Docket Number 2004D-0462

January 19, 2004

To: Division of Dockets Management (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Rm 1061
Rockville, MD 20852

From: Robert M. Winslow, MD
Sangart, Inc. and the University of California, San Diego

Re: "Guidance for Industry. Criteria for Evaluation of Oxygen Therapeutics as Red Blood Cell Substitutes"

The Blood Division should be congratulated on production of this very extensive and very helpful guidance document for the development and clinical testing of potential new "Oxygen Therapeutics." My comments are few.

1. Section III. B. Safety Considerations
    a. I believe the sentence, "For hemoglobin-based oxygen carriers, the recommended testing scheme is based on the hypothesis that one cause of toxicity involves the interaction of oxygen radicals or iron with cellular metabolism," gives an unbalanced emphasis on potential toxicity. There is no question that cell-free hemoglobin can act as an oxidant, and potential harmful effects have been demonstrated in vitro and in cell culture. However, I am not aware of data supporting the hypothesis that this is a principle, or even important source of *clinical* toxicity. Instead, both preclinical and clinical trials with hemoglobin-based solutions since 1949 have shown a clear pattern of physiological effect: elevated systemic and pulmonary pressure, reduced cardiac output and bradycardia. This triad correlates with arteriolar vasoconstriction and reduced tissue oxygenation. The mechanism of this effect is still not completely clear, although as you point out later, progress is being made and hypotheses have been offered including NO scavenging and hyperoxic vasoconstriction. In the absence of universal agreement on this issue, I believe FDA should require clear demonstration whether this triad has been eliminated, including relevant animal studies, with controlled volume status, comparing test article with purified (or stroma-free) hemoglobin to serve as a positive control. Clinical trials with any product that has not solved this problem should not be encouraged
2. Section III. B.3.b. Bacterial Transolcation
    a. The connection between the architectural changes in intestinal microvilli and the danger of bacterial translocation may be overstated.

2004D-0462 C1

3. Section III C.
    a. This section should be careful not to equate optimal properties of red blood cells (hemoglobin level, P50, for example) with cell-free oxygen carriers. A wealth of data now indicate that the mechanisms of oxygen transport for cellular vs. acellular oxygen carriers are very different, and no assumptions should be made about measures of potency. Rather, sponsors must develop their own measures of potency and be prepared to defend the rational for their use. P50 and hemoglobin levels are not sufficient (or even relevant) measures of potency. The goal of these products is to oxygenate tissue, and the surrogates used for red blood cells must not be assumed to apply to cell-free solutions. Insofar as O_2 content is used as a marker for efficacy, any measure of in vivo O_2 content must account for any loss of O_2 capacity due to heme oxidation.
4. Section III C.
    a. FDA should be aware that its recommendations reach beyond the developed world. In many parts of the world blood is either not available or unsafe. Therefore, FDA should consider that showing safety and efficacy equivalent to blood may be sufficient for approval.

Guidance for Industry

Criteria for Safety and Efficacy Evaluation of Oxygen Therapeutics as Red Blood Cell Substitutes

DRAFT GUIDANCE

This guidance is being distributed for comment purposes only.

Submit comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

Additional copies of this draft guidance are available from the Office of Communication, Training and Manufacturers Assistance (HFM-40), Suite 200N, 1401 Rockville Pike, Rockville, MD 20852-1448, or by calling 1-800-835-4709 or 301-827-1800, or from the Internet at http://www.fda.gov/cber/guidelines.htm.

For questions on the content of this guidance, contact, contact Toby Silverman, M.D. at 301-827-3524.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
October 2004

Contains Nonbinding Recommendations

Draft – Not for Implementation

Table of Contents

I. PURPOSE AND RATIONALE FOR RECOMMENDATIONS **1**

II. SCOPE OF RECOMMENDATIONS **1**

III. BACKGROUND DISCUSSION **2**

- **A. General** **2**
- **B. Safety Considerations** **2**
- **C. Efficacy Considerations** **6**
  - 1. Local Effects/Regional Perfusion 7
  - 2. Perioperative Indications 8
  - 3. Trauma 8

IV. RECOMMENDATIONS **9**

- **A. Preclinical Evaluation** **10**
  - 1. Characterization of the Product 10
  - 2. Animal Safety Testing 11
- **B. Clinical Evaluation** **13**
  - 1. General 13
  - 2. Elective Surgery 15
  - 3. Trauma 16

Contains Nonbinding Recommendations

Draft – Not for Implementation

Guidance for Industry

Criteria for Safety and Efficacy Evaluation of Oxygen Therapeutics as Red Blood Cell Substitutes

This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the appropriate FDA staff. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. PURPOSE AND RATIONALE FOR RECOMMENDATIONS

The development of oxygen therapeutics for a variety of clinical applications has progressed rapidly in recent years. The potential benefits of an oxygen therapeutic include universal compatibility, immediate availability, and long-term storage. This guidance document provides you, as a sponsor or investigator, with suggested criteria for testing the efficacy and safety of oxygen therapeutics as substitutes for red blood cells, and guidance on the design of clinical trials to assess risk/benefit ratio of such use.

The term "blood substitutes" has often been used to describe oxygen therapeutics, although to date, these products are not replacements for whole blood, either in terms of its many components or the duration of oxygen-carrying capacity. While this guidance document is restricted to use of oxygen therapeutics as substitutes for red cells, that is not the only indication being evaluated for these investigational new drugs. This guidance should not discourage innovation in the development of appropriate endpoints for and the design of clinical trials for other uses of oxygen therapeutics.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA's guidances means that something is suggested or recommended, but not required.

II. SCOPE OF RECOMMENDATIONS

We, the FDA, are providing current recommendations applicable to the development and preclinical or clinical evaluation of oxygen therapeutics as blood (red blood cell) substitutes. This guidance, when finalized, will supercede the "Points to Consider on the Safety Evaluation

of Hemoglobin-Based Oxygen Carriers" dated August 27, 1990, and replaces the draft "Guidance for Industry: Efficacy Evaluation of Hemoglobin- and Perfluorocarbon-Based Oxygen Carriers" dated September 1997. This guidance is based, in part, on presentations and discussions at a workshop entitled "Criteria for Safety and Efficacy Evaluation of Oxygen Therapeutics as Red Cell Substitutes" held on September 27-28, 1999. The workshop was sponsored by FDA's Center for Biologics Evaluation and Research (CBER), the National Heart, Lung, and Blood Institute, the Department of Defense, U.S. Army Medical and Material Command, and the Armed Services Blood Program Office. The transcript of this workshop is available on the CBER Web Page at www.fda.gov/cber/minutes/workshop-min.htm. We would like to acknowledge the participation and contributions of the members of the workshop steering committee, workshop panelists, and other speakers whose names are listed in the transcript of the workshop proceedings.

For general guidance, please consult the FDA Web Page, www.fda.gov. This site contains information and Internet addresses for other relevant FDA Guidance documents, International Committee on Harmonization (ICH) documents, and direct links to the homepage for CBER.

III. BACKGROUND DISCUSSION

A. General

Oxygen therapeutics are derived from hemoglobin- or fluorochemical-based compounds. The starting material for hemoglobin-based oxygen carriers may be a stroma-reduced hemoglobin, commonly referred to as stroma-free hemoglobin (SFH), chromatographically purified hemoglobin obtained from sources including outdated human blood or bovine blood, or recombinant hemoglobin. Stable and functional oxygen therapeutics are produced from these starting materials by various chemical and/or genetic manipulations. The resulting products include intra-tetrameric cross-linked hemoglobin, polymers of hemoglobin tetramers (intra- and inter-cross-linked), hemoglobin tetramers conjugated to non-protein macromolecules, or genetically stabilized tetramers.

Fluorochemical-based oxygen therapeutics are compounds in which hydrogen atoms on cyclic or straight chain hydrocarbons are replaced with fluorine. Some compounds also contain other atoms such as bromine. These compounds are emulsified in electrolyte solutions containing surface-active agents (surfactants) (perfluorochemical emulsions). Fluorochemical-based compounds are capable of dissolving large quantities of oxygen (without binding) at high concentrations of inspired oxygen.

B. Safety Considerations

A number of new and largely unresolved safety-related problems have arisen during the preclinical and clinical development of the current generation of hemoglobin-based products and perfluorochemical emulsions. The mechanisms of various observed

toxicities have not been elucidated fully. There is evidence to suggest that unmodified hemoglobin, which is filtered through the renal glomerulus, is nephrotoxic. This evidence suggests that unmodified hemoglobin (i.e., native tetrameric hemoglobin, mw - 64,000) should not be present in the final product and should be removed to the maximum extent possible. However, nephrotoxicity does not explain all the effects observed when hemoglobin-based oxygen carriers are infused. For hemoglobin-based oxygen carriers, the recommended testing scheme is based on the hypothesis that one cause of toxicity involves the interaction of oxygen radicals or iron with cellular metabolism. Thus, the modified oxygen carrying capacity of hemoglobin-based oxygen carriers could affect safety as a result of excess oxygen supply to cells and tissues, deficient supply, or oxidative effects of the hemoglobin moiety itself. Similarly, the toxicities observed in animals following administration of perfluorochemical emulsions may also be clinically significant. Activation of complement and procoagulant cascades may affect the safety of perfluorochemical emulsions and result in dose limiting toxicities in clinical practice. For both hemoglobin-based oxygen carriers and perfluorochemical emulsions, it is hypothesized that the effects seen are in part the result of activating any number of triggered enzyme or cellular systems.

Current oxygen therapeutic research efforts are aimed at gaining a better understanding of reactions in humans and developing safe and effective products. Following is a list of toxicities and laboratory findings known or thought to be associated with the use of hemoglobin-based oxygen therapeutics. The list is not intended to be all-inclusive, and it also includes some hypothetical toxicities that have not been demonstrated experimentally or clinically, but which, nevertheless, merit consideration.

1. Vasoactivity

Almost all hemoglobin products, regardless of their molecular weight distribution, are associated with vasoactivity in humans and in animal models. The etiology and clinical consequences of the vasoactive property of hemoglobin are still controversial and not completely understood. One hypothesis is that the interactions between cell-free hemoglobin and nitric oxide (NO), the endothelium-derived relaxing factor, may be a primary event that contributes to a vascular inflammatory response progressing to multi-organ failure. Other hypotheses suggest that cell-free hemoglobins modulate adrenergic receptor sensitivity and stimulate endothelin-1, a peptide with vasoconstrictor activity. Even modified hemoglobin products with small effects on blood pressure may result in significant vasoconstriction and increased vascular resistance.

2. Cardiac Toxicity

Administration of a hemoglobin-based oxygen carrier has been reported to be associated with the development of myocardial lesions characterized by mild to moderate focal and multifocal degeneration and/or necrosis of cardiac myocytes in certain animal models. The lesions have been reported to be seen 24-48 hours

after a single infusion of product. Left ventricular myocardium near the base of the papillary muscle is most prominently affected, with other affected regions including the interventricular septum and the right ventricle. Animal species most commonly affected include rhesus monkey and pig. There are significant animal species differences in sensitivity to the effect of the oxygen therapeutic; dogs, sheep, and rats did not develop the lesion after single infusion of the hemoglobin-based oxygen carrier.

3. Gastrointestinal Toxicity

a. Discomfort.

Symptoms reported in clinical trials include nausea, vomiting, dysphagia, or generalized abdominal pain. These symptoms are believed to be related to NO scavenging by hemoglobin, causing localized spasm throughout the gastrointestinal tract.

b. Bacterial Translocation.

Animal experiments indicate changes in the architecture of the intestinal microvilli within minutes of infusion of some hemoglobin-based oxygen carriers. These experiments suggest the possibility that the incidence or the severity of bacterial translocation across the gut epithelium may be increased.

4. Pro-inflammatory Activity

Infusion of hemoglobin in small animals stimulates monocyte procoagulant activity resulting in disseminated intravascular coagulation. Early in vivo studies in rabbits, but not in other animal species, demonstrated pulmonary arteritis and thrombotic lesions possibly related to procoagulant activity. In vitro studies on the effect of hemoglobin on leukocytes in whole blood demonstrate that in the presence of other blood components, leukocytes release pro-inflammatory cytokines such as tumor necrosis factor (TNF) and interleukin-8 (IL-8).

5. Oxidative Stress

Numerous in vitro and some in vivo reports suggest that hemoglobin solutions may induce an oxidative stress. Oxidative stress may, in part, be explained by the ability of hemoglobin to serve as a source of toxic oxygen species and/or the ability of hemoglobin to remove nitric oxide, an important component of normal antioxidant mechanisms. Indirect evidence in humans of such effects is seen with reports of increased enzyme activity including creatine phosphokinase (CK), lactic dehydrogenase (LDH), and pancreatic enzymes, lipase and amylase (see below).

6. Pancreatic and Liver Enzyme Elevation

Reports of elevated pancreatic and liver enzymes in a number of animal models of exchange transfusion suggest possible free-radical mediated injury. Increased levels of amylase and lipase after hemoglobin infusions in animals and humans have been frequently observed. The pattern and magnitude of lactate, CK, and LDH elevations seen in animal studies are similar to those reported in humans and also suggest possible free-radical mediated injury.

7. Endotoxin Synergy with Hemoglobin

Endotoxin, a bacterial product, and hemoglobin have been shown to exert synergistic toxicity when hemoglobin is given in a clinically relevant dose as a resuscitative fluid. Direct biochemical interactions between hemoglobin and endotoxin have been shown to reduce clearance of endotoxin from the circulation and to enhance lethality in some animal models of sepsis. This is a worrisome feature since these products may be given in some instances to patients with ongoing infectious processes or to trauma victims with contaminated wounds.

8. Neurotoxicity

Although experimental data suggest that acellular hemoglobins scavenge NO and may inhibit NO-related neurotoxicity, numerous published reports implicate free hemoglobin in the degenerative changes in the brain in experimental models of subarachnoid hemorrhage. Direct neurocytotoxicity of modified hemoglobin is suggested by pre-clinical studies in which neurons in culture were killed by the addition of hemoglobin, leaving glial cells intact. In one clinical report, use of an acellular hemoglobin product was an independent predictor of an unfavorable outcome at three months following acute ischemic stroke. The mechanism of this clinical toxicity has been hypothesized to be, in part, due to the potent vasoconstrictor effect of endothelin-1 which was increased in a dose-dependent manner by the administration of the acellular hemoglobin product.

<u>Toxicities known or thought to be associated with one or more of the current perfluorochemical emulsions include the following:</u>

1. Thrombocytopenia

The basis for this side-effect in animals and humans is not fully understood, but may be related to metabolism and normal clearance process of these compounds. Fluorochemical compounds may lead to some changes in the surface characteristics of platelets and subsequent uptake of the altered platelets by the liver and the spleen. Platelets may appear "functional" in aggregation and bleeding time measurements in vitro, but may potentially have a short half-life in circulation even though platelet production is normal.

2. Complement Activation and Cytokine Release

At present, although a hypothetical toxicity, there are no experimental data to suggest that complement activation occurs with the use of perfluorochemical emulsions.

3. Reticuloendothelial Blockade

After intravenous administration, the droplets of perfluorochemical emulsion are taken up by the reticulo-endothelial system. It has been hypothesized that administration of perfluorochemical emulsions may affect ability to clear circulating bacteria.

4. "Flu-like" Symptoms

Administration of perfluorochemical emulsions in humans has been associated with the development of flu-like symptoms and transient elevations in proinflammatory cytokines. The etiology of this phenomenon is not known.

5. Central Nervous System Effects

Cerebrovascular accident has occurred in the context of administration of perfluorochemical emulsion. The pathophysiologic basis for the association has not been elucidated.

C. Efficacy Considerations

In addition to the safety considerations described above, oxygen therapeutic products present several issues relating to efficacy. Efficacy endpoints may be direct measures of clinical benefit (improved survival, alleviation of symptoms) or they may be laboratory measurements or physical signs expected to correlate meaningfully with clinical benefit. The latter are referred to as surrogate endpoints and, once validated, are especially important in the case of oxygen therapeutics since direct demonstration of efficacy is likely to be very difficult (as it has been for red blood cells, per se). Validation of a surrogate endpoint for a therapy includes generation of clinical data demonstrating that effects of the therapy on the surrogate endpoint are reasonably likely to predict clinical benefit. *See* 21 CFR 601.41. Factors of importance when considering acceptability of surrogate endpoints include feasibility of using direct clinical measurements, risk/benefit assessments, and perhaps most importantly, knowledge and understanding of the disease and of the agent.

There has been extensive clinical experience with red cell transfusion, resulting in a practical appreciation of relevant indications, benefits, and risks. There is also an extensive collection of data on red blood cells, the anemic state, and their interaction,

resulting from years of basic and applied research. Thus, although it is not possible to document the clinical benefit of all red cell transfusions with specific endpoints, the available knowledge relevant to such transfusions support use of surrogate endpoints such as the P_{50}, the oxygen content and the hematocrit as suitable endpoints to demonstrate efficacy of red cell transfusions in clinical practice and in some clinical trials. Currently, we do not consider these surrogate endpoints to be acceptable as measures of the effects of hemoglobin- and perfluorochemical-based red cell substitutes, because knowledge of the effects of hemoglobin- and perfluorocarbon-based red cell substitutes and of the interaction of these agents with various clinical states is rudimentary. Further, no oxygen carrier presently approved by FDA has all the properties of the human red cell, nor are any two products identical. We recommend that the endpoints used in clinical studies of these agents be selected with these caveats in mind.

Under certain circumstances, oxygen therapeutic agents may qualify for Fast Track designation. Criteria and considerations for Fast Track designation may be found in "Guidance for Industry: Fast Track Drug Development Programs. Designation, Development, and Applicataion Review" dated September 1998. The guidance document may be found at http://www.fda.gov/cber/guidelines.htm.

There are several potential indications for oxygen therapeutics. Below, we discuss the following three such uses for these products: 1) local effects/regional perfusion, 2) perioperative indications and 3) trauma. As noted earlier, this discussion is not intended to represent the only approaches to evaluating clinical use of oxygen therapeutics, nor are investigators required to accept these categories.

1. Local Effects/Regional Perfusion

This category might best be defined by considering two examples: perfusion during coronary angioplasty and enhancement of tumor radiosensitivity. Perfusion, via the central lumen of a catheter used for percutaneous transluminal coronary angioplasty (PTCA), is an FDA-approved indication for a perfluorocarbon preparation (Fluosol). The data that supported this approval included clinical studies utilizing surrogate endpoints of left ventricular function that had been validated as clinically relevant by recognized cardiologic investigations. Future studies for this indication could conceivably utilize similar clinical trial design, with specific endpoints appropriately updated. The rationale for use of oxygen carriers (systemically administered) in therapy of neoplasms is based on the observation that increased tumor tissue oxygen tension will increase the sensitivity of tumors to radiation or to chemotherapy more than that of normal tissue. Demonstration of increased oxygen tension in the target tumor can function as an important supporting argument for efficacy, but would not be likely to serve alone as the primary endpoint. Ultimately, we recommend that the endpoint used to establish efficacy be similar to that used in evaluation of cytotoxic agents for the stage and type of cancer under investigation.

2. Perioperative Indications

Sponsors must monitor their investigations and evaluate pertinent risks to research subjects. (21 CFR 312.56.) In the case of investigational oxygen therapeutic products for proposed perioperative indications, such risks include the risks to subjects receiving the oxygen therapeutic in lieu of allogeneic transfusion (*e.g.*, inferior perfusion, undesirable hemodynamic responses, and other adverse drug reactions). In situations where red blood cells are used, reduction in the use of allogeneic blood is a surrogate endpoint for the avoidance of the risks associated with the use of allogeneic blood including, but not limited to, viral transmission, incompatibility, etc. In the past, we have accepted reduction in the use of allogeneic blood (for instance, in the indications for erythropoietin). However, use of both allogeneic transfusion and oxygen therapeutics entail risks. Therefore, safety is a critical element in any evaluation of oxygen therapeutic products for elective surgical use. In the setting of elective perioperative use, a mere delay in the requirement for allogeneic transfusion without reduction in the use of allogeneic red blood cells would probably not be considered of benefit to patients. The category of peri- and post-operative use of oxygen therapeutics includes situations such as hemodilution (with or without autologous predonation) and intra- and post-operative replacement. We recommend the investigators be aware of the present lack of objective criteria to define a broadly applicable transfusion trigger and strive to develop and validate physiologic markers of efficacy for individual oxygen carriers.

A trial to obtain an elective surgical indication alone, without evaluation of the product in unstable patients in a trauma setting, is unlikely to assure the safety of an oxygen therapeutic in elective surgical patients who become unstable or in trauma patients. While an oxygen therapeutic may appear to be safe in patients who are euvolemic and anemic, it cannot be assumed that such an oxygen therapeutic would be safe in hypovolemic or unstable patients, either surgical or trauma.

3. Trauma

In trauma, mortality is an unambiguous endpoint that many consider to be the most meaningful of the potential indications related to clinical benefit of oxygen therapeutics. Although short-term (e.g., 24-48 hours) survival is helpful in assessing the physiologic activity of an oxygen therapeutic, long-term survival is the primary clinical benefit of interest to the patient and the patient's family. The benefit of short-term survival is limited if it does not lead to long-term survival. There is insufficient information at present to correlate short-term survival with long-term survival for oxygen therapeutics.

In designing clinical trials for a potential trauma indication in the hospital setting, we recommend that you consider designs where patients are able to provide

consent for themselves or where consent can be obtained from a legally authorized representative. In the hospital setting, the use of an oxygen therapeutic would not be expected to result in a survival advantage over the use of red blood cells. Rather, oxygen therapeutics may be interchangeable with red blood cells in the short-term. Many, if not all, recipients of the oxygen therapeutic will need administration of other transfusion blood components. A clinical trial to demonstrate noninferiority, as opposed to superiority, of an oxygen therapeutic to blood in terms of mortality might not qualify for exception from informed consent under 21 CFR 50.24.

Historically, many researchers have considered field use as the most likely situation in which oxygen therapeutics could improve survival. However, in major urban areas with rapid transit to definitive care, there may only be a small percentage of patients for whom use of an oxygen therapeutic might provide a survival benefit. Some of the most seriously injured patients will die in spite of rapid availability of optimum definitive care and other, less seriously injured, patients will survive with current resuscitation measures. It is difficult to select deliberately and prospectively the small population of trauma patients for whom use of an oxygen therapeutic may provide a survival advantage. If a clinical trial is not designed to select deliberately and prospectively for this small subset of patients who are likely to benefit from treatment, a large number of patients would likely need to be tested for a survival advantage. Accordingly, many more subjects would receive an oxygen therapeutic agent than are likely to benefit from such use; therefore a careful overall safety assessment would be appropriate. In addition, for trauma patients who have also sustained head injuries, the heterogeneity in the severity of head injury may lead to mortality outcomes independent of the effect of blood loss or the use of an oxygen therapeutic agent.

In rural areas or other situations where there may be prolonged delay to definitive care, there may be greater potential for an oxygen therapeutic to provide clinical benefit than in urban settings. Such studies are difficult to control and may pose complications of trial design and data analysis, due, for example, to practical considerations such as differences in the length of time required to transport a patient to the hospital. Nevertheless, in the rural setting, a temporary treatment that sustains adequate tissue oxygenation and aerobic metabolism prior to control of bleeding and/or prior to obtaining cross-matched blood may provide a clinical benefit.

IV. RECOMMENDATIONS

Given the considerations described above, we provide the following recommendations for assessing the safety and efficacy of oxygen therapeutic products:

A. Preclinical Evaluation

We recommend that you consider the following general categories of safety testing when developing oxygen therapeutic products: 1) characterization of the product and 2) animal safety testing.

1. Characterization of the Product

We recommend that you perform a physicochemical characterization of the product using available modern technology. This may include, but need not be limited to, the following:

a. Oxygen capacity (P_{50}, Hill coefficient, Bohr, Chloride and CO_2 effects). It would be most useful to determine the entire curve of bound oxygen as a function of P_{O2}, at least over a physiologically relevant range (40-120 mm Hg).
b. Optical spectrum
c. High pressure liquid chromatography (HPLC) (size and charge-based), polyacrylamide gel electrophoresis (PAGE), sodium dodecylsulfate-PAGE (SDS-PAGE), iso-electric focusing (IEF)
d. Lipids and lipid phosphate content and identification
e. Endotoxin level
f. Free iron content
g. Methemoglobin, sulfhemoglobin , carbonmonoxy hemoglobin levels
h. Colloid osmotic pressure, viscosity measured at various temperatures to mimic mild and moderate hypothermia
i. pH
j. Identification and quantitation of electrolytes and trace metals

We recommend using in vitro biological assays with appropriate test systems that may include, but need not be limited to, the following:

a. tests for generation of oxygen radicals
b. tests for activation of triggered enzyme/cell systems (e.g., the complement/kinin/coagulation cascades, macrophage/neutrophil/platelet activation, mediator release [such as histamine, thromboxane metabolites, leukotrienes, interleukins]).

The measurements used in these stages of testing may not involve all of the items listed or all stages of product manufacture. However, we recommend that you perform a sufficient number of tests on a sufficient number of independent batches to assure that the characterization is accurate, and that the properties of the product are consistent from batch to batch.

We recommend that you make an appropriate subset of these measurements on every lot of product and set appropriate specifications (criteria) for acceptability.

Similarly, we recommend that you choose an appropriate subset of these measurements for inclusion in any stability study on the product that should be applied initially and after suitable storage intervals and under suitable storage conditions including accelerated, high temperature storage conditions.

For a hemoglobin-based oxygen carrier, we recommend that you develop a potency assay that reflects the biological activity sought in clinical studies. For use as an oxygen therapeutic in lieu of blood (blood substitute), such a potency assay should include a measure of the ability of the hemoglobin product to load, carry, and unload oxygen reproducibly.

2. Animal Safety Testing

We recommend that you consider the following points when planning and executing safety studies of oxygen therapeutics in animals:

General Recommendations Regarding Toxicology Testing

a. Perform studies on several animal species and should include late effects. A large animal, such as the dog or pig, should be included.
b. Design toxicology studies to induce toxic effects in the animal at some dose level.

Evaluation of Immune Responses to Oxygen Therapeutic Products

We recommend that you test for an immune response to the product, e.g., development of IgG or IgE response to product administration or appearance of delayed hypersensitivity on repeated exposure.

3. Use of Animal Models in Toxicity Testing

a. Volume overload and exchange transfusion experiments are of limited value in assessing safety. Though such experiments can contribute to determination of the dose/toxicity relationship or serve as a model for a blood replacement indication, they may not adequately reflect the circumstances in other clinical or clinical transfusion situations.
b. While meaningful information can be obtained from studies in normal animals, special animal models will likely be needed to obtain a complete safety profile. The animal model, fully instrumented to measure cardiac and pulmonary function, should be stressed so as to resemble the clinical use of the oxygen therapeutic (*e.g.*, volume depleted for resuscitative indication; ischemic model for PTCA; repetitive

administration for sickle cell disease; septic shock model for Systemic Inflammatory Response Syndrome [SIRS]; cardiopulmonary bypass model for cardiac surgery). We recommend that concomitant medications and other agents be included, *e.g.* contrast agents with hemoglobin-based oxygen carriers for PTCA indications. Controls should include use of approved oxygen carriers and plasma expanders.

c. You should consider a model designed to produce reperfusion injury. Such a model would be a relevant test of clinical situations that involved ischemia.

4. Important Observations in Animal Tests

a. Evaluation of effects of hemoglobin solutions on microvascular circulation and on endothelium.

b. Serum creatinine or blood urea nitrogen (BUN) levels alone may not suffice for evaluation of nephrotoxicity. Rather, evaluation of renal function is more appropriately evaluated by utilizing a battery of tests including, but not limited to: direct pressure or flow measurements to detect changes in the renal arterial bed (e.g., vasoconstriction); a combination of clearance tests (inulin or other suitable agent), and histological examination to evaluate glomerular function and structure. Because of the variable amounts of tubular secretion of creatinine in some animal models, the creatinine clearance will not be a satisfactory measure of glomerular function.

c. Measurement (and characterization) of tubular proteinuria or enzymuria to determine the status of tubular function or to detect tubular damage.

d. Blood chemistry assays, including: alanine aminotransferase (ALT), aspartate amino transferase (AST), CK, Troponin I or Troponin T, LDH, creatinine, BUN, and electrolytes. It is recognized that hemoglobin will cause interference with colorimetric assays.

e. Hematological studies, including: hematocrit, hemoglobin, white blood count (WBC) with differential, platelet count, prothrombin time (PT), activated partial thromboplastin time (PTT), fibrinogen/fibrin split products, factor VIII.

f. Gross and microscopic examination of all vital organs post-mortem.

We recommend that measurements of reliable markers of oxidative damage be incorporated in animal models performed as part of the preclinical evaluation of hemoglobin-based products. You should evaluate hemoglobin-based oxygen carriers in simple biochemical tests of pro-oxidant potential. We are recommending this with the recognition of the complex interrelated properties of hemoglobin and the various manifestations of injury thought to be mediated by iron and heme-mediated free-radical reactions.

We suggest that you perform studies in a primate model to evaluate cardiac toxicity. Such a model should be sensitive to detecting degenerative changes in cardiac myocytes. Histologic evaluation of sections of myocardium should include papillary muscle and interventricular septum including the conduction system.

We recommend that you evaluate hemoglobin-based oxygen carriers for time- and concentration-dependent effects on neurons in culture. You should perform studies in animal models of stroke and/or head injury at a time when the blood-brain barrier has been demonstrated to be open.

For stroma-reduced hemoglobin used as source material for further chemical modification, you should determine the precise levels and effect on hemoglobin potency and stability of residual red cell enzymes that may interfere with oxygen-carrying and/or redox activities of the hemoglobin products.

We recommend that you evaluate and resolve interference of hemoglobin solutions with measurements of clinical laboratory parameters for all relevant clinical laboratory instrumentation. Colorimetric interference with a number of clinical laboratory assessments that are important for individual patient management may occur with hemoglobin-based oxygen therapeutics. Manufacturers of oxygen therapeutics should anticipate ongoing support of clinical laboratories and evaluation of the effects of either hemoglobin-based oxygen carriers or perfluorochemical emulsions on new instruments or methods of analyte determination.

Regarding flurochemical products, we recommend that sponsors test for fluorochemical-related effects on platelets, in particular the normal survival time in circulation and markers of platelet function.

B. Clinical Evaluation

1. General

We recommend that initial studies in humans use a low dose in well-hydrated normal volunteers. For the hemoglobin-based oxygen carriers, consideration may be given to concomitant phlebotomy, depending on the initial administered volume of the hemoglobin-based oxygen carrier. You should monitor subjects carefully for at least circulatory and immune function. You should also monitor subjects receiving either perfluorochemical emulsions or hemoglobin based oxygen carriers for inflammatory mediators, *e.g.*, complement/kinin/coagulation cascade, histamine release, thromboxane metabolites, and leukotrienes. If safety is clearly established in the initial phase of investigation, the dosage in normal volunteers may be carefully increased, with monitoring as indicated above. When

safety has been established in normal volunteers, appropriate patients may be considered, with the understanding that:

Patients may be more vulnerable to adverse effects of the product than are normal, well-hydrated, healthy subjects

The specific interactions (if any) between hemoglobin-based oxygen carriers or perfluorochemical emulsions and the disease process are poorly understood.

Clinical situations where the use of blood is indicated differ, making it difficult to generalize results obtained from a trial conducted in one setting to other settings. Separate safety and efficacy data are therefore generally necessary for each clinical setting in which an oxygen therapeutic is needed and for which an indication (claim) is sought. The indication for use section of the package insert is expected to include information about the clinical setting and the patient population. We recommend that you prospectively define the indications sought for the use of an oxygen therapeutic before trials are performed, and these claims should be amenable to study using outcomes that are direct measures of clinical benefit or validated surrogates.

We recommend a clinical development plan that includes safety and efficacy assessments in both trauma and elective surgical settings to gain a full understanding of the adverse event profile of an oxygen therapeutic. We believe an evaluation in both trauma and elective surgery will provide a full understanding of both the benefits and the risks of oxygen therapeutic use in the broadest spectrum of situations in which such products may be used. The agency believes, however, that clinical development plans for elective surgical indications alone may be appropriate in situations where the relative benefits of such use outweigh the risks. (See Section III.C.2.) Comments are invited regarding other appropriate claims, endpoints, and assessment tools.

We recommend that clinical studies to evaluate the safety of oxygen therapeutics include assessments for all organ systems with particular emphasis on those organs and tissues known to be affected by one or more of these products as noted above. We recommend that you conduct such studies initially in controlled settings such as elective surgery before embarking on studies in unstable surgical patients or unstable trauma patients. Because many of the adverse events seen to date with oxygen therapeutics are qualitatively similar to adverse events that occur in the general surgical population, we recommend that you design clinical trials to capture a numerical increase and/or an increase in the intensity of adverse events above the underlying background rate/intensity of such events.

We recommend that you exercise care in designing clinical trials to evaluate potential clinical utility in the treatment of patients with head trauma or in clinical settings that may be associated with disruption of the blood-brain barrier. We

recommend that you make provisions for complete neurologic assessment (before and after administration of any oxygen therapeutic) using the same trained clinical investigator to conduct the evaluations to minimize inter-observer variability in assessment.

2. Elective Surgery

We recommend that you evaluate oxygen therapeutics in patients with a wide variety of co-existing pathophysiologic processes to assess the safety of the products in a wide variety of co-morbid conditions. In a phase III surgery trial, we recommend that the population enrolled should reflect the characteristics of the population likely to undergo that particular surgery in clinical practice. In addition, as these products are likely to be used broadly in general surgery, the surgery studied in a phase III trial should be one in which the enrolled study population also reflects the characteristics of the general surgical population.

We recommend that you consider studies in which complete avoidance of allogeneic transfusion during and after surgery would be considered one of the efficacy endpoints. To this end, we recommend that you conduct concentration/dose toxicity trials to determine the maximum tolerated dose of an oxygen therapeutic so that future patients will not be administered a toxic dose in attempts to avoid allogeneic transfusion. In this regard, you are encouraged to use non-linear mixed-effects modeling (NONMEM). If the primary efficacy endpoint is avoidance of allogeneic red blood cell transfusion, we recommend that you also evaluate total allogeneic exposure (all allogeneic blood components administered) as a secondary endpoint. While it may not be possible to substitute completely an oxygen therapeutic agent for red blood cells in all patients undergoing elective surgery, a suitable trial design should specify and confirm enrollment of patients requiring two or more units of red blood cells.

We recommend that you develop and validate clinical guidelines that are routinely available at the bedside for dosing both individual oxygen therapeutics and red blood cells in the face of circulating oxygen therapeutic agents. In patients treated with an oxygen therapeutic agent, hematocrit will not reflect accurately the clinical status of the bleeding patient. Hemoglobin-based oxygen therapeutics may be oncotically active, thereby precluding the use of routine measures such as total hemoglobin as a reflection of the need for additional transfusion/infusion. Perfluorochemical emulsions are functionally distinct from red blood cells in the manner in which they load, carry, and unload oxygen and will therefore need careful consideration of dosing recommendations and infusion criteria.

Of course, sponsors must assure that clinical trial subjects and investigators are fully informed about the risks of an oxygen therapeutic, given that there are also uncertain risks associated with the use of red blood cells in clinical practice. See

21 CFR 312.53(c)(vi)(*d*), 312.55(b) Because a clinical trial to assess the relative safety of an oxygen therapeutic compared to red blood cells with statistical significance could require a very large sample size, at this time we are willing to accept a modest level of uncertainty as to whether the oxygen therapeutic is actually as safe as red blood cells.

3. Trauma

You are encouraged to evaluate and validate surrogate markers that may correlate with the mortality endpoint. Surrogate markers/endpoints may be laboratory measurements or physical signs that are expected to correlate with clinical benefit. At present, there is no general agreement about the usefulness of individual, easily measured surrogate markers to predict clinical outcome in trauma.

We recommend that in a setting where blood is available, a trial to evaluate clinical noninferiority of an oxygen therapeutic to blood should provide for exclusive use of the oxygen therapeutic in lieu of red blood cells at least until bleeding is controlled. While noninferiority to red blood cells in clinical outcome (mortality and durable morbidity) may be sufficient for an indication (claim) in trauma, such a clinical trial will require a large sample size to assure that widespread use of the product is very unlikely to affect adversely mortality, durable morbidities, or other safety endpoints. As noted previously under section III.C., Efficacy Considerations, since many patients may not be able to provide informed consent and their legally authorized representatives may not be available, the clinical trial design must take into account the possibility that the patient population enrolled in the study might not adequately represent the patient population presenting at the hospital in actual clinical practice.

In a direct study of safety and effectiveness of an oxygen therapeutic in the field setting, it would be expected that current approved asanguinous resuscitative solutions would be used in the control population and that survival would be the primary endpoint. To support a labeling claim in the field setting, survival (primary endpoint) in subjects receiving the oxygen therapeutic should be superior to survival in subjects receiving control. If the available data support the potential of the product for direct benefit to subjects, a field trial may meet the criteria for exception from informed consent under 21 CFR 50.24. It should be noted that in the urban setting, a large clinical trial would likely be needed to test for a superior survival outcome unless a subset of subjects with massive bleeding could be identified at baseline. It may be possible to collect information about red cell mass at baseline to identify such a subset of subjects.

Alternatively, an indication (claim) of efficacy where blood is not available or cannot be used might also be supported by data from clinical trials in the hospital setting where blood is available and used in the control arm of a comparative study (see section III.B.3). It may be difficult to extrapolate data generated in the

Emergency Room/Operating Room setting where blood is available to the field situation where definitive control of bleeding is often not possible. Nevertheless, an oxygen therapeutic agent demonstrated to be safe and effective in the treatment of trauma or acutely massively bleeding patients in a hospital setting might be expected to provide an advantage over current asanguinous resuscitative fluids when definitive care is delayed and blood loss is life-threatening.

email Stacie or Yen Lee about archival retention SOP SOP on SOPs	file cabs 10	Reg.	Clinical