HBOC was initially given as fixed amount there after LR and HBOC were administered to keep MAP above 60 mmHg. The pigs were anesthetized, paralyzed and warmed. They were ventilated with 100% O2. The amount of hemorrhage resulted in an estimated 50% loss of blood volume. Infusion of HBOC increased MAP to 60 mmHg in 1.6 minutes whereas infusion of LR took 7.4 minutes to increase pressure to that same level. Only 1/10 pigs given LR survived 129 minutes, while 7/7 pigs given HBOC survived (page 40 of 165). Heart rate was very high (page 37 of 165).

Questions to be addressed.

1a) Does the animal model adequately simulate RESUS prehospital trauma setting? Yes, although the technical measurements are more sophisticated.

1b) To what extent do the preclinical data support use in humans in the RESUS trial? The increased survival to 129 minutes support use of HBOC in humans.

2) Is the study well designed? Yes it is well designed.

3) Do the dosing schedules and rates of administration simulate the RESUS prehospital trauma setting? The dosing schedule is initially to a fixed volume and to maintain MAP above 60 mmHg. The pressure goal is less then RESUS.

4) Does the type of monitoring simulate the RESUS prehospital trauma setting? The type of monitoring is more invasive than RESUS.

5) Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital trauma setting? No new endpoints were examined nor was survival beyond 129 minutes.

6) Can all animals entered into the study be accounted for? No new endpoints were evident.

7) Are there aspects of the model that confound interpretation and/or relevance to the prehospital resuscitation? The use of paralytics, anesthesia and ventilation along with 100% O2 is problematic.

8) Is the absence of similar adverse events reported in preclinical studies with HBOC-201 reassuring in terms of the RESUS prehospital trauma setting? The increased survival to 129 minutes with HBOOC is important.

9) Have the preclinical requirements for exception from informed consent been met? 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. The increased survival to 129 minutes support tat HBOC may be useful.