Chapter 39
International Consortium for Development of Hemoglobin-Based Oxygen Carriers, Oxygen Therapeutics and Multifunctional Resuscitation Fluids–A White Paper

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39.1 Background

Today, allogeneic donor blood transfusion has evolved as a life-saving treatment for many acute anemic conditions. In developed countries, safe donor blood supply is generally adequate for routine clinical demands. However, in situations where demand greatly exceeds supply (e.g., natural or man-made massive disasters), matched donor blood is not immediately available (e.g., remote locations, battlefield, a rare blood type) or blood transfusion is not an option (e.g., certain religious group or patients with an unusual antibody status), currently there is no alternative treatment.

Based on the post-WWII experience, it is estimated that approximately 20 % of battlefield casualties are potentially salvageable (IOM 1999). The single most cause of death in battlefield casualties is hemorrhage. Therefore, there is greatest opportunity for reducing morbidity and mortality in this group if a safe and battlefield usable 'blood substitute' is available. In recognition of the potential life

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saving benefit, the US Department of Defense, Office of Naval Research, is supporting development of a multifunctional resuscitation fluid (MRF) that may contain a low volume electrolyte solution, an oxygen carrier and coagulation factor(s) in a ready to use battlefield friendly package (ONR 2010).

Of note, blood transfusion carries a risk of disease transmission (e.g., AIDS, hepatitis, malaria, STD, etc.) and certain non-infectious risks (e.g., clerical errors, transfusion reactions, TRALI, immuno-modulation). In sub-Saharan Africa, supply of safe donor blood is scarce because of high prevalence of HIV/AIDS (as much as 30 % in some countries or approx 25 million people) and other transmittable diseases among the donor population (e.g. malaria, trypanosomiasis, leishmaniasis) and inadequate donor blood screening due to limited resources (WHO 2008). Moreover, more than half a million women die each year of severe post-partum hemorrhage representing up 50 % of maternal death in some countries in Africa and Asia due to shortage of safe blood (WHO 2007). Even in the developed countries, safe donor blood is in greater demand as elderly population increases (who more likely to have surgery requiring transfusion) while eligible donor pools decrease due to stagnation in population growth and emergence of newly identified transfusion transmittable pathogens (e.g., vCJD, H1N1 and West Nile viruses, etc.) (Alter and Klein 2008).

Further, allogeneic donor blood can only be used in patient with compatible antibody status requiring typing and crossmatching before use. Donor blood is limited in supply and can only be stored for 5 weeks under refrigerated conditions. In addition, there is ongoing debate that blood transfusion (especially with older blood) may be harmful in certain situations (Alter 2008; Stowell 2010).

Considering these facts, there is a great need to develop universally compatible and readily available alternatives to allogeneic donor blood (red blood cells) for use especially when transfuable blood is not available or an option.(Weiskopf and Silverman 2013). A ‘red blood cell substitute’ that is safe and effective in saving lives by adequate oxygen delivery and tissue oxygenation preserving vital organ functions during the severe hypovolemia and other acute anemic conditions would be highly desirable.

39.2 Development Status of HBOC, Oxygen Therapeutics (OT) and Multi-Functional Resuscitation Fluids (MRFS)

Over the last 30 years, hemoglobin-based oxygen carriers (HBOCs) have been in development as safe and clinically effective therapeutics (‘red cell substitutes’) for treatment of hemorrhagic shock, acute anemia, ischemia and other conditions. For several HBOC candidates, preclinical studies were generally positive and some leading products have been tested in Phase III clinical trials, a final stage of development process (reviews by Kim 2004; Jahr 2011). However, observations of some serious adverse events (SAEs) including severe hypertension, MI, stroke and

Table 39.1 FDA summary of adverse events reported in HBOC clinical trials (modified from Silverman et al. 2008)

	Baxter		Biopure		Hemosol		Northfield		Sangart		Somatogen	
Cohort	T	C	T	C	T	C	T	C	T	C	T	C
Number of subjects	504	505	708	618	209	192	623	457	85	45	64	26
1. Death	78	61	25	14	1	4	73	39	2	0	*	*
2. Hypertention	76	38	166	59	113	75	*	*	7	1	8	0
3. Pulmonary Hypertension	1	0	3	0	*	*	*	*	*	*	*	*
4. Chest pain/chest tightness	*	*	21	16	*	*	*	*	*	*	6	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	4	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	645	195	23	1	*	*			36	6
16. Liver, LFTs abnormal	27	8	20	5	8	0	*	*	57	20	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

T HBOC treated group
C Control solution treated group
* No information available
Note Apex and Enzon also conducted clinical trials but data were not reported

death in recent HBOC clinical trials (Silverman 2009, Table 39.1) and a highly controversial Meta analysis that HBOCs are associated with increased risk of MI and death (Natanson 2008) are hampering further development of HBOCs as viable therapeutics. Recent workshops organized by NIH and FDA (Estep et al. 2008; Silverman 2009; NIH 2011) discussed current issues and provided recommendations on directions of future HBOC research and development.

The causality of HBOCs for the observed SAEs has not definitively been established. To elucidate the pathophysiologic mechanisms of AEs observed with HBOCs, it is essential to understand how HBOCs affect key organ systems and their physiologic functions not simply in normal subjects but in patients. Studies conducted in models of healthy young animals have failed to predict the pathophysiologic responses observed in actual patients who often are older and present with multiple co-morbid conditions (e.g., diabetes, hypertension, cardiovascular diseases, etc.). It is essential that preclinical safety studies be conducted in animal models that closely simulate target patient conditions. To further the development, investigations are required to establish causality of the observed serious adverse events (SAEs) and to test HBOC used and determine the pathophysiologic mechanism involved. Only when armed with accurate knowledge of pathophysiologic mechanisms of adverse events (AEs), may appropriate modifications be made to the current HBOC products or develop a new generation of safer products.

More recently, Mozzarelli (2011) presented a more open view on the strategies for designing a new generation of safer and effective products. Certain HBOCs are also being developed as oxygen therapeutics (OTs) targeted for treatments of ischemic tissues and organs (e.g., ischemic heart/limb, ischemic stroke). In addition, because many civilian and military hemorrhagic trauma victims are presented with coagulopathy, MRFs that contain procoagulation agents are also in development.

39.3 Current Issues and Barriers

The current impediment in the progress of HBOC development is in large part due to insufficient scientific understanding of some critical mechanisms. How an individual HBOC formulation, when administered intravenously, interacts with the host mechanisms in heightened or compromised state by disease, surgery or traumatic injury especially presented with concurrent underlying co-morbid conditions (e.g., hypertension, diabetes, cardiovascular diseases). It is an extremely complex dynamic process involving multiple cellular, tissue and organ systems which are ultimately integrated into the whole systemic response and its fate. To help facilitate HBOC development, a NIH-NHLBI organized working group workshops in 2006 (Estep et al. 2008) and 2011 (NIH 2011) and identified some of the key issues holding up the progress of the field (Table 39.2) and made a series of recommendations (Table 39.3). In addition, there are some inherent limitations in a traditional industry-centered collaboration model (Kim 2011).

Some of the key issues are:

- Animal models did not predict adverse effects observed in clinical trials Results of most preclinical animal studies conducted with various candidate HBOC products were generally positive. However, preclinical animal models did not predict the AEs observed in clinical studies. Therefore, there is a need to identify/develop animal models that are relevant to target patient conditions and

Table 39.2 Important issues to be addressed in HBOC development (Estep et al. 2008; NIH 2011)

- Development of animal models that more closely simulate human clinical conditions with co-morbidities (e.g., diabetes, hypertension, cardiovascular diseases)
- Development of new improved HBOCs (e.g., HBOCs with reduced vasoactivity and oxidative reactions, enhanced circulation time and shelf life)
- Investigation of mechanisms of cardiovascular and cerebrovascular events observed after blood substitutes infusion
- Significance of cardiac lesions observed after HBOC infusion
- Role of reactive oxygen species (ROS) in the etiology of human clinical side effects
- Exploration of interactive effects between blood substitutes infusion and concurrent fluid, drug and anesthetic therapies
- Effect of concurrent stress, particularly local or systemic inflammation, in response to blood substitutes infusion
- Investigation of the cause of bradycardia associated with HBOC administration
- Further study of mechanism and clinical significance of vasoactive effects of HBOCs
- Evaluation of the mechanism and clinical significance of gastrointestinal distress, pancreatic toxicity and liver and pancreatic enzyme elevation
- Comparative assessment of the antigenic and immunomodulatory properties of different blood substitutes
- Effects of formulation excipients
- Further study of the distribution and metabolism of HBOCs

Table 39.3 2006 NIH workshop recommendations (Estep et al. 2008)

- Exploration of the mechanism(s) of adverse side effects that have been observed during the clinical testing of HBOC formulations. Particular priority should be given to the investigation of cardiovascular and cerebrovascular events. The use of animal models with compromised cardiovascular systems and/or altered physiology in the evaluation of HBOC solutions is highly encouraged
- Further evaluation of the distribution and metabolism of different Hb derivatives, especially with reference to the role that these factors play in the etiology of adverse events and the determination of functional intravascular persistence
- Continued research into the physiology of oxygen delivery by acellular formulations ranging from subcellular to global levels of response, with emphasis on the microcirculation in different tissue beds
- Assessment of whether enhanced generation of ROS after HBOC infusion is responsible for clinically observed adverse events in humans
- Evaluation of the impact of HBOC formulation excipients on product toxicity and stability
- Development of new Hb active entities with improved adverse event profiles and enhanced intravascular functional persistence
- Identification and use of improved models for the comparative assessment of HBOC formulation safety and efficacy. Such models should be predictive of response in humans, incorporate stress conditions and be used to systematically evaluate the effect of variation in Hb structure, biochemistry and physical chemical properties
- Production and distribution of highly purified HBOC solution(s) in sufficient quantity to support the research and testing advocated elsewhere in this report
- Comprehensive assessment and reporting of the adverse events and physiologic response of HBOC solutions evaluated in clinical trials, recognizing that such an assessment would require the permission of commercial manufacturers and collaboration with the US FDA
- Development and validation of a noninvasive method for the routine clinical assessment of critical organ oxygenation to better inform decisions to transfuse HBOCs and blood

to employ more sensitive tests that could detect molecular and cellular dysfunction as well as organ-specific toxicities and systemic abnormalities.

- Limited availability of test materials (HBOCs/OTs)
  One well recognized issue in HBOC development is limited availability of test HBOC solutions for conducting independent investigations. Because most HBOC products are not yet marketed, independent investigators have difficulty obtaining test HBOC products to conduct evaluations. To allow more independent evaluations of candidate HBOC products, 2006 NIH-NHLBI Workshop (Estep et al. 2008) recommended that supply of sufficient amount of well characterized standardized HBOC formulations be made available to the general research community. To help accomplish that recommendation, NIH awarded a SBIR contract to a company that now make its products available for investigators albeit at a cost. However, because AEs and toxicities have been observed with different HBOC products, it is important that more than one product be made available to investigators. In addition, as deemed necessary certain experimental (as well as marketed) procoagulation products (e.g., platelets, fresh frozen plasma, cryoprecipitate, rFVIIa anti-fibrinolytics) should also be made available for development of MRFs. Therefore, this consortium will invite producers of HBOC/OT/MRF products to participate knowing that this will be a NIH/FDA guided pathway for possible regulatory approval. As such, the FDA will be invited in these deliberations and expected to provide advice/guidance to create a validated pathway for potential approval of products.
- Causality of HBOC in observed SAEs
  Cause(s) of the SAEs observed in HBOC clinical trials have not been thoroughly investigated. This is in part due to lack of detailed and objective information/data regarding the nature/circumstances of observed AEs. Currently, major HBOC developmental efforts are led by few companies that adopt a traditional industry-centered research model. This approach is a largely a 'closed' system in which most data (especially negative data) are kept confidential among the close collaborators only. The exact nature of negative results is not generally made available to a wider group of independent unbiased investigators. Therefore, outside researcher are often deprived of the opportunity to the timely investigation of SAEs and other side effects/toxicities. This 'closed' approach significantly hampers expeditious development of possible resolutions.

39.4 The HBOC/OT/MRF Research Consortium, a Way Forward

To facilitate progress of HBOC development, we propose a HBOC research consortium of key leading academic investigators and select HBOC producers from US, Europe and Asia. The consortium will serve as a think tank and a

coordinating body for collaborative efforts in investigation of the key unresolved scientific issues that are hampering further progress in HBOC development. The HBOC research consortium will facilitate development of viable HBOC products through concerted efforts of the some of the world's leading experts in the field. To encourage constructive discussions/solutions for key unresolved issues, the consortium will adopt an open communication policy and objective and transparent processes in the conduct of research. The goal of the consortium is to foster orchestrated collaborations and constructive discourse and to breakdown barriers that impede development of viable HBOC products.

Some key goals of the consortium are:

- Foster collaboration for expeditious resolutions of key unresolved issues in HBOC development.
- Coordinate collaboration to minimize unnecessary duplications/redundancies for maximum efficiency and conserve resources.
- More objective and transparent evaluation of candidate products by independent investigators exploiting state of the art methods to investigate physiological and biochemical mechanisms.
- Facilitate information/data exchange and prompt and timely dissemination of research findings through open presentations, publications and other media.
- Serve as a central 'library' for HBOC research and other relevant information obtained from public sources or voluntarily provided by authors, study sponsors and publishers. (copy right issue will be openly discussed and negotiated).
- Identify and secure funding for HBOC research/development (e.g., national and international, public and private funding agencies).
- Foster young investigators to enter into the field.
- Identify an optimal HBOC formulation and/or develop a new viable product in the next 10 years, including basic science, translational and FDA approved Phase 1 clinical trials.
- FDA will be invited to participate from the ground level to provide advice in design of experiments, protocol development and formulating guidelines to ensure that required preclinical and clinical studies are performed according to Good Laboratory Practice (GLP) and Good Clinical Practice (GCP) and other regulatory guidelines. This FDA guided product development approach will serve as a 'validated' pathway to eventual product approval.

To achieve the stated goals, the consortium will utilize a multi-disciplinary approach. The core groups of the consortium will be US-based but to maximize 'brain power' for more expeditious development, a select group of leading international experts will also be included (see Table 39.4). In addition, to maximize the probability of successful outcome (discovery/identification of a successful product), the consortium will evaluate multiple candidate products with distinct characteristics. The candidate HBOC products will be studied (for selected projects) by member investigators with no conflict-of-interest issues with the products being tested. All investigators agree to participate in the consortium will be asked to disclose any conflict-of-interest issues. If found a clear conflict, he/she

Table 39.4 Some potential projects (investigators will be invited based on relevant expertise)

- Systems biologic approach to investigation of HBOC-mediated AEs (e.g., hypertension/vasoconstriction, enzyme abnormalities, cardiac abnormalities, etc.) utilizing molecular, genomic and proteomic analytical tools as HBOC interaction with cells/organs is dynamic multi-faceted process necessitating collaborative efforts of multi-disciplinary experts
- Robust global safety evaluation of HBOCs/OTs/MFRs via total body assessment based on organ proteomics and clinical assays
- Temporal and between group comparison of key physiological parameters before, during and after infusion of control and test HBOC/OT/MRF agents using in vivo analytical tools including single photon emission computed tomography (SPECT)
- Role of HBOCs in radical mediated toxicity and organ dysfunction in hemorrhagic shock/resuscitation
- Mechanism(s) of HBOC-mediated vasoconstriction/hypertension and it relationship to observed AEs and organ dysfunction
- Toxicities or harmful interactions between HBOCs and a patient's underlying disease
- Study of mechanisms of cell-free HBOC-mediated AEs/SAEs
- Role of vascular endothelial dysfunction (including NO and endothelin response and barrier function)/inflammation on physiological response to HBOCs
- Pathophysiologic relationship of post-trauma/hemorrhage immunosuppressive conditions and HBOC-mediated AEs
- Development of MFRs that include a crystalloid solution, oxygen carrier and procoagulant agents
- Others deemed necessary and appropriate

will be withdrawn from participation or conduct of certain studies. Qualifying investigators will submit a specific research proposal studying a selected HBOC/OT/MRF product(s) according to a format adopted by the consortium in consideration of potential funding sources (including a full budget proposal within a proposed direct cost cap). Most relevant high priority projects/investigators will be selected and included in the final consortium research proposal to be submitted to an appropriate funding agency. Ethics Committee, Data Safety Monitoring Board and strict adherence to local Institutional Review Board policies will be enforced as appropriate.

39.4.1 Proposed Activities of Consortium

- Coordination of collaboration in a concerted manner to bring about investigation with efficient use of resources.
- Identify and define highest priority issues/areas to resolve in HBOC/OT/MRF research/development for the consortium investigator to undertake.
- Evaluation of several distinct multi-product candidates (e.g., acellular and cellular HBOCs, OTs, MRFs, etc.).

- Data mining of literature (and possibly relevant FDA database if proper arrangement can be made) for in-depth analyses utilizing system's biology approach.
- Identify and develop avenues/means to undertake collaborative research including possible source of funding.
- Data/information exchange/workshop on focused topics.
- Identify and develop standardized methods/assays/tools/test HBOCs and specialty reagents and quality standards for preclinical and clinical tests.
- Repository for test HBOCs/OTs/MRFs, preclinical and clinical study data, relevant literature and regulatory information/advice.
- Others as deemed appropriate.

Specific terms of collaborative activities including nature of projects, execution of experiments, data management/dissemination, copyright/IP and other issues will be defined in a written Memorandum of Understanding (MOU).

39.4.2 Organizing Members

- Hae Won Kim, Ph.D., Brown University, Providence, RI, USA.
- Jonathan S. Jahr, MD, UCLA, Los Angeles, CA, USA.
- Andrea Mozzarelli, Ph.D., University of Parma, Parma, Italy.
- Hiromi Sakai, Ph.D., Department of Chemistry, Nara Medical University, Kashihara, Japan.

The core of the consortium will be U.S.-based. Dr. Hae Won Kim (Brown University, Providence, RI) and Dr. Jonathan Jahr (UCLA, Los Angeles, CA) will serve as co-Directors and share responsibilities in the overall management and coordination of consortium activities. Additional members with expertise in selected areas of interest will be included once priority areas/projects are determined (see Table 39.4, for potential projects). Specific roles and responsibilities of each consortium member will be defined in a written MOU agreement.

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