Summary of a review of preclinical studies of the use of HBOC-201 in the treatment of hemorrhagic shock.

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Biopure, producer of a glutaraldehyde-polymerized bovine hemoglobin, has come forward with a request for permission to conduct a national clinical trial of its product, HBOC-201 in prehospital trauma patients under the waver of informed consent mechanism. The FDA has had the product on clinical hold for two years because of excess hypertension, myocardial infarctions, strokes, heart failure, cardiac arrests, hypoxemia, and oliguria in previous clinical trials. The company presents a series of animal trials as evidence that the material can be lifesaving in hemorrhagic shock. Studies that address the known toxicities of hemoglobin solutions are conspicuously absent.

Hemorrhagic shock is the second most common cause of death from injury in both civilian and military experience. In civilian experience, about half of all deaths occur in the field. The range of mechanisms and energies associated with vehicular, industrial, penetrating, and fall-related injuries means that some casualties die instantly or very quickly after injury of wounds that are unlikely to respond to first aid. Many others will need care but such care can be delayed for hours without threat to life. For those in between, the size of the deficit in vascular integrity determines the rate of blood loss and the time of onset of shock and death. The distribution of injury size in a trauma population is continuous but with many more minor injuries than severe ones.

Surgical trauma models attempt to capture a "representative" point on the continuum of injury severity. In the article by King, Cohn & Proctor (J Trauma 2005; 59:553-562) fluid percussion of the brain and volume removal were used to model the situation of simultaneous acute brain injury and ongoing severe bleeding. At the modeled levels of injury severity, removed volume, and resuscitation used, the combination of Ringer's lactate and the HBOC did restore mean blood pressure and maintain perfusion. Under the same conditions, the HBOC alone did not increase perfusion and Ringer's lactate followed by blood and mannitol infusion did not restore pressure. However, the model required giving Ringer's continuously while the blood pressure was low. This led to the administration of fluid equal to 30% of body weight, equivalent to more than 5 gallons of fluid in a 150 pound human. This fluid administration led to hemodilution and brain swelling, preventing the animals from getting off the ventilator and leading to their early euthanasia. Thus, while the model provided an objective measure of the effect of three modes of treatment, it seems biased in defining the "standard of care" as an unmodulated administration of Ringer's, mannitol and blood that led to massive brain swelling.

The demonstration that a treatment such as an HBOC can work in a narrowly defined clinical situation is not the same as showing that it does work when used more generally in situations that cannot be clinically differentiated from the situation studied. This is the difference between efficacy and effectiveness. Injury in populations is a continuum, bleeding in individuals is frequently ongoing, field resuscitation resources are necessarily

limited, and the dynamics of perfusion and shock in patients are constantly changing. It is hard to know how the treatments would work in the face of ongoing hemorrhage, when the relationship between volume lost and volume administered is confused and clinical signs such as blood pressure and heart rate are altered by the drug.

In the real world of trauma care, the fraction of patients that will benefit from such treatment is probably small, a few percent at best. However, the presence of injury and hypovolemia will raise the fraction of patients having bad reactions to the HBOC by creating unusual patterns of altered perfusion, bacteremia, endotoxemia, and inflammation. Thus, the model probably overestimates benefit and previous clinical experience almost certainly underestimates the toxicity of these products. The end result is that the net benefit of giving an HBOC is likely to be less than the toxicity observed in the earlier human trials and far less then their toxicity when given to trauma patients.

I have read the papers in the binders provided by the FDA and find that the studies presented in support of the product do demonstrate that the product can work under restricted circumstances. The studies also show moderate vasoactivity, with substantial reductions in cardiac output under a variety of situations that delay correction of lactic acidemia. None of the studies explore the safety of the materials in the presence of bacteremia or endotoxemia or look at co-existing brain injury in useful ways.

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Papers on the Wilford Hall Model:

1. Henderson CL, Anderson CM, Sorrells DL, Wilson BJ, Dick EJ, DiGeronimo RJ. The use of a hemoglobin-based oxygen-carrying solution (HBOC-201) for extracorporeal membrane oxygenation in a porcine model with acute respiratory distress syndrome. Pediatric Critical Care Medicine 2004; 5:384-90.
2. McNeil JD, Smith DL, Jenkins DH, York GB, Josephs JD. Hypotensive resuscitation using a polymerized bovine hemoglobin-based oxygen-carrying solution (HBOC-210) leads to reversal of anaerobic metabolism. J Trauma 2001; 50:1063-1075.
3. Fitzpatrick CM, Savage SA, Kerby JD, Clouse WD, Kashyap VS. Resuscitation with a blood substitute causes vasoconstriction without nitric oxide scavenging in a model of arterial hemorrhage. J Am Coll Surg 2004; 199:693-701.
4. Sampson JB, Davis MR, Mueller DL, Kashyap VS, Jenkins DH, Kerby JD. A comparison of hemoglobin-based oxygen carrier HBOC-201 to other low-volume resuscitation fluids in a model of controlled hemorrhagic shock. J Trauma 2003; 55:747-754.
5. York GB, Eggers JS, Smith DL, Jenins DH, McNeil JD, Mueller D, Josephs JD, Kerby JD. Low-volume resuscitation with a polymerized bovine hemoglobin-based oxygen-carrying solution (HBOC-201) provides adequate tissue oxygenation for survival in a porcine model of controlled hemorrhage. J Trauma 2003; 55:873-885.

1. Does the animal model adequately simulate the RESUS prehospital trauma setting? No. The model is a fixed-endpoint (MBP=30) blood removal and variable volume resuscitation lab experiment. In the field, injuries and blood loss will be highly variable, and for the majority of seriously injured casualties, bleeding will be ongoing. Resuscitation in the field will be limited by problems with vascular access and potentially by limited amounts of fluid.
2. To what extent do the preclinical data support use in humans in the RESUS trail? Known toxicities of hemoglobins, such as vasoconstriction with reduced cardiac output were seen in all uses of the HBOC. In low dose this was associated with a v. fib. death and at higher doses with liver injury and oliguria. Experiments are short term and in young animals.
3. Is the study well designed? The studies are designed as a demonstration, not as an investigation. Having found doses of the HBOC that worked and doses that did not work, they compared the doses of HBOC that did work with discredited ways of using Ringer's lactate or Hypertonic Saline.
4. Does the type of monitoring simulate the RESUS prehospital trauma setting? The authors specifically state (abstract: "when HBOC-201 is administered as a primary resuscitation fluid in hypotensive protocols, common clinical markers for determining adequacy of resuscitation may not be useful."
5. Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital setting? No,

they found a way to use the drug in a "controlled hemorrhagic shock" model that was associated with good outcome. Most of the other fluids they tried had equally good outcomes. They claimed an advantage for the low volumes of HBOC but did not try the other fluids in equivalent low volumes.

6. Can all animals in the study be accounted for? Animal numbers are accountable in all of the published studies.
7. Are there aspects of the model that confound interpretation and/or relevance to prehospital resuscitation? As mentioned, this is a model of controlled bleeding and resuscitation whereas the major problem of field medicine is that severe bleeding is usually uncontrolled.
8. Biopure's Biologics Licensing Application (BLA) for use of HBOC-201 in elective surgery reported individual line listings of adverse events in each of it Phase 2 and Phase 3 clinical trials. Imbalances against the test product, some of which were statistically significant, were evident for many of these events which included hypertension, myocardial infarction, stroke, heart failure, cardiac arrest, hypoxemia, and oliguria. Is the absence of similar adverse events reported in preclinical studies with HBOC-210 reassuring in terms of the RESUS prehospital trauma setting? No these are juvenile animals with excellent cardiovascular reserve.
9. Have the preclinical requirements for exception from informed consent been met? Not to my mind.

Papers on the Ken Proctor Models:

1. King DR, Cohn SM, Proctor KG. Resuscitation with a hemoglobin-based oxygen carrier after traumatic brain injury. J Trauma 2005; 59:553-562.
2. Malhotra AK, Kelly ME, Miller PR, Hartman JC, Fabian TC, Proctor KG. Resuscitation with a novel hemoglobin-based oxygen carrier in a swine model of uncontrolled perioperative hemorrhage. J Trauma 2003; 54:915-924.
3. Malhotra AK, Schweitzer JB, Fox JL, Fabian TC, Procter KG. Cerebral perfusion pressure elevation with oxygen-carrying pressor after traumatic brain injury and hypotension in swine. J Trauma 204: 56:1049-1057.
4. Maxwell RA, Gibson JB, Fabian TC, Procter KG. Resuscitation of severe chest trauma with four different hemoglobin-based oxygen-carrying solutions.

1. Does the animal model adequately simulate the RESUS prehospital trauma setting? The papers by Proctor describe good scientific investigations and the data are well reported and thoughtfully evaluated. Only the most recent paper, #1 above, constitutes a formal trial of a model. Its results are scientifically interesting and clinically useful as an example of what not to do. In the model, animals are simultaneously brain injured and blood volume withdrawn to simulate the common situation of brain injury and hemorrhagic shock. The HBOC increases blood pressure to increase cerebral perfusion pressure but does not lead to additional bleeding because it is not a free hemorrhage model. The standard of care arm replaces about two liters of blood loss with about 20 liters of Ringer's replacement solution leading to brain swelling and a poor outcome. I do not think

that this represents either the standard of care or even a useful study design except to demonstrate what happens if one treats to an unachieveable goal.

2. To what extent do the preclinical data support use in humans in the RESUS trail? Models do not address the questions of safety.
3. Is the study well designed? It is a good physiologic study that points out what can happen.
4. Does the type of monitoring simulate the RESUS prehospital trauma setting? No
5. Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital setting? No
6. Can all animals in the study be accounted for? Yes
7. Are there aspects of the model that confound interpretation and/or relevance to prehospital resuscitation? Yes, the controlled hemorrhage.
8. Biopure's Biologics Licensing Application (BLA) for use of HBOC-201 in elective surgery reported individual line listings of adverse events in each of it Phase 2 and Phase 3 clinical trials. Imbalances against the test product, some of which were statistically significant, were evident for many of these events which included hypertension, myocardial infarction, stroke, heart failure, cardiac arrest, hypoxemia, and oliguria. Is the absence of similar adverse events reported in preclinical studies with HBOC-210 reassuring in terms of the RESUS prehospital trauma setting? The juvenile animals in the study are a poor model for cardiovascular hemoglobin toxicity and the other toxicities were generally not assessed.
9. Have the preclinical requirements for exception from informed consent been met? Not to my mind.

Papers related to the Freilich Model:

1. Gurney J, Philbin N, Rice J, Arnaud F, Dong F, Wulster-Radcliffe M, Pearce B, Kaplan L, McCarron R, Freilich D. A hemoglobin based oxygen carrier, bovine polymerized hemoglobin (HBOC-201) versus hetastarch (HEX) in an uncontrolled liver injury hemorrhagic shock swine model of delayed evacuation. J Trauma 2004; 57:726-738.
2. Arnaud F, Hammett M, Asher L, Philbin N, Rice J, Dong F, Pearce B, Flournoy WS, Nicholson C, McCarron R, Freilich D. Effects of bovine polymerized hemoglobin on coagulation in controlled hemorrhagic shock in swine. Shock 2005; 24:145-152.
3. Dong F, Hall CH, Golich SA, Philbin NB, Rice JP, Gourney J, Arnaud FG, Hammett M, Ma X, Flourney WS, Hong J, Kaplan LJ, Pearce LB, McGwin G, Ahlers S, McCarron R, Freilich D. Immune effects of resuscitation with HBOC-201, a hemoglobin-based oxygen carrier, in swine with moderately severe hemorrhagic shock from controlled hemorrhage. Shock 2006; 25:50-55.
4. Philbin N, Rice J, Gurney J, McGwin G, Arnaud F, Dong F, Johnson T, Flournoy WS, Ahlers S, Pearce LB, McCarron R, Freilich D. A hemoglobin-based oxygen carrier, bovine polymerized hemoglobin (HBOC-201) vers hetastarch (HEX) in a

moderate severity hemorrhagic shock swine model with delayed evacuation. Resuscitation 2005; 66:367-378.

5. Johnson T, Arnaud F, Dong F, Philbin N, Rice J, Asher L, Arrisueno M, Warndorf M, Gurney J, McGwin G, Kaplan L, Flourney S, Apple FS, Pearce LB, Ahlers S, McCarron R, Freilich D. Bovine polymerized hemoglobin (hemoglobin-based oxygen carrier-201) resuscitation in three swine models of hemorrhagic shock with military relevant delayed evacuation – Effects on histopathology and organ function. Crit Care Med 2006; 34:

1. Does the animal model adequately simulate the RESUS prehospital trauma setting? The trial reported in the lead paper is not interpretable. The data as presented in the paper are not internally consistent (see Table 1, the hemorrhage volumes do not correspond to the EBV % s) but they suggest that the trial was not well conducted. The weights of the animals in the HEX group varied markedly (SEM was 44% of the mean, meaning the SD had to be greater than 100% of the mean) and the hemorrhage volumes in the major treatment group is more than a quarter less than the controls. This means that HBOC treated animals lost 25 or 26% of their blood volume and were in class II shock while the control groups lost 32 or 35 or 38% of their blood volumes and were in class III shock.
2. To what extent do the preclinical data support use in humans in the RESUS trail? It does not
3. Is the study well designed? It wasn't carried out as designed.
4. Does the type of monitoring simulate the RESUS prehospital trauma setting? No. Invasive hemodynamics are not available in the field.
5. Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital setting? No.
6. Can all animals in the study be accounted for? Probably
7. Are there aspects of the model that confound interpretation and/or relevance to prehospital resuscitation? Lack of consistent data.
8. Biopure's Biologics Licensing Application (BLA) for use of HBOC-201 in elective surgery reported individual line listings of adverse events in each of it Phase 2 and Phase 3 clinical trials. Imbalances against the test product, some of which were statistically significant, were evident for many of these events which included hypertension, myocardial infarction, stroke, heart failure, cardiac arrest, hypoxemia, and oliguria. Is the absence of similar adverse events reported in preclinical studies with HBOC-210 reassuring in terms of the RESUS prehospital trauma setting? Young animals and not comparable groups.
9. Have the preclinical requirements for exception from informed consent been met? No.

Model of Geoffrey Manley

1. Knudson MM, Lee Seong, Erickson V, Morabito D, Derugin N, Manley GT. Tissue oxygen monitoring during hemorrhagic shock and resuscitation: a comparison of lactated Ringer's solution hypertonic saline dextran, and HBOC-201. J Trauma 2003; 54:242-252.
2. Lee SK, Morabito D, Hemphill JC, Erickson V, Holcroft JJ, Derugin N, Knudson MM, Manley GT. Small-volume resuscitation with HBOC-201: Effects on cardiovascular parameters and brain tissue oxygen tension in an out-of-hospital model of hemorrhage in swine. Acad Emerg Med 2002; 9:969-976.
3. Manley GT, Hemphill JC, Morabito D, Erickson V, Holcroft JJ, Derugin N, Knudson MM. Small-volume resuscitation with hemoglobin substitute HBOC-201: Effect on brain tissue oxygenation. Pages 311-317 in Dunn and Swartz, Eds; Oxygen Transport to Tissue XXIV, Kluwer Academic/Plenum Publishers, 2003.

1. Does the animal model adequately simulate the RESUS prehospital trauma setting? No. Low severity, fixed pressure model in healthy young animals.
2. To what extent do the preclinical data support use in humans in the RESUS trial? No. Data show that low volume HBOC does not improve cardiac output despite higher blood pressures.
3. Is the study well designed? The study shows that in the face of moderate shock and several forms of resuscitation that polarographic liver PO2 and muscle PO2 are the same.
4. Does the type of monitoring simulate the RESUS prehospital trauma setting? No. Paper is about invasive oxygen monitoring.
5. Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital setting? We are not going to use invasive oxygen monitoring in the field. Polarographic tissue PO2 monitoring gives different results than other types of monitoring such as palladium mesoporphyrin fluorescence quenching.
6. Can all animals in the study be accounted for? It appears so.
7. Are there aspects of the model that confound interpretation and/or relevance to prehospital resuscitation? Short term, mild shock, young animals.
8. Biopure's Biologics Licensing Application (BLA) for use of HBOC-201 in elective surgery reported individual line listings of adverse events in each of it Phase 2 and Phase 3 clinical trials. Imbalances against the test product, some of which were statistically significant, were evident for many of these events which included hypertension, myocardial infarction, stroke, heart failure, cardiac arrest, hypoxemia, and oliguria. Is the absence of similar adverse events reported in preclinical studies with HBOC-210 reassuring in terms of the RESUS prehospital trauma setting? Study uses mild shock and young healthy animals.

9. Have the preclinical requirements for exception from informed consent been met? Not in my mind.

Model of the Carolina Resuscitation Research Group

1. Katz LM, Manning JE, McCurdy SL, Pearce LB, Gawryl MS, Wang Y, Brown C, Carolina Resuscitation Research Group. HBOC-201 improves survival in a swine model of hemorrhagic shock and liver injury. Resuscitation 2002; 54:77-87.
2. Manning JE, Katz LM, Brownstein MR, Pearce LB, Gawryl MS, Baker CC, and the Carolina Resuscitation Research Group. Bovine hemoglobin –based oxygen carrier(HBOC-201) for resuscitation of uncontrolled, exsanguinating liver injury in swine. Shock 2000; 13:152-159.
3. Manning JE, Katz LM, Pearce LB, Batson N, McCurdy SL, Gawryl MS, Baker CC. Selective aortic arch perfusion with hemoglobin-based oxygen carrier-201 for resuscitation from exsanguinating cardiac arrest in swine. Crit Care Med 2001; 29:20672074.

1. Does the animal model adequately simulate the RESUS prehospital trauma setting? Not really. The models combine massive initial blood loss which is fatal in 30 minutes in the untreated controls with massive fluid replacement which may not be possible in the field. The end result is a model which rapidly exchanges the blood volume for asanquinous fluid which is rapidly fatal in the absence of oxygen carrying capacity. In the RESUS trial, these patients will die before care arrives.
2. To what extent do the preclinical data support use in humans in the RESUS trail? They show that an oxygen carrier can potentially be lifesaving in a small group of patients.
3. Is the study well designed? The study confirms what we already know, that conventional fluids do not work in the face of massive hemorrhagic injury, leading to rapid hemodilution and death.
4. Does the type of monitoring simulate the RESUS prehospital trauma setting? Invasive cardiac monitoring used throughout.
5. Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital setting? No.
6. Can all animals in the study be accounted for? Yes
7. Are there aspects of the model that confound interpretation and/or relevance to prehospital resuscitation? The model starts with instrumented animals. It is unlikely that an EMT is going to be able to start an IV on a patient that has lost 50 ml/kg as such patients are in profound class IV shock with every vessel collapsed.
8. Biopure's Biologics Licensing Application (BLA) for use of HBOC-201 in elective surgery reported individual line listings of adverse events in each of it Phase 2 and Phase 3 clinical trials. Imbalances

against the test product, some of which were statistically significant, were evident for many of these events which included hypertension, myocardial infarction, stroke, heart failure, cardiac arrest, hypoxemia, and oliguria. Is the absence of similar adverse events reported in preclinical studies with HBOC-210 reassuring in terms of the RESUS prehospital trauma setting? Again, young animals with short-term followup.

9. Have the preclinical requirements for exception from informed consent been met? No.

Models of resuscitation in rats

1. Handrigan MT, Bentley TB, Oliver JD, Tabaku LS, Burge JR, Atkins JL. Choice of fluid influences outcome in prolonged hypotensive resuscitation after hemorrhage in awake rates. Shock 2005; 23:337-343.
2. Hayward R, Lefer AM. Administration of polymerizd bovine hemoglobin improves survival in a rat model of traumatic shock. Methods Find Exp Clin Pharmacol 1999; 21:427-433.

1. Does the animal model adequately simulate the RESUS prehospital trauma setting? There are two different models in the two papers above, with the Handrigan paper using moderate hemorrhage and hypotensive resuscitation with a long follow-up while the Hayward paper uses Noble-Collip drum shock. The Handrigan paper is the more relevant to the questions at hand but was designed to look at the problems of maintaining soldiers injured in isolated locations with very long evacuation times.
2. To what extent do the preclinical data support use in humans in the RESUS trial? They do not. The Handrigan study shows the worst outcome with the HBOC. The Hayward paper shows a survival benefit only with the middle dose, a result that would be hard to duplicate in the field with continued bleeding.
3. Is the study well designed? The Hayward study is underpowered.
4. Does the type of monitoring simulate the RESUS prehospital trauma setting? The Handrigan study used death as an endpoint. This will be possible in the RESUS study.
5. Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital setting? Neither study does identify clinically useful endpoints.
6. Can all animals in the study be accounted for?
7. Are there aspects of the model that confound interpretation and/or relevance to prehospital resuscitation? Long treatment times in Handrigan and not a hemorrhage model in Hayward.
8. Biopure's Biologics Licensing Application (BLA) for use of HBOC-201 in elective surgery reported individual line listings of adverse events in each of it Phase 2 and Phase 3 clinical trials. Imbalances against the test

product, some of which were statistically significant, were evident for many of these events which included hypertension, myocardial infarction, stroke, heart failure, cardiac arrest, hypoxemia, and oliguria. Is the absence of similar adverse events reported in preclinical studies with HBOC-210 reassuring in terms of the RESUS prehospital trauma setting? Again young animals and short-term models.

9. Have the preclinical requirements for exception from informed consent been met? Not in these studies.

Overview of all of the published studies:

1. Do the animal models adequately simulate the RESUS prehospital trauma setting? No. All of the models are simplifications, usually representing severe hemorrhage, but in healthy young animals with a high degree of cardiovascular reserve.
2. To what extent do the preclinical data support use in humans in the RESUS trail? The models are inadequate in that they do not explore the limits of safe dosing or situations in which poor outcomes occurred.
3. Is the study well designed? The animal studies are at times thoughtfully designed as in the earlier papers by Proctor in Memphis. However, the article by King, Cohn & Proctor mentioned above ignored their earlier work suggesting that mild pressors improve outcome and used a gross simplification of the standard of care that resulted in the administration of Ringer's lactate equal to 30% of body weight with resulting brain swelling that clearly biased the outcome.
4. Does the type of monitoring simulate the RESUS prehospital trauma setting? No. In fact several of the articles mentioned that the vasoconstrictive activity of the HBOC made conventional monitoring unuseful.
5. Does the experiment identify endpoints and/or biomarkers that correlate with favorable outcome and which can be used in the RESUS prehospital setting? No. None of the studies explored or evaluated non-invasive endpoints for their relationship to outcome.
6. Can all animals in the studies be accounted for? Generally yes, but arbitrary endpoints, such as euthanizing the animals that could not be weaned at 6 hours make the studies less useful.
7. Are there aspects of the models that confound interpretation and/or relevance to prehospital resuscitation? The fixed volume or fixed pressure nature of many of the models does not duplicate the human trauma population with broad range of injuries. Even some of the "free" liver hemorrhage models are manipulated to control rates of volume loss.
8. Biopure's Biologics Licensing Application (BLA) for use of HBOC-201 in elective surgery reported individual line listings of adverse events in each of it Phase 2 and Phase 3 clinical trials. Imbalances against the test product, some of which were statistically significant, were evident for many of these events which included hypertension, myocardial infarction, stroke, heart failure, cardiac arrest, hypoxemia, and oliguria. Is the absence of similar adverse events reported in

preclinical studies with HBOC-210 reassuring in terms of the RESUS prehospital trauma setting? Healthy young animals are at reduced risk for the vascular complications. Exposure to bacterial infections or endotoxemia were not tested. The brain injury model is given massive quantities of Ringer's in the control arms so the safety of the HBOC is not compared to a reasonable control.

9. Have the preclinical requirements for exception from informed consent been met? No. The animal models have not tested the product for many of the known toxicities.