Hemoglobin-based Oxygen Carriers

Current Status and Future Directions

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SEMISYNTHETIC or synthetic preparations of hemoglobin, now termed hemoglobin-based oxygen carriers (HBOCs), have been in development as an alternative to erythrocytes for several decades. Nonclinical and clinical studies of HBOCs have raised questions about their safety. Similarity of the serious adverse event profiles among these products has raised questions regarding the possibility of common underlying mechanism(s) of toxicity despite differences among these molecular preparations. The observed serious adverse events have presented an obstacle to development because they raise concerns about the relative benefit/risk of these biologics.

A conference sponsored by the Food and Drug Administration (FDA) and National Institutes of Health (NIH) was held April 29–30, 2008 to (1) review the existing publicly available information about the characteristics and clinical profiles of the products that are or were in development; (2) discuss the potential mechanisms of toxicity both for the whole organism or by specific organ system(s); and (3) consider the feasibility of, obstacles to, and ethical issues related to future clinical trials of HBOCs.

This report summarizes the scientific presentations and opinions of the speakers at the conference. A transcript of the meeting is available.‡§ Appendix 1 contains a list of the members of the Planning Committee. Appendix 2 contains a list of the Speakers. Appendix 3 summarizes information about conflict of interest available to the FDA at the time of the workshop.

Overview of the Workshop

The workshop was divided into four sessions. Session I provided a forum for a scientific overview of oxygen physiology, general biochemical and physiologic characteristics of HBOCs, nitric oxide physiology, and characteristics of nonclinical studies of HBOCs. Presentations in Session II provided an overview of current information about the safety and efficacy of HBOCs that have been evaluated in clinical trials, the regulatory and ethical framework for clinical trials of HBOCs, and unresolved issues related to publicly available information from commercially sponsored studies of HBOCs. FDA and industry representatives discussed data from clinical trials of HBOCs that are publicly available from peer-reviewed literature, press releases, or presentations at public meetings. Session III was divided into two parts. In the first part, functional aspects of HBOCs as a class of therapeutic agents were discussed, with particular emphasis on common clinical findings, need for additional research into mechanisms of toxicity, clinical trial design issues, and clinical settings where benefit might outweigh safety issues. In the second portion, organ-specific aspects of HBOC safety were addressed. Panelists discussed possible mechanisms for observed safety issues and the pros and cons of sensitive and specific biomarkers for evaluation of organ-specific toxicity. Session IV was devoted to seeking a way forward in development of HBOCs. Presentations were made on new modifications of HBOCs, effects of HBOCs on the microvasculature, molecular biologic reengineering of HBOCs to achieve desired physiologic effects, development of preclinical models, and focused clinical trial designs were discussed. Panelists then discussed ethics, the feasibility of designing new molecules, various clinical trial designs, and the need for HBOCs in civilian and military settings and in ex-U.S. sites.

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‡ Day 1 (April 29, 2008): Available at: www.fda.gov/BiologicsBloodVaccines/NewsEvents/WorkshopsMeetingsConferences/ucm092010.htm. Updated May 19, 2009. Accessed July 6, 2009.

§ Day 2 (April 30, 2008): Available at: www.fda.gov/BiologicsBloodVaccines/NewsEvents/WorkshopsMeetingsConferences/ucm106954.htm. Updated May 19, 2009. Accessed July 6, 2009.

Session I: Setting the Stage

Moderator: Joseph Fratantoni, M.D., Vice President Medical and Clinical Development, Maxcyte, Inc., Gaithersburg, Maryland.

Speakers noted that regulation of oxygen transport/oxygen physiology, including physiologically appropriate oxygen affinity and cooperativity, and hypoxic vasodilatation appear to be fundamental to appreciating the complexities of HBOCs and to understanding how a patient's organ and whole body physiology adjust to hypoxia. Hypotheses with varying degrees of experimental validation have been published to explain the phenomenon of hypoxic vasodilatation including (1) release of adenosine triphosphate (ATP) and binding of ATP to an endothelial cell receptor, resulting in increased circulating nitric oxide and vasodilatation, (2) allosteric release of nitric oxide by sulfhydryl-linked nitric oxide that occurs as the red blood cell (RBC) deoxygenates,[1] (3) partially oxygen-saturated hemoglobin acting as a nitrite reductase,[2] and (4) metabolic autoregulation of blood flow.

Nitric oxide, a major systemic vasodilator, is a short-lived molecule that is produced by, among other cell types, endothelial cells, and it is released and diffuses both luminally and abluminally. The importance of nitric oxide in modulating downstream effects of acellular hemoglobin was discussed. Some investigators have challenged long-held assumptions that nitric oxide scavenging by HBOC results in impaired blood flow and that oxygen binding of HBOC should match that of RBC.[3,4] They hypothesize that the presence of low-oxygen-affinity hemoglobin intraluminally disrupts the oxygen gradient from red blood cell to vessel wall and leads to vasoconstriction. Other researchers have emphasized the extent to which the heme moiety in acellular hemoglobin is subject to oxidation. Spontaneous oxidation of hemoglobin within the protective environment of the red blood cell is effectively reversed by naturally occurring intracellular reducing agents. Outside of the red blood cell, however, hemoglobin undergoes spontaneous oxidation and is subject to the effects of oxidant agents such as hydrogen peroxide and the effects of interaction with nitric oxide. Chemical modifications to the hemoglobin can lead to unexpected and significant changes in susceptibility to oxidation and thereby to destabilization of the product. Protective antioxidant strategies have recently been a focus of the HBOC community.

Conventional single and repeated-dose safety and toxicity testing of the various HBOCs in preclinical animal models have not been able to predict the adverse outcomes frequently observed in clinical trials. Evaluation of HBOCs to date has been performed in two environments: (1) conventional safety testing in healthy normal animals under good laboratory practice-compliant conditions; (2) "academic" or efficacy testing settings that are performed under non-good laboratory practice and non-International Conference on Harmonzation (ICH) standards.[5-8] The effects observed in conventional studies conducted in healthy, normal (inbred) young animals do not mimic those of clinical tests of HBOCs, many of which have been conducted in populations with elderly, diabetic, atherosclerotic, hypertensive patients with significant comorbid conditions who are undergoing elective surgical procedures. A prominent feature of these populations and conditions is the presence of endothelial dysfunction, including impaired nitric oxide response and exaggerated endothelin response.[9-11] Introduction of an HBOC into the circulation likely worsens preexisting endothelial dysfunction, probably by exacerbation of the impaired nitric oxide availability. Limitations of conventional animal toxicity studies include feasibility and applicability of dose escalation and repeat dosing, failure to detect infrequent events, and failure to establish possible synergy of HBOCs with preexisting disease.

Session II: Clinical Framework and Current Data

Moderator: Barbara Alving, M.D., M.A.C.P., National Center for Research Resources, National Institutes of Health, Bethesda, Maryland.

Some unresolved issues that have hampered the development of HBOCs have included difficulty in defining clinical benefit and defining and assessing clinically meaningful, readily measurable efficacy endpoints, understanding certain safety parameters such that a safe dose of products can be administered, and defining an acceptable benefit:risk profile for each clinical indication in studies where subjects provide informed consent and in studies where informed consent cannot be obtained. Table 1 contains publicly available information about the physicochemical characteristics of eight commercially sponsored HBOCs and a listing of clinical situations in which these HBOCs were evaluated.

Publicly available information (peer-reviewed journals, press releases, meeting presentations, *etc.*) on the safety of HBOC products studied in a variety of clinical settings was presented. Of the eight commercial products, data were available in the public domain for six. The safety data (table 2) formed the basis for subsequent discussions during the workshop. Important caveats regarding these data are described in the footnote.

To place existing clinical trial data into context for further discussion, the concept of risks and benefits was introduced in the framework of general ethical considerations and two FDA regulations (Code of Federal Regulations, CFR) governing investigational use of an unapproved drug product (21 CFR 312) and investigational use of an unapproved drug product in situations when obtaining informed consent is not possible (21 CFR 50.24). The latter recognizes the need for such research when it is necessary to obtain the required data. Under one ethical framework devised by Drs. Emanuel, Wendler, and Grady, there are a number of hierarchically important and universal requirements that must be fulfilled for clinical research to be ethically acceptable.[12] These include the need first for the research to have

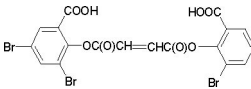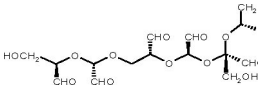**Table 1. Biochemical and Biophysical Properties of Hemoglobin-based Oxygen Carriers in Development**
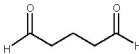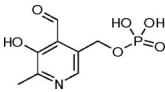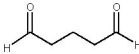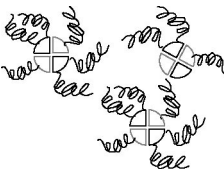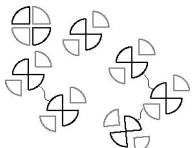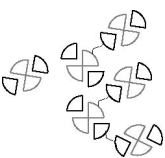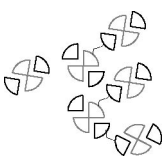
Hemoglobin Name, Brand Name, Company Source	Molecular Size (kDa)	% Tetramer	Chemical Modifier
HbA$_0$ (Normal Human Hb)	64	100 (dissociates when acellular)	None
DCLHb (Hemassist®) Baxter (development terminated)	64[52]	96–98 (2–4% ditetramer) (≤1% unmodified tetramer)[53,54]	Bis-(3,5-dibromosalicyl) fumarate αα–intramolecular crosslinking
MP4 (Hemospan®) Sangart	95[55]	0	Maleimide PEG No intramolecular crosslinking Surface conjugation/polymerization (PEG = polyethylene glycol #6 (5,000 kDa))
Pyridoxylated Hb POE – conjugate (PHP) + Catalase & SOD Apex Biosciences	106 (peak average molecular weight by size exclusion chromatography[53,57]	0 (<0.5% unmodified tetramer)[53]	– pyridoxal-phosphate crosslinking α-carboxymethyl,ω-carboymethoxy-polyoxyethylene Pegylation 16% polymerization between PEG-Hb tetramers 90% catalase and SOD activity of lysed RBCs
O-R-PolyHbA$_0$ (Hemolink®) Hemosol (development terminated)	32 to >500[58,59]	34–42% stabilized tetramer and 54–62% polymers (5% unstabilized tetramer)[58,59]	O-raffinose β intramolecular/intermolecular crosslinking
PolyBvHb (Hemopure®) Biopure Bovine Hb	130–500[58,61]	<5% stabilized/unstabilized tetramer[58]	Glutaraldehyde αα intramolecular/intermolecular crosslinking
PolyHb (Polyheme®) Northfield Human Hb	Average 250[62]	<1%[17,58]	Pyridoxal-phosphate intramolecular crosslinking Glutaraldehyde intermolecular crosslinking
rHb1.1 (Optro®) Somatogen (development terminated)	64[54]	—	Fused aα globin chains αArg141 > Gly mutation (c-terminal fused to βVal1 > Gly1 (N-terminal) β Asn-108 > Lys 108 mutation (function- increases P$_{50}$ to 30 mmHg)
PEG-Hemoglobin Enzon (development terminated)	~130	NA	Succinimidyl carbonate PEG-modified bovine Hb No intramolecular crosslinking Surface conjugation/polymerization (PEG = polyethylene glycol #12 (5000 kDa))

Table 1. Continued

General Structure(s)	Hemoglobin Concentration and Solution	P50 (mmHg)	Clinical Studies
		26.6[49,50] (< 10 when acellular, dissociated)[51]	
	10% in modified LR[53,54]	32[53]	Normal volunteer, cardiac surgery, high–blood loss surgery, perioperative use, elective abdominal aortic aneurysm repair, stroke, critical illness, renal dialysis, US (in-hospital) trauma, European (prehospital) trauma
	4.4% in modified LR[13,55,56]	5*[55]	Normal volunteer, orthopedic surgery, prostatectomy, prevention of hypotension, treatment of hypotension
	10%[56]	20[53]	Treatment of nitric oxide–dependent shock, sepsis
	10% in modified LR[56,59]	39[60]	Normal volunteer, coronary artery bypass graft with intraoperative autologous donation
	13% in modified LR[58]	40[56,58]	Normal volunteer, sickle cell anemia, cardiac surgery, general/urologic/gynecologic/plastic and reconstructive/major vascular surgery, orthopedic surgery, liver resection, elective abdominal aortic aneurysm repair with acute normovolemic hemodilution, percutaneous coronary intervention
	10% in ?[17,19]	26–32*[17,19]	Normal volunteer, elective abdominal aortic aneurysm repair with acute normovolemic hemodilution, in-hospital trauma and urgent surgery, prehospital trauma
	8% in ascorbate-containing phosphate buffered saline[53,54]	33[54]	Normal volunteer, intraoperative, cardiac surgery with or without acute normovolemic hemodilution
	4–6%*	14 (strongly dependent on temperature and hemoglobin concentration)[53]	Radiation sensitization

* Slides presented at the workshop; *Grey wedges* = α-globin chains; *black wedges* = β globin chains.

Hb = hemoglobin; LR = lactated Ringer's solution; NA = not available; PEG = polyethylene glycol; P50 = partial pressure of oxygen (mmHg) at which hemoglobin is 50% saturated at pH 7.4 and base-excess 0 at 37°C; RBC = red blood cell.

Table 2. Summary of Adverse Events Reported in the Literature or Publicly Available

Cohort	Apex Test	Control	Baxter Test	Control	Biopure Test	Control	Enzon Test	Control	Hemosol Test	Control	Northfield Test	Control	Sangart Test	Control	Somatogen Test	Control
Number of subjects	Not reported		504	505	708	618	Not reported		209	192	623	457	85	45	64	26
1 Death	*	*	78	61	25	14	*	*	1	4	73	39	2	0	*	*
2 Hypertension	*	*	76	38	166	59	*	*	113	75	*	*	7	1	8	0
3 Pulmonary hypertension	*	*	1	0	3	0	*	*	*	*	*	*	*	*	*	*
4 Chest pain/chest tightness	*	*	*	*	21	16	*	*	*	*	*	*	*	*	12	0
5 Congestive heart failure	*	*	0	1	54	22	*	*	0	2	17	20	*	*	*	*
6 Cardiac arrest	*	*	*	*	17	6	*	*	1	1	14	9	*	*	*	*
7 Myocardial infarction	*	*	6	1	14	4	*	*	14	7	29	2	2	0	*	*
8 Cardiac arrhythmias/conduction abnormalities	*	*	23	17	153	100	*	*	1	1	*	*	15	5	1	1
9 Cerebrovascular accident, cerebrovascular ischemia, TIA	*	*	*	*	16	3	*	*	2	1	3	1	*	*	*	*
10 Pneumonia	*	*	*	*	35	22	*	*	*	*	27	21	*	*	*	*
11 Respiratory distress/failure	*	*	*	*	22	12	*	*	*	*	21	17	*	*	*	*
12 Acute renal failure	*	*	1	3	10	4	*	*	2	2	*	*	*	*	*	*
13 Hypoxia, cyanosis, decreased oxygen saturation	*	*	*	*	76	35	*	*	1	1	*	*	*	*	3	1
14 Hypovolemia	*	*	*	*	19	4	*	*	*	*	*	*	*	*	*	*
15 Gastrointestinal	*	*	51	31	345	195	*	*	23	1	*	*	57	20	36	6
16 Liver, LFTs abnormal	*	*	27	8	20	5	*	*	8	0	*	*	*	*	6	3
17 Pancreatitis	*	*	11	0	5	3	*	*	1	0	*	*	*	*	*	*
18 Coagulation defect, thrombocytopenia, thrombosis	*	*	*	*	45	17	*	*	1	0	13	4	*	*	*	*
19 Hemorrhage/bleeding/anemia	*	*	33	22	108	55	*	*	1	1	20	17	*	*	*	*
20 Sepsis, septic shock, MOF	*	*	2	2	15	6	*	*	0	1	26	20	*	*	*	*
21 Pancreatic enzyme inc	*	*	13	4	3	0	*	*	*	*	*	*	*	*	*	*
22 Lipase increase	*	*	29	9	48	12	*	*	19	2	*	*	8	4	7	1
23 Amylase increase	*	*	48	45	*	*	*	*	35	20	*	*	7	2	4	1

Adverse events categories—terms included in each category:

1. Death.
2. Hypertension, blood pressure increased, hypertensive crisis, systolic hypertension, systemic vascular resistance (SVR) increased, malignant hypertension, postoperative hypertension, systolic blood pressure increased.
3. Pulmonary hypertension.
4. Chest pain, chest tightness.
5. Congestive failure- cardiac, cardiac failure, cardiorespiratory failure, left ventricular failure, pulmonary edema, acute circulatory failure, rales, cardiac index decreased, cardiac output decreased, central venous pressure (CVP) increased, fluid overload, dyspnea.
6. Cardiac arrest, cardiopulmonary arrest, ventricular fibrillation.
7. Myocardial infarction.
8. Arrhythmias and conduction abnormalities.
9. Cerebrovascular accident, cerebral infarction, hemiparesis, hemiplegia, monoparesis, transient ischemic attack (TIA), reversible ischemic neurological deficit (RIND), transient cerebrovascular event.
10. Pneumonia, pneumonia-Klebsiella, pneumonia-pseudomonas, pneumonia-staphylococcus, aspiration pneumonia, pneumonitis.
11. Acute respiratory distress syndrome (ARDS), respiratory distress, respiratory failure.
12. Acute renal failure.
13. Hypoxia, decreased oxygen saturation, cyanosis.
14. Hypovolemia.
15. Gastrointestinal (GI) pain, GI pain-upper, esophageal spasm, vomiting, abdominal distension, dyspepsia, dysphagia, eructation, vomiting-aggravated, postoperative nausea, retching, abdominal pain-lower, nausea-aggravated, nausea, ileus.
16. Liver function tests (LFTs) abnormal.
17. Pancreatitis.
18. Coagulation disorder, disseminated intravascular coagulation (DIC), thrombocytopenia, thrombocythemia, activated partial thromboplastin time (aPTT) prolonged, Bleeding time prolonged, fibrinogen decreased, D-dimer increased, prothrombin level decreased, petechiae, purpura, thrombosis, arterial thrombosis-limb, deep venous thrombosis, pulmonary embolus, thromboembolism, thrombophlebitis-deep, PT change.
19. Anemia, duodenal ulcer hemorrhage, gastric ulcer hemorrhage, rectal hemorrhage, exsanguination, ulcer hemorrhage, intraoperative hemorrhage, postoperative hemorrhage, secondary anemia, hemoglobin decreased, vaginal hemorrhage, and anemia aggravated.
20. Sepsis, septic shock, multiple organ failure (MOF).

Not all clinical trials conducted by commercial sponsors have been published, and the published results are not synonymous with line listings that would be found in a comprehensive final study report. For each paper, editorial decisions were made about what information should be included or excluded and data presentation (numbers versus percentages), making derivation of number of subjects experiencing an event and aggregation of information to derive a comprehensive list of adverse events difficult and potentially incomplete. Not all studies were controlled. Not all enzyme elevations were captured as adverse events; in some instances, the number of subjects experiencing enzyme elevations was not captured. Differences in reporting methods may have resulted in counting subjects more than once in each category of events (row).

References for data in Table 2: Baxter[22–24,35,63–71]; Biopure[69,72–79]†; Hemosol[80–82]; Northfield Laboratories[17,83–85]‡; Somatogen[28,86,87]; Sangart.[15,16]

* No information available; † Available at: www.fda.gov/ohrms/dockets/ac/cber06.html#BloodProducts. Accessed March 15, 2008. ‡ Available at: www.northfieldlabs.com. Accessed March 15, 2008.

social (scientific and medical) value and scientific validity and to be performed under conditions where subject selection is fair. Other considerations include the need for a favorable benefit:risk ratio, informed consent, respect for potential and enrolled participants, and independent review of the research. The requirement for prospect of direct benefit to the subjects enrolled in a trial with exception from informed consent is explicitly stated under 21 CFR 50.24; however, all elements of the assessment (conceptual support, preclinical evidence, and clinical studies in other settings and other populations) generally apply to all clinical research. The considerations stated explicitly in 21 CFR 50.24 reflect the complex assessment that must be made, an assessment that requires experienced judgment in conjunction with rigorous scientific evidence about the prospect for benefit.

Presentations of clinical data were made by representatives of five commercial sponsors; Sangart, Northfield, Prolong Pharmaceuticals (reporting data from Enzon), Biopure, and Apex. In addition, Tim Estep (President, Chart Biotech Consulting, LLC, Erie, CO) presented publicly available data from Baxter/Somatogen, though not as a representative of the companies. Most presenters emphasized that each product had unique characteristics and properties and should be considered individually rather than collectively.

Sangart, Inc., San Diego, CA

Sangart[13–16] is developing a polyethylene glycol (PEG)-conjugated hemoglobin, MP4 (MalPEG-hemoglobin). This larger molecule is characterized by (1) high oxygen affinity that limits oxygen release in arterioles and decreases rates of oxidation; (2) increased oncotic pressure; and (3) a formulation of lower hemoglobin concentration. The development of this molecule is based on the theory of autoregulation, wherein high oxygen unloading in arterioles leads to vasoconstriction. Based on preclinical data, Sangart hypothesizes that MP4 might preserve functional capillary density in anemic and shock states and reduce the incidence of hypertension and other adverse events, arguing that nitric oxide scavenging does not explain all HBOC-related adverse events.

Six clinical trials in elective surgery, chronic critical limb ischemia, and prevention of hypotension have been and are being performed. Safety results of a phase 2 trial in orthopedic surgery[14] showed an imbalance in cardiac and vascular events, including bradycardia and blood pressure elevation; gastrointestinal events including nausea; cardiac rhythm, including bradyarrhythmias; and pancreatic enzymatic activity (lipase and amylase) with greater frequency after administration of product than placebo (Ringer's acetate). Data were presented suggesting that the small increases in mean arterial pressure may be attributable to the hyperoncotic volume-expansion properties of the product rather than nitric oxide scavenging.

Northfield Laboratories, Inc., Evanston, IL

Northfield manufactures Poly-SFH-P, a glutaraldehyde polymerized stroma-free human hemoglobin product (PolyHeme®) and intends to seek an indication for when blood is not available, as might occur at the scene of injury, during transport to definitive care, or in the hospital, because of religious objection, blood incompatibility, or shortage. Northfield has conducted six clinical trials in 1,133 subjects, 674 of whom have received the product. The results of a phase 2 trial in 171 subjects retrospectively compared against historical control data from Jehovah's Witness patients undergoing elective surgery and who had sustained significant blood loss leading to low or extremely low red blood cell hemoglobin concentrations,[17,18] and the randomized pivotal phase 3 trauma trial, conducted under the provisions of 21 CFR 50.24, were presented. The phase 3 study was designed to assess through extrapolation the benefit of Poly-Heme® to the intended population for whom blood would not be available for prolonged periods of time; it was not designed to demonstrate that PolyHeme® could be used in place of blood.[19] The study had a dual superiority/noninferiority design.[20] Results of mortality assessments in the Intent-to-Treat (ITT; as randomized), As-Treated (AT; as treatment received), and Per Protocol (PP; without major protocol violations) populations were presented. In the prespecified ITT population, the mortality for the test group was 13% (47 of 350) and 10% (35 of 364) for the control group, failing the prespecified 7% noninferiority boundary. Northfield expressed the opinion, however, that the appropriate analysis was the PP analysis because this analysis excludes both subjects who received a treatment other than that assigned and those who were enrolled in violation of entry criteria. Subjects in the PolyHeme® group were reported to have had on average lower blood pressure before randomization and more severe neurologic findings, more severe coagulopathy at randomization, and more severe injuries than subjects in the control arm.

Serious adverse events in this trial were reported for 40% (141 of 349) test subjects and 35% (126 of 365) control subjects. The most common serious adverse events that occurred in excess in test subjects included pneumonia, multiple organ failure, hemorrhagic shock, respiratory failure, hypercoagulable state, coagulopathy, and myocardial infarction. *Post hoc* adjudication of cardiac events by a blinded subcommittee of the data monitoring committee suggested that myocardial infarction in victims of trauma is much more common overall than previously believed and did not differ between treatment groups.

Enzon Pharmaceuticals, Inc., Piscataway, NJ

Enzon developed a PEG-HBOC (polyethylene glycol-conjugated hemoglobin) bovine product, with the goal of development to increase tumor oxygenation to en-

hance radiosensitivity of susceptible tumors. The most common side effects were reported to be mild hypertension in 24%, dysphagia in 24%, nausea in 24%, and vomiting in 12%. Esophageal spasm was effectively prevented/well-managed with an atropine-like antispasmodic agent Levesinex. Clinical development of this PEG-hemoglobin was halted in the late 1990s. Subsequently, Prolong has been developing PEGylated drugs hypothesizing that attaching one or more PEG (polyethylene glycol moieties) to a protein molecule could improve therapeutic and functional characteristics of biopharmaceuticals, including acellular bovine hemoglobin.

Biopure Corporation, Cambridge, MA

The Naval Medical Research Center (NMRC) and FDA have presented results of clinical trials of Biopure's candidate acellular hemoglobin product, HBOC-201, a glutaraldehyde crosslinked bovine hemoglobin, at the December 2006 Blood Products Advisory Committee meeting (Bethesda, MD). At the April 2008 workshop, Biopure presented a reanalysis of safety signals arising out of two pivotal phase three studies in elective surgery noting that data from the larger of the two studies, HEM-0115,[21] are representative of all safety signals seen in the pooled safety database arising out of 21 clinical trials. Biopure acknowledged that there was an imbalance against HBOC-201 for adverse events and serious adverse events and presented an analysis of the clinical and preclinical data with respect to mechanisms. Biopure concluded that the safety signals did not reflect product toxicity *per se* and that the serious adverse events were the result of a combination of patient comorbidity, clinical management differences, and underdosing with HBOC-201 with ongoing anemia and ischemia on the one hand and overdosing with HBOC-201 with volume overload and heart failure on the other.[21] In the HEM-0115 trial, 59% of patients allocated to the HBOC arm avoided red cell transfusion; however, their total hemoglobin concentration was less than that of the group given red cells, which might have affected both efficacy and safety results.

Biopure maintained that vasoconstriction was not the primary factor in the emergence of serious adverse events. They presented preclinical animal studies evaluating vital organ and skeletal muscle blood flow and tissue oxygen tension in animals undergoing up to 50% exchange transfusion to show that there was increased vascular resistance in skeletal muscle but no evidence of vasoconstriction in heart, brain, and kidney. Biopure also presented studies in pigs and a phase 2 study in humans undergoing percutaneous coronary intervention to show that HBOC-201 affects mean arterial pressure but not coronary function. Coronary blood flow, left ventricular end-diastolic pressure, electrocardiographic findings, and cardiac output were not affected by use of HBOC-201, and left ventricular function, as assessed by pressure-volume loops, was maintained with infusion of oxygenated HBOC-201 directly into the coronary artery.

Biopure stated, although no data were presented, that there have been no issues regarding management of hypertension or about myocardial infarctions and other adverse events/serious adverse events in over 480 patients treated in South Africa.

Biopure concluded that given the adverse events observed in clinical trials of HBOC-201 compared with red blood cells in elective surgeries, further clinical trials against red blood cells are not warranted. They further concluded that HBOC-201 should be evaluated in trials where blood is not immediately accessible or an option.

Baxter Healthcare Corporation, Deerfield, IL (including Somatogen)

Baxter manufactured a diaspirin $\alpha\alpha$-crosslinked human tetrameric hemoglobin product, DCLHb, and later recombinant tetrameric hemoglobin products based in part or whole on molecules previously developed by Somatogen. Baxter presented a history of the development of DCLHb focusing on properties and factors potentially affecting the outcome of human phase 3 clinical trials.

During preclinical testing of DCLHb, Baxter identified several safety concerns, including the development of cardiac lesions in some animal species, transient liver pathology, gastrointestinal effects, jaundice, and vasoactivity. In primates, the myocardial lesions were described as focal or multifocal, minimal to moderate myocardial degeneration characterized by cytoplasmic swelling and vacuolization of myofibers occurring primarily in the left ventricle and septum. The lesions, which resolved with time, exhibited a threshold and a plateau maximum with dose. Morphometry studies in rhesus monkeys suggested that an average of 1.3% of the myocardium was susceptible to damage. The lesions were not associated with changes in cardiac enzymes, and there were no functional deficits detected in pigs with the lesion. Many tested interventions, including pharmacologic interventions, had no effect on the development of the cardiac lesions. However, based on work with the recombinant molecules, polymerization of the hemoglobin product and reduction of the rate of nitric oxide scavenging mitigated lesion development. The relationship of these cardiac lesions to human pathology is unclear.

Vasoactivity was overtly manifested as an increase in systemic blood pressure that was highly correlated with hemoglobin extravasation and nitric oxide scavenging and tended to manifest and maximize rapidly at low doses.

Baxter conducted two phase 3 trials evaluating the use of DCLHb in the treatment of traumatic hemorrhagic shock.[22-24] One trial was conducted in the prehospital setting with use of product in lieu of blood. The second trial was an in-hospital evaluation of DCLHb administered in addition to standard of care including red blood

cells. The in-hospital phase 3 trial was halted because of excess mortality in the test arm. The prehospital study was halted early with a higher, but not statistically significant, point estimate for mortality for DCLHb. Pancreatitis was noted as one important safety finding for DCLHb. Increased blood pressure and uncontrolled bleeding did not systematically occur among trauma subjects administered DCLHb in the in-hospital trial.

Unpublished data from subsequent preclinical studies in traumatic hemorrhagic shock showed that administration of relatively small volumes of DCLHb after resuscitation with large volumes of crystalloid may have an adverse impact.

rHb1.1, first developed by Somatogen and later acquired by Baxter, had clinical properties and toxicities similar to DCLHb. To reduce the incidence of cardiac lesions, vasoactivity, and gastrointestnial effects, a second generation recombinant product, rHb2.0, was developed with reduced interaction with nitric oxide. Subtherapeutic doses administered to subjects enrolled in early phase 1 studies resulted in complement activation. Baxter abandoned development of HBOCs.

Estep concluded that HBOC solutions have multiple properties that are important to tissue perfusion and oxygenation, including oxygen transport, vasoactivity, oncotic pressure, viscosity, and fluid volume, and that these properties might have very different dose response characteristics.

APEX Bioscience, Inc., Chapel Hill, NC

Apex manufactures a pyridoxylated chemically modified human hemoglobin that is conjugated with polyoxyethylene as a nitric oxide scavenger intended for the treatment of distributive shock. Nitric oxide is hypothesized to be the final common mediator in nitric oxide induced shock independent of etiology and redundant cytokine pathways, with direct toxic and pathophysiologic actions.

Results of a small study of continuous infusion of pyridoxylated polyoxyethylene-conjugated hemoglobin in the treatment of subjects with volume refractory, pressor-dependent systemic inflammatory response syndrome were presented. Mean arterial pressure increased, and heart rate decreased with administration of product, and median times to first withdrawal of conventional vasopressors among survivors was shorter in pyridoxylated polyoxyethylene-conjugated hemoglobin-treated subjects than in control subjects. Mortality rates at days 10 and 28 were not different among treatment groups. Although perhaps not statistically different for this small study, the absolute difference in mortality on day 10 was 18% (48% for control *vs.* 30% for pyridoxylated polyoxyethylene-conjugated hemoglobin); however, on day 28 the mortality was similar for the two groups (57.6% for pyridoxylated polyoxyethylene-conjugated hemoglobin and 58.6% for control). There was a statistically insignificant increase in days alive and free from cardiovascular dysfunction and mechanical ventilation favoring pyridoxylated polyoxyethylene-conjugated hemoglobin, without an increase in the need for certain medical interventions for liver, kidney, coagulation, or central nervous system complications. Blinded review and adjudication using prespecified criteria did not confirm the excess of cardiac events identified by investigators who were not blinded to treatment allocation. Apex concluded that a blinded, adjudicated review of all cardiac events will be performed using prospective definitions in future studies, and that the patient population should be at high risk of dying due to shock unresponsive to standard of care such as dopamine or norepinephrine.

During the question and answer session, Tom Fleming, Ph.D. (Department of Biostatistics, University of Washington), at the request of the session moderator, provided a biostatistical critique of the commercial presentations. He noted the following beliefs. (1) Absence of an adverse safety signal in a single clinical trial does not equate to an absence of harm; there must be sufficient data to rule out a major morbidity occurring at a clinically unacceptable rate, or excess mortality. (2) Any noninferiority margin must be justified; the effect of the active comparator on outcome must be known so that the level of benefit that can be lost (noninferiority margin) without also losing clinical significance of the outcome with the test product can be determined. (3) Whether to use the ITT population or the PP population for analysis is an area of debate for noninferiority trials because a difference in adherence to the protocol between the active comparator and the experimental treatment can affect observation of the true difference. (4) Any *post hoc* analyses, although interesting and hypothesis-generating, must be viewed with great caution; an ITT analysis is the most reliable indicator of causality even though ITT analyses do not take into account all the clinical exigencies of each individual patient.

Session III: Clinical Findings and Mechanisms

Session III was divided into two sessions. The first session was devoted to discussion of clinical findings and mechanisms of toxicity. The second session was devoted to discussion of organ-specific aspects of safety findings. Panel members were permitted to present brief position papers. The sessions concluded with question and answer periods.

Session IIIa: Functional Aspects of the HBOCs as a Class

Moderator: Harvey Klein, M.D., Chief, Transfusion Medicine, National Institutes of Health, Bethesda, Maryland.

Harvey Klein, M.D., summarized four overarching questions for the panel. (1) Can information about the safety and efficacy obtained from clinical trials in one setting be applied to another? (2) Given what is known about the biochemistry and pharmacology of the current and previous HBOCs, can safety information obtained from the study of one HBOC be used to inform safety and risk assessments for a different HBOC? (3) Are there toxicities or harmful interactions between these molecules and a patient's underlying disease(s) that are common to all of these molecules? (4) Are there lessons from what was heard in Sesion II for designing future trials?

Differences in mechanism of death and prognosis in blunt trauma and penetrating trauma were discussed by Demetrios Demetriades, M.D., Ph.D. (Professor of Surgery, Department of Surgery, Division of Trauma and Critical Care, University of Southern California, Los Angeles, California). He noted that blunt trauma rarely results in hemorrhagic death within 1 h. Excluding head injury, the mortality in blunt trauma for patients with systolic blood pressure less than 90 mmHg is approximately 20%, whereas the mortality rate in patients with penetrating trauma and systolic blood pressure less than 90 mmHg is approximately 33%. Segregating blunt and penetrating trauma into two separate clinical studies because of the difference in prognosis, enrolling victims of penetrating trauma (excluding head injured patients) with a systolic blood pressure less than 80 mmHg, and using HBOCs on a "compassionate" basis were discussed.

Daniel Freilich, M.D., C.D.R., M.C., U.S.N. (Naval Medical Research Center, Combat Casualty Directorate, Silver Spring, Maryland) discussed strategic considerations in the design of clinical trials to evaluate the current generation of HBOCs for trauma. He noted that preclinical data should steer HBOC clinical trial design for trauma and summarized 16 studies performed in preclinical models of controlled hemorrhage, uncontrolled hemorrhage, traumatic uncontrolled hemorrhage with or without concomitant traumatic brain injury and concluded that there was a significant potential benefit of HBOC (specifically HBOC-201 from Biopure) to reduce mortality in severe hemorrhage.‖ Optimization of study design and practice guidelines, inclusion of a target population with severe hemorrhagic shock and high mortality, and risk minimization strategies should enable clinical evaluation of the current generation of HBOCs with reasonable risk.

John Holcomb, M.D. (Commander, US Army Institute of Surgical Research, Brooke Army Medical Center, San Antonio, Texas) identified trauma-induced coagulopathy as an independent predictor of increased mortality and discussed an algorithm using physiologic parameters of heart rate, systolic blood pressure, pH, and hematocrit

‖ http://www.fda.gov/ohrms/dockets/ac/06/transcripts/2006-4270t1-01-BPAC.pdf. Accessed July 6, 2009.

that has relatively high predictive ability for massive transfusion among patients with all four predictors. He noted that massively transfused patients were at particular risk of dying necessitating rapid diagnosis and intervention. Dr. Holcomb presented data suggesting that increasing the plasma and platelet to red blood cell ratio decreases early death due to hemorrhage and improves overall survival from 41% to 74% at day 30.[25] These results were seen despite equal numbers of units (21) of RBC transfusions. Dr. Holcomb noted that, although significant attention has been paid to rapid restoration of oxygen delivery with transfused hemoglobin, one must not neglect the plasma and platelet transfusions as well.

Steven Cohn, M.D. (University of Texas Health Science Center, Houston, Texas) discussed the potential use of HBOCs when blood is not available in both prehospital and hospital settings and the need to consider the risk of mortality and morbidity of not using an HBOC in these circumstances.

Ed Norris, M.D., M.B.A., F.A.H.A. (Associate Professor, Department of Anesthesiology and Critical Care Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland) expressed his belief that HBOCs can serve a critical unmet need when blood is not available or is refused.

Charles Natanson, M.D. (Critical Care Medicine Department, National Institutes of Health, Bethesda, Maryland) presented the results of his meta-analysis of 16 publicly reported randomized, controlled trials of five HBOCs (Baxter, Biopure, Hemosol, Northfield, and Sangart).[26] These 16 trials involved 3,711 patients in several clinical settings. All five HBOC products have as common features the ability to bind nitric oxide and to interfere with nitric oxide function, suggesting that all five HBOC products are likely to result in similar toxicities. A test for heterogeneity of the results for these trials was not significant for either mortality or myocardial infarction and data were combined by using a fixed-effects model. Overall, there was a statistically significant increase in the risk of death and myocardial infarction. Subgroup analysis of the studies indicated that the increased risk was not restricted to a particular HBOC, a particular indication, or a particular control solution. Dr. Natanson concluded on the basis of this analysis that HBOCs are associated with a significantly increased risk of death and myocardial infarction and that further clinical trials of these products should not proceed unless the underlying mechanisms of toxicity have been elucidated and corrected.

Mitchell Fink, M.D. (President and CEO, Logical Therapeutics, Inc., Waltham, MA) presented information about the beneficial and deleterious effects of modulation of the Nitric oxide-guanylyl cyclase pathway. He also noted that high blood lactate levels in trauma victims probably are not associated with changes in blood flow to tissue but rather are related to the presence of elevated circulating catecholamine levels; in experimen-

tal animals subjected to hemorrhagic shock, hyperlactatemia is abrogated by β-adrenergic blockade.

Edward Sloan, M.D., M.P.H. (Department of Emergency Medicine, University of Illinois at Chicago, Chicago, Illinois) discussed the two trials of DCLHb in traumatic hemorrhagic shock. General conclusions based on revised trauma score (RTS) and the impact of traumatic brain injury on ability to study HBOCs and on effects of DCLHb on blood pressure, base deficit, lactate, and shock index were as follows. (1) Blood pressure did not differ over time in hemorrhagic shock patients based on treatment assignment, and the putative pressor effect of DCLHb was not consistently observed. (2) Incidence of markedly elevated blood pressure did not differ among treatment groups. (3) Base deficit and lactate data did not demonstrate differences based on treatment group even though patients who expired had larger base deficits and higher lactate levels than those who survived. (4) Shock index defined as heart rate/systolic blood pressure of at least 1 clinically detects uncompensated shock and possible death. (5) DCLHb use did not alter the ability of shock index to predict mortality. Inclusion of patients with traumatic brain injury who presented with a Glasgow Coma Scale of 3 increased the study mortality by 63%, suggesting that patients with Glasgow Coma Score of 3 should be excluded from traumatic hemorrhagic shock trials. Traumatic hemorrhagic shock patients with a low RTS from 1 to less than 4 have a very low Trauma and Injury Severity Score survival probability (less than 30%). Optimization of clinical trial entry criteria based on RTS might be needed to better identify patients with an intermediate injury severity and intermediate survival probability.

Gus Vlahakes, M.D. (Professor of Surgery, Harvard Medical School, Division of Cardiac Surgery, Massachusetts General Hospital, Boston, Massachusetts) presented data from a phase 2 trial of HBOC-201 *versus* red blood cells after cardiac surgery. This study of up to three infusions and 120 g of total of product demonstrated a savings of only 0.5 units of allogeneic red blood cells. Dr. Vlahakes discussed the vasoconstrictor properties of HBOCs, noting that, although hemoglobin avidly binds nitric oxide and virtually all HBOC preparations elevate systemic and pulmonary vascular resistances, nitric oxide binding and increased vascular resistance have not been shown to override metabolic autoregulation. Nitric oxide binding might therefore be of therapeutic benefit in some clinical settings such as trauma and cardiac surgery although titration to a blood pressure endpoint might lead to underresuscitation of recipients. Dr. Vlahakes raised the question of the effect of HBOC on vulnerable plaque because myocardial infarctions have been an issue in many clinical trials.

During the question and answer period, panelists were asked about clinical situations where, despite the safety problems with the current generation of HBOCs, clinical trials might be conducted. There was a wide variety of opinions on this matter, ranging from trials exclusively in patients with close to 100% likely mortality to statements of the need to select patients very carefully (young, no comorbid conditions), to considerations of use in traumatic hemorrhagic shock. Panelists generally agreed that in view of the current safety of blood, it would be difficult to evaluate HBOC products against allogeneic red blood cells in an elective surgical setting.

A number of panel members agreed that much more research needs to be done to elucidate the effects of nitric oxide scavenging by HBOCs, with suggestions for additional research in animal models of disease and pilot phase 2 clinical trials. Modulating nitric oxide-guanylyl cyclase pathway has resulted in beneficial and adverse effects depending on the direction of modulation. Some panel members commented that all HBOCs should not be considered alike. Others noted that the meta-analysis performed by Dr. Natanson raises significant questions that necessitate further study. A number of panelists agreed that given the relative safety and general acceptability of red blood cells for transfusion, an important population for study would be those patients for whom red blood cell transfusion is not an alternative or is not available, even if such a study would be logistically difficult.

Session IIIb: Organ-specific Aspects of Safety
Moderator: Richard Weiskopf, M.D., Professor, University of California, San Francisco, California.

Andrew Baines, Mitchell P. Fink, Mark Gladwin, Joseph E. Parrillo, Raymond F. Regan, and David C. Warltier each discussed toxicity of a specific organ (system) and there followed a period of questions and answers and panel discussion.

Renal: Andrew Baines, M.D., Ph.D., University of Toronto, Toronto, Ontario, Canada. Historically, renal damage was associated with early, unpurified hemoglobin preparations, likely owing to presence of monomers and dimers. Crosslinking of current preparations have made acute renal failure generally uncommon with HBOCs. The incidence of stage 1 and stage 2 acute kidney injury[27] in HBOC trials has been variable, with a more than 25% glomerular filtration rate decrease in studies performed by Hemosol and Baxter, whereas glomerular filtration rate increased in studies performed by Biopure and Somatogen[28] and did not change in a study performed by Sangart using iohexol determination of glomerular filtration rate. However, there are limitations on ability to diagnose renal injury by using only serum creatinine values because of the delayed response to injury, the nonlinear relationship of serum creatinine to the glomerular filtration rate, and the general absence of information about the initial glomerular filtration rate and serum creatinine, resulting in ascertainment bias.[29] Dr. Baines remarked that better markers of renal disease are needed, such as urinary *N*-acetylglucosamine, a

marker of inflammation, or KIM-1, a marker of proximal tubular changes,[30] or neutrophil gelatinase-associated lipocalcin (NGAL).[31] He also noted that animal models do not correlate well with human disease in that it is very difficult to reproduce acute renal injury that mimics human response. Dr. Baines concluded by noting that there are only very limited data from reported studies to determine how many subjects had stage 1 and stage 2 kidney injury and no data regarding long-term follow-up of subjects receiving either HBOCs or red blood cells.

The toxic effects of heme appear to be mediated through reactive oxygen species and inflammatory pathways rather than through nitric oxide pathways.[32] Dr. Baines noted that because it is difficult to know definitively whether HBOC products are associated with nephrotoxicity, it is difficult to answer a question about mechanism of toxicity. Nevertheless, his supposition is that the HBOCs do all have a common effect on kidney related to their ability to generate reactive oxygen species and redox reactions related to the availability of iron.

Gastrointestinal: Mitchell Fink, M.D., President and CEO, Logical Therapeutics, Inc., Waltham, Massachusetts. At least five of six HBOCs for which public information is available have been associated with GI-related adverse events and at least three HBOCs have been associated with at least biochemical evidence of acute pancreatitis. The three most consistent gastrointestinal complications have been evidence of pancreatic injury, evidence of hepatocellular injury, and esophageal spasm presenting as chest pain.

With the advent of computerized tomography, the diagnosis of pancreatitis has become easier because radiologic criteria for the diagnosis and grading of severity have been developed. These include extent of perfusion abnormality in the pancreas, extent of pancreatic necrosis, and the degree of fluid sequestration in the retroperitoneum. Pancreatic imaging permits reproducible grading of the severity of acute pancreatic changes and prediction of mortality risk. The biochemical diagnosis of acute pancreatitis, which has not changed, is based on the circulating concentrations of the pancreatic enzymes, amylase and lipase. In particular, an increase in circulating levels of lipase is considered prima facie evidence of acute pancreatic injury. The diagnosis of acute pancreatitis is therefore based on combined anatomic and biochemical damage.

Clinical evidence of pancreatitis has been documented by increased circulating concentrations of amylase and lipase, and less frequently, by the presence of clinically overt pancreatitis. Evidence of hepatocellular injury has been documented almost entirely by increases in circulating activity of transaminases. Esophageal spasm has been a consistent finding, presenting as classic chest pain. Dr. Fink suggested that the biochemical changes of pancreatic and hepatocellular injury are signals of the potential of HBOCs to produce rare but clinically significant cases of massive hepatocellular damage and massive acute necrotizing pancreatitis if the products were used in large numbers of patients.

It was suggested that the pancreatic changes are related to nitric oxide scavenging by hemoglobin, which is known to cause (1) spasm of the sphincter of Oddi leading to increased intraductal pressure in the pancreas and (2) diminished or impaired pancreatic microvascular perfusion leading to pancreatic ischemia. The combination of intraductal hypertension and pancreatic ischemia is very likely to be associated with development of acinar cell damage and the induction of pancreatic inflammation and pancreatitis. It was also noted that it is possible that liberation of reactive oxygen species in the pancreatic milieu might result in redox-mediated damage to the pancreatic parenchyma, also leading to pancreatitis.

Dr. Fink noted that whether or not HBOCs produce gastrointestinal changes by a similar mechanism of action is an open question but that there appears to be a fairly strong and consistent signal of biochemical abnormalities indicative of acute pancreatic injury.

Cardiovascular: David Warltier, M.D., Ph.D., Chair, Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, Wisconsin. HBOCs that scavenge nitric oxide increase vascular resistance in most vascular beds (kidney, skin, pancreas, liver, brain, right ventricle, but not left ventricle) and increase mean arterial pressure, decrease cardiac output secondary to the increased afterload (not due to decreased inotropic state), and decrease heart rate (only partially due to the baroreceptor reflex). An HBOC that does not bind nitric oxide (rHb 2.0) did not change canine vascular resistance in any of the organs examined (J. Craig Hartman, Gilead Colorado Inc., Boulder CO; personal verbal communication, April 2008).

Several HBOC products are associated with the development of myocardial lesions in sensitive species such as rhesus monkey or swine. These lesions are very small, diffuse, punctate, degenerative left ventricular lesions with vacuoles and nuclear lysis appearing in cardiac myocytes. These changes are similar to those observed with administration of sympathomimetic amines or chronic infusion of N(G)-nitro-L-arginine methyl ester (L-NAME), a nonspecific inhibitor of nitric oxide synthase. The lesions are associated with only very small changes in cardiac enzymes. Baxter has reported that administration of recombinant hemoglobin that does not bind nitric oxide reduces, but does not eliminate, the development of these cardiac lesions.[33]

Several clinical trials have revealed an increased incidence of myocardial infarction in patients given HBOCs. The occurrence of myocardial infarction may not be due to the vasoconstrictive effects of HBOCs but rather may be due to some other as yet undetermined mechanism. Nitric oxide protects the myocardium against ischemia and reperfusion injury. Reducing bioavailability of nitric

oxide could block endogenous cardioprotective effects. It was noted that there may be differing definitions of myocardial infarction among clinical trials. Troponin as an isolated marker of myocardial injury is not specific for myocardial infarction but will be increased in patients with myocardial injury due to other causes, necessitating additional clinical and laboratory findings to verify the diagnosis. The concept was expressed that the etiology of this disturbing finding of increased incidence of myocardial infarction should be better elucidated before further clinical trials with HBOCs, considering the similar effects of different HBOC products in the cardiovascular system.

It was also noted that although the HBOCs differ biochemically and physiologically the incidence of myocardial infarctions is of concern and may be the most sensitive indicator that nitric oxide bioavailability may be reduced.

Dr. Weiskopf speculated that the effect of nitric oxide scavenging on platelet function and coagulation[34] might be the single most important effect of HBOC products and that this interaction could be related to the effects of HBOCs on myocardial ischemia and infarction. Dr. Gladwin added that it was very likely that infusion of a potent nitric oxide scavenger would result in activation of the coagulation system.

Central Nervous System: Raymond Regan, M.D., Department of Emergency Medicine, Thomas Jefferson University, Philadelphia, Pennsylvania.[35–37] $\alpha\alpha$-Crosslinked hemoglobin appears to behave very much like native hemoglobin in the central nervous system. Although HBOC products appear to be neurotoxic *in vitro*, owing to release of iron rather than to the hemoglobin itself, data about *in vivo* effects are very sparse. It therefore remains an open question whether HBOCs are neurotoxic in the context of an open blood-brain barrier as occurs with traumatic brain injury or stroke. Of particular concern is that the various outcome measures used in animal studies, although reasonable measures, are not sensitive to the neurotoxic effects of hemoglobin and that the timing of evaluation may be too early to detect any signal. Some sensitive markers of neuronal damage produced by hemoglobin, including protein carbonyls, malondialdehyde, and 8-OH-2-deoxyguanosine were not measured in the studies. Hemoglobin is a rather slowly acting neurotoxin because its toxicity appears to be facilitated by oxidation, release of its heme moieties, and their degradation, liberating iron. Therefore, the appropriate time to evaluate neurotoxicity *in vivo* is 24–72 h after HBOC administration, not earlier. Clinical data about neurotoxicity are also very sparse, suggesting the need for further preclinical studies looking at relevant oxidative injury markers at relevant time points before clinical evaluation in patients suffering traumatic brain injuries or stroke is undertaken. Dr. Regan further stated that given the current state of knowledge and until further preclinical testing had been

done, he would exclude patients with significant traumatic brain injury (TBI) and patients with any blunt trauma from study of HBOC products. He did not, however, define a specific Glasgow Coma Scale score that could be used as an exclusion criterion for significant TBI for HBOC trials. He would exclude all patients with altered mental status, as well as those with any abnormality on neurologic examination or computerized tomography scan. He would not exclude from an HBOC clinical trial patients with penetrating trauma that did not involve the brain.

Shock: Joseph Parrillo, M.D., Professor of Medicine, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Camden, New Jersey. Dr. Parrillo summarized the Weil-Shubin classification of shock – hypovolemic, cardiogenic, extracardiac obstructive, and distributive. Contrary to past assumptions that the treatment of septic shock was not time-sensitive, it has been found that regardless of the type of shock condition, the timing of reversal of shock is a critical component in outcome.

Pulmonary: Mark Gladwin, M.D., Chief, Pulmonary and Vascular Medicine Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland. There are few data regarding the pulmonary effects of HBOCs. Clinically, pulmonary hypertension, and pneumonia have been identified as adverse effects of some HBOCs. Pulmonary hypertension may have contributed to the increased incidence of cardiac arrest observed with some HBOCs, and conceptually, the occurrence of pneumonia, sepsis, and pancreatitis with multiorgan failure may contribute to an increased risk of adult respiratory distress syndrome (ARDS).

Examining consequences of other circumstances where free hemoglobin is present in the circulation may be instructive.[38] Pulmonary hypertension is an increasingly recognized complication of chronic hemolytic anemia, occurring in up to 33% of patients with sickle cell anemia. It has been hypothesized that oxygen itself results in vasoconstriction of the arterioles and that enhanced oxygen delivery by HBOCs to the arterioles rather than nitric oxide scavenging by the HBOC is responsible for the vasoconstriction associated with use of HBOC products. This mechanism does not explain the pulmonary hypertension that occurs with administration of an HBOC product because oxygen is a vasodilator in the pulmonary circulation. Second, use of a nitric oxide synthase inhibitor, NG-mono-methyl-L-arginine (LNMA), resulted in significant adverse effects in septic patients, a finding that should inform clinical trials with HBOC products that have nitric oxide scavenging properties.

Patients with paroxysmal nocturnal hemoglobinuria (PNH) suffer from many of the same symptoms as subjects administered HBOC products, including gastric dysmotility, thrombosis, pulmonary hypertension, and fa-

tigue independent of total hemoglobin concentration. These changes appear to be related to the presence of circulating hemoglobin that disrupts the cell-free zone adjacent to the vascular endothelium in flowing blood and which mediates nitric oxide consumption. Experimentally, in patients with sickle cell anemia, there is a dose-related inhibition by hemoglobin of forearm blood flow response to administered nitroprusside, a nitric oxide donor. Further, hemoglobin has been shown to activate platelets directly and to blunt the ability of nitric oxide to inhibit platelet activation.

More study on the pulmonary safety issues related to HBOC administration is needed, with particular emphasis on assessing nitric oxide-mediated pathways.

Session IV: Finding a Way Forward

Moderator: George Biro, M.D., Ph.D., Professor Emeritus, University of Ottawa and University of Toronto, Toronto, Ontario, Canada. In this session, the presenters addressed possible strategies to mitigate HBOC toxicities and potential approaches to the continuation of clinical trials.

Nitrite Reductase Activity of HBOCs

Dr. Gladwin focused on nitrite as an intravascular endocrine reservoir of nitric oxide.[39] His group has presented a hypothesis that hemoglobin is an allosterically regulated nitrite reductase with maximal nitric oxide generating "enzymatic" activity as it undergoes transition from oxy (R) to deoxy (T) states.[40] Increased forearm blood flow in human subjects infused with nitrite was presented as evidence of the nitrite reductase ability of hemoglobin in humans. Data were also presented to show the hypertensive effects of HBOC infusion in wild-type mice, which was not present in NOS3⁻ mice (mice congenitally deficient in endothelial nitric oxide synthase). In other experiments, pretreatment with nitric oxide breathing, which increased nitrite concentrations in blood in mice, prevented the systemic hypertension caused by infusion of murine tetrameric hemoglobin without causing methemoglobinemia. Similar results were observed by simple treatment with nitrite. It was concluded on the basis of these experiments that nitrite interacts with HBOCs to inhibit vasoconstriction while maintaining oxygen delivery. These data suggest that hemoglobin is a nitrite reductase that generates nitric oxide at low oxygen tension to modulate vasodilatation and cellular respiration and mediate cytoprotection after ischemia–reperfusion.

Engineering of Safer and Stable HBOCs

Protein engineering strategies to meet safety and efficacy requirements for HBOCs were discussed by John Olson, Ph.D. (Professor of Biochemistry and Molecular

Biology, Rice University, Houston, Texas).[41-45] It is possible to modulate hemoglobin affinity for oxygen (P50), rate of nitric oxide scavenging, resistance to auto and chemically-induced oxidation, and rate of heme loss. Hemoglobins produced with reduced nitric oxide scavenging decrease the HBOC-induced increase of blood pressure.

HBOCs and Microvasculature/Effect of Viscosity

Marcos Intaglietta, Ph.D. (Professor, Department of Bioengineering, Microhemodynamics Laboratory, University of California, San Diego, California) discussed microcirculation and the possible interactions between nitric oxide scavenging by hemoglobin (vasoconstriction) and the viscosity of blood. He suggested that increased plasma viscosity may generate nitric oxide, thus potentially ameliorating the scavenging of nitric oxide by hemoglobin. He suggested that increasing plasma viscosity with appropriately engineered HBOCs could overcome the physiologic effects of their scavenging of nitric oxide. The functional capillary density of hamster skin window chamber model increases with infusion of both non-oxygen carrying molecules and HBOCs of increased viscosity.[5,46,47]

Endogenous Scavengers of Hemoglobin

Dominik Schaer, M.D. (Clinic for Internal Medicine, Department of Medicine, Medical Clinic Research Unit, University Hospital, Zurich, Switzerland) focused on the interactions of HBOCs with endogenous mechanisms of hemoglobin clearance, namely haptoglobin and the monocyte/macrophage cell surface receptor, CD163, which have largely been unstudied with regard to modified hemoglobin. An important and unrecognized novel function of haptoglobin is to control the cell-free hemoglobin induced hypertensive response, as shown in a dog model of pharmacologic haptoglobin induction. Chemical modifications of hemoglobin within its central cavity and on its surface appear to determine the predominant mechanism by which systemic and local HBOC clearance can occur. Intramolecular crosslinking between β-globin chains, irrespective of molecular size, preferentially facilitates haptoglobin binding, normalization of hypertension, and systemic vascular resistance in haptoglobin-expressing dogs. CD163 interaction and clearance by circulating macrophages and resident tissue macrophages is dependent on the molecular size of HBOCs such that molecular size correlates inversely with CD163 binding, cellular uptake, and clearance. The potential for certain chemical modifications on hemoglobin to facilitate multivalent binding interactions with haptoglobin could potentially alter blood rheologic properties and could contribute to ischemic toxicity associated with certain HBOCs. Endogenous mechanism of hemoglobin clearance should be taken into account when designing and evaluating HBOCs.

Better Targeting of Nonclinical Studies

Joy Cavagnaro, Ph.D., D.A.B.T., R.A.C. (President, Access BIO, Boyce, VA) noted that the following should be considered in nonclinical evaluation to improve value: emphasis on animal species that reflect human physiology, emphasis on models that can mimic human vascular compromise, physiologic adaptation in important target organs, evaluation of more than one animal model to address specific questions, organ blood flow and distribution should be understood as part of the nonclinical evaluation of any HBOC, similarly organ specific differences in vasoconstriction must be understood with individual HBOCs. Nonclinical assessment is an iterative process. The design of highly specific nonclinical programs for HBOCs can be achieved and improved on the basis of the vast array of knowledge gained from clinical experiences and an understanding of normal and pathologic physiology.

Statistical, Ethical, and Clinical Trial Design Considerations

Three speakers addressed questions about interpretation of existing clinical data and implications for future clinical research directions. Each expressed his own opinion about the implications of existing data, but these opinions do not necessarily reflect a consensus of opinion about this class of products.

Statistical Considerations

Thomas Fleming, Ph.D. (Professor, Department of Biostatistics, University of Washington, Seattle, WA) discussed the role of meta-analysis in understanding safety (and efficacy) of a class of drugs, and aspects of safety assessments in clinical trials.[48] Because clinical trials with HBOCs have had various designs with various primary endpoints in several clinical circumstances and because there is only a limited database for HBOCs, the advantage of a meta-analysis is the creation of a sufficiently sized cohort to enable an appropriate analysis of risk:benefit. Ordinarily a meta-analysis including more than one pharmaceutical requires that they have similar mechanism of action for efficacy and safety. Knowledge about HBOC mechanism of action and toxicity is just emerging, making it difficult to know whether data can be pooled for a meta-analysis. However, only limited data are available in the public domain; therefore, pooling of data to discern patterns becomes a necessity. Although there is some heterogeneity in terms of myocardial infarction, it is difficult not to accept that there is a signal and that the signal applies to all the agents that have been studied. Dr. Fleming expressed his opinion that HBOCs should be evaluated in settings where the benefit:risk ratio is maximized as in populations at high risk of dying (hemorrhagic shock, etc.) or where blood is not an option or available.

Ethical Considerations

Ezekiel Emanuel, M.D., Ph.D. (Chief, Clinical Bioethics Department, National Institutes of Health, Bethesda, MD) discussed ethical considerations for HBOCs, noting that they must fulfill the several requirements for ethical clinical trials,[12] but he focused on his views regarding social value, scientific validity, and risk:benefit.

The social value of infusing an HBOC lies in the potential need for HBOCs: avoiding the complications of RBC transfusions, availability of oxygen-carrying capacity when there is an urgent, life-threatening blood loss and no RBC supply, and saving money at no higher risk level. However, Dr. Emanuel questioned the need for HBOCs, given the safety and availability of red cells, including in trauma and military settings, although it is difficult to quantify the risk of future potential for transmission of infectious disease vectors, whether there is an RBC availability problem, and the low likelihood that HBOCs will cost less than RBCs.

With regard to scientific validity, Dr. Emanuel expressed his opinion that further clinical trials with HBOCs should be designed to test for superiority, rather than noninferiority, against red cells, allowing for the possibility of testing for equivalence in the unlikely condition that the HBOC would cost substantially less than blood. It was acknowledged that there may not be a way to conduct a superiority trial.

With regard to risk:benefit, Dr. Emanuel expressed his view that the information presented and discussed in the workshop regarding risk:benefit ratio did not offer a positive profile for any HBOC.

Approach to Clinical Trial Design

Jeffrey L. Carson, M.D. (Professor and Chief, Division of General Internal Medicine, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School of Medicine, Camden, NJ) commented that some HBOC clinical trials had been plagued by a relatively poor adherence to the trial design and suggested that this could be ameliorated by the use of pilot trials. He further suggested that, given that data regarding RBC efficacy are very sparse, future trial design should consider (1) appropriate control comparator, (2) noninferiority *versus* superiority design, (3) choice of primary endpoint, (4) clinical population/setting, and that these interact with each other. For example, he thought that comparison of an HBOC to crystalloid requires demonstration of superiority, whereas a noninferiority design might be appropriate if the comparator is red blood cells. He suggested that because allogeneic blood is relatively safe and currently not in short supply, the endpoint of any such clinical trial should be clinically meaningful, either mortality or a major predefined morbidity. He viewed that reduction in allogeneic transfusion, unless a significant reduction, would not be an appropriate endpoint.

Dr. Carson's preferred settings would be religious objection to blood transfusion, trauma (prehospital, with conversion to blood after hospital arrival), and perhaps a trial in intensive care units should increased age of stored blood be shown to reduce safety relative to blood stored for shorter periods of time, all with composite endpoints of mortality and major morbidity.

Summary and Conclusion

This report summarizes the scientific presentations and opinions of the speakers at a conference sponsored by the FDA and NIH on April 29–30, 2008 to (1) review the existing publicly available information about the characteristics and clinical profiles of the products that are or were in development, (2) discuss the potential mechanisms of toxicity both for the whole organism or by specific organ system(s), and (3) consider the feasibility of, obstacles to, and ethical issues related to future clinical trials of HBOCs.

The effects observed in conventional studies conducted in healthy, normal (inbred) young animals do not mimic those of clinical tests of HBOCs, many of which have been conducted in populations with elderly, diabetic, atherosclerotic, hypertensive patients with significant comorbid conditions who are undergoing elective surgical procedures. A prominent feature of these populations and conditions is the presence of endothelial dysfunction, including impaired nitric oxide response and exaggerated endothelin response.

Results of clinical trials and organ-specific aspects of safety were presented and discussed to endeavor to respond to the following questions.

(1) Can information about the safety and efficacy obtained from clinical trials in one setting be applied to another? (2) Given what is known about the biochemistry and pharmacology of the current and previous HBOCs, can safety information obtained from the study of one HBOC be used to inform safety and risk assessments for a different HBOC? (3) Are there toxicities or harmful interactions between these molecules and a patient's underlying disease(s) that are common to all of these molecules? (4) Are there lessons from what was discussed for designing future trials?

A variety of opinions were expressed regarding these issues; however, most speakers thought that the most substantial challenge for the field remains finding clinical situations where there is a favorable balance of benefits and risks for hemoglobin-based oxygen therapeutics and finding appropriate methods for evaluating the efficacy and safety of HBOCs in those settings.

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Appendix 1: Steering Committee

1. Abdu Alayash, Ph.D. (cochair), Chief, Laboratory of Biochemistry and Vascular Biology, Center for Biologics & Research (CBER), Food and Drug Administration (FDA), Rockville, MD

2. Paul Buehler, Pharm.D., Ph.D., Division of Hematology, Office of Blood Research and Review, Center for Biologics Evaluation and Research, Food and Drug Administration (FDA), Rockville, MD

3. Jay Epstein, M.D., Director, Office of Blood Research and Review, Center for Biologics Evaluation and Research, Food and Drug Administration (FDA), Rockville, MD

4. Joseph Fratantoni, M.D., Vice President Medical and Clinical Development, Maxcyte, Inc., Gaithersburg, MD

5. Mark Gladwin, M.D., Chief, Pulmonary and Vascular Medicine Branch, National Heart, Blood, and Lung Institute, National Institutes of Health, Bethesda, MD

6. Sara Goldkind, M.D., Office of the Commissioner, Food and Drug Administration, Rockville, MD

7. Jonathan Goldsmith, M.D.

8. Jerry Holmberg, Ph.D., Senior Advisor for Blood Policy, Executive Secretary of the Advisory Committee on Blood Safety and Availability, US Department of Health and Human Services (DHHS), Office of the Secretary, Office of Public Health and Science, Rockville, MD

9. Harvey Klein, M.D. (cochair), Chief, Transfusion Medicine, National Institutes of Health, Bethesda, MD

10. George Nemo, Ph.D., Acting Deputy Director, Division of Blood Diseases and Resources, National Heart Lung and Blood Institute (NHLBI), Bethesda, MD

11. Alan Schechter, M.D., Chief, Molecular Medicine Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD

12. Toby Silverman, M.D., Chief, Clinical Review Branch, Division of Hematology, Office of Blood Research and Review, Center for Biologics & Research (CBER), Food and Drug Administration (FDA), Rockville, MD

13. Richard Weiskopf, M.D., Professor, University of California, San Francisco, CA

Appendix 2: List of Panelists

Session I

Moderator: Joseph Fratantoni, M.D., Vice President Medical and Clinical Development, Maxcyte, Inc., Gaithersburg, MD

1. Abdu Alayash, Ph.D., Chief, Laboratory of Biochemistry and Vascular Biology, Center for Biologics & Research (CBER), Food and Drug Administration (FDA), Rockville, MD

2. George P. Biro, M.D., Ph.D., Adjunct Professor, Department of Physiology, University Of Toronto, Ontario, Canada

3. H. Franklin Bunn, M.D., Hematology Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

4. Alan Schechter, M.D., Chief, Molecular Medicine Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD

Session II

Moderator: Barbara Alving, M.D., M.A.C.P., National Center for Research Resources, National Institutes of Health, Bethesda, MD

1. Toby A. Silverman, M.D., Chief, Clinical Review Branch, Division of Hematology, Office of Blood Research and Review, Center for Biologics Evaluation & Research, Center for Biologics Evaluation & Research (CBER), Food and Drug Administration (FDA), Rockville, MD

2. Sara Goldkind, M.D., Office of the Commissioner, Food and Drug Administration, Rockville, MD

3. Peter Keipert, Ph.D., Vice President of Clinical And Regulatory Affairs, Sangart, Inc., San Diego, CA

4. Steven A. Gould, M.D., Chairman and CEO, Northfield Laboratories, Evanston, IL

5. Abraham Abuchowski, Ph.D., President and COO, Prolong Pharmaceuticals, Monmouth Junction, NJ

6. A. Gerson Greenburg, M.D., Ph.D., Vice President, Medical Affairs, Biopure Corporation, Professor Emeritus of Surgery, Brown University, Providence, RI

7. Tim Estep, Ph.D., President, Chart Biotech Consulting, Boulder, CO

8. Joseph DeAngelo, M.S., Chief Development Officer, Apex Bioscience, Inc., Chapel Hill, NC

Session IIIa

Moderator: Harvey Klein, M.D., Chief, Transfusion Medicine, National Institutes of Health, Bethesda, MD

1. Steven Cohn, M.D., University of Texas Health Science Center, Houston, TX

2. Demetrios Demetriades, M.D., Ph.D., Professor of Surgery, Department of Surgery, Division of Trauma and Critical Care, University of Southern California, Los Angeles, CA

3. Mitchell P. Fink, M.D., President And CEO, Logical Therapeutics, Inc., Waltham, MA

4. Daniel Freilich, C.D.R., M.C., U.S.N., Naval Medical Research Center, Combat Casualty Directorate, Silver Spring, MD

5. John B. Holcomb, M.D., Commander, US Army Institute of Surgical Research, Brooke Army Medical Center, San Antonia, TX

6. Charles Natanson, M.D., Critical Care Medicine Department, National Institutes of Health, Bethesda, MD

7. Edward J. Norris, M.D., M.B.A., F.A.H.A., Associate Professor, Department of Anesthesiology and Critical Care Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD

8. Edward P. Sloan, M.D., M.P.H., University of Illinois at Chicago, Department Of Emergency Medicine, Chicago, IL

9. Gus J. Vlahakes, M.D., Professor of Surgery, Harvard Medical School, Division of Cardiac Surgery, Massachusetts General Hospital, Boston, MA

Session IIIb

Moderator: Richard B. Weiskopf, M.D., Professor, University of California, San Francisco, CA

1. Andrew Baines, M.D., Ph.D., University of Toronto, Ontario, Canada
2. Mitchell P. Fink, M.D., President and CEO, Logical Therapeutics, Inc., Waltham, MA
3. Mark Gladwin, M.D., Chief, Pulmonary and Vascular Medicine Branch, National Heart, Blood, and Lung Institute, National Institutes of Health, Bethesda, MD
4. Joseph E. Parrillo, M.D., Professor of Medicine, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Camden, NJ
5. Raymond F. Regan, M.D., Department of Emergency Medicine, Thomas Jefferson University, Philadelphia, PA
6. David C. Waltier, M.D., Ph.D., Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, WI

Session IV

Moderator: George Biro, M.D., Ph.D., Professor Emeritus, University of Ottawa and University of Toronto, Ontario, Canada

1. Jeffrey L. Carson, M.D., University of Medicine and Dentistry Of New Jersey, Robert Wood Johnson Medical School, Camden, NJ
2. Joy Cavagnaro, Ph.D., D.A.B.T., R.A.C., President, Access Bio, Boyce, VA
3. Ezekiel Emanuel, M.D., Ph.D., Chief, Clinical Bioethics Department, National Institutes of Health, Bethesda, MD
4. Thomas Fleming, Ph.D., Department Of Biostatistics, University of Washington, Seattle, WA
5. Marcos Intaglietta, Ph.D., Professor, Department of Bioengineering, Microhemodynamics Laboratory, University of California, San Diego, CA
6. John Olson, Ph.D., Professor of Biochemistry and Molecular Biology, Rice University, Houston, TX
7. Dominik Schaer, M.D., Clinic for Internal Medicine, Department of Medicine, Medical Clinic Research Unit, University Hospital, Zurich, Switzerland
8. Gus Vlahakes, M.D., Professor of Surgery, Harvard Medical School, Division of Cardiac Surgery, Massachusetts General Hospital, Boston, MA
9. David Warltier, M.D., Ph.D., Department Of Anesthesiology, Medical College Of Wisconsin, Milwaukee, WI

Appendix 3: Statements of Conflict of Interest

The following information was available to FDA at the time of presentation on April 29–30, 2008

Disclosures of conflicts of interest reported to the FDA:

Abdu Alayash–None

Barbara Alving–None

Andrew Baines–Hemosol: research grant 1995–1996; Natural Science and Engineering Research Council of Canada: currently collaborating with R. Kluger to test the efficacy of new HBOC

George Biro–None

H. Franklin Bunn–Sangart, Inc.: Member of the Scientific Advisory Board

Jeff Carson–Biopure: Consultant 2006–2007; Northfield Laboratories: Consultants 1999–2003; published Gould SA, Moore EE, Hoyt DB, Ness PM, Norris EJ, Carson JL, Hides GA, Freeman IH, DeWoskin R, Moss GS: The life-sustaining capacity of human polymerized hemoglobin when red cells might be unavailable. *J Am Coll Surg* 2002; 195:445-52

Joy Cavagnaro–None

Stephen Cohn–Baxter, Biopure, Hemosol: past research grants, consultant (no interactions with these companies for more than 5 yr)

Joseph De Angelo–Apex Bioscience, Inc.: employee and officer, stock options; patent inventor in the field of hemoglobin-based therapeutics

Ezekiel J. Emanuel–None

Tim Estep–Baxter: Consultant 1996, current financial interests, not acting as Baxter representative at workshop, will primarily discuss testing of diaspirin crosslinked hemoglobin (DCLHb; HemAssist; an intramolecularly crosslinked human hemoglobin), will focus on DCLHb properties and factors potentially affecting the outcome of human Phase III clinical trials

Mitchell Fink–None

Joseph Fratantoni–None

Daniel Freilich–The views expressed in written and oral presentations during this workshop are those of Daniel Freilich, C.D.R., M.C., U.S.N., and do not necessarily reflect the official policy or position of the Department of Navy, Department of Defense, nor the U.S. Government; NMRC and Biopure Corp. (Cambridge, MA) have a Cooperative Research and Development Agreement (CRADA) for evaluation of HBOC-201 in trauma clinical trials. In addition, HBOC-201 has been provided to NMRC researchers, including Dr. Freilich, at no cost under a Materials Transfer Agreement (MTA). There has been no transfer of funds in any of these agreements. NMRC has transferred funds to Biopure (through USAAMDA and Fleet Industrial Supply Center) for purchase of HBOC-201 and vaso-activity-attenuated HBOC (VA-HBOC) formulations for testing in preclinical and clinical studies. NMRC has filed an IND application to the FDA to test HBOC-201 in a prehospital traumatic hemorrhagic shock government-sponsored and -funded trial called *RESUS*. Dr. Freilich has no financial or other competing interests related to any HBOC research

Mark Gladwin–Patent: coinventor on an NIH government patent for the use of sodium nitrite for cardiovascular indications; Patent application: inventor on an NIH government patent application for a method of use patent for nitric oxide and N2O3 generator hemoglobin-based blood substitutes

Sara Goldkind–None

Steven Gould–Chairman and Chief Executive Officer of Northfield Laboratories Inc. and a scientific founder of Northfield

A. Gerson Greenburg–Biopure Corporation: Vice President of Medical Affairs

Marcos Intaglietta–Sangart, Inc.: Consultant, Financial Interest

Harvey Klein–Baxter, Biopure, Somatogen: Consultant (not in the last 5 yr); Sangart, Inc.: NIH materials transfer agreement

Charles Natanson–Hemosol Biopharma: Consultant, 2004; Research study with Sangart, Inc. (materials transfer agreement); Co-author patent application, nitrites as therapeutic agents to prevent hemolytic injury

John Olson–None; many of his former graduate students and post-doctoral students have worked or are still working for companies in the blood substitute field

Joseph Parrillo–None

Raymond Regan–Sangart: research contract 2003; no subsequent contact

Alan Schechter–None

Toby Silverman–None

David Warltier–None

Richard Weiskopf–Alliance Pharmaceutical: consultants 1998–2000; Somatogen: consultant; research grant: phase I trial, 1997–1998; Hemosol: chair, independent safety review committee 2003

Disclosures of conflicts of interest were not received from the following individuals: Abraham Abuchowski, Demetrios Demetriades, Thomas Fleming, John Holcomb, Edward Norris, Dominik Schaer, Edward Sloan, Gus Vlahakes, and Robert Winslow