CENTER FOR BIOLOGICS EVALUATION AND RESEARCH, FDA

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OFFICE OF THE SECRETARY AND OFFICE OF PUBLIC HEALTH AND SCIENCE, DHHS

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LIST OF PARTICIPANTS

JOSEPH C. FRATANTONI, M.D.
Maxcyte

H. FRANKLIN BUNN, M.D.
Brigham and Women's Hospital,
Harvard Medical School

ABDU I. ALAYASH, Ph.D.
Office of Blood Research and Review, CBER, FDA

ALAN N. SCHECHTER, M.D.
Molecular Medicine Branch, NIDDK, NIH

GEORGE P. BIRO, M.D., Ph.D.
University of Ottawa and
University of Toronto

BARBARA ALVING, M.D.
MACP National Center for Research Resources, NIH

TOBY A. SILVERMAN, M.D.
Office of Blood Research and Review, CBER, FDA

SARA F. GOLDKIND, M.D., M.A.
Office of the Commissioner, FDA

ROBERT M. WINSLOW, M.D.
Sangart, Inc.

STEVEN A. GOULD, M.D.
Northfield Laboratories, Inc.

ABRAHAM ABUCHOWSKI, Ph.D.
Prolong Pharmaceuticals

A. GERSON GREENBURG, M.D., Ph.D.
Biopure Corporation

TIMOTHY N. ESTEP, Ph.D.
Chart Biotech Consulting

JOSEPH DE ANGELO, M.S.
Apex Bioscience, Inc.

C O N T E N T S

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P R O C E E D I N G S

(8:30 a.m.)

OPENING REMARKS

MR. GOODMAN: Good morning, everyone. We should get started. I'm Jesse Goodman, Director of the Biologics Center at FDA, and it is really our pleasure to have you here for this scientific meeting. You know, I would really like to thank NHLBI and George Nemo and Simone Glynn for helping sponsor this as well as the Office of the Secretary. And I'm just going to make a few brief introductory comments and then so is Simone.

As you can see from the program, this meeting has really assembled a terrific group of experts to consider the data, including about 40 people from academia, government, and industry. There is over 300 participants signed up from many countries. Now, to preface these concerns about safety of HBOCs in general, have increased over time based on accumulative clinical experience including with newer products.

The purpose of this workshop which FDA began

organizing several months ago was to bring forth and have this discussion. As many are aware, on the day before this workshop, a net analysis was published on diverse products. We've already heard considerable commentary on this, methods and perspectives of this analysis, and whatever you think we should certainly consider that review as part of the broad picture in our discussion here today.

Safety concerns about various candidate products are not new. FDA reviewers have identified potential concerns and carefully considered all available data in making their decisions about individual studies. Some of them have been allowed to proceed. Some have not been allowed to proceed. And we have been criticized for both, as being too restrictive, or as being not restrictive enough.

As we review and discuss the data today, we shouldn't lose track that there is tremendous unmet medical need here, whether on the highways of the United States, the battlefields, people who can't be transfused because of failure to immunologically cross match, people who for religious reasons don't want blood. There is a

tremendous unmet medical need.

We need you to help us improve and define and advance the science to better predict safety and efficacy of these products. We must better understand the basic and pre-clinical sciences to minimize risk. But even then, nothing, whether a clinical trial or an approved product can be risk free. Without progress, there can be no benefits to those who remain in need every single day.

It is our hope that the presentation and discussion at this workshop will contribute to finding fast forward for further development of these products, but only as appropriate, based on risk benefit analysis of all available relevant data. Scientists both at FDA and NIH working with others will continue to be engaged in helping advance scientific understanding and developing tools for safety and efficacy evaluation of the HBOCs.

So I thank you for coming here today, for your contributions, and for your deliberation and input and also for your consideration of all views on the data and the subject. So with that, I'll turn it over to Dr. Simone Glynn, thank you very much.

(Applause)

MS. GLYNN: Good morning. And it is a pleasure to welcome you on behalf of the National Heart Lung and Blood Institute, at this workshop. The NHLBI is proud to be a co-sponsor of this workshop and as you know, the institute has reported basic research on Oxygen Carrying Red Cell Substitutes for more than 30 years. So I just wanted to remind you or inform you that the institute recently released a strategic plan to serve as a guide for its research and training programs for the next 5 to 10 years.

And the process initially involved a series of thematic, strategic, planning meetings, involving members of both the extramural and the intramural research communities. And one such group concentrated on issues related to global blood safety and availability. And one of the major recommendation from this group was the need to develop alternatives to standard allergenic donor blood, which included the development of safe and effective hemoglobin based oxygen carriers.

So we followed that at the institute by another working group in 2006. And this working group was tasked with formulating research recommendations for basic

research studies on -- again, on hemoglobin based oxygen carriers. And a number of recommendations were provided including the need for basic studies to elucidate the mechanisms of adverse reactions primarily the cardiovascular and the cerebral vascular systems, with hemoglobin based oxygen carrier formulations.

The need to conduct studies on the distribution and metabolism of different hemoglobin derivatives, research into the physiology of oxygen delivery, at the level of the microcirculation and the production and distribution of highly purified hemoglobin based oxygen carrier solutions for use by the scientific community. And if you have not seen it, and if you are interested there is a summary of this working group meeting that has been published in this month's issue of *Transfusion*.

So at the Institute, we are very much interested in the outcome of this workshop, which will review available scientific data and gather informed opinions regarding the safety of the hemoglobin based oxygen carriers in a variety of clinical settings. And the information which emerges from this workshop will serve as a basis, we hope, for further studies to advance the

field.

The institute remains very much committed to supporting meritorious research in this area and we certainly look forward to an exciting and productive workshop review over the next couple of days. So thank you.

(Applause)

MR. HOLMBERG: Welcome. I'm Jerry Holmberg. I'm the senior advisor for Blood Policy. And I just want to welcome you on behalf of the secretary and also the Office of Public Health and Science and the Assistant Secretary for Health. I think that as we look at some of the advances that have occurred over the last 30 years, as Simone mentioned as the interest, I think that we have to really reflect on the safety and availability of the products and how are we moving on the various products that are out there.

I think that one of the things that we really have to be concerned about is the safety of any product that we make available to the American public. And so I do greatly appreciate all the support, the research support that is provided by NIH, NHLBI, and also the

regulatory review that is undertaken by the Food and Drug Administration.

When Simone mentioned about 30 years of moving ahead and -- and the strategic plan, I just quickly was thinking about well, 30 years, let's say, that would have been back in 1978. And what -- one of my comments that I wanted to make this morning was that I think we have been talking about hemoglobin carriers for probably greater than we have had an energy crisis. And I think that that is a true statement.

And so we really have to be able to analyze the information that is provided today. And without taking too much time, I just want to thank you all for coming here. I really appreciate and look forward to the discussions that take place today. I'm going to turn the meeting over to Dr. Fratantoni.

(Applause)

SECTION I

WORKSHOP OVERVIEW AND HBOC UPDATE

OVERVIEW OF THE WORKSHOP

MR. FRATANTONI: Well, good morning. As many of you know I was heading up the review in research aspects of blood substitutes for CBER for a number of years until I left the field in '96 and it was truly an honor to be asked to come back and work with the planning committee and moderate this session at this very important meeting.

I've got a number of general ground rule announcements to make and then I want to talk a little bit about how the meeting is organized. Get the housekeeping out of the way first. Standard comment, we have a very full agenda. Going to have to ask speakers to pay attention to the length of talk and the moderators will work with you on this. We have a warning light system and will try to keep on time as best as we can.

Ask attendees to help in that way also by coming back as soon as possible after breaks and lunch. Would -- after the breaks and -- and if you were outside this morning saw that there is a bell that will announce that the break time is over. Lunch is available on the level above here. The Natcher Cafeteria and it is a -- it is fairly large and we hope we will be able to move people through there in the one hour that has been allotted for

lunch.

After the session today, the area above the auditorium, again up at the atrium level, will be available for social gathering, and people can meet there, have discussions, until the building closes at 6:30 p.m.

We would ask that all press questions for FDA be directed to Karen Reilly. And there's Karen Reilly. Want to make finally, something that requires special mention, the organizers want to call attention to the work done by the administrative staff in preparing for this meeting and a special mention to Jennifer Sharpe, Rhonda Dawson and Jim Durum. We'll just give a little hand.

(Applause)

MR. FRATANTONI: Okay, now regarding the meeting itself, can't start without pointing out that the title for the meeting Hemoglobin Based Oxygen Carriers, HBOC, that term was developed at the first FDA NIH Workshop on Safety in 1990. At that time FDA saw that there were some questions that couldn't be answered with data that they had at hand. We pulled together a workshop that led to the first points to consider on safety, and the term HBOCs came out of that meeting.

And this meeting as you can see from your program, there are four sessions. In this first session, we are -- presenting an overview of basic material underlying the HBOCs. In the second session, it is listed as clinical experience and after an introduction by FDA of some of the technical matters and -- and ethical matters there will be presentations from representatives from industry with their clinical data. There will be a discussion after that as there will be after each session.

Session III is divided into two parts. The first will be a series of brief presentations and then panel discussions, moderated by Dr. Klein. And these will be aiming at considering the -- the class effects, these similarities or dissimilarities between the various preparations that have come to be known in recent years.

The second part moderated by Dr. Weiskopf is going to be primarily aimed at discussing safety, primarily at an organ specific manner. And last session, Session IV moderated by Dr. Biro is going to be looking at some way forward looking at biochemical strategic ways of doing things safely and yet learning about the properties and efficacies of these products.

There is the one change in Session IV that I will call to your attention now. The first speaker in Session IV will be Dr. Emanuel. The other speakers will be as -- as listed. Regarding the discussions, written question will be accepted from the audience and will be presented to the Panels at the end of each session. Index cards for writing these questions are in your folders. If you have written the question, please raise you hand, between speakers and there are people who will pick up the cards and bring them forward.

For Session I, I would ask that the questions regard clarification of the factual material that will be presented here, issues of interpretation and analysis will be better served in the later sessions. There are disclosures regarding conflicts of interest. These are provided by the speakers and the list of these are again, in your folder. We encourage speakers to make any information -- any pertinent information available as it is appropriate.

I'm going to call -- I'm going to -- on the first speaker now, the first presentation is on Overview of Oxygen Physiology. It is by Dr. Frank Bunn. He is

Professor of Medicine at Harvard Medical School and at the Brigham and Women's Hospital.

OVERVIEW OF OXYGEN PHYSIOLOGY

MR. BUNN: Thanks, Dr. Fratantoni. I -- it's a pleasure to be here. The -- it is certainly a meeting I've been looking forward to. The -- I have a couple of disclosures. I'm a member of the SAB at Sangart and formally I was a -- had a similar role at Somatogen. When we talk to medical students about oxygen homeostasis, I think that it is almost compulsory to begin with the Fick equation, which says that the oxygen delivery to either the whole organism or to a organ or -- or tissue within the organ is a product of three independent variables. The blood flow, the oxygen carrying capacity of the blood, the hemoglobin concentration, and the unloading of oxygen from -- from the hemoglobin, which is a function of the oxygen-binding curve, so that the -- these three independent variables are controlled in very different ways.

The -- there is complex regulation of blood flow, the erythropoietin is the major hormone that drives red cell production. And the placement of the oxygen binding curve is -- is determined in human red cells by levels of 2,3-DPG and -- and PH.

Now, when we think about hypoxia and adaptation to hypoxia, there are a number of organismal changes that occur acutely with -- and some of these are very obvious. Increased cardiac output, pulmonary basal constriction, systemic vasodilation, one can call -- refer to the third item as hypoxic vasodilation, increased ventilation, and then at a metabolic level, there is a shift to anaerobic glycolysis. And a change in the -- in the position of the oxygen-binding curve, immediately as a function of PH and then soon thereafter changes in -- in red cell 2,3-DPG.

The -- these are events and phenomenon that have been known for a long time. What is a bit more recently appreciated is that accompanying these immediate changes are delayed adaptations to hypoxia that are a result of programming of gene expression. So that there is an induction of genes that will make new blood vessels. Neovascularization, which complements the hypoxic

vasodilation. There is Tyrosine hydroxylase, rate-limiting step in dopamine synthesis will increase the carotid body function. Induction of glycolytic enzymes, induction of erythropoietin, these are all mediated by the transcription factor HIF, hypoxia inducible factor.

Now, the increased cardiac output is of course, a direct correlate to what I showed in the previous -- previously in the Fick equation. The induction of erythropoietin as well, and the lowering of oxygen affinity so that there is -- so that all three elements of the Fick equation are encompassed in these changes with -- adaptations to hypoxia.

Now, hypoxic vasodilatation is a topic that I am sure will be visited a number of times during these two days. Because it is -- it is fundamental to understanding how patients or -- who have -- might be in need of oxygen carrying blood substitute, how that their physiology adjusts at an organismal and tissue level. And the mechanism underlying hypoxic vasodilatation has been a subject of great interest and to some -- to some degree controversy.

There are three major mechanisms. These were shown in a slide that I borrowed from the Alabama group who have recently published on this topic. There -- the -- for a number of years one possible mechanism for sensing and signaling hypoxic vasodilation was ATP release from red cells as they profuse hypoxic tissue. The -- and as a result the -- an ATP receptor on endothelial cells would generate nitric oxide for vasodilatation.

Jonathan Stamler and his group at Duke University have promoted the notion that there is a -- a sulphyderal linked nitric oxide, SNO derivative of hemoglobin reactive beta 93 on hemoglobin that altruistically will release nitric oxide as red cells undergo de-oxygenation. And even though a very tiny proportion of hemoglobin would be -- be the SNO derivative, it would suffice to allow for a vasodilation at a point in which it is needed in relation to local hypoxia.

The -- this paper by Isabel et al, reports a transgenic or actually a knock in mouse model, where the beta 93 cysteine in -- in -- is been replaced by an alanine. And they find that there is no change in cardio

dynamics. And no evidence that there is any alteration in hypoxic vasodilation with this important mutated hemoglobin circulating in the mouse. So that that provides some fairly strong evidence against the importance of SNO as a regulator and a vasomotor tone in response to hypoxia.

Mike Gladwin and his group at the NIH and collaborators elsewhere propose that nitrite is a source of a nitrogenous compound that would impact on the vasomotor tone. And the -- with the idea that hemoglobin particularly when it is partially saturated with oxygen can function as a nitrite reductase. And I think we will be hearing more about that from -- in Alan Schechter's talk. And -- and as well as others.

Now, getting back to the Fick equation, it's -- it's -- obviously, hypoxic vasodilatation is an important determinant of blood flow to -- to the needy tissue, hypoxic tissue. And that is going to be determined by the oxygen unloading as well as the hemoglobin concentration. Now, the nitrite reduction then would be a way in which hemoglobin can mediate the release of a -- a nitro --

nitrogenous compound to orchestrate and -- and enable as the -- vasodilation to occur.

More relevant to our topic for the next two days, the HBOCs, is that NO can be -- is a substance which can be readily scavenged by free hemoglobin in the circulation. And so there is an issue as to whether or not NO scavenging could impact adversely on vasomotor tone causing vasoconstriction and reduction in blood flow. And this is a topic which I know it will be thoroughly aired during -- during the next two days.

Now, in the terms of designing an optical -- optimal hemoglobin substitute there are a number of important criteria. Prolonged survival in the circulation, physiologically appropriate oxygen affinity, colloid osmotic pressure, slow rate of auto oxidation and minimal NO scavenging. And what -- what I want to talk about are three of these briefly. One would be the prolongation of -- survival in the circulation. I will first talk about that. Then I will talk briefly about NO scavenging. And then finally the issue of what is the appropriate oxygen affinity for optimal delivery with a hemoglobin based blood substitute.

I -- I can't escape going into some ancient history. I -- I first began research with Jim Yantal (phonetic), my mentor at Thorndike Lab at Boston City Hospital, who died last year. And my first research project was on specifically dealing with how free hemoglobin in this -- in the plasma is handled by the kidney. And I actually finished this work when I was in -- I drafted into the Army at Fort Knox, Kentucky. I was in the -- at the Army Research Lab there.

And the studies that we did focused on the mechanism by which hemoglobin is filtered by the kidney. And the hypothesis we worked off of was that the free hemoglobin particularly when it is dilute in the circulation -- disassociates from its tetramer into identical half molecules alpha beta dimers. And that it seemed logical that the filtration of hemoglobin through the glomerulus might be a function of this disassociation process.

It is -- it is clear that albumin with a molecular size similar to hemoglobin tetramer is not filtered through the glomerulus, where hemoglobin readily is. So the -- the thought was then that the mechanism by

which you see hemoglobin emerge in the urine with high concentrations in the plasma was related to the extent to which it disassociated into dimers which were more readily filtered.

So to test that hypothesis we used a bi-functional sulphyderal reagent, basically a methyl ether, to crosslink hemoglobin at the beta 93 sulphyderal groups to -- to -- to -- and that would keep -- keep hemoglobin from disassociating. Sandy Simon at New York had -- had -- had shown that this -- this reagent worked quite well to prevent hemoglobin disassociating into dimers. Then as a control used that mono-functional reagent, N-Ethylmaleimide.

And what we showed was that in -- in rats, treated with BM -- BME hemoglobin that the -- on this log scale, you can see that the retention of the hemoglobin in the circulation of the rat was considerably longer than that with either unmodified hemoglobin or not shown here, hemoglobin modified with a mono-functional reagent. So there was a -- the cross-linking then resulted in a marked prolongation of the half-life of the hemoglobin.

And when the rats were nephrectomized there was no difference between normal and BME hemoglobin, indicating that the difference in the survival had to do with renal excretion. Same -- same was observed with dogs, treat -- treated -- hemoglobin treated with BME or normal.

So what the conclusion from -- from this, then was that that the hemoglobin filtered through the glomerulus as an alpha beta dimer and then once it -- it -- it got into the tubule it could be metabolized by the proximal tubule. And till that capacity was overloaded, then you would get free hemoglobin in the urine.

So obviously, it was an important -- important in developing and I -- hemoglobin based blood substitute to prevent this from happening. And in fact, this is a partial list of hemoglobins that have been developed through the years to be tested as oxygen carrying blood substitutes. And all of them are cross-linked so that this transit through the glomerulus is prevented.

Now, I would like to mention -- go on to talk about nitric oxide. It has been -- it has been a -- an assumption and a very reasonable assumption that NO

scavenging is a critically important issue in the use and application of hemoglobin based blood substitutes. Obviously, free hemoglobin in the circulation will have access to the endothelium to a greater extent than circulating -- the laminar flow of circulating red blood cells. And therefore, NO that is produced at the local endothelial level could -- could readily be scavenged and that may have deleterious effects on blood flow.

The Somatogen company a number of years ago, developed a -- a cross-linked hemoglobin which was the result of a isopeptide bond created between the two alpha globins and at various lengths of lysine residues were inserted by genetic engineering to make for a di-alpha goblin subunit that -- that would prevent the hemoglobin from disassociating.

And this as expected had -- this di-alpha hemoglobin had a prolonged circulation in the -- compared to free hemoglobin that is -- that -- native hemoglobin that can disassociate. Now, Doug Lemon and John Olson decided to look in depth at -- at the issue of nitric oxide scavenging. And so what they did was to make mutants in the heme pocket which -- significantly reduced

the uptake of nitric oxide through the hemoglobin and the -- the conversion of -- of NO to nitrate with oxidation of the heme-iron.

And so what they did was ask whether or not these modified hemoglobins which were -- they showed very -- very elegantly by stop flow analysis did retard NO binding. Whether they might have any effect, physiological effect, on blood flow in the -- in the animal. And so here is a -- a diagram showing the inverse relationship between the ability of the hemoglobin to scavenge nitric oxide and the increase in blood pressure noted.

And you can see that it is clear that unmodified hemoglobin were -- was -- had -- had a -- had a marked pressure response whereas -- genetic modifications that reduced NO uptake, reduced that effect. Now, the question then remains how important this NO scavenging is and what can be -- and if it is important what can be done about it and I believe that we will hear quite a bit more on this issue during this meeting.

A second faith based assumption is that oxygen binding of HBOCs should match that of the red blood cell.

I actually had a -- my first grant was from the Army to work on this 40 years ago. And I gave up on it with the cross-linked hemoglobin that I showed you because it had such high oxygen affinity. I thought it will be worthless as a oxygen carrying blood substitute. I had -- I sort of took it as an article of faith that the hemoglobin that circulated in the plasma should match that of the red cell and have a p50 of 26 torr, in order for there to be efficient oxygen unloading to tissues.

But the -- this thinking has been challenged and revisited in a major way by Bob Winslow who has -- postulated that the red blood cells are -- are designed to deliver oxygen in an orderly way to minimize undue vasoconstriction by virtue of facilitated diffusion and a gradient from the red blood cell to the endothelial cell. And when tissue oxygen retention is -- is low, that is in other words, where there is high oxygen consumption, that the -- it -- the oxygen carrier has to be poised in such a way as to not trigger oxygen dependent vasoconstriction.

And -- so that this can be illustrated I think in a couple -- a few illustrative slides here. Just think about a -- a micro vessel whether it is an arterial or a

initial capillary, one -- one which is subjected to regulation of vasomotor tone. And subjected to -- therefore, subjected to hypoxic vasodilation. You can see that with -- with the laminar flow of red blood cells there is diffusion of oxygen to the surface of the endothelial -- endothelium and that there is a gradient and so that the oxygen concentration around the red cells can be greater than that, that impaction of the endothelial cell. And this -- this is a -- in a physiologic system, this will allow for a certain maintenance of appropriate vasomotor tone.

Now, if we -- we then flood a hemoglobin based oxygen carrier into the system, and that would be done, say in a patient with severe blood loss, or where the red -- circulating red cells may be marked decreased, you have hemoglobin with a capability of unloading oxygen right at the level of the endothelial cell. And to that extent the oxygen tension at that cite is -- may -- may well is increased particularly if the hemoglobin has an oxygen affinity similar to that of whole blood.

So that the -- the -- so that you are going to get an increase in oxygen retention at the endothelium and

what this is going to do if the P50 is close to physiologic, is going to cause vasoconstriction. So you are going to get a narrowing of the lumen of that blood vessel and impairment of blood flow. Now, the -- the -- this problem can be offset by infusion of a hemoglobin that has a high oxygen affinity, where there is less unloading of the oxygen from the oxygen -- oxygen carrying hemoglobin, the HBOC.

And -- and therefore the P02, a level of the blood vessel will be sufficiently low so as not to engage vasoconstriction. So this is a paradigm which I think is one that is worthy of considerable pursuit. And I think I'm going to stop with that and hopefully some of these point will be revisited and -- and better amplified in this -- in future talks. Thank you.

(Applause)

HBOCS: BIOCHEMICAL AND PHYSIOLOGICAL PERSPECTIVES

MR. FRATANTONI: The next presentation will be on the biochemical and physiological perspectives of the HBOCs and this is given by Dr. Abdu Alayash, who is the

Chief of the Laboratory of Biochemistry and Vascular Biology at CBER, at FDA. Abdu?

MR. ALAYASH: Thank you, Joe. My presentation will basically focus on as Joe said, the title indicate some biochemical, physiological properties of some HBOCs that we had a chance to work on them. The work is largely done here at CBER. There were such programs that we have been involved with some 18, 19 years ago. Hopefully, some -- some basic aspects will transpire from this -- this presentation. And will be hopefully some use as you deliberate with these important products.

So let me start just with the overall -- I'm sure many of you have seen this slide before. The different approaches that have been used by industry to modify these hemoglobins. And as you can see, we have basically two classes of product. The fluorocarbon based and the hemoglobin based products. We are not obviously going to talk about the fluorocarbons anymore.

The hemoglobin based -- hemoglobin is basically derived from the red cells, outdated blood, chemically modified and the modifications either takes the form of -- either cross-linking -- cross-linking and the surface of

the protein is decorated with some non-protein molecules. Or in some cases the protein is pulverized. In some indication of the -- at least at the research stages now, the hemoglobin is encapsulated with lipid bilayer.

The purpose of modifications is primarily to serve two really basic issues. As Dr. Bunn indicated is obviously to stabilize the tetramer. The tetramer -- the hemoglobin as it released from the red cells when in free form, will break down into dimers. So the idea is to either to stabilize it in the tetrameric form or the polymeric form.

Today, you are going to hear representation of these approaches from Baxter, which is the original (inaudible) hemoglobin, Apex and Sangart. They will be presenting some data on the conjugated hemoglobin, and of course, Northfield and Biopure opted for the pulverized hemoglobin.

Okay. In spite of the bad press, the -- some people in the community, actually believe that there are some promising therapeutic value for these products. But unfortunately as the slide indicate, we are facing a number of issue regarding the toxicity. But if you check the

literature these days this is the list that you will come up with, vasoactivity and hypertension GI side effect, pancreatic effect and so on and so forth.

The common thread in all of these reactions is really the -- the -- the -- the issue is either triggered or emanated from the healing prosthetic wound of hemoglobin. And an example of that is -- was vasoactivity. As Bunn had indicated it is very simple reaction between hemoglobin and nitric oxide. But if you really biochemically through some of these events, carefully you can also again, see the role of heme in these reactions.

And anyway you look at it, with the nitric oxide or reactions of hemoglobin without molecules, heme will be oxidized. This is really the main theme of my talk. Regardless, whether it is nitrous oxide or oxidants and so on and so forth. So what drives oxidation? Inside the red cells, and outside the red cells? And as you know, hemoglobin spontaneously oxidizes even within the red cells to a number of species, ferric or the mat, which is non-functional, or even sometimes ferra which is even little bit toxic.

But as you know, in the red cells, we have a very efficient and sematic machinery that reduce the hemoglobin back to its previous functional form. When we have hemoglobin free outside the red cells, of course, you can't control the hemoglobin. Hemoglobin will -- will oxidize spontaneously. And additionally, the hemoglobin will -- the oxidation itself will be actually enhanced by a number of factors, including as you said, the spontaneous oxidation.

If you leave hemoglobin on a bench for 10, 15 hours and you look back at it, it will turn little bit brownish. But it is just the rusting, the oxidation. And of course, the activity with nitrous oxide will also oxidize the hemoglobin to certain extent. And of course, the oxidant, but of course, we have no shortage of oxidants. And incidentally, even the hemoglobin itself when it auto-oxidizes produces oxygen. And can actually if you leave it for long time it will self destruct.

Additionally, in our case, the way you modify the hemoglobin, the manufacturing that goes into producing the hemoglobin, in some cases, can actually enhance the oxidation. In some other cases, may slow it down. And the

net result of all of this of course, we can have the effect -- the effectiveness of these hemoglobins. When you accumulate methemoglobin, methemoglobin doesn't carry oxygen. Of course, if it oxidizes fully you break up the hemoglobin. That may actually lead to some issue with the -- with the safety.

I'm going to choose two examples on the manufacturing and on the oxidant. Very briefly. This is the story of hemoglobin that we had to chance to actually look at it. It is human-linked manufactured by Hemosol and they give us this some few years back. And we had some agreement with them. The common scientific name is polymerized hemoglobin. What I am trying to do here is show you how chemical modifications in some cases could actually lead to some undesirable destabilization of the -- of the product.

This cartoon summarized the story. What they tried to do is basically treat the hemoglobin, which is extremely purified form of hemoglobin, A0, with sugar which is a trisaccharide raffinose. And this sugar, before they added to the hemoglobin, they oxidized it to open up the grains, added to the hemoglobin and of course, the sugar

will bind to 3-amino acid here in this space which is known as the 2,3-DPGs pocket.

They had done initial work to indicate that the actual cross-linking had occurred. And in fact, if you look at the HBLC in our hands also it looks in a polymeric form. Because the sugar not only goes in the DPG pocket, the sugar actually modifies some of the amino acids on the surface and it produce a polymer.

But if you look at the typical oxygen titration curve here, if you look at typical A0, you get this nice sigmoidal curve. And if you use fresh blood and of course, the curve is shifted. And is again nicely sigmoidal in nature. But if you look at the product which is produced from that addition of that sugar on purified hemoglobin the result is this bizarre form of oxygen. Look at this curve. It is almost linier. Has no sigmoidal nature. Doesn't saturate.

In fact, even if add pure oxygen to it, it would not saturate. So clearly there is something wrong in the chemistry of the hemoglobin. At this rate it would have done something wrong and we tried to sort of get to the bottom this issue, to sort of try to understand what

actually went wrong. Two things emerged from an extensive study that we published.

One of them, was the heme itself, if you know the heme is protein and iron, usually sit in the center. Well, we found out that this hemoglobin is actually the -- the heme itself is distorted. The iron instead of being in the middle, it is actually tilted. And that would lead to break up of the heme and iron will be released. And we picked this up with an EPR technique.

The other problem with this product is that their protein as you know, go -- spontaneously they transition from the fully oxygenated to the non-oxygenated. The R and the T form. This hemoglobin is actually locked in the T form, the de-oxy form. And this makes plain -- funny shape polybural curve because you -- you paralyze the hemoglobin in the T4. Remember almost most H factor are to certain extent in the T form. This particular hemoglobin it appears to be actually frozen in one form.

We went down. We broke in the hemoglobin. Now, if they are at the top, we thought if we can take these six fraction, pull them out, look at their properties and if we pull out the bad fraction, maybe if we can put it together

we can fix the problem. Couldn't do that. We've broken the hemoglobin into small peptide looking for the reagent.

Where did the reagent go? Remember the reagent was supposed to go here? Unfortunately, we found it bound to (inaudible) amino acid completely different 60-93. And here is the mass fact data to confirm that the masses of the sugar is actually on 60-93, which is way from the area that the reagent was supposed to be here. It was supposed to be here, here, and here.

And here is some calculation of that, the masses to convince ourselves that we are actually looking at the sugars on -- on the wrong side. And here is the close up. The sugar found here, and ironically the pieces of the sugar, not the full sugar, which is called (inaudible) product, we find that on a completely different amino acid.

The point in all of this, the reagent didn't go in the area where it is supposed to go, create some destabilization, pulled some water from the cavity and that may explain the unusual activity. That we unfortunately, didn't have enough of the material to do it. And now, to sort of relate the chemistry to the animal and the story end and there. But the point in here is that when you saw

a reagent on your hemoglobin you really need to know what you've done to the protein. From simple experiment, you can actually pull a lot of quite important information.

One more story here, on the -- oxidation. And as I said oxidation can occur by oxidants, by hemoglobin (inaudible) on -- on oxidants. And here is a story that we published recently, which is really very simple story. What we did here and I guess -- remember pure oxide is available physiologically even small amounts can do it.

So we took the hemoglobin, we treated with hydrogen peroxide in 1:1 ratio; very little of hydrogen peroxide. And we found out again, using mass spec, we found out that actually the oxidation of a handful of amino acids is always consistent. Each time you do the experiment we find 60-93 again, is oxidized, irreversibly. And 60-112 and tryptophan and the infamous methionine 55.

Now, when I say reversibly, when you talk to protein chemists, this is unheard of. To actually use little hydrogen peroxide, add it to the protein and you convert sisteic to sisteic acid. Normally you would require a huge amount of hydrogen peroxide and more powerful oxidant. The reason for that is very simple here.

What pure oxide did actually radicalize the hemoglobin. And we know that. We have done that. With the pure oxide they create a radical, radical what? Protein and irreversibly damage the protein. The point in all of this, even very little oxidant or actually hemoglobin, can actually radicalize your hemoglobin.

Okay, so the question is we can do chemistry from now, until eternity but obviously there will be a time when you need to ask the question do these simple test-tubes reaction really occur and leave a -- an animal? Does oxidation occur and whether these reactions can actually compromise the ability of hemoglobin to carry oxygen? And more importantly, if it leads to some toxicity?

So we did the following. We've chosen -- and again, depends when you do these experiments, you really need to be careful as far as the choice of amino model, and the extent of your -- your -- your search of the facts. So what we are doing here, we had two identical species. The rat, which we know ahead of time, the rat has the ability to somatically produce ascorbic acids, which is a very powerful reducing agent. While the guinea pig, of course,

unfortunately, like humans are -- are not able to produce hydrogen peroxide.

So we infuse these animals with 50 percent exchange transfusion with the same protein which is commercially available oxyglobin. And we looked at the -- the oxidation. But before that you can see in the rats, they maintain normal level of ascorbate, which is very high ascorbate. Nitric oxide guinea pig, after transfusion drop it remain very low. And how about the hemoglobin and circulation? From information of the oxidation that you can see the value almost maintain normal level of -- of hemoglobin, functional hemoglobin, very little oxidation. The -- the guinea pig, almost 50, 60 percent of the hemoglobin turned into met, which is very similar to what actually Bahagas (phonetic) reported years ago. And similar to some clinical data which we published recently in humans.

So what happens with the hemoglobin? The question is out of this oxidation, if you like, we know that hemoglobin will end up with some changes. Can we actually find that in the blood? So we pulled the blood of the animal and we looked at the oxidant modification in the

guinea pig. Nothing happens in the rat. And we see here, this is at four hours. This is at 24 hours, and you can see both the alpha and the beta subunits have undergone oxidant modification very similar though we don't have very definitive answer to the oxidation pattern that I showed -- the some of the p-amino acids. So clearly, in the physiology, these things do occur.

We looked for toxicity in the tissue and we've seen very similar to what Baxter and other people have seen. Transient (inaudible) changes and so called, the heart -- cardiac collisions, kidney damage. The rats and the guinea pig are slightly different, so to speak, but we are looking at more sensitive biomarkers to actually relate the chemistry we saw in circulation to the tissue.

The other part of my talk is, do these products deliver oxygen after the oxidation that we have seen in -- in circulation? And we have been looking for a really reliable tissue biomarker. And recently, as Dr. Bunn had indicated, we stumbled on very valuable biomarker. And that is of course, the hypoxia inducible factor, which is -- which is a transitional factor, can control the responses to the hypoxia controlled large number of genes.

Now, they have -- and low oxygen or rather high oxygen is normally degraded through an enzymatic pathway, ultimately by the proteasome. If low oxygen helps binds to the beta subunit, transmit it to the nucleus and bind to the DNA and trigger the activation of a number of key important genes. And these are of course, alpha (inaudible) genes, beta glycolytic genes, (inaudible) genes and so on and so forth. So the point in here, we need something sitting in the cells to tell us whether really oxygen deliver -- being delivered by the hemoglobin. And this is really one thing that we have there, which is an oxygen sensor, you know.

So what happens if the hemoglobin comes with oxygen? Can we see any changes in the genes and the -- and the other responses? Go back to the rat and the guinea pig and here you are looking at the functional ferritin. We pulled the hemoglobin from the circulation and look at the different heme, total heme concentration and other species, but we really concentrating on the ability of hemoglobin as time goes by, clear. And of course, hemoglobin loses the ability to carry oxygen. As you can see they have in the kidney is going up.

And late, in hours you can see a nice coalition between the two. This particular hemoglobin was able to suppress, HIF in the early stages, which means oxygen presumably being delivered. And in the guinea pig, we see -- we see similar thing, but you can see clearly towards the end, hemoglobin turned into absolutely nothing but a cluster of modified hemoglobin and you can see the HIF is extremely high.

The genes, here we are looking at -- at -- at hemoglobin slightly different experiment but we are focused on the rat. Because the rat is basically, cleaner than guinea pig. This is to control oxidation. We didn't want to compound our experiment. Here we comparing the same hemoglobin versus starch, hetastarch. It is 80 percent ET. Which is to chill, exaggerate the hypoxia.

You can see here, with an unoxygen carrying volume expander, the huge increase in the eco-gene and of course, it goes down after some times. This again, the kidney and of you can look at the hemoglobin, there is some suppression early, which is again about 10, 12 hours. Then you can see the EPO rebound to higher level.

Interestingly, few years ago, some in industry thought that this is a new property of hemoglobin, which is induction of erythropoietin but in reality what happens of course, when EPO rebound, it has usually lost the ability to carry oxygen. Hemoglobin has been oxidized and by this time of course, has been cleared. Here, we are looking at the erythropoietin, which basically corresponds with the genes.

Recently, we looked at a rather sensitive organ which is the mitochondria. And here we are looking at cytochrome oxidase, which is -- terminal oxidase in the mitochondria. And it just happens that this protein is also controlled by HIF. Here we are looking at the glycolytic metabolism in case of the -- the same animals, of course. And what happens during normoxic, one subunit of the hemoglobin -- of -- sorry, of the cytochrome oxidase, COX4 1, transformed to COX4 2. Don't confuse it with COX inhibitor. This is cytochrome oxidase.

And what happens, the reason for this because when the mitochondria transfer that, you know, from 4-1 to 4-2, it is to maximize the electron transfer and -- and -- and what you see here again, the -- piece -- piece of HIF

starts to fuse around -- increase in the COX4, to very little initially in the case of the oxyglobin. Again, at the mitochondria level where every molecule of oxygen really counts, you can see that the hemoglobin at least, the first 10 hours was able to do what it was supposed to do.

Okay, so clearly, I hope I have convinced you that -- that heme-oxidation is really critical here. And I know that there are a number of people here in the room, and ourselves who started early to think of ways and means to control it. And number of people here in the room, including John Olson and ourselves, and particularly John, started using simple protein type models, which is myoglobin and later on hemoglobin, to reengineer the hemoglobin pocket. The heme pocket, where -- and I presume that these oxidants interact. We, more recently started looking naturally occurring actually, hemoglobin that could have some nice chemistry that we can obviously later on translate it into human.

Recently, we looked at the (inaudible) hemoglobin, which you throw anywhere at it, or oxidant react very, very slowly. The idea is here, that of course,

when -- so use that clever chemistry in nature to hopefully do that in human situation. Number of approaches people have tried to put the enzymes from the red cells back to the hemoglobin either cross-linked or not and the whole idea is to control oxygen -- oxygenation or oxidation, rather.

We have recently, we used ascorbate. Of course, as we said it is an important reducing agent, selenium. Even the green tea actually, has some antioxidant property. This is an area that -- and -- or part of the control of oxidation which is heptaglobin, CD 163, which has been really ignored in recent years. This is more recent interest of ours and you are going to hear more from Dominik Schaer who came from Switzerland who will talk about this a little bit more.

But here is a little cartoon which show of course, the conventional thinking that heptaglobin of course, rightfully binds with the dimers and CD 163 also combined with some of these dimers or the tetramers. We actually recently shown that the tetramer even some tetrameric species within the polymerized hemoglobin can be picked up by CD 163 or -- or they heptaglobin. And if we

modify the surface of the protein, you can actually enhance either pathway. Or you can enhance both pathways for the clearance. Again, you are going to hear more on that issue tomorrow.

So in summary, almost all HBOC will undergo oxidation. There is no way you can control it and you can actually radicalize the hemoglobin as I have indicated to you because of this transition. If however your HBOC can withstand NON (phonetic) cells, and oxidants (inaudible). All by addition of some of these additives to control slow oxidation, you may actually get away with it. And you can keep the hemoglobin intact and deliver some oxygen.

Finally, the people who actually did the work in my life are listed here. And I would really sincerely like to thank colleagues here with me on the organizing committee for a stunning job and helping us in putting the workshop together. Thank you very much.

(Applause)

MR. FRATANTONI: Dr. Bunn mentioned that we would be talking about nitric oxide physiology, and now for a more detailed discussion of that, Dr. Alan Schechter. He is the Chief of the Molecular Medicine Branch, of the National Institute of Diabetics, Digestive and Kidney Diseases. Alan?

MR. SCHECHTER: I realize that the time is late and I will try to go through this rapidly. I would like to thank the organizing committee, including myself, for inviting me to present here today. Can we have the lights down please? Thank you. What I will try to do is give a general view of -- for the non-specialists of nitric oxide physiology, pharmacology, and -- and pathology. And just to point of disclosure, that I -- I am a co -- co-inventor of a patent from -- by National Institutes of Health for the use of nitrite salts in the treatment of cardiovascular diseases.

Background slide, most of you probably are familiar with this. The nitric oxide is believed to be the major systemic vasodilators, short-lived free radical which is multiple balanced states, which can undergo reactions with many low and high molecular weight

biological compound. For the purpose of this meeting, the fact that nitric oxide can be rapidly destroyed by hemoglobin, a fact that has been known since the very first discovery of -- of nitric oxide by Octivity (phonetic) in the mid-1980s, it was used as an assay for nitric oxide for many years, leads to a paradox about its bioactivity, because of the expectations of the vast amounts of hemoglobin in the body, intracellular and extracellular would destroy virtually all the nitric oxide.

As we have gradually realized over the last 10 or 15 years, the physiological and pharmacological potential of nitric oxide depends upon the balance between destruction and preservation and perhaps transport of nitric oxide through hemoglobin.

The nitric oxide paradigm, which was worked out in the mid-1980s by Furchgott, Ignarro, Murad, and Macata (phonetic), the first three of whom won the Nobel Prize a few years ago, for basically, the ideas described in -- in this cartoon is that either shear stress in the vasculature at the very top of the slides, with certain hormones like (inaudible) acting through its receptor can activate a nitric oxide synthase, NOS, in endothelial

cells, which converts arginine to citrulline, freeing nitric oxide, much of which diffuses into the smooth muscle below the endothelium and activates guanylate cyclaseto, an active form, which compares GTP and cyclic GMP. And through processes involving calcium fluxes, causes smooth muscle relaxation.

It was also realized but only really studied intensively in the last 10 to 15 years that nitric oxide also diffuses luminally as well as abluminally into the vascular system and that this process of the NO reactions within the vasculature obviously contributes very greatly to determining the balance of nitric oxide in -- in -- in the body.

The functions of nitric oxide as I indicated are enormous. The regulation of vasodilatone was the first to be described in that initial work that lead to a Nobel Prize but quickly it was realized that there were many other important functions including platelet out-gauge and attachment, changes in circulating selectins and other -- other proteins in activation of super-oxide and the whole complex of reactions involving oxygen radical chemistry.

In addition, it was soon realized that in addition to the NOS in endothelial cells, there were NOS -- there were other NOS enzymes, one in neuronal tissue, the end NOS, which was -- is involved in producing NO in the neuro system which important for neuro transmissions, as well as other processes. And another -- and a third NOS, the iNOS, in macrophage is the inducible NOS, which is important in fibrocytosis and destruction of various pathogens.

And so all in all, nitric oxide I believe that the publication of 75 or a 100,000 papers does indicate some importance is -- is considered a -- a topic of great biomedical interest. My own background for the last 30 years in hemoglobin and sickle cell hemoglobin led -- led to my interest in nitric oxide because of the interactions that were known from long time ago and some recently postulated interactions between hemoglobin and nitric oxide.

Many of these reactions were actually first described in the 19th century, but it was only at the beginning of the 20th century, during the first World War in that -- that the study of oxy-hemoglobin reaction with

nitric oxide to lead to methemoglobin nitrate was first studied in -- in detail by Coleman (phonetic) and Rao (phonetic.) And others, for reasons having to do with the use of gases during the First World War.

A second reaction was intensively studied in the '50s and '60s with the advent of EPR, spectroscopy, the reaction of NO with deoxy-hemoglobin to give nitrosoheme hemoglobin with NO hemoglobin with nitrosohemoglobin which I will mention again later. And the third -- a third reaction which as Frank Bunn alluded to was postulated about 12 years ago, primarily by Jonathan Stemlyn, his colleagues at Duke University, who suggested that oxy-hemoglobin could also react with NO to modify the conserved beta 93 cysteine compound which is called S nitrosohemoglobin or SNO hemoglobin and they postulated a -- an important homeostatic function for their -- of great -- and it became the -- the postulate had great theological interest, in that it was -- the idea was that SNO hemoglobin was allosterically controlled and it is dissociation under hypoxic conditions could free NO and lead to increase in -- in blood flow and increased oxygen delivery to compensate for hypoxic conditions.

However, from the very beginning this hypothesis was very controversial but it did serve a function of getting me and many other investigators into the field who were interested in hemoglobin into the field at that time. And in particular, just about then, in '96 and '97, I was fortunate that I initiated a collaboration with Mark Gladwin who had joined the NIH in Critical Care Medicine and Richard Cannon of the National Heart Lung and Blood Institute.

And we began to try to look at the question in humans and virtually all the work I mentioned before about SNO hemoglobin was done in animals and we were not sure how relevant this was to human beings. We decided to -- to investigate the reactions of NO with hemoglobin under physiological conditions and we chose the inhalation methodology of delivering NO, which had been approved by FDA based upon the work of Lawrence A. Paul and others. And it has been approved for use in the treatment of -- of certain pulmonary conditions.

And we were able with the help of Critical Care Medicine nurses, and other staff to have a number of normal volunteers and then later on sickle cell patients

and other individuals breath nitric oxide for varying periods and analyze the -- the changes in hemoglobin chemistry that occurred in these individuals. And so you can see under what we considered physiological conditions, what the relevant reactions were.

I will just say, I have long advocated the importance of clinical research of studying phenomenon in humans and I -- I think this is a very good example that -- that things that we found in humans were not necessarily the same as were reported in animals. In any case, the -- the paradigm was have an individual for a couple of hours at rest, we measured nitric oxide derivatives in arterial and venous blood, the individual then breathed nitric oxide at -- at 60 or 80 parts per million for a -- two hours.

We measured the changes in these metabolites and then the inhalation was stopped and then the values went back to -- to zero. And we set up assays for all what we believed were the important NO adducts at this time. And some of the results we got then, and these studies including the use of nitric oxide inhibitors, exercise to -- to test various physiological hypothesis, which I won't

go into now, was that we found when individuals breathed, these were normal individual breath nitric oxide for two hours, we got significant increases in nitrosohemoglobin but very interestingly a statistically significant arterial venous difference in all three of these experimental situations.

No increase in SNO hemoglobin which values we got in our assays were much lower than were being reported from further south of here. And no AB differences. But interestingly we found that as you would expect there were marked increases in nitrate. In fact, we have inferred that most of the methemoglobin in the body comes NO reaction. The NO reaction with the oxy-hemoglobin to give methemoglobin nitrate and in addition, how with small increases in nitrate levels with generally significant AP differences both before and after inhalation.

And on this -- on the basis of these studies of normal volunteers we postulated that NO inhalation might lead to NO -- systemic NO increases but that the likely fact is for potential physiological delivery if it occurred, were -- was NO hemoglobin and nitrite. Both of these hypothesis were not warmly accepted. In vitro the

half-life of NO hemoglobin is very long, 100 or more hours. And so it did not seem that it could act physiologically and many investigators said nitrate cannot be under physiological condition converted to -- to NO.

But in any case, we believe that data and we wrote a short review and by that time for the New England Journal of Medicine, I think 2003, in which we postulated that the basic overview of nitric oxide pharmacology, physiology, pathology is show in this -- this cartoon that all ordinarily erythrocytes are fairly much immune from interaction with nitric oxide because of unstirred layers, Liao (phonetic) and others, Kim Shapiro, had studied the diffusion and for a variety of hydronamically -- I won't go into this, probably a little diffusion under normal conditions of the NO into the red cell.

With pharmacology such as with nitric oxide inhalation, we postulated a number of reactions the possibility of these anti -- oxygen reductase reduction that (inaudible) has been studying, might contribute to NO formation. But in particular, in terms of red cell, we believed that the major resultant of large scale NO administration as with the inhalation was the formation of

nitrosohemoglobin and that could disassociate under -- under physiological conditions to free nitric oxide, perhaps through SNO hemoglobin.

With the idea that we suggested was SNO hemoglobin was an unstable reactive intermediate in nitric oxide metabolism of the red cell. But also that -- that nitrite itself could be formed in the vascular system and nitric oxide could be converted to free NO.

In contrast, we were just beginning to do studies then and from other data that suggest to us that the cell free hemoglobin could be very different, that the NO produced by NO syntheses in the wall of that blood vessel would quickly react with the largely ferrous cell free hemoglobin, convert that to ferric and nitrate and lead to a relative NO deficiency and cause constriction of the vessels and perhaps contribute to some of the pathology of -- of various cardiovascular diseases.

This hypothesis -- this article led to five letters of protest brought by Dr. Stambler and four of his colleagues, five separate letters brought by four of his colleagues saying that none of this could be right. But still five years later, I think what we said here is

basically the view we still have. We often initiated studies and this is with Andre Dejam of nitrite levels in humans in plasma and red cells. There was a lot of controversy in the literature. Again, the value bring reported especially for animals, were immensely higher than we were seeing in human normal and individuals and patients.

And we had to work out, largely done by a post doctoral fellow, who is now at the Bingham under Dejam, method both of assaying for nitrite and stabilizing if we felt a stabilization system that we could get stable values of 24 hours. This is -- these data are from a paper in Blood two or three years ago, in which we found that now measurements whole blood nitrite levels in just about a dozen human beings as with regard to 117 animals per liter red blood cells are close to 300 animals per liter suggesting that most of the intravascular nitrite is in the red cells but a significant amount in the plasma and that using these methods, the stabilization solution, one could study nitrites in a systematic way, other than many ideas that it might be a risk factor for various cardiovascular diseases.

We also with our usual propensity to use PowerPoint to draw cartoons did another one of the --this zoom is the interaction of the nitric oxide in the vascular system with the nitric oxide produced by iNOS undergoing reaction with many oxidants reductants to give a whole variety of balance dates which I represent here as N-Oxy and these various balance dates which include proxy nitrite and NO2 and N2O3 and many others can either react to nitrate proteins, tyrosine groups or sulphydral groups or lipids -- could this -- this significant amounts of free NO, as well as oxidation to nitrite which can be reduced to NO by (inaudible) oxidase at low PH or NO and nitrite can go into the red cell and undergo a -- some of the reactions I have been describing.

I don't have time to step through these reactions except to say that the two uncertain issues of importance in this schema is first whether or not there is some nitric oxide being produced within the red cell. Dr. Kelman (phonetic) and his colleagues have published a number of papers over the last few years suggesting that red cell membranes have eNOS activity and convert largely to NO. Other groups have not been able to confirm that

but if it is true and it may require certain conditions of substrate, it would be very important to in understanding where the NO -- NO and nitrite in the red cell comes from.

The other aspect is that the -- there is still a question of how the NO can get out of the red cell after any or all these reactions. And this is still controversial. There is an interesting recent suggestion by Kim Shapiro and Gladwin that N2O3 is the active form for efflux in the red cell but I think that that -- those analyses are still ongoing.

But at this point, given our physiological interest in all this, we ask the question is nitrite a vasodilator. We know and this has been known for a long time that vasodilator -- in vasodilators aortic rings have high concentrations. In fact, Dr. Sommerwise (phonetic) did studies like this in 1930 -- well, not with Eric Rings (phonetic), but with -- in animals and people, the studies were done in 1930s at Brigham and in the 1950s by Dr. Brownwald (phonetic) NIH.

The nitrite vasodilates lung profusion models at concentrations of 100 micromolare. I took the byzantine (phonetic) oxidase and the levels but there were papers

appearing as recently as three or four years ago, that suggested that there was no vasodilator activity of nitrides.

So we began a collaboration with a group at Loma Linda, Gordon Powers and Chris Hunter and their colleagues and we infused -- they infused nitrite into hypoxic newborn sheep and you can see that blood pressure fell with the infusions. And it fell to a great extent that hypoxic animals than normal animals, suggesting that the hypoxia enhanced this. A very important correlative of this was the measurement of exhaled NO in these sheep and we could when we infused nitrite into an artery, we could see NO being exhaled which was direct proof that nitrite was being converted to NO, was not acting by some other mechanism.

Eventually, a few clinical studies were done with the FDA IND with low levels of nitrite were infused step wise into a few individuals and we found an increase in forearm blood flow which correlated with changes in whole blood nitrite and we could follow the time course of that seeing the effects were almost immediately if seen

laterally, but only started occurring after a minute or two in the contra-lateral arm.

And so the conclusion of this work was that nitrite irons caused vasodilatation and physiological concentrations and may contribute to hypoxic vasodilatations. Nitrites probably induced to NO by deoxy heme-proteins and other nitrite reductase mechanisms. And Dr. Gladwin will go into this I think tomorrow in his lecture. Nitrite may be relatively stable tissue and blood source and bioactive NO. And nitrite may be useful for administration by inhalation or infusion as a therapy for various patho-physiological states characterized by a lack of NO.

Now, the time is late and I see the red light so say -- with just to say we have been recently been examining the effect of a ascorbate and the dehydro ascorbate which are in equilibrium on -- on some of these reactions. And we can show, and there is a paper that has just been published in biochemistry by my colleagues (inaudible), that DHA can oxidize HBNO to methemoglobin and presumably free the NO as you can see the time -- the time course of this. And that DHA can increase nitrite

levels in erythrocytes and we again, we have worked out a proposed mechanism of this.

Time is late and I won't go into this. You can look at this in your hand out. The only point I would make that I think of general importance for this group, is that other factors like ascorbate or perhaps urate can also affect these reactions and so inferences from studies of pure hemoglobin solutions like the NO hemoglobin solutions are not necessarily valid for what occurs physiologically especially in the red blood cells.

Lastly, it is important that I spend two minutes just mentioning hemolysis. I have worked for 30 years in sickle cell disease and this summarizes the fact that we know that hemolysis is a very important part of sickle cell disease, into vascular hemolysis. And it -- and again, we know that NO, as I said it is a destroyed by hemoglobin on diffusion, limited reaction.

This slide which I made up several years ago, before I was -- got involved in HBOC question, we obviously knew and actually I think it was Bob Winslow who suggested to me in 1993, 15 years ago, that a lot of the problems in the HBOCs was indeed due to nitric oxide. And

this was before I'd even paid any attention to nitric oxide.

And this again is just the part of the schemata but what I wanted to -- is that this idea has been taken by Mark Gladwin and his colleagues Martin Steinberg and others into the idea that -- that sickle cell disease may actually have two distinct components. A vasoinclusive components, related to intracellular polymerization. I wouldn't call it erythrocytic sickle, I would call it intercellular hemoglobin mass polymerization.

And the factors of sickle cell disease that are affected by this include the, obviously the hemoglobin level -- include the hemoglobin level for destruction of red cells, the terminal arterials, vasal (inaudible) pain crises, acute chest syndrome and other symptoms. But that was a distinct subset of symptoms including pulmonary hypertension, pryposim, glycolysis and now, there is some data for stroke as well, that is related to the hemolytic component.

Up until now, hemolysis has been largely known as sickle cell disease. Most patients are very well compensated and do not need transfusion. But this -- this

approach to it -- to the disease suggests that there are two sets of symptoms which are due to quite different distinct patho-physiological mechanisms.

Lastly, just Frank Bunn alluded to the paper that has impressed, I think may come out this Friday or a month from now, of Nature Medicine from Isabel Patel, in towns at UAB which they made a knock in, replacing beta 93 cysteine with an alanine and you see a change in SNO hemoglobin, but there was no other phenotype in these animals. And in fact, when -- when one does, the classic aortic ring assays with either normal mouse hemoglobin or the without the beta 93 there are in -- in pulmonary arteries of rabbits there are no differences at all.

And the conclusion of this is that under these circumstances there is no evidence that SNO hemoglobin has any physiological importance. And I think all the papers that have come out recently about the important of SNO hemoglobin in blood transfusion, in sickle cell disease and diabetics and pulmonary hypertension have to be thoroughly reevaluated on the basis of this very important finding.

And then just to conclude that nitrite ions cause vasodilatation, physiological concentrations contribute to hypoxic vasodilatation and a relatively stable tissue and blood sources of bioactive NO for endocrine delivery. Nitrite is reduced by deoxyhemoglobin and possibly other heme proteins myoglobin, hemoglobin, et cetera. NO hemoglobin may also be a source of bioactive NO and SNO hemoglobin appears to be an unstable intermediate in NO reactions with hemoglobin.

Cell free hemoglobin and acute and chronic anemias may contribute to pathology by reaction with either NO or nitrite ions. And administration of NO by inhalation of nitrite by inhalation or infusion may compensate for pathology related NO deficiencies. And already there is work in the HBOC field. A recent paper from (inaudible) Paul along these lines. So I think the -- we still have a big problem with NO biochemistry with the blood substitutes. But the -- there is a possibility of how robust it is, I don't know, that the -- there may be a solution. Thank you.

(Applause)

MR. FRATANTONI: I remind people that if you have any questions for these speakers, write them on cards, raise your hands, someone will come down and get the card. The final speaker is going to address the topic of non-clinical studies, the animal models why they do work, why they don't work, a question that's very important one for this conference, is Dr. George Biro, who is the Emeritus Professor at the University of Ottawa, adjunct professor at the University of Toronto. George?

NON-CLINICAL TESTING: STRENGTHS AND LIMITATIONS

MR. BIRO: Good morning, ladies and gentlemen. I'm gratified to have received this invitation to talk and I'm especially gratified to follow the distinguished speakers who have set up an excellent groundwork for what I'm about to say. What I'm going to say has little to do with the molecular and sub-cellular aspects. I'm going to talk about mostly really old-fashioned animal physiology. And what I would like to say is that I left -- retired from the University of Ottawa from which a recent editorial on HBOCs has emanated. I left the University of

Ottawa in 1998, I think, worked for Hemosol for about 4 years. Consulted Hemosol for about two more, and went into retirement and been dragged back into the arena.

I set myself three questions for this talk. I wanted to think about the conventional -- the really conventional and old-fashioned ICH and GLP compliant testing for safety into whole animals only. I didn't want to address issues like in vitro testing or the standard issues, only about the things that apply to safety to HBOCs.

Secondly, I wanted to look at the academy laboratory experiments using unique resources that are mostly available only at universities and research institutes and I'm going to use some examples without using actual data. And lastly, I wanted to pose the question of why is it that the safety testing on animals has failed to predict convincingly what has been observed in the clinical trials. So first of all, whole animals only and these were all healthy, normal animals, single dose safety.

The largest problem with single dose safety testing in animals using HBOCs is the fact that there is a

very limited applicability to use multiples of the intended clinical dose. The blood volume is limited and there is obviously an issue of interpretation when you're replacing or adding a very large fraction of the blood volume.

There have been two experiments in which practically all of the blood volume has been replaced. One set of experiments by Hemosol replaced 95 percent of the blood volume and studied the animals over the subsequent seven days. All survived in the presence -- in the virtual absence of red cells. Second experiment was done by Baxter and they replaced practically all 98 or 99 percent of the red cells and they also survived. The standard model is to do a standard toxicity panel biochemical histological testing and looking at immediate and short term as well as delayed results.

It is possible using these studies to complete a limited study of the mechanism of effects, but by and large these are small animals and the ability to study mechanisms is quite limited. It is possible to do pathology on these, but it is likely that with a single exposure, a single infusion, there are not likely to be

very obvious pathological changes. And there is a possibility to do limited pharmacodynamic and pharmacokinetic studies and these are useful and it is also useful if animal models of relevant disease have been involved; so far, none have.

Repeated dose toxicity is the standard toxicology paradigm. Again, they use whole animals and healthy, normal ones, usually litter mates or at least animals of similar age, size, and of the same species. I would like to offer you an example of one of these, which was conducted by Hemosol, and this was presented at the society for toxicology some years ago as an example for this. Sprague-Dawleys rat were given daily intravenous infusions of Humulin (phonetic) of 10, 20, and 30 milliliters of kilogram. So these represent about 5, 10, and about 20 or 30 percent of the blood volume on 14 consecutive days.

The infusions were either Humulin 10 percent in Ringer's lactate or pentastarch 6 percent in physiological saline. At the time this was done, X10 (phonetic) was not yet on the market. So this was the control, which is obviously not an ideal or appropriate control. But we

wanted to use the then available clinical colloid.

The standard panel linked with the hematological clinical resident -- clinical chemistry grows in microscopic examination of all organs, veterinary observations, feed consumption and weight gain, coagulation, special staining by Prussian blue of all the organs for ferric iron, special staining of the testes for spermatogenesis, and quantitative measurement of tissue iron in various target organs.

What I want to emphasize here, is what is the magnitude of the exposure and burden in these animals? At 30 milliliters per kilogram per -- 30 milliliters of Humulin per kilogram, they are exposed to 3 grams of hemoglobin, which represents a cumulative exposure of 14 days or 42 grams which is 5-1/2 -- 5-1/4 times the blood volume. The volume is 30 milliliters and it is 420 milliliters, 800 times the blood volume -- sorry -- this is 5 times the hemoglobin mass.

Iron was given at 11 milligrams which is 20 percent of the total body iron and almost 3 times over the 14 days of the total body iron. Globin was 500 -- 5 times total globin mass and porphyrin was 22 times the normal

daily turnover, so these are massive overloads, none of these animals died, all of them survive to the intended time of sacrifice and they were tested and they were a variety of abnormalities, obviously, clinical chemistry was abnormal, liver function tests were abnormal.

Hematology was abnormal, but the interpretation of this changes is difficult because we don't know whether the change in liver function, for example, is due to the disposal of the large amount of globin, or the large amount of porphyrin.

The change in feed consumption may be due to the enormous intravenous protein load, which may play havoc with the hypothalamic signals of satiety and hunger. So it is possible to achieve in these animals a large, manifold multiple of the clinically intended single dose. In larger animals it is possible to make repeated observations and establish a time course for recovery.

There is now out in the public domain one set of experiments in which healthy normal pigs, sheep, as well as dogs and rats have been exposed to Baxter's hemoglobin and subjected to extensive histopathological examination, which revealed widely dispersed small diffused

degenerative changes, which were not associated with global changes in either global ventricular function or troponin release.

The perplexing thing is the apparent species specificity. Rats and dogs do not appear to exhibit these lesions, pigs, sheep, and primates do. The mechanism is not understood, but it is possible or probable that there is nitric oxide improvement. And we do not understand the significance of species specificity. So overall, conventional safety testing on large animals -- the procedures are uniform, because there is extensively tested and produced standard operating procedures, the population is homogenized. The rats, and for example, they are often litter mates, same size, same age, and the analysis is mostly done by aggregation.

The variability is minimized because of the aggregation and the large and massive overload may detect common events. The laboratory and the pathology, given long enough exposure for pathology to develop, may help to understand the significance of the clinical chemistry and hematology changes and may allow the determination of reversibility of these changes.

The limitations are that these studies are of limited generalizability. You cannot generalize very easily. They may fail to detect the rare and infrequent events. Unless diseased animal models are used, you cannot identify possible synergistic effect between the disease and the exposure to the agent. So what they may fail and likely do fail is that it fails to identify the specific monitoring requirements for the clinical trials.

Now, my second question was to look at the academic experiments using these unique resources and expertise in university and research institute labs. What I find is this is a wonderland of applied physiology, sophisticated, extremely well-developed methods are used in highly competent and technically excellent health, hence, and they generally are aimed at demonstration of efficacy. They may indicate safety issues, but the major issue in these experiments is to look at the efficacy, and they are comparing with controlled studies.

They use very expensive -- extensive hemodynamic monitoring with additional unique measurements such as oxygen availability in the tissue. Models are reproducing physically relevant conditions such as normovolemic

hemodilution, bleeding shock, and delayed resuscitation, animal models such as the spontaneous hypertensive rat, blood flow measurements, both total cardiac output and regional distribution of blood flow, microcirculatory observations using very sophisticated methods and critical oxygen delivery estimates.

The setup and special skills obviously are not available in CROs, and therefore these are not GLP compliant. Unique measurements are available using palladium porphyrin, phosphorescence, platinum microelectrodes and polarographic electrodes to measure tissue PO2 and this distribution within the tissue.

Whole animal hemodynamic and oxygen dynamics can be done in clinically relevant models such as shock and resuscitation and combine, for example, with observations in the conjunctiva microcirculation. Very sophisticated, quantitative microcirculatory observations are made in whole animals through windows mostly looking at accessible parts like skin and the skin pouch.

Global and regional myocardial function can be measured and region can be combined with regional and organ blood flow measurements in whole animals. And these

are often combined with tissue oxygen measurements in accessible tissues, such as skeletal muscle. Organ function in other tissues, pancreas, kidney, and in fact animals have been subjected to severe hemorrhagic shock and resuscitation to see if there is amelioration of post ischemic microcirculatory change.

In hearts, in pigs with previously imposed critical coronary stenosis, simulating coronary artery disease has been measured. Again, global and regional myocardial function and blood flow distribution to see what is the effect of an HBOC in the presence of a critical coronary stenosis. But measurements of tissue PO2 have been generally indicative of reasonably well preserved tissue PO2. These have shown that there is reasonable recovery from shock and resuscitation when an HBOC was used.

Quantitative observations have shown differences between the microcirculatory parameters and in the HBOC-resuscitated animals and in controls. Pancreatic organ function has been seen to recover well to post ischemic conditions in the presence of HBOC. And critical coronary stenosis in the presence of HBOC has allowed extreme

hemodilution down to a level of a hematocrit of only two percent, whereas animal's hemodilution with albumin would show cardiac failure and die at a hematocrit of about six or seven percent.

In standard hemodynamics and blood flow distribution models, blood flow was measured in all organs, and in the myocardium, blood flow was reasonably well preserved in HBOC hemodiluted animals whereas blood flow has impaired in starch hemodiluted animals. Critical oxygen delivery after similar sequential hemodilution to extremely low hematocrits was shown to have the same critical oxygen delivery level as animals that had been diluted with either/or old or fresh red cells.

Sangart and Intaglietta have shown microcirculatory observations in animals hemodiluted to extremely low hematocrits and compared it with the same degree of hemodilution with decorated albumin. Again, the hemodilution with the HBOC was beneficial. Brain blood flow and oxygenation was measured in the caudate nucleus of rats hemodiluted with an HBOC, and the tissue PO2 in the caudate nucleus was actually increased with maintained blood flow in the presence of an HBOC.

And eventually the HBOC effect of a pharmacological dose of the same dose of an HBOC injected or infused into spontaneous hypertensive rats and the control was the Wistar-Kyoto rat, showed that quantitatively the same dose of Humulin increased the blood pressure bore in a spontaneous hypertensive rat than in the Wistar-Kyoto rat.

So the strength of these sophisticated experiments in academic labs and research institutes are that there are direct indications of efficacy in a really reasonable acceptable criteria. Critical oxygen delivery, critical hematocrit, and there are also indirect indication of safety issues, such as organ or tissue vascular microcirculatory resistance.

Microcirculatory resistance is greatly increased. We have an indication of a possibility of a safety issue. Very powerful tools have been brought to this investigation. Variables of clinical interest have been used in models of clinical relevance and observations were made in highly stressed intervals in contrast to the standard conventional toxicology model.

Pharmacodynamic analysis can be conducted and

the investigation is extended to organs of high physiological importance, the heart, the brain, the kidney, pancreas, and the liver. The limitations are actually very specific to the experimental model. There is limited generalizability if the study is conducted in those organs which do not receive a large proportion of the cardiac output and in which the blood flow distribution is not coupled to the metabolic need or other function such as secretory or excretory function.

These studies if they are specific to organs that do not receive the overwhelming majority of the cardiac output, neglect the affected compromise models in morbid conditions, unless they are specifically included and they really have not been.

And focusing a single organ deflects the whole body physiological adjustments that occur in response to any stress. I would remind you of Dr. Bunn's first slide in which the first box is the cardiac output and right under the cardiac output is blood flow distribution. Cardiac output is less important than its distribution.

The striking observation about these is that on balance, they offer strong support for beneficial effects

of the HBOC against the controls used. The benefits are seen even in models simulating clinical conditions, for example, hemorrhage and resuscitation, or organ ischemia, or arterial stenosis. In all but two of the studies, actually three -- in all but two of the studies, the control is a non-oxygen transporting colloid. Two of the studies, used either shed red -- shed blood or old and stored red cells and nearly all used healthy normal animals. Only one study that I know of used the spontaneous hypertensive rat.

I was going to say more, but I'm going to cut it short. There has been a failure of the non-clinical testing. The failure has been that by and large these testing showed beneficial effect against the control and failed to predict the adverse clinical outcomes. The published academic experiments have also shown benefits have failed to predict the published outcome. Why? Because there is a great discrepancy between the non-clinical testing and the clinical testing, healthy normal inbred young animals.

In the clinical studies there is a huge heterogeneity of such subjects, age, co-morbid conditions,

procedures, et cetera. By and large, the adverse clinical outcomes have been seen more frequently in the aged, in the diabetic, in the atherosclerotic, in the hypertensive, and these adverse clinical outcomes were occurred in this population, which represented a large proportion in these trials than their prevalence in the normal population. I thought that there may be one probable answer to the question of why the animal testing and the human testing diverged.

Endothelial dysfunction not what you thought I was referring to occurs in diabetes, in atherosclerosis, in hypertension, ageing, and others. So the endothelium is an extremely important organ, 20 years ago it first got recognized that the endothelium is very important. Recently there was a review about the endothelial dysfunction in which they quoted, "A 100 years ago textbook of the principles and practice of medicine in which it was said that the age of your arteries defines how long you will live and how well."

The recent review modified this by saying that the health of your endothelium defines how long you will live and how well. Endothelial dysfunction, I am going to

ask you to throw away the hardcopy of my slides. I will have a new set tomorrow. Endothelial dysfunction will amplify and exacerbate all the effects that these are supposed to show because they interfere with NO, because they make other vasoactive substances more important.

In conclusion, conventional testing has not been very useful, unconventional testing has failed to predict and the animal experiments should emphasize evaluation of the HBOC effect on important target organs with appropriate attention to physiological adjustments.

Organ blood flow is regulated by an enormously complex interplay of multiple vasodilator mechanisms. These regulate or autoregulate the supply to meet the metabolic demand. The hypertensive effects of HBOCs are also multifactorial and they mediate the vasodilator mechanisms mostly through NO, which are rendered less effective, less bioavailable. And in many highly prevalent human diseases, endothelial dysfunction, which is really manifested in a priori impairment of the NO response. Endothelial dysfunction and HBOCs together exacerbate and make each other worse. Thank you.

(Applause)

MR. FRATANTONI: I have not received any card -- I am receiving a card with question. Okay, I've just got a single question with me. Let me read this one and I'll just give it to one speaker to handle and then we'll go to a break as -- there are more questions coming. Okay, well then can I ask the speakers to join me up here on the panel, we'll just spend a few minutes with these.

Okay, I'm going to hand these questions out to a couple of (off mike) and there is a general question here that we just -- obviously there is one question that asks, will there ever be a substitute as good as your own autologous whole blood; that I think is what this meaning is about. So hopefully we have some approach to that.

Okay, I've got a question for Dr. Bunn. Since HBOC or tetramer injection does not produce systemic vasoconstriction in specialized mouse, you know, the negative imbalance, why is HBOC oxygen delivery vasoconstriction above the (off mike)?

MR. BUNN: I think this is a good point. I

don't really have anything to add to what the NOS knockout mouse is an argument I suppose that one could use. I don't think there is any question that vasoconstriction is seen with HBOC administration, and so the question is, you know, how much of a contributor is nitric oxide to this. And I think that's hopefully something we'll gain further insight during the meeting about.

MR. FRATANTONI: For Dr. Schechter, does arginine feeding effect nitric oxide balance?

MR. SCHECHTER: Thank you. I thought -- that is arginine feeding?

SPEAKER: Yeah.

MR. SCHECHTER: Sorry, it is not clear. I suspect not, there have been a number of reports that it does, there is at least one company that supplies arginine candy bars in health food stores and they are apparently widely used. The levels of arginine in the blood are much higher than the KM, the enzyme would indicate as necessary through maximal reduction of NO from the arginine.

People have argued may be within the endothelial cells or other places the levels are lower and by raising the arginine levels one can increase the levels and insert

specific tissues where NO is synthesized. There have been a few reports of benefit from arginine administration, but they have not been tested in very large scale controlled studies. I think the verdict is out. I'm a little dubious, but I think we need control trials to establish whether or not it does have value.

MR. FRATANTONI: The question to him -- towards the entire panel, I think it's a question that comes up a lot. Could you explain the difference between vasoactivity and hypertension? Abdul, you want to try that first?

MR. ALAYASH: I tried to explain that before and I was (inaudible) in my simple-minded biochemical mind vasoactivity refers to constrictions of blood vessels as a consequence of that is the hypertension, but to those of us here with a better physiological background could actually explain that. Alan, can you explain it? I mean did I get it right or wrong?

MR. SCHECHTER: Okay. Vasoactivity and hypertension. Well, this is standard physiology. The blood pressure is the result of the interaction between the cardiac output and the peripheral resistance. In

almost all studies, the rise in blood pressure is accompanied by the calculated resistance because that's the one method you can measure. You can measure cardiac output, you can measure blood pressure, and you can therefore calculate peripheral resistance which translates into -- this is one of the things I wanted to say -- translates into a constriction of the arterioles principally, unless the blood viscosity changes.

Resistance comprises the constriction of the blood vessels, hindrance and viscosity of the blood. Generally, blood viscosity declines not a great deal from the normal hematocrit of 45. It increases exponentially when you go up the hematocrit at 45. So vasoconstriction accompanies vasoactivity, accompanies the rise in blood pressure in using an HBOC, unless the conditions are such that you really cannot determine the peripheral resistance.

Beyond that, all I can say is that there is not a single factor that determines the blood pressure. There is a host of vasodilators, nitric oxide one, every organ, especially, the ones in which blood flow is coupled to metabolic rate sequence a variety of signals and

mediators, the heart, the brain, adenosine is one of the important mediators which is a vasodilator and work synergistically with nitric oxide. In addition, there is a host of vasoconstrictors. The simple adrenal system, endothelin, angiotensin, and there is evidence that HBOCs affect every one of those.

MR. FRATANTONI: Okay, Dr. Bunn you have a question. We'll make this our last one.

MR. BUNN: This is from Dr. Simone, Texas Tech. Do you think that besides the mass the charge on the protein is important in glomerular filtration? Hemoglobin tetramer can also cross the glomerular barrier because it has more -- these more electropositive charge than albumin. This is a very good point. In fact this came up in some detail at Somatogen, where they engineered hemoglobins with different charges with the hypothesis that the more electropositive, the hemoglobin that -- you might get more filtration and it was tested by double-charge mutations to quite rigorously and the result was that that there was not a significant impact of hemoglobin charge on this glomerular filtration.

MR. FRATANTONI: Thank you. We're going to stop

there. I'm not getting to all the questions, the time is not going to allow that, so I'll apologize to anyone whose question we did not reach. We're going to -- now it's -- I've got 10:40; we'll reconvene at 5 minutes before 11:00. I want to thank all the speakers for getting this off to a great start.

(Applause)

(Recess)

SESSION II -- CLINICAL EXPERIENCE WITH HBOCS

MS. ALVING: Could you please take your seats now so we can begin the next session?

(Pause)

MS. ALVING: Okay. Our next session is going to be on "Clinical Experience with Hemoglobin Based Oxygen Carriers."

I will be the moderator for the panel this afternoon. I'm Dr. Barbara Alving. I'm the director of the National Center for Research Resources, which is one of the 27 institutes and centers of NIH. In interest of full disclosure, I once worked for the FDA and I am a

retired Army Colonel, worked at Walter Reed Army Institute of Research, and thought about the products or potential products that were needed for trauma and for soldiers, and I leave it at that. I'm now a civilian and still very interested in these products and interested in the products for both civilians and soldiers.

And also I think we are very interested as federal -- a federal agency working closely together meaning FDA, NIH, in what is the best way to proceed. And that's going to come out, I think, tomorrow with further discussion. This panel and this session is really going to be about hearing what has been going on in the clinical trials, what has been the experience, and we're -- I think, we're very fortunate to have representatives from several of the companies here to speak about clinical trials.

But first I'd like to introduce Dr. Toby Silverman, and she is going to provide some introduction. She is from the Office of Blood Research and Reviews, CBER, FDA.

Also I'll remind speakers and all of us that we are going to speak very clearly and loudly, and if you

cannot hear, please raise your hand in the back to remind the speakers.

(Pause)

INTRODUCTION TO THE ISSUES

MS. SILVERMAN: All right. Okay, can everybody hear me? Fantastic, I'll hold this one here too.

As Dr. Alving said, I'm -- my name is Toby Silverman. I'm the branch chief in the clinical review branch in the Office of Blood Research and Review in CBER, FDA.

My group has evaluated all of the hemoglobin-based oxygen carriers that have come before FDA over the years. I'd like to introduce you to the issues, and I'd like to set the tone here by saying that we will be discussing settings and indications that either have been studied or have been contemplated.

We'll try to set the stage for defining clinical benefits, and after that, endpoints. And then very briefly we'll talk about some unresolved issues. So let's start with settings and indications.

First, how and under what conditions will hemoglobin-based oxygen carriers be used? These are some of the things that people have thought about over the years. It been proposed for initial resuscitation as a bridge to transfusion, as a transfusion alternative, as oxygen therapeutics in various states such as ischemic state stroke, medical anemia.

Some have thought about them as adjuncts, adjunctive therapy, particularly for radiation therapy, and others yet have thought about them of as treatment of pressor-dependant septic shock or SIRS.

There've been a lot of questions about where and by whom such products will be used: Battlefield situations, accident scenes, in transport vehicles, in the hospital, in the emergency room, in surgery, whether elective or urgent, in the ICU, in the oncology ward, in the cath lab on the medical ward.

And some have even thought about using these in physician's office. Other questions that have been raised have been who will control the distribution. Will they be controlled and distributed from the pharmacy, from the blood bank, both, neither?

There had been some questions about medical oversight issues, initial and total dose of product, monitoring of use and utilization review. Certainly there have been issues of clinical laboratory measurements and interference with some perhaps critical laboratory parameters for patient care, and then questions about transfusion or infusion reactions.

Studies that have been conducted in potential indications have included perioperative use, general surgery, orthopedic surgery, GU, GYN cardiac, some with and some without acute normovolemic hemodilution, for the purpose of evaluating these products with transfusion avoidance or reduction in allogeneic transfusion.

Studies in trauma have been conducted and have been proposed for the pre-hospital setting, for the pre-hospital setting into the hospital, and in the hospital. Products have been studied or/and being studied for hemodynamic stabilization, for example, pressor-dependant sepsis and SIRS, in renal failure and in a post-surgical critically ill patients. And these products have been studied in ischemic events, ischemic settings including percutaneous coronary intervention and stroke.

So let's start by trying to define a clinical benefit and the first question I'd like to ask is what's the target?

Well, there are some potential benefits to these products to include, in general, universal compatibility, immediate availability, stability on long-term storage including at room temperature, the fact that these products are pathogen-inactivated or pathogen-reduced, and then in general, an avoidance or reduction of allogeneic red blood cell transfusion.

Potential clinical benefits include oxygen delivery, resuscitation from hemorrhagic shock, treatment of ischemia, radiation sensitization, and again other pharmacologic effects, including taking advantage of the pressor effect of these agents and hemodynamic stabilization.

So let's talk a little bit about endpoints, how do you measure such clinical benefits?

Well, there are -- I work for FDA, so we have to consider some regulatory concepts. We deal with the concept of substantial evidence of effectiveness as defined by the Food Drug and Cosmetic Act and here is the

quote, "Evidence consisting of adequate and well-controlled investigations by experts, qualified by scientific training and experience, to evaluate the effectiveness of the drug involved on the basis of which it could be concluded that the drug will have the effect it purports to have under the conditions of use prescribed, recommended or suggested in the labeling."

The Public Health Service Act Section 351 states that licenses for biologics are issued upon showing that the product meets standards designed to ensure continued safety, purity, and potency. And the concept of potency has long been interpreted to include evidence of effectiveness. All hemoglobin-based oxygen carriers are biological drugs. So they're subject not only to the FD&C Act, but also to this provision of the PHSM.

So let's talk about some general endpoint considerations. First, sample sizes must be sufficient to permit adequate assessment of risk versus benefit of use. FDA has said generally separate safety and efficacy data are necessary for each clinical setting for which an indication is sought.

Now what's an indication? And indication is the

beneficial effect or effects as determined in clinical investigation or investigations. And the claim should include the setting or settings in which the use of the product is indicated.

General efficacy considerations include an increase in survival, prevention or slowing of disease progression, in other words a decrease in morbidity, or some measurable symptomatic relief. And the real question here is how to apply these general considerations to HBOCs?

In order to do that, CBER has just put out one points to consider in 1990 to which you heard Dr. Fratantoni elude, and then draft guidance in 1997 on the efficacy evaluation of hemoglobin and perfluorocarbon-based oxygen carriers, and then in 2004 draft guidance on criteria for safety and efficacy evaluation of oxygen therapeutics as red blood cell substitutes.

Efficacy and safety considerations are context-specific, and we've talked about some of the contexts, elective surgery and trauma, but the one I haven't talked about yet is blood not available, not appropriate or not acceptable, either due to objections in the use of blood,

religious or non-religious, or hemolytic anemias, blood incompatibility, and so forth.

There are other indications that I've alluded to ischemia, as in coronary ischemia and stroke, radiation sensitization, and hemodynamic stabilization are taking advantage of the pressor effect of some of these products.

So how do we measure efficacy? Well, in the various guidance documents, FDA has noted that the population should reflect the clinical population likely to undergo that particular surgery, this is for elective surgery. And the protocols should specify and confirm enrolment of subjects with high transfusion need.

Finally, the hemoglobin based oxygen carrier and the control, which would probably be red blood cells, must be administered for appropriate and evidence-based reasons. Endpoint considerations include reduction and/or avoidance of allogeneic red blood cells, which is a surrogate for risk reduction, including the risk associated with allogeneic red blood cells, which include non cross-matching compatibilities, theoretical immune suppression, transmissible infectious diseases, outcomes related to the age of stored blood, and whatever are

known.

Red blood cell transfusion avoidance however does not equate to avoidance of all allogeneic risk. And a delay in allogeneic transfusion without reduction and use of allogeneic red blood cells would not be considered a clinical benefit.

In trauma, some general considerations include -- include the following. Evaluation of clinical outcomes is quite difficult, because of the uncontrolled conditions, variations of the site and extent of injuries, the duration of hypertension, hypoperfusion and hypothermia, and the time interval between injury and access to definitive care.

There are issues related to the difficulties in classifications of trauma severity and the methods for assessing total body oxygen debt to improve evaluation of shock severity, and the success of resuscitation are not currently available.

FDA has said that mortality is an unambiguous endpoint, that's true. And long-term survival, what the good quality of life is the clinical benefit of interest to the patient and the patient's family. But 30-day

mortality is not a sensitive measure of the impact of an oxygen therapeutic agent used for early resuscitation.

And present information is insufficient to correlate short-term survival with long-term survival for oxygen therapeutics for a number of reasons. Again, inadequate classifications of trauma severity, the methods for assessing total body oxygen debt to better evaluate shock severity in the success of resuscitation are not currently available, a kind of a circular problem here.

Let's talk a little bit about blood not available, appropriate or acceptable, general considerations. I think it's -- people would agree that it's difficult to devise a single clinical trial that would address all of the situations where blood might not available, or appropriate or acceptable. There is a diversity of clinical situations.

For example, transfusion of avoidance versus other intended uses, when one talks about blood incompatibility in hemolysis, that's not the same as religious objection. The urgency of need is difficult to define and the medical versus the surgical situations would need to be defined.

With these considerations in mind, FDA has suggested that studies in both remote field trauma and elective surgery are needed in order to understand adequately the benefits and risks of oxygen therapeutics in the broadest spectrum of transfusion situations where such products might be used.

However, even that approach does not address the benefit to risk ratio of use in certain settings. For example, there is theme here, ischemia, cardiac, CNS, or other radiations sensitization, or hemodynamic stabilization and taking advantage of the pressor effect.

Studies in both remote field trauma and elective surgery also might not answer fully the question of whether an oxygen therapeutic is as safe as red blood cells in a setting where both are available and the patient is not clinically stable. And the decision whether to use an oxygen therapeutic await the brief time until allogeneic blood is available might actually be quite difficult.

So let's talk a little bit about safety. This is the topic of most of the meeting today. Clinical evaluation of safety, efforts to ensure the quality and

completeness of the safety database should be comparable to those made to support efficacy. And this can -- maybe found, this citation may be found in the guidance for industry on pre-marketing risk assessment.

Evaluation of hemoglobin-based oxygen carriers in diverse populations with the wide variety of comorbid conditions -- you heard Dr. Biro has talked this morning, and so the -- this should be fairly self-evident that studying at a variety of comorbid conditions is important.

And the study plans should be designed to capture new or novel adverse events, and changes in the frequency and severity are the mild, moderate, and severe of adverse events of both the background rate or intensity of those events. And there should be pre-specified stopping rules.

In general, there've been a number of toxicities noted for hemoglobin-based oxygen carriers to include, as you've heard earlier, cardiac toxicity with degenerative lesion seen in the left ventricular myocardium in susceptible species such as swine or monkey.

We don't know what the relevance of this is to humans. Myocardial ischemia has been observed clinically.

Vasoactivity of the product -- many of these products or most of these products are vasoactive, which probably related, at least in part, to the scavenging of nitric oxide by hemoglobin.

Gastrointestinal effects have been noted to include discomfort, nausea, vomiting, diarrhea, dysphagia, generalized abdominal pain, and there is experimental evidence of enhancement of bacterial translocation across gut epithelium.

These products have proinflammatory activity including procoagulant activity and DIC, and release of procoagulant (inaudible) by simulating leukocytes experimentally. Oxidative stress is a consideration as you heard from Dr. Alayash's talk. Many of these products have been associated with elevations of pancreatic and liver enzymes. And there may be an adverse synergy of free hemoglobin with bacterial endotoxin, and finally neurotoxicity has been raised as a safety concern.

I'm going to show some slides here of eight commercial products. Data are available in the public domain for six.

FDA reviewed these data, which were obtained

from peer-reviewed publications, press releases, and testimony presented at the December 2006 Blood Products Advisory Committee meeting. There are a lot of caveats. For each product, data are presented aggregated from all reviewed studies.

This is not by any stretch of the imagination meta-analysis. Controls varied from study to study, and some of the studies I'm going to show you were not controlled. Not all clinical trials conducted with the reviewed products have been published.

Results presented here are not synonymous with line listings of the type that would be reported to FDA in a comprehensive final study report. And this leads to another set of caveats, aggregating information to derive a comprehensive list of adverse events may not give a completely accurate tally of all adverse events that occurred.

Now those of us who did this work made every effort not to count a subject more than once for each category of event which will be represented by a table row. It is possible though that subjects may have been counted more than once because of the reporting methods

used in the publications.

In some instances, the number of subjects was back calculated from reported percentages. In these instances, the denominator was assumed to be the number of subjects in each cohort -- that assumption may not be correct. Not all enzyme elevations were captured as adverse events. And the number of subjects with enzyme elevations into clinically significant ranges was not captured uniformly or was not reported at all.

In some instances, only means and standard deviations, not the number of subjects contributing to the data set, were captured. Now let's take a look at some of these.

Here are the eight companies, Apex -- they are in alphabetical order, so nobody is up for particular description -- Apex, Baxter, Biopure, Enzon, Hemosol, Northfield, Sangart and Somatogen.

Two of the companies did not report anything in the public domain. Those are Apex and Enzon. And I believe that you'll be hearing from representatives after this talk. Large studies -- large numbers of subjects are included in the Baxter, Biopure, Hemosol, and Northfield

databases.

The number of deaths -- there is an imbalance in the number of deaths, with the exception of Hemosol, in for Baxter, Biopure, Northfield, and Sangart, Sangart reporting two deaths versus zero. Hypertension is a fairly -- is a common feature among these products for those that have reported it, and there is an imbalance for Baxter, Biopure, Hemosol, Sangart, and Somatogen.

Of importance cardiac events, yesterday there was publication to discuss myocardial infarction. There is an imbalance for Baxter, Biopure, Hemosol, Northfield, and Sangart. And then there is a -- there are imbalances in terms of cardiac arrhythmias for the same companies.

So you see that there are some cardiac events of importance and there is an imbalance in deaths. Now we also took a look at pancreas and liver. And as I remind -- I'd like to remind you that not all of the numbers were captured here, Baxter reported a number of cases of frank pancreatitis including hemorrhagic pancreatitis.

There is a small imbalance for Biopure and only one case of pancreatitis was reported in literature for Hemosol. There are excursions in lipase and amylase for

these companies, and in some cases these were reported as pancreatic enzyme elevations.

And then a number of these companies have showed changes in the AST or ALT or other liver function tests as you see here. This captures all of the other adverse events to include CNS, respiratory, renal, GI, coagulation, and sepsis and septic shock. What I would like to point out to you is that there is an imbalance in terms of CVA for this company, and a smaller number reported in the literature for Hemosol.

There are imbalances for pneumonia, for respiratory failure, hypoxia and cyanosis, a large imbalance for gastrointestinal events. This category of coagulation defect includes the citation of thrombocytopenia, but also the general category of coagulation defect.

And there is an imbalance again for those that have reported these events. I'd like to bring to your attention, this last one, sepsis, septic shock, multiple organ failure, to show you that there are some imbalances in the literature in terms of this endpoint including Northfield over here, and I'm sure that Dr. Gould will be

discussing this later.

So this is a more comprehensive view of the overall safety database for -- that's in the literature. There are some unresolved issues that I'd like to bring to people's attention. We've already eluded to them, the role of public versus proprietary research. There is an urgent need here for better scientific understanding of the chemistry, the redox biology and the pathophysiology of acellular hemoglobins as you heard in the first session today.

Of particular importance is defining a clinical benefit, and once defining a clinical benefit assessing clinically meaningful, readily measurable efficacy endpoints. And I think that there is a critical need for developing predictive surrogate markers of efficacy; we don't have any right now. There is also a critical need to understand clinical safety in terms of dosing and maximum tolerated dose.

We need to define an acceptable benefit to risk profile for each clinical indication based on all of the above, both in studies where subjects are able to provide informed consent, and most particularly in studies where

informed consent cannot be obtained.

And finally, I think that there is a critical need for defining a logical, clinical development program for these products. And with that, I'd like to turn it over to Dr. Alving.

(Applause)

MS. ALVING: Thank you, Toby.

Our next speaker is going to be Dr. Sara Goldkind. And she is the senior bioethicist at the FDA and in the GCP program in the Office of the commissioner.

(Pause)

RISK-BENEFIT CONSIDERATIONS IN CLINICAL TRIALS IN THE CONTEXT OF 21 CFR 50.24 AND CFR 312

MS. GOLDKIND: Okay. Good morning.

I'd like to continue to build upon some of the points that Dr. Silverman began to address in her presentation and what I am specifically going to focus on are risk benefit considerations in trials.

And I was asked to focus my remarks on how do we understand risk benefit considerations related to our

regulatory dictates 312 and 50.24 which Toby just introduced, and I will go through those further.

But what I would like to do is to bring to your consciousness that while we'll be discussing risk benefit considerations within the context of our regulations, really what we're talking about are ethical concerns. We're talking about ethical considerations for the protection of human subjects, who'll be in these trials and that's really what's captured in the regulations.

And I'm going to present a framework, one of many good frameworks for the discussion of ethical research and the analysis of whether or not research is ethical.

This framework was established by doctors Emanuel, Wendler and Grady. And you have an article in your packets which discusses this in more detail. And Dr. Emanuel will be here tomorrow and will be discussing hemoglobin oxygen carriers more specifically within the context of some of these specific attributes.

I'm going to look at these attributes more generally so that I can give you a framework in which to think about risk benefit across our regulatory spectrum.

And I'm not going to discuss all of these attributes. I've highlighted favorable risk benefit ratio, because that's what I'm going to focus on.

But I am going to touch upon what we've described as an unmet need, which is listed as number one, social value is the way Doctors Emanuel, Wendler and Grady referred to it. But it's really the scientific and medical unmet need.

And I'm also going to discuss the interplay between unmet need, scientific validity, and the favor of how we understand risk benefit ratio, and how we see the risks, and what we think are reasonable risks in relation to the benefits within -- touching upon those two first attributes.

Now, Doctors Emanuel, Grady and Wendler added an additional attribute, which they called collaborative partnership, which they described in a different article, and that is not listed here. However, it is pertinent when we look at research that involves the exception from informed consent.

And I'll touch upon that very briefly later. So what are some important caveats to my talk and important

messages? One is that before we start to even think about risk benefit ratio, we have to first satisfy conditions of social value and scientific validity.

In other words, we have to convince ourselves if there is a compelling unmet need, and that the protocols that we are designing have intrinsic scientific validity, and can answer the questions that they are posing. And prior to even thinking about what are the risk benefit ratios, we have to be very clear on those first two points.

What are the associated risks may be significantly affected by the chosen study population, and you'll see this, I think, play out as I go through the talk. And can the risks be minimized by studying a less sick population?

So can you study a consenting population that's sustained trauma instead of studying a group of trauma patients who are not conscious? Will that have an effect on the scientific validity of the study, and will it have an effect on the generalizability of the results?

And of course as Toby mentioned, some of this relates to what is the indication for which the products

been studied, what's the intended use populations once it's out on the market?

This is just a schematic of what've I said, and we have begun talking about what kinds of information we can bring to the table to assess risks and benefits, and we look to very -- to different disparate bodies of information to inform us about the risks and benefits for those populations that we will have under study.

We look at preclinical animal models and whether or not, as we've heard discussed earlier by Dr. Biro, as those are translatable or not, we look at healthy human adults. If possible, we look at adults or children with a different disease or less severe presentation of the disease under study, if possible.

And this is of course true for most clinical research that we bring to bear a wide array of information to help us assess the risks and benefits. We may or may not be able to use some of this information to translate, under these circumstances we've already talked about, some limitations of the animal models, some potential complications with generalizability of information if you use a less sick population rather than the population

who'd be the intended use population.

So now looking at the regulations, what do they have to say about risks and benefits? Part 312 is our investigational new drug application regulations. And it has a variety of components to it; I'm going to only discuss a few aspects that relate to risks and benefits.

And -- its many requirements protect the safety and welfare of human subjects and they include that the scientific quality of the investigation and whether it can yield data capable of meeting statutory standards from marketing approval is essential.

Talks about the essential components of the scientific design, the protocol in relation to its stated objectives, which I mentioned earlier, sponsor and investigator responsibilities, safety reports and it requires compliance with parts 50 and 56, which are informed consent regulations in our IRB regulations, which I'll mention just in passing as we go. But I wanted you to see how the regulations interdigitate with each other for the protection of human subjects.

And 312.23, more specifically looking at the content and format of an investigation of new drug,

requires a brief summary of previous human experience with the drug. If the drug has been withdrawn from an investigation or marketing in any country for any reason related to safety or effectiveness, and any risks of particular severity or seriousness anticipated on the basis of the toxicological data in animals or prior studies in humans with the drug or related drugs which essentially reiterates the schematic that I showed earlier.

And another way to sort of get at what the regulation say about risks and benefits is to look at what are some of the reasons, and this is not an exhaustive list by far, for putting a study on clinical hold essentially stopping the study. And one reason would be because human subjects are or would be exposed to an unreasonable and significant risk of illness or injury.

And the plan or protocol for the investigation is clearly deficient in designed to meet its stated objectives. And part of what I think we're tasked with is to work out more clearly what we think are unreasonable and significant risks of HBOC products and when are they offset by compelling benefits.

So our regulations require informed consent in all but two situations, and I'll be discussing one today. And that is when you accept informed consent for the purposes of emergency research, the so-called 50.24 regulation.

And protocols involving the exception from informed consent have to be performed under both the regulations 50.24, which are -- which is a complex set of requirements, and also they have to comply with the regulations for either an investigational new drug or an investigational device, and I'm not going to talk about IDEs today since HBOCs fall under an IND application.

And the -- when a protocol is submitted, that harkens to a 50.24; it has to be labeled and identified as a protocol that includes subjects who are unable to consent. And such protocols have to satisfy the requirements of both 50.24 and the applicable IND or IDE regulations in order for FDA to grant that the exception from informed consent study can go forward.

And this is actually been the reason why the vast majority of protocols that have been submitted with the exception from informed consent have not gone forward.

We've had over 60 applications since 1996, and only about a third of them have proceeded forward and that is because they either have failed to meet the requirements of 50.24, the requirements of the investigational of new drug application.

And in addition, the reviewing IRB has to satisfy criteria for approval for research under the IRB regulations part 56. So there are three sets of regulations that would be applicable to studies done with the exception from informed consent.

Now what are some of the criteria that the IRB would have to satisfy if it looks at a study? I've only listed a few, the few that are pertinent to these remarks, and that is the IRB has to determine that the risks to the subjects are minimized and they really do look at the study design to assess that.

The risks to the subjects are reasonable in relation to anticipated benefits, and when the IRB makes that assessment, it considers only those risks and benefits that may result from the research as distinguished from risks and benefits of therapies that subjects would receive even if not participating in the

research.

In other words, it's looking at the research interventions specifically. And it looks at whether the selection of subjects is equitable among many other criteria. Now, as I mentioned earlier, 50.24, the exception from informed consent regulation is a complex regulation, it has component in its -- components in it that honor what I would call the principle of beneficence which is doing for the subjects who are enrolled.

And components in it which focus on, what I would call the principle of autonomy, which is honoring the self-determination of the subjects enrolled which may seem like an oxymoron because the subjects are unconscious.

But it really emphasizes trying to obtain informed consent if at all possible, trying to obtain informed consent from legally authorized representatives in doing a series of public disclosure and community consultation activities prior to the onset of the trial.

And those -- the community consultation activities, I would say, is where this collaborative partnership that doctors Emanuel, Grady and Wendler talk

about. In other words, there's a partnership between the researchers and the community. And how does the community understand the risks and benefits, what are they told to consider as part of understanding the protocol is significant in this situation.

So looking specifically though at issues related to benefits, 50.24 requires that the subjects be in a life-threatening situation that available treatments are unproven or unsatisfactory.

So these are very dire circumstances -- and that the evidence supports the prospect of direct benefit to the subjects. So these subjects are already based upon the fact that they are in a life-threatening situation and available treatments are unproven or unsatisfactory, are in very, very extreme conditions.

But yet because we accept informed consent, we ratchet up the requirement for proof of benefit. In other words, the prospect of direct benefit has to be supported prior to being able to allow these trials to go forward, and we'll talk now about what that means.

And then, it's worth noting that while FDA recommends data monitoring committees in a series of

different circumstances, this regulation actually requires the establishment of the DMC by the sponsor, and this is one more area where you can see that there is yet additional oversight to the evaluation of risks and benefits that of course occurs once the trial is in progress. But it occurs in an ongoing fashion.

So the prospect of direct benefit is supported by conceptual proof of concept in vitro assays, pre-clinical evidence, animal studies, clinical studies done in other settings or with other populations, other countries, and while this -- as I had mentioned before with my schematic is certainly applicable to all clinical research, this has to be very clearly articulated in these submissions.

And the risk benefit assessment for 50.24 requires that the risks are reasonable in relation to the benefits, and are evaluated in association with the medical condition, standard therapy if there is any, and the proposed intervention or activity.

And this needless to say, is a complex assessment that requires the experienced judgment in conjunction with rigorous scientific evidence.

Now just to be complete, there is one additional subpart to the IND regulations, which is not a frequently used subpart, but it also talks a bit and gives us a sense of what has come to fore, in terms of risks and benefits. So I wanted to include it here.

And this subpart relates to drugs that are intended to treat life-threatening and severely debilitating illnesses and it's a -- an interesting interplay, if you will, between the recognition that there is unmet need, which is certainly how 50.24 was established, after great public discussion about the unmet and scientifically unproven therapies that have been used in the emergency setting.

And here, this particular subpart was established because there is a recognition that in certain circumstances, certain limited circumstances, there might be the expedition of the development, evaluation, and marketing of new therapies intended to treat persons with life-threatening and severely debilitating illnesses, especially where no satisfactory alternative therapy exists.

Now this is for subjects who can provide

informed consent. But it's still placed to the issue of what is the unmet need, scientific validity, and the risk benefit evaluation. So the FDA is willing to exercise the broadest flexibility in applying the statutory standards while preserving appropriate guarantees for safety and effectiveness and also for human subject's protections.

And so it is -- in this subpart there is a definition of life-threatening and is severely debilitating. And its circumstances, in which the likelihood of death is high, unless the course of the disease is interrupted, and the disease or condition is potentially fatal where the endpoints of the clinical analysis is survival. And severely debilitating would mean that the disease or condition causes major irreversible morbidity.

So these are life-threatening and severely debilitating. And the procedures reflect the recognition that physicians and patients are generally willing to accept greater risks or side effects from products that treat life-threatening and severely debilitating illnesses when they know -- then they would normally accept from products that treat less serious illnesses and this comes

out of the regulation specifically.

It also recognizes that the benefits of the drug need to be evaluated in light of the severity of the disease being treated. And what this regulation does is it allows early consultation with FDA and reviewing officials and to reach -- to review and reach an agreement on the design of necessary preclinical and clinical studies.

So there is intense consultation early on with the FDA review divisions and there is a meeting at the end of Phase I to review and reach agreement on the design of Phase II, controlled clinical trials with the goal of that such testing will be adequate to provide sufficient safety and efficacy data to support a decision on its approvability for marketing.

So you're trying to expedite the course of the study, but again, without compromising safety and effectiveness data and without compromising human subjects' protections, given the extreme unmet need.

And FDA will consider whether the benefits of the drug outweigh the known and potential risks of the drug and the need to answer remaining questions about

risks of the drug taking into consideration the severity of the disease and the absence of satisfactory alternative therapy.

So in essence, what I try to do in these remarks is to, number one, show that ethics undergirds our regulations and an understanding of risk-benefit assessment is a component of our regulations and that an ethical understanding of a protocol interdigitates with both the scientific knowledge that's available as well as unmet need.

Thank you.

(Applause)

PRESENTATIONS FROM INDUSTRY: PROPOSED CLINICAL INDICATIONS FOR HBOCS AND CLINICAL TRIAL EXPERIENCE TO DATE

MS. ALVING: Thank you very much for a very clear overview. We're now going to begin presentations from industry and discuss the proposed clinical indications for hemoglobin-based oxygen carriers and clinical trial experience to date.

We will have two presentations, I think, and

then break for lunch, and I would like to ask each of you to write any questions that you have on cards. We will collect that set of cards before lunch, and then for the other set of speakers we'll collect more during the afternoon and before the break, and then all of us will have a panel discussion and that way you can sort of remember what's going on.

So please feel free to write your questions and then just before we break for lunch we'll find out those individuals who will collect the cards and keep them.

So Dr. Keipert will you please -- you're the first one up to the plate here, Dr. Keipert. Dr. Keipert is going to be representing Sangart and he will discuss the development of hemospan, and Dr. Keipert also is -- had a former history in the military. I think he worked with Dr. Bob Winslow at Letterman, then you came to Alliance and he is now the vice-president of clinical and regulatory affairs at Sangart.

Thank you.

MR. KEIPERT: Thank you.

DEVELOPMENT OF HEMOSPAN

MR. KEIPERT: Well, obviously you were all expecting Dr. Winslow to be here and unfortunately he had an urgent medical, personal issue that he had to take care of which prevented him from coming. So, he entrusted his slides to me and I will try to do my best to convey the message that he would have wanted to get across at this meeting.

So there are three main points I think that we want to get across this morning. The first one is a very fundamental one and that's that all HBOCs are not the same. There are very unique properties to some of these solutions and it is important to keep this in mind when we look at evaluating, you know, results across multiple studies.

Second of all, over the years, starting off with a lot of search at UCSD and academia and previous to that research in the military, there has been a history of using the knowledge that has been acquired so far to rationally develop the physical and chemical properties of hemospan, to try to address some of the issues and limitations that have plagued some of the early first-

generation hemoglobin products.

And then I think the third message that is really important that Sangart has been stressing is that demonstrating safety both pre-clinically and more importantly in the clinic is of utmost importance and that's the first priority and that efficacy then and the other broader clinical indications are secondary to that.

So, let's talk a little bit about HBOCs and how they are different, of course you're all familiar with the very early work, the first-generation products were simply cell-free hemoglobin solutions starting from hemolysate which were then purified to remove the stromal lipids.

Second-generation products, then were addressing the issue of excretion, rapid renal excretion of these unmodified hemoglobins, so chemical modification was used to prevent dimerization of the hemoglobin tetramer in an alternative approach to prolong circulating time was to polymerize these hemoglobins into macromolecules using a variety of different crosslinking agents.

Roughly, grouped here under a third-generation heading would be the more homogenous products that have been developed commercially by companies in recent years.

These have been purified and produced in general under GLP conditions and the design was really to look at why were some of these early solutions vasoactive and one of the products, which has a lot of history of course is the alpha alpha-hemoglobin here that was developed by the Army and then also subsequently developed by Baxter.

Then finally, I think the work at Sangart has sort of evolved into what we're now loosely calling a fourth-generation product based on all of the knowledge about vasoconstriction that has been developed. I think Sangart and Dr. Winslow, and it was mentioned already this morning in the introductory talks, have come up with sort of a new theory of oxygen transport and how this may impact vasoactivity, and then design the molecule knowing what we now know about physiology of oxygen transport and vasoactivity.

So just a brief refresher course in terms of physiology, I'm going to present some data here comparing alpha alpha-hemoglobin -- this an alpha alpha-hemoglobin prepared by Sangart using their GLP/GMP manufacturing facility -- compared to hemospan in the rat model. But it is important just to remember the basic physiology here

for vascular resistance, which is pressure divided by flow.

And as a general statement, these terms are often intermittently tossed around and I think it's important to point out that if there is vasoconstriction, the vessels constrict in diameter, typically you will see an increase in mean arterial pressure. However, there are other ways that pressure can increase, for example, if there is enhanced fluid, if there is fluid loading, which doesn't necessarily imply that the vessel diameter has changed.

So let me just show you the experiments that were done. This is the standard rat hemodilution model looking at baseline conditions. This is mean arterial pressure. Here we do a 50 percent exchange transfusion with hemospan compared to alpha alpha-hemoglobin and you can see here the typical rise in blood pressure.

You will note there is a slight increase in pressure that we see with hemodilution with hemospan. However, when we then go further and look at cardiac output -- this was an important parameter that was also mentioned this morning -- we can see that in the case of

the hemospan animals there is actually an increase in cardiac output.

We have never seen a decrease in cardiac output contrary to what was mentioned in the FDA article for this workshop, but with alpha alpha-hemoglobin here you do see a decrease in cardiac output.

And then when you calculate systemic vascular resistance, then you see the biggest differential where with hemospan you see a very slight decrease, but more importantly you see the traditional significant increase in systemic vascular resistance.

Now, is hemoglobin concentration itself an indicator of oxygen delivery? So here, you have a standard oxygen delivery plot here, there is hemoglobin concentration. So if you start off at a normal systemic hemoglobin of about 14 or 15, here is your normal delivery of oxygen when you have a normal resting animal with the normal cardiac output.

If you now do a progressive hemodilution down to very low levels, you remove about 50 percent of the red-cell mass and if you do this with a variety of colloids these are non-oxygen carrying colloids -- voluven is a

hetastarch, 5 percent albumin or pentastarch -- you can see this grouping of data here showing the reduction in DO2 as you would expect because of the reduction in hemoglobin concentration. And you can draw sort of a general fit through those points.

If we now compare in the same experimental model a variety of different hemoglobin solutions, here is first-generation unmodified stroma-free hemoglobin, here is the alpha alpha-hemoglobin, and here is a gluderaldehyde polymerized hemoglobin preparation. Again all of these prepared under the GMP conditions at Sangart.

And you can see that even though the hemoglobin concentration has been increased so there is plasma hemoglobin present in terms of the delivery of oxygen it falls below the line -- and it says if these hemoglobins were delivering at much lower hemoglobin levels. And when we then compare hemospan, we find in the same model that if falls directly on this curve where the predicted hemoglobin level gives you the predicted level of delivery of oxygen.

Similarly, in the same type of model we have looked at the lactate concentrations, vis-à-vis

vasoconstriction to see if those correlate. So, on the X-axis you have systemic vascular resistance and here you have lactate concentration. Once again the grouping of colloids used to hemodilute the animals -- you can see here lactate levels and low vascular resistance.

As we now look at the various hemoglobin solutions, stroma free hemoglobin, the alpha alpha and the polymerized, you can see how you're starting to create this curve going up here or vascular resistance progressively increases as you do -- as you use these earlier generation products.

When the same experiment is done with hemospan, once again it behaves much more like a colloid, it comes into the line at the predicted place where you would expect it to and you do not see the increase in lactate.

So are these deleterious effects of some of the HBOCs as a result of nitric oxide scavenging? Then there is going to be a lot of talk at this workshop on nitric oxide.

Unfortunately, the literature is not completely consistent, some early work or some work in the 1998 was published from the group with Intaglietta and at Sangart

looked at a variety of different hemoglobin solutions that have a different nitric oxide binding, and they were able to show that even though they all bind nitric oxide to some degree there is not a consistent increase in blood pressure or hypertension reported.

However, Olson's group working with a group at Somatogen and this slide was also presented earlier today; they were able to show that there is a correlation with vasoactivity and oxygen affinity and nitric oxide binding.

So the literature is not entirely clear on this, unfortunately, neither of these early studies included a full analysis of the vasoconstriction or the hemodynamics, because they did not evaluate cardiac output.

So is there an alternative to nitric oxide scavenging to try to explain vasoconstriction? Well, going back to sort of basic physiology principles, oxygen supply needs equal oxygen demand in a balanced physiological system and this has been published many years ago by several authors.

It has been known for quite a while that excessive amounts of oxygen can be toxic because of generation of oxygen radicals or nitric oxide degradation,

and oxygen oversupply causes protective vasoconstriction. The phenomenon of cerebral vasoconstriction in response to hyperoxia has been well known for many years.

Also the plasma, as was mentioned earlier today presents a diffusion barrier for oxygen diffusing from the red cells through the plasma space into the tissues.

Well this gets dramatically altered. This entire scenario gets dramatically changed when you put a cell-free hemoglobin as an oxygen carrier into the plasma space, because you now augment the oxygen availability in the plasma through a process that's been described as facilitated diffusion and this has been worked on extensively early on by Wittenberg and Scholander and other authors.

So how much plasma oxygen does the hemoglobin actually carry? If you compare the plasma dissolved oxygen, of course, under normal air breathing conditions, solubility of oxygen and plasma is exceedingly low. So, it is typically ignored in most calculations and only -- people only worry about what the red cell carries.

However, the minute you put cell-free hemoglobin into the plasma compartment, here you can see that with

only a plasma concentration of 1.4 grams per deciliter of cell-free hemoglobin that moves you up the curve and becomes equivalent to having that same animal or subject breathing high levels of inspired oxygen and having an arterial PaO2 of about 600 torr.

So this is quite a tremendous impact by putting this cell-free hemoglobin in the plasma compartment.

So is oxygen itself hemodynamically active? This is another experiment. These are recent data that have not been published yet. This is in an awake rat, these are fully instrumented rats, but they're conscious, they are restrained, they are allowed to sit and have a stable baseline and then the only thing we do at this point is we change from room air to 100 percent oxygen into the breathing apparatus that the animal is breathing from.

And here you can already see an increase in systemic blood pressure, an immediate fall in cardiac output, and then if we look at vascular resistance you can see an increase in vascular resistance. This is simply the only perturbation if the animal is switching from breathing room air to breathing oxygen.

And if you look at these curves, so the people who have done work in animals, in rats with hemoglobin solutions, this is very similar to what has been seen with many of the early generation HBOC products.

So this lead Dr. Winslow working with Kim Vandegriff, Marcos Intaglietta at UCSD over several years looking at all of this data to come up with this so called autoregulatory theory to design hemoglobin molecule that will potentially avoid this problem.

One feature of the hemospan is that it is a larger molecule to decrease the diffusion, it provides more physiological viscosity, and Dr. Intaglietta's lab has shown recently how viscosity is becoming a more and more important feature, but maintaining a patent microcirculation and by being a large molecule potentially also minimizes any extravasation.

The hemoglobin in contrast in many of the earlier products also has a high oxygen affinity, has a low P50, which may seem a little counterintuitive since blood has a P50 of about 25, 26 torr. But by doing this we have to remember this oxygen is being released in the plasma compartment, the HBOC is in the plasma space.

So, by having a high oxygen affinity it limits how much oxygen is going to be released in the large arterials and then hold on to that oxygen until it gets down into the microcirculation, so it can be released at the capillary level where it is needed. And by having a higher oxidation this also tends to protect the hemoglobin molecule from autooxidation.

Finally, the preparation the way it is formulated has a lower concentration. It is approximately 4 grams per deciliter. This lower concentration will also help to reduce toxicity and also lower cost.

And finally, hemospan has an elevated oncotic pressure. Part of this is due to the fact that it has a pegalation shell (phonetic) around it, but that provides very good plasma expansion and it's actually quite a potent volume expander.

So, all of these properties have gone into the rational design of this new molecule. So now I'll talk a little bit about the development of hemospan. This is a pictorial representation of what the molecule looks like. Here is the hemoglobin tetramer with the four heme groups and then with very specific maleimide linking chemistry

there are six or seven of these polyethylene glycol chains.

These are 5,000 molecular weight PEG molecules that are very specifically attached to these residues on the surface. In a very recent work from Sweden, using small angel X-ray diffraction or scattering, has shown this model of what the molecule actually looks like where the hemoglobin tetramer is sort of buried in the center here, and these blue dots represent space-chilling regions for the PEG. You can see that the PEG has wrapped itself around the hemoglobin with some clustering at each end and then these white dots represent the water molecule that is held in that PEG chain. So it's encapsulated in this shell of polyethylene glycol with water molecules.

The preparation is exceedingly pure; here is a size exclusion chromatogram. Here you can see the unmodified hemoglobin and the pure hemoglobin peak and then after chemical modification and the PEGalation you can see here a very homogenous peak separated from the unmodified hemoglobin.

As I mentioned it has a very low P50, it's a high affinity molecule by design. Has a P50 of

approximately 5 or 6 torr so it's a very left-shifted curve that of course fully saturates in the lung. Some other key properties, I mentioned the concentration, its currently formulated at about 4.3 grams per deciliter. It's formulated in standard Ringer -- in a standard Ringer's lactate type electrolyte solution. P50 is approximately 5 to 6 torr and the half life in humans now from a variety of studies is dose dependant and depending on the dose, ranges from about 20 to 24 hours, in surgical patients up to approximately 36 hours in volunteers.

This graph -- this data is a study that was presented -- that was published two years ago by Amy Tsai in Dr. Intaglietta's lab in blood, and it's a very unique experiment because it enables to measure -- make actual direct measurements of very vascular nitric oxides, so these are actual concentration measurements of nitric oxide made just around a blood vessel where the blood vessel diameter is also being measured. This is in the microcirculation window chamber model that Dr. Intaglietta lab has developed.

So here you can see under normal resting conditions, here is your periovascular nitric oxide and

then here is your arterial diameter. If we give L-NAME -- sorry this is somewhat blocked here -- this is L-NAME, you can see that as you would expect there is an increase here, hypertension and vasoactivity occurs.

If we now look at some hemoglobin solutions this is the typical alpha alpha-hemoglobin solution, this is the polymerized hemoglobin solutions. You can see the behavior is similarly that you have an increase in pressure. This is mean arterial pressure going to the right; you see an increase in pressure coincident with a decrease in the actual diameter of the arterials.

However, when we measure hemospan, you can see that despite the fact that hemospan can bind nitric oxide, and so the binding is shown by the drop from the line, it binds nitric oxide, we do see a slight increase in blood pressure because of the volume expanding properties of this material, but here you can see that in opposition to these hemoglobin solutions the blood vessels are actually slightly dilated.

So we do not see vasoconstriction despite the fact that because of the oncotic properties we do see an increase in pressure. So other physiological properties

and/or in summary these properties show that the high oxygen affinity reduces release of oxygen in the resistance of arteriolar vessels and thereby prevents vasoconstriction.

The facilitated plasma oxygen diffusion targets this oxygen delivery to the hypoxic or ischemic tissue which is really where you want that oxygen to go. Looking in the microcirculation, we have been able to show that we preserve functional capillary density. This is the definition used to define those capillaries that still are patent and have red cells flowing through them. In a variety of models of extreme hemodilution and anemia or hemorrhagic shock there is no decrease in cardiac output seen in these models both in anemia and in shock. So then coming to the final section of the talk, safety is first, efficacy is secondary.

Clinical trial design, trauma is clearly a very exciting indication that the whole world views these products to be ideally suited for. Sangart has done a lot of work in swine models of hemorrhagic shock and the data are very dramatic. We always see improvement in acid-base balance and resuscitation in these animals, thereby, our

chemistry is much improved. But it is a very heterogeneous patient population, Baxter and Northfield have ventured into this our patient population for clinical trials and have obviously encountered, you know, challenges in doing that not the least of which we just heard about is the problem with informed consent.

The second big indication that this field has been talking about for years is of course the blood substitution idea to randomize patients when they have reached some kind of a predefined transfusion trigger. Public health would suggest that this might be the greatest use in the future of these products to try to avoid transfusion.

Possibly these products may be shown to be safer and more acceptable than blood. This of course remains to be proven in pivotal studies.

The trouble in this indication is there are no agreed upon or even ethically possible ways to strictly define transfusion triggers and a lot of the transfusion literature is plagued with this problem where you try to define triggers and of course they are never agreed upon. The clinicians tend to transfuse based on a whole gestalt

of what this patient has presenting to them. Age, underlying morbidity, how much blood loss has occurred, what's the patient's hemoglobin, are they a smoker, et cetera.

So Sangart's approach has been to focus on albeit perhaps a more defined and specific indication but to look at a specific physiological parameter in a clinical trial setting where we believe the results will be much more interpretable. Possibly, this will mean that there would be a more limited efficacy signal, but I think with the history in this field, it's more important to demonstrate a good safety profile and a well-defined clinical study and not worry about the scope of the potential clinical indication that you're initially targeting.

I'm just going to list the clinical studies that Sangart has performed. Of course the early first and ninth study was done at the Karolinska in Sweden. This was followed up by a dose escalation study, a 1b/2 study in 20 orthopedic surgery patients was also done is Sweden. Both of these studies have been completed and both have been published in the literature.

The third study, this was the first multi-center study done in six hospitals in Sweden in arthroplasty patients, hip and knee arthroplasty and fracture and a total of 90 patients were randomized.

This column is listing only the actual hemospan treated patients. So we haven't listed the controls here for these studies. This study is also complete and has been published. A study in the U.S. and radical prostatectomy patients has just recently completed enrolment so it's a small Phase II study and 20 subjects, total. We also did a pharmacokinetic study in Sweden to compare the pharmacokinetic profile of two different vendors of PEG, two different formulations. This study recently completed enrolment.

We're doing a pilot study in Sweden in patients with chronic critical limb ischemia looking at blood flow and tissue PO2 in the ischemic foot. This study is underway and three of eight treated patients have been completed, and then we come to our two Phase III studies. These are multi-center pivotal Phase III studies being done in Europe.

One of these studies completed enrolment last

month and the second study we anticipate will complete enrolment probably next month. So I'll tell -- I'll just say a few words about the Phase II study, this was published in anesthesiology two years ago in 2006. This study was initiated in 2005, and was published about a year later after it was completed.

So in terms of efficacy, the easiest way to view this in terms of timing during surgery as to look at the fraction of patients that remained normotensive. The primary endpoint in this study was to look at the avoidance of hypotension, to prevent from occurring. So the first dose was administered here just prior to spinal anesthesia, patients underwent spinal anesthesia and then the surgical repair, and then here you can see how in the control group approximately 80 percent or more than 80 percent of these patients eventually exhibited at least one episode of hypotension.

When we look at the two groups treated with hemospan, we had two doses either one unit or two units of hemospan. You can see early on right after spinal anesthesia, the pharmacological effect of the anesthetic is similar in all groups, but then remarkably when surgery

starts and you started to have surgical bleeding, the hemospan groups then are statistically different from the control group where you have very stable hemodynamics and you don't see the hypotension that you see in the controlled group.

There were three serious adverse events. You can see here they all happen to occur in the treatment groups. One was an 84-year-old male; he was undergoing a second time, a revision arthroplasty. After surgery he wasn't doing very well and ended up with a massive aspiration. Upon autopsy, they discovered that he had a massive incarcerated inguinal hernia, which was probably the reason for his lower-quadrant pain when he came to the hospital in the first place.

Second patient was an 89-year-old female. She was hypertensive, had very high blood pressures in surgery and immediately post-op-surgery already at baseline; probably was even above and beyond the limit that had been set by the protocol, but nevertheless, was allowed in the study. She came in for an acute fracture repair.

After surgery, because of the high blood pressure, they had a variety of cardiology consults, and

one cardiologist then decided to give her intravenous clonidine to lower the pressure. It dropped her systolic pressure from 240 down to 80 and the Holter monitoring then showed that she infarcted at that point and later on had a second infarct, which then led to her death on postoperative day 9.

Finally, the only patient in this study that was in the primary hip patient population, which is what is being done in Phase III. This patient was actually completely asymptomatic. The event is listed as an MI. It was found only because we were collecting troponin measurements on these patients. The lab data came back with the troponin being elevated, but the patient had no symptoms. The EKG was completely normal. There were no CK-MB changes. The patient was then sent for cardiology consult and, you know, has done very well and eventually ended up getting treated. But the patient had actually not disclosed her cardiac history in order to get into the trial to get her hip done.

So these were the only three SAEs in these patient population and they were all deemed to be unlikely related to treatment.

Now this is one new slide which is not in your handout and I have asked the organizers to print out a new set of handouts which will be available tomorrow because I have made some very minor changes to a few slides and I have added this one. And the reason for adding this slide is because of the recent med analysis that was just published, which unfortunately, incorrectly reported the results from this Phase II study by focusing only on the primary hip population, which was 74 of the 90 patients total in that study.

So I'll show you the correct data here. If we look at all patients in the study, there were two deaths as I just showed you on the SAE table. There were 2 out of 59 deaths in the treatment group versus 0 out of 31, both of these patients or one of these patients had an MI, the other one was massive aspiration due to the incarcerated bowel.

Unfortunately, in the med analysis this was listed as 2 out of 46 and 0 out of 28, because they use the denominator from the primary hip sub-population. This study has also been published in the literature, so I thought I would add this in as well, 30 patients, there

were no deaths and no MIs in this patient population and I can share with you although this is not published yet, but the study -- the data has been collected in the 20 patients done. In the prostatectomy trial, there were also no events. So if we now add up all of the Phase II data that we have to date, we end up with 2 out of 89 patients that died versus 0 out of 51 in the controls, and of course, we have the two MIs again versus zero. So I just wanted to put that up to correct that mistake.

Finally, a few words to talk about the European Phase III program.

Sangart went to Europe and got scientific advice both at the national level from Sweden and in the U.K. This is typically considered informal scientific advice and then we also took it one step further and went to the scientific advisory working party of the EMEA to get formal scientific advice on the design and the end points for these Phase III trials.

So all of this was discussed with Quintile's, our CRO and with the EMEA and overall, this design was very similar to what had been done in that multi-center Phase II trial in Sweden. The main change is that rather

than going against a crystalloid control we now decided to use the standard colloid which is typically used in Europe for volume replacement which is the low-molecular-weight hetastarch known as voluven.

The proposed indication based on this Phase III program is to try to get hemospan indicated as oxygen carrying plasma expander to prevent and treat hypovolemia in patients undergoing hip arthroplasty with spinal anesthesia, so very analogous to what we did in the Phase II.

The two studies in total will enroll 830 patients. The EMEA recommended that we run two complimentary trials because we're asking for prevention and treatment, they wanted us to evaluate these separately in two different trials. So one is a prevention trial where the first unit of investigational product is given at induction of spinal anesthesia just like it was done in the Phase II.

The second unit then is given at a lower blood pressure trigger, but not all the way down to the defined level of hypotension, because we're trying to prevent the patient from getting to that level of hypotension.

Subsequently, after they get two units, the clinicians introvert the standard of care. They can give vasopressors, they can give volume or blood depending on what the patient needs.

The second trial is a treatment protocol. Here, we actually wait until the patient achieves the defined level of hypotension. The regulators insisted that if you want to treat something you have to actually allow the even to occur first and then you have to treat it to show that you can reverse the event and then show for how long you can keep it reversed.

So here the first unit is given at the first trigger. If the trigger occurs again, the second unit is given and then subsequently it's standard of care. Both trials are very complementary because they all have identical data collection, all safety parameters, all lab data is identical in both. The only difference here are the actual triggers when the dosing occurs. These are multi-center studies.

We have 39 clinical sites that have contributed to these trials. You can see here the six countries. Of these 39 sites, 8 of them are sites that have actually

been able to contribute to both protocols. These were sites that reached the maximum enrollment allowed within the given study for a single site and then they were rolled over to contribute into the other protocol. So we have 36 unique hospitals, a total of 39 sites.

We also have an independent data safety monitoring board for this study. The first meeting was held in October when they reviewed data from one-third of the patients enrolled in both trials. They recommended that there were no safety imbalances and that we should proceed. The second meeting was held after two-thirds of the patients were enrolled. This was held in February of 2008.

I would like to point out that the DSMB is comprised of two senior anesthesiologists and a cardiologist. The reason we've put a cardiologist on the board is because of the history in the field and the concern about cardiac events. These Phase III trials are being run with not only 12 EDCG monitoring, but also 24-hour Holter monitoring.

So we have a lot of ECG data and a lot of Holter data, so we had a cardiologist on the board as well. The

609E the larger treatment protocol, we just completed enrolment in March of last year and the prevention trial we expect to complete enrolment most likely next month.

So with just some potential applications here, of course restitution of tissue profusion shock is one area that is very exciting based on all the preclinical work. Maintenance of hemodynamic stability is the indication that Sangart has selected as a more achievable goal so that we can demonstrate safety in a more controlled patient population as a first indication. Temporary oxygen transport when blood is not available, unsafe, or not accepted. This of course was the original hold the Grail that this field was going after, but because of the safety of blood showing that your product is at least as safe as blood has become a really significant regulatory hurdle.

And then finally, companies are moving into this arena looking at targeting oxygen transport to specific organs or tissues and of course you have sickle cell, heart, brain, liver, gut, a variety of indications you can go after. Potential limitations of hemospan because it has a lower hemoglobin concentration, this would limit its

use in extreme cases of anemia, and likewise because of the elevated oncotic pressure, you know, dosing and volume administered has to be done carefully to avoid the potential for volume overload.

So finally in conclusion, I would just like to restate again that all HBOCs have significant differences. Oxygen supply to the resistance vessels must be controlled to prevent vasoconstriction.

Interaction with nitric oxide does not completely explain the physiology and the vasoactivity although it certainly a component and safe and effective oxygenation of that risk tissue, we believe, is an attainable goal.

Thank you very much.

(Applause)

MS. ALVING: Thank you very much, Dr. Keipert.

Our next speaker is going to be Dr. Steven Gould and Dr. Gould has been working in this area as part of Northfield Laboratories of which he founded I believe in 1993, and he is currently the chairman and CEO of Northfield.

So we're going to hear today about the clinical

development of polyheme.

Thank you so much.

MR. GOULD: Thank you, thank you, Dr. Alving.

CLINICAL DEVELOPMENT OF POLYHEME

MR. GOULD: Good morning everyone. It's a pleasure to be here. I'll get you out time for lunch. I'm sure we'll have a delicious meal upstairs.

My slide they're showing here, they are not up on the screen. Okay. Here we go. We'll have the lights down please. So I think this session was well set up by Dr. Silverman and Dr. Goldkind. I particularly enjoyed Dr. Silverman's comments talking about some of the complex issues related to particular clinical settings, and design, and execution of trials.

I'm going to take you through our approach over the years. From the outset we have focused on a single clinical setting, seeking the clinical indication and the treatment of life threatening hemoglobin levels when red blood cell, the standard care unavailable. This indication addresses a critical unmet medical need. Dr.

Goodman this morning really preempted this slide. I'll just run through it again. There are a variety of settings where blood is needed by the red blood cells and not available starting certainly in the pre-hospital setting either at the scene or during transport and then he covered most of these. We talked about blood shortages either in this country or on a global basis.

I want to set up the scenario -- what we mean by unavailability that's really shown on the slide. We're talking about patient at-risk-of dying due to life-threatening hemoglobin levels who need oxygen counter replacement when there is no available alternative.

And that does pose some unique challenges to the design, execution, and interpretation of these trials, and I really want to run through today how we have approached that during our development.

This is just a one quick slide for those who may not be familiar with what human polymerized hemoglobin or polyheme is, and there are a couple of unique characteristics for -- excuse me -- for us one unit of polyheme equals 50 grams of hemoglobin. Again if you're not familiar with that number that's the mass of

hemoglobin function delivered by a unit of donated blood cells.

So as a surgically based team from the outset, we wanted to provide continuity and not cause any confusion. It consists 500 ml of volume and a 500 gram per deciliter concentration, P50 is slightly rightward shifted due to pyridoxal-phosphate. The other unique characteristic is a tetramer, unmodified tetramer concentration of 1 percent or less.

I think Dr. Alayash, in talking about the various approaches to modification, I think made the point that everyone in this field a number of years ago came to the conclusion that unmodified tetramer is probably not the way to go. And our product has a short life in excess of one year. So let me give an overview of our clinical experience and then really review much of our data with you.

This slide lists all of our studies today. I want to make a comment here as ways we go through -- most of my slides are similar. I've amended a number of them and updated them since we submitted these about 10 days ago and the full presentation we posted on our website

later today if the people want everything, then I shall today.

So total of 1,133 patients, 674 of whom have received polyheme. I'm going to focus today on the acute blood loss and trauma studies starting with these three in the hospitalized trauma individuals which demonstrate the efficacy of polyheme and the treatment of profound blood loss at life threatening hemoglobin levels. And we're served as the basis for our larger randomized control trial starting at a pre-hospital setting.

So let me begin with this data, 171 patients represent all of the patients included in the three trials. On the prior slide, they were published in a single report in the Journal of the American College of Surgeons in 2002. So these were severely injured patients and informed consent was obtained from the patient or a family member.

They all sustained substantial blood loss and arrived at the hospital where they received routine care other when transfusion was indicated. They received polyheme in lieu of red cells with rapid infusion above the 20 units as necessary. The goal was to provide rapid

massive infusion since we felt that's the way that blood was to be used.

Efficacy was assessed based on hemoglobin concentrations and mortality, with a comparison, using a retrospective historical comparison to data in literature in patient with acute blood loss who refused transfusion due to their religious beliefs.

Now, just to walk you through this equation actually explains the protocol.

Normally, the total hemoglobin concentration in an individual is only the hemoglobin carried by the red blood cell that's all we have. When one adds any plasma hemoglobin, in this case polyheme, you have two components and they are added up, so the total is the sum of those. So the protocol is for patients to bleed red cells and not receive red cell transfusions.

That is possible because we are infusing an alternative form of hemoglobin namely the polyheme. And in an effort to maintain an adequate total concentration of hemoglobin, and as we go through the data it is possible to take a sample of the patient's blood after infusion, separate the red cells and the plasma, and make

very precise measurements as to what is being carried in each compartment.

So for those that may not be clinicians, these are the guidelines and the NIH consensus conference it is hard to believe that was 20 years ago in 1988, lot of us were here then. The key is to keep the total concentration in the seven to ten the therapeutically desirable range and again for those that may not be familiar, hemoglobins of 3 grams or less are almost uniformly fatal.

So let me start with the data. This slide shows the impact of infusing unified unit increments of polyheme on the plasma hemoglobin. So we have the hemoglobin concentration on the y-axis, the dose on the x-axis, and we see that essentially each unit raises the hemoglobin concentration approximately a gram which is the same increment that we see when one infuses a unit of red cells in a stable setting.

The plateau is somewhere around six. Remember, the concentration in the bag is ten, so this relates to equilibration and fluid shifts.

The next slide is an important one showing the

relationships of the total and the red cell hemoglobin. Once again we have the hemoglobin concentration on the y-axis and the dosing starting with the pre-infusion level on the x-axis. This green barrier is the seven to ten, the therapeutically desirable range. The dotted line is the critical 3 gram per deciliter level that would be considered life threatening.

At pre-infusion, the mean hemoglobin is 9 grams per deciliter. This is important clinically. It means that the surgeons at the time when they made the decision did a very good job. Transfusion or infusion was not started at the 15, it was not started at 5. The red line for the first time, I believe, represents the natural history of untreated blood loss, and that patients who bled and also required replacement of up to 20 units of blood did not receive red cells.

So we see that very quickly the red cell hemoglobin falls not only below the 7 but somewhere beyond 6 units. Fall below the 3 gram per deciliter level, clearly unacceptable. In contrast, the total hemoglobin is well maintained within the 7 or 10 therapeutically desirable range.

The mean red cell hemoglobin out here is 1.7. The mean total hemoglobin is 7.2. It is this delta that is the physiological increment provided by the polyheme. The next slide I want to show you the data on 40 individual patients you had red cell hemoglobin less than 3, again we have that -- sorry -- the concentration on the y-axis, and each bar here represents an individual patient. Once again the horizontal line is the 3 gram level. We've arranged in decreasing order the red blood cell hemoglobin for all 40 down to the lowest patient at 0.2.

And the lighter shade of red is the plasma, so that the height of the column represents the total hemoglobin. So in every case the total hemoglobin was not only above 3, above 5, and most of them above 6 and 7. Again this is most striking patient the total hemoglobin is 0.5.

Based on that one should not see mortality due to inadequate hemoglobin concentration, the data summarized here, the mortality based on the lowest red cell hemoglobin for the 171 patients in this study and the comparison I mentioned in the 300 historical patients from

Jeff Carson -- I know Jeff is in the audience.

Jeff took this data from his database and more than 2,000 patients with religious objection and it is the best available data in the literature where the lowest hemoglobin and the outcome was recorded on the initial basis.

So what we see is that mortality increases as hemoglobin falls in both groups of patients, which is what one would expect from the normal physiology. However, the rate of increase is very different, and in a logistic regression, analysis which is in the manuscript, we see that the difference becomes statistically significant at a hemoglobin of 5.2.

That number is important because Richard Weiskopf who is also among the audience that help us data showing that in young healthy men undergoing elective surgery cardiovascular compensation is adequate in a normovolemic state down to at least a hemoglobin of 5. So we think this is consistent with what's known about the physiology.

The most striking observations are in the 40 individuals who had red cell hemoglobin of 3 grams or less

-- if we look over here we see that virtually all of them died. There were only seven patients whoever get to 2 and none are alive if the hemoglobin is 1.

We see that there are 12 patients, nine of whom survive, 75 percent survival, and these are long-term 30-day survivals.

So these data allow us to observe that polyheme can be infused in bleeding patients in rapid massive infusion that the infusion can increase the hemoglobin concentration in the recipient like a unit of red cell transfusion; that during ongoing hemorrhage, polyheme can maintain an adequate hemoglobin concentration without the need for red cell transfusion; and the data showed that it can support survival at life threatening red blood cell hemoglobin levels and offer potential benefit when blood is unavailable.

So the data formed the basis for our U.S. multi-center trauma trial which has received considerable attention. I want to take you through all of the data now for both efficacy and safety for that trial. So this really was a landmark study. It's the first U.S. study to begin treatment starting in the field with HBOC. This was

a randomized control prospector trial. Importantly, this was open label. Open label to physician investigators, virtually all personnel involved, and a large number if not the majority of the patients as well.

It was conducted under reg 50.24 that we heard about earlier. Importantly, there were four interim safety analyses conducted with stopping rules by the IDMC and the study did go through the completion. So the purpose of the study was to assess the outcome of patients treated with polyheme versus the standard of care. A word about the logistics, the map shows the distribution of the 32 level on trauma centers around the country and while it's not the largest study ever done, we feel it was one of the most if not the most complex with literally a cast of 1,000 and we appreciate the efforts of all involved to complete the study.

So eligibility, again, our goal was to enroll severely injured patients who were bleeding and in shock. It is not as easy to do this in the field as it is sitting around a conference table, so the inclusion criteria will raise systolic blood pressure of 90 mm of Hg or less, that's the traditional textbook definition of shock. In

adult patients, excluding criteria were a neurological score, a GCS of 5 or less and patients that were considered "unsalvageable," it's a subjective call by the paramedics to avoid enrolling patients who would represent futile resuscitation or non-preventable deaths.

This is the study design, the time points are shown from the point of injury across the bottom so the paramedics arrived at the scene, assess the patient, the patient met the enrolment criteria, a single sealed envelope was opened, the patient was randomized to either controlled or polyheme.

Let me walk you through -- so for polyheme for the first time and HBOC was infused starting at the scene of injury that continued during transport, which in this case was relatively short -- I'm going to show you the numbers, it's a largely urban center -- and continued upon arrival at the hospital up to a dose of 6 units of polyheme or an interval of 12 hours following injury.

If the patient had the need for oxygen counter replacement beyond the 6 units they received stored red cells. After 12 hours, they received stored red cells. The standard of care -- and this was an important

distinction -- received crystalloid or salt water, starting at the scene and during transport. And upon arrival at the hospital if necessary they had immediate access to stored red cells. And after the 12 hours both groups are getting stored red cells. The interval or follow up in the study was 30 days.

So, I want to emphasize that the comparison here is polyheme versus crystalloid and red cells because of the circumstances of the trial and because the duration of follow up there is no way to separate out, and what some have considered as a trial of polyheme versus salt water. Really is polyheme versus standard of care, which includes red cell transfusion.

Based on that the primary efficacy endpoint was day 30 mortality with the unusual situation of dual primary endpoints not co-prime; and dual primary endpoints have superiority and non-inferiority.

Superiority is the usual parameter in randomized trials. Non-inferiority occurred again because of the control, early access to blood and the potential that was recognized at the outset that the observed benefit in this situation might not be as great as if patients had been

enrolled in situations of prolonged unavailability of red cells and a longer period to definitive care.

You had to balance the practical aspects of competing the trial with the reality. So the implications of just doing -- now just do a primary endpoint -- that's shown here.

Under a superiority outcome, polyheme would be able to be used in place of red cells. Non-inferiority is different. Unlike traditional non-inferiority trials where the test article is being evaluated to be used in place of the control, in this instance the understanding was that with a non-inferiority outcome, polyheme would not be used interchangeably with red cells.

It would be used when red cells were unavailable and the observed data would be used to extrapolate benefit to setting of true unavailability of red cells in patients with prolonged delay to availability of red cell therapy. So let me start to review the data, this shows the baseline characteristics. This is pretreatment.

This is at the time of enrolment for both groups. We see they are well matched for age, gender, mechanism of injury penetrating versus blunt, systolic

blood pressure, ISH which is an injury of severity score, and transport time.

Again as I mentioned, the transport times were relatively brief, 26 minute was the median in both groups. So the period before the control arm had access to blood was only 26 minutes.

Now, the bottom of the slide here points out a potential imbalance in the patients. These are the values for the prothrombin time, the partial thromboplastin time, and I have highlighted them, because there was a statistically significant difference in the baseline coagulation status with higher numbers in the polyheme arm.

That is important because the presence of a coagulopathy at the time that a trauma patient present is known to signify a poor outcome and increased likelihood of mortality. I'm going to report the result in two analyses populations that were pre-specified in both the protocol and the statistical analysis plan or SAP.

Of the 720, there were six patients who received no treatment whatsoever so they are excluded. The 714 is the total number of patients that were enrolled, treated,

and they are analyzed as randomized. This is the primary analysis population.

The second population I'm going to review is the "per protocol." The 590 patients that were appropriately enrolled and treated according to the protocol followed all criteria. The difference between these is 124 patients with major protocol violations related to inappropriate eligibility enrollment or treatment not according to the protocol. And again that was described in the statistical analysis plan.

So here are the actual data for the two populations. If I start with the "as randomized," the "as randomized" did not need the primary endpoint. There were 47 deaths out of 350 versus 35 of 364. This just exceeded the boundary for the non-inferiority outcome. If we look at the "per protocol," there were 31 versus 29 deaths and this fell well within the agreed upon boundary for non-inferiority. The difference is the patients with the protocol violations where were we see 16 versus 6 or 10s or 10 of the total death of 12 deaths occurred in patients with protocol related violation.

I'm going to discuss that. I want to make a

comment about per protocol. We believe and I think the literature supports that for non-inferiority trials the per protocol population represents the clearest opportunity to look for a treatment effect, because things are well matched and the patients are treated appropriately.

In fact the ICH guidance talks about the per protocol population being a preferred analysis population for non-inferiority trials.

Now although 30-day mortality was the primary input, I also want to show you the result for day 1 mortality and I understand the comments that Dr. Silverman made on her slides, but we think day 1 mortality is an important time point to observe, because with an indication of unavailability, we think that that's an important and substantial period to provide support for patients until they have access to definitive care.

So again if we look at the randomized -- as randomized group we see there were 35 versus 27 deaths. If you look at the per protocol, at the end of 24 hours, these were patients who were treated for up to 12 hours without receiving red cells there were two fewer deaths in

the polyheme arm and the rate was 7 percent or identical.

Once again the major difference was in the patients with the protocol violation. So what's really going on we are repeatedly asked what is the story with the patients with the protocol violations. First, as we see there are more patients in the polyheme arm than in the control arm and the percentage is actually 20 percent versus 15 percent of the enrolled patients. And there are more violations per patient.

In addition, we looked in the protocol violation group at a number of indicators of poor outcome and they are listed here. The patients with the lowest blood pressure in the field, patients with abnormal neurological function or traumatic brain injury, patients with baseline coagulopathy, I already told you about that, and patients both of these represent those with the highest score for injury severity including patients with chest injury. In every instance, each one of these there are more polyheme patients with these predictors of poor outcome prior to treatment.

So we think this is an important bit of evidence that helps account for the outcome and the contrast

between the per protocol and the protocol violation patients.

Let me turn to safety. And as I mentioned there is a fair bit of new information in the slide. Overall, as we see virtually every patient in the study had an AE which is what one would expect in the trial of seriously injured patients. And just over one-third had serious adverse event. In both instances, the numbers are slightly higher in polyheme patients. This is a traditional way of expressing the most common adverse events that occurred in greater than 20 percent of the patients in either one of the groups. And we see the types of things that occurred.

Now AEs if you're not familiar with the regulatory reporting it can be anything. So pyrexia, a fever is an adverse event. What's more meaningful is to look at serious adverse events.

And this slide reports the most common SAEs that occurred in more than 2 percent of the patients. And I want to emphasize that categories on the left represent what we call "pooled preferred terms." The reason we did that if we looked at the individual preferred terms for

each SAE, the numbers are much lower.

So we thought it was more meaningful to pool things into clinically relevant groupings to give you a representation of what's going on. Again the numbers are slightly higher in the polyheme. The largest delta is in the coagulopathy, 4 percent versus 1 percent.

But I refer back to the difference at baseline when these patients were enrolled. I think the point of interest on the slide for many were the myocardial infarction -- reported as SAEs there were 10 in the polyheme versus 2 in the control.

On the next slide, I've summarized -- there were two additional patients that were reported to have had myocardial infarctions by the principal investigators. They were classified as AE, so the total number would be 11 versus 3, and these are the verbatim terms that were actually used by the investigators on their case report form.

Now, I also want to show you this information. We paid careful attention to try and assess cardiac issues in these patients. These are the numbers for patients, who at any time in the study had an abnormal

electrocardiogram or an abnormal biomarker either CK-MB or troponin I.

What is striking is that despite the low total incidents of 2 percent of MIs is reported by the investigators, 14 out of 720, up to 79 percent of the patients had either an abnormal electrocardiogram or an abnormal biomarker.

So in order to reconcile that, try and reconcile that discrepancy, we assembled a subcommittee of the IDMC prior to unblinding of the data to object of the review the data on all 720 patients on an objective basis and categorized every patient in to one of the following four categories.

And this is new data we have not shown before. Probable MI or plausible MI, indeterminate MI, or absent MI, the distinction between and probable and plausible is based on how many of the piece of information coincides. Probable MI would be if there was evidence by EKG and by a biomarker with predefined criteria, a plausible Mi would be, if there were just one of those.

So as we look at this data, I think it's useful information there is a considerable debate in the literature about how one can or should diagnose myocardial ischemia particularly in trauma this maybe the first large objective approach in trying to define that.

And what we see is that just over half of the patients in each arm had some evidence of myocardial infarction. If we look at indeterminate there were more patients in the control arm in whom that analysis with indeterminate, meaning there was insufficient evidence to say they did or did not.

If we look at absent MI there were more patients in the PolyHeme round in whom there was definitive evidence to say there was no MI than the controlled. So we do think this is very useful to supplement, it's a post-doc analysis, but in a very careful objective way, it supplements the PI assessments in an unblinded trial.

In addition, this slide looks at other pooled cardiovascular events related to categories of either a pump failure, Dysrhythmia, or cerebral ischemia, these

categories are based on presentation that was made at a recent BPAC meeting to look at these sorts of events.

Lastly, we have other clinically important AEs again pool-preferred terms and we selected these based on their discussions, draft 2004 guidance that was mentioned earlier today.

So in summary, let me try and bring all this to a closure. This clearly was a complex study, and we want to make sure everybody understands what we stated today. In the, as randomized group, the result did exceed the primary end point.

If we focus and consider the data from the patients in whom the protocol was followed as specified, namely the protocol population, we did meet the primary end point.

Lastly, it was indeed a higher incidence of AEs in patients receiving PolyHeme compared to the control group receiving crystalloid and red cells. Although as I mentioned earlier, the planned indication would be for the treatment of patients when red blood cells was not available, not in place of red cells.

So as a final slide to bring this to closure, based on the totality of our data, we believe that the evidence provides -- demonstrates the potential for PolyHeme to provide a survival benefit and life-threatening hemoglobin levels with an acceptable safety profile, and a favorable benefit-to-risk ratio when an oxygen carrier is required and red cells are not available with the ability to address a critical unmet medical need. Thank you.

(Applause)

MS. ALVING: Thank you very much Dr. Gould. Will you please raise your hand if you are collecting the white forms? Those who are collecting them please raise your hands, please write your notes. Dr. Gould you have given us food for thought but not enough so we are going to have to break for lunch and come back at about five until two.

Lunch is upstairs in the cafeteria, there is also a -- no don't go there; go upstairs if that's the best place to go.

(Whereupon, at 12:55 p.m., a luncheon recess

was taken.)

A F T E R N O O N S E S S I O N

(2:00 p.m.)

MS. ALVING: Good afternoon, we are going to get started again, and our first speaker for the afternoon is Dr. Abraham Abuchowski, and he is the president and CEO of Prolong Pharmaceuticals. And Prolong means "PEGylated proteins," and he really developed PEGylated hemoglobin while at Enzon, and I believe he is continuing this work now.

So we are delighted to hear you talk. Thank you.

HBOCS: CURRENT STATUS AND FUTURE DIRECTIONS

MR. ABUCHOWSKI: Thank you very much. Well, I know everybody is going to be awake because we didn't have a very heavy lunch so --

(Laughter)

MR. ABUCHOWSKI: -- we are going to move forward here, for those just landed on the planet of PEGylation is

our technology that we use to attach polyethylene glycol to proteins has a very unusual dynamic properties, and it improves the product from in vivo as well from a physical-chemical characteristics. And it is today the only FDA approved delivery system for protein therapeutics.

Little bit of background this was a technology that I developed for my Ph.D. thesis. I have records. And following that work we worked on a lot of different products there and started a company called, "Enzon." I was there from 1983 to 1986, and there we developed the first three approved products using the PEGylation technology. They were Adogen, which was used with respect to -- it was the first product approved for a genetic deficiency disease.

It was used to correct enzyme deficiency of adenosine deaminase in children who have severe combined immune heart deficiency, the bubble boy kids. It successfully worked. The importance of this product, who tries to be the first product approved with this technology, is its longest running toxicology experiment with this particularly technology.

These children have been getting once a week doses of this product for 20 years now. And there's been no toxicology. The other product was Oncospar, which was used as a treatment for acute mucoblastic leukemia in children, especially in children, who had ongoing immunity response to the protein that was being used. This is now the main state treatment for leukemia.

And lastly was a PEG-INTRON, which we licensed to Schering Plough, this is a PEGylated interferon, and it is also currently marketed. So get on to what we did with PEG-hemoglobin, it seemed to us that obviously hemoglobin was a logical application for PEGylation extend the circulating life minimize the toxicities. Do everything that PEGylation does.

We started the work in the early '90s, I think around 1991, and proceeded to develop a product and tested it pretty broadly in a variety of different animal species to look at the effect that the product has on various organ systems, toxicity, and even some efficacy experiments. And we developed a pretty extensive toxicology dose at CA, which we then used to move forward

into phase I, and phase I(b) studies. And we looked at a total of about 60 patients and that actually takes us all the way to about to 1996.

The specifications at the time for the PEG-hemoglobin and the P50 range from about 14 to 20 as we were developing our formulations, and the concentrations also ranged between 4 and 6 percent depending on the application we were using at the time, and it was basically in a phosphate buffer, buffered saline. Methemoglobin was below 10 percent it was stable for slightly more than 6 months at minus 20, and only about 7 days at 4 degrees.

And you know, we have since improved the formulation now, in my second iteration at Prolong. We now have a reformulated product that is stable at 37 degrees for a month, so, and for a year, at least a year now at room temperature.

So I'll go through a series of experiments done in animals there are actually many more than I am presenting, but these are kind of the highlights of the various experiments that were performed leading up to the

phase I study. First was a 30 percent exchange transfusion in a dog, and here we looked at the PK.

Circulating half life was about 60 hours for the product and the dog. And there appeared to be no significant alterations in the cardiovascular function, did not see any hemoglobin urea, or any gross morphological renal damage, or any changes in the blood chemistry.

And then we looked at -- a more difficult model, which is a dehydrated hypovolemic hemorrhagic shock model, where the animals were first dehydrated there were withheld from food and water for 48 hours and actually experienced about a 5 percent drop in their blood volume during that time. And then we hemorrhaged them at 25 mills per kg for an hour

Following that, they were infused with PEG-hemoglobin, and what we found S-GPT and S-GOT. We did see on live histology, some centrilobular necrosis, but that seems to be consistent with the type of injury that we caused and not due to the PEG-hemoglobin. And the history of the rest of the organs was normal.

Down the study was the effect on hemodilution here at Yorkshire pigs, 10 kilo pigs. We did 80 percent exchange in six pigs, all the animals survived. Their heart rates were normal, their mean arterial pressure did increase slightly during the infusion, but they returned to normal with 6 to 8 hours. And there were normal blood Ph and other parameters of Peo2 E CO2.

We did see presence of this renal tubular -- tabular cells cytoplasmic vacuoles. And vacuolated macrophages in the spleen, that was thought to be due to the processing of all the protein that was injected. We -- 80 percent of their blood volume is not PEG-hemoglobin and it's got to go somewhere.

In the rabbit we looked at again effects here, we dozed that 10 mil ad 20 mil per kg. In the rabbit half live is about 43 hours, again no hemoglobin urea, no changes in the daily urine volumes. They were, they stayed in the normal range. We saw again these small vacuoles in the renal tubular epitheliam.

Again the end characteristics of protein absorption, and there was no significant pathological

changes in the liver or the spleen. And then we looked at some other species as well. Looking at doses at 10 mil per kg and 20 mil per kg. We looked at the renal plasma flow and the glomerular filtration rate in dogs.

Tubular function studies in rats and glomerular filtration rate and urine analysis in rabbits. And we saw no adverse effect on renal function in any of the animal models.

We also wanted to check whether there was any effect, interaction with human blood components as we are going to giving it to people. We want to make sure we didn't affect any of the testings that were done, so we did test with various concentrations, it did not induce or inhibit any blood coagulations, did not activate lymphocytes, or monocytes, or neutrophils, basophils, or platelets.

And interestingly it did reduce the effect of endotoxin activation. This is kind of a bit of a positive effect on monocytes, and that occurred as a dose dependent manner.

In terms of toxicity work, we did acute overload experiments where up to 67 percent of the whole blood volume was overloaded. So this was top load, so we put two-thirds again blood volume in to the animal. And then we did multiple overload studies where there were infused with 15 mils per kg, every other day for seven doses total, and then a series of experiments where we did an exchange transfusions and hemorrhagic shock.

And overall, we did not see any over toxicity or changes in behavior and appetite or weight gain. There were some mild changes in blood chemistry, hematology, and urine analysis. But these were all transient, they occurred at the beginning of the study, and then stopped, and again vacuolization as observed in various tissues.

And but again likely due to the degradation process of PEG-hemoglobin again there is a awful lot of protein being administrated to these animals.

So from there with that data we did a phase 1 study and normal volunteers is an escalating dose study, in 34 patients that goes from 1.6 up to 8.33 mils per kg.

8.33 mils per kg is approximately a 500 mil bag of PEG-hemoglobin.

Now the only side effect that we noticed was that the high dose, where there were some gastrointestinal spasm and principally that was esophageal spasm. But that was able to be prevented and managed using a antispasmodic agent called Levsinex.

Now, at this time we were thinking what we were going to do with this product, and as we wanted to move forward in the clinical development and we did not wish to use this product as a blood substitute because the thought was, it's going to be very difficult to compete with whole blood.

So we had to look for indications that, but there was a therapeutic opportunity where whole blood was not indicated. And at the same time the thought was that maybe an oxygen carrier was inappropriate in terms of the return for these products, and I see it is already changing a little bit because simply because the protein was carrying oxygen didn't mean that it delivered oxygen.

And it could very easily just be acting as a plasma expander with no delivery characteristics. And so I saw -- I was trying to use the term, "oxygen therapeutic," which I think is probably more appropriate than a oxygen carrier. But we thought of a lot of different applications, for an oxygen-carrying agent, where there would be therapy, which would not require a competition with whole blood, just providing list here (inaudible) others have been presented as well I don't want to go over everything again.

So we decided that maybe two opportunities or multiple formulations depending on the applications used. First formulation is the one I presented all the data on and that was PEG-hemoglobin and buffered sailing and another preparation is the same PEG-hemoglobin in the hypertonic saline solution.

The first the buffered saline was used for radio sensitization, this is again the PEG-hemoglobin that was used at Enzon and this was all done at Enzon. And in the animal models that we tested, we substantially increased tumor oxygenation in a number of animal models, tumor

models, sarcomas and Lewis Lung Carcinoma, Sarcoma, and the like here, and we did find that it had an ability to radio sensitize tumors.

In all these studies not only were the tumors hyperoxygenated, but we had a much better effect following radiation therapy in those animals that were treated with PEG-hemoglobin. So that was the impetus to move forward to do a phase I B study looking at radiosensitization as the indication.

So we did an open-label study, and patients with metastatic disease, 33 patients again ranging from 2 mil to 8 mil per kg, 8 mil being at 500 cc bag. And the most common side effects, and this was primarily in the 8 mil per kg, were some mild hypertension, dysphasia again, nausea and vomiting, but nevertheless the results were positive enough that it was continued -- recommended for continued study

At this point, I actually left Enzon and went on my way as a consultant, but then in 2005, I opened up Prolong Pharmaceuticals to continue this work. This work was actually dropped by Enzon in 1998, so the product just

never had a chance to move forward again and continue to be tested

So we decided to continue it as Prolong now, and this time we developed a different formulate version, which we called "aftershock," and this specifically formulated with hypertonic salts to treat severe hypovolemic shock. And the idea here is that we don't fill up the animal, but we use a single unit dose to resuscitate the animal because it is primarily targeted for military applications to be used in the field where you need just finite volume to resuscitate a soldier and keep them stable until they can get him to appropriate places.

So the work that we have been doing is at the Virginia Commonwealth University Reanimation Engineering Shock Center under Kevin Ward, and he had -- has developed a model, which measures a whole body oxygen debt in the pig.

And basically the way the model works is the animals are highly instrumented we actually take readings of oxygenation of all the organs, blood chemistries,

pressures, but the main elements that have enriched it in is oxygen debt and mean arterial pressure, so that's the main one I am providing.

But basically the animals are led to a oxygen debt, a uniform oxygen debt of 80 ccs per kg, or 80 millimeters per kg -- no 80 cc per kg, I am sorry. And the intent here is to make the all the animals uniform rather than bleeding a fixed amount of blood, because when you bleed a fixed amount of blood, you don't get the same oxygen debt between animals.

And to normalize the animals, we wanted to actually measure the oxygen debt, go to a fixed point and then compare against various other products. So we compared against packed red blood cells, hetastarch, and Oxyglobin.

And as you can see, once we get to the resuscitation point, Oxyglobin and hetastarch do virtually nothing. We do get repayment of oxygen debt from packed red cells, we actually get better repayment of oxygen debt with whole blood, for some reason. And -- but with PEG-hemoglobin, we not only see a repayment of oxygen debt,

but the hemoglobin get straight on oxygenating, I guess the oxygenating bunny I guess, I don't know.

They keep straight on oxygenating, and in the case of the mean arterial pressure, again we see the pressures drop, and as you can see the pressures drop pretty quick, but yet there are still hemorrhage throughout this point. Pressures don't change much, what we are -- I was kind of getting to is the oxygen debt, but you can see the packed red cells hetastarch, Oxyglobin and PEG-hemoglobin maintains a very nice mean arterial pressure.

So this turns out to be a very nice model where we are continuing to work the model looking at different formulations, there will be a publication sometimes soon from VCU on the model, and the application of the product.

Now, it does not -- one of the issues we do get back mean arterial pressure, but it is not due to ways of constriction, because when we look at the micro circulation under the tongue of these animals, we started the baseline -- you know very nice circulation at the end of the hemorrhage everything has collapsed, we start

putting in PEG-hemoglobin and starts to recover, and by the end of the experiment microcirculation is identical to baseline.

So we are not causing ways of constriction, or opening up the circulation and providing the oxygen appropriately.

I just wanted to touch a little bit about manufacturing, because we are talking about an HBOC or an Oxygen therapeutic really in a scientific sense, and I think this, at some point in time we are going to arrive at a successful product.

And once you do, the real issue is how you are going to manufacture this product, then either some sobering numbers, because the manufacturer of hemoglobin is going to be on a scale that that is going to dwarf every other biotechnology products of magnitude.

Because a single dose of this product, is 20 to 40 grams is the dose, and if you are looking at a million doses we are talking about 40 metric tons of hemoglobin as a starting material for a rational-sized product, and that means that in order to extract that much hemoglobin you

have to start with almost 800,000 actually about closer to a million liters of blood a year, and process it to get that amount of hemoglobin.

And then to turn all that hemoglobin into a PEG-hemoglobin, you still need another 40 metric tons of activated PEG. So the manufacturing exercise is difficult as the development exercise.

So let me conclude with some thoughts that -- and it has been a theme coming into this meeting here, which is that probably will not be a single blood substitute that's going to work universally for all applications.

I think that each application has its own dynamics that has to be dealt with, and they are going to be specific formulations of product, probably some products that haven't even been discussed today, with unique properties to deal with the various issues that occur during achievement of various diseases. So they are going have to be formulated, for these specific applications.

And you know, we have to have a clear therapeutic effect at some point in time when these products that says, yes, they are good to use, or no they are not good to use. So again, neither of shattering thoughts, but that's a common theme. And so let me thank you very much for this opportunity.

(Applause)

MS. ALVING: Thank you so much for your talk. Please remember write questions you have on note cards and if you could write the name of the person whom you would like to address these, that would be excellent and then we will collect them just before a break.

Our next speaker is Dr. Gerson Greenberg, and he is currently the vice president of Medical Affairs, of Biopure. He's had very long and distinguished career in surgery and is currently professor of Surgery Emeritus, at Brown. And also was very involved with Hemosol, and in launching some of their initial clinical trials.

MR. GREENBERG: Thank you. Can everyone hear me? Good afternoon, Dr. Alving, and yes, thank you for inviting us to participate here, it really is a pleasure to join this august body and thus be able to discuss some of our issues. My colleagues and I really do thank the FDA and NIH organizers for providing us the opportunity to discuss our experiences with HBOC-201 at this meeting.

Moreover, I would like to recognize the difficult tasks faced by the FDA daily with respect to approving trials and marketing of drugs when -- within defined parameters of safety and efficacy. The opportunity to enter into scientific discourse and debate is welcomed, and in my allotted time it is my intention to inform, enlighten, and challenge some closely held views.

It's reflected in the agenda, it is been suggested the HBOCs as a class have in common an adverse safety profile that is presumed to arise from a common mechanism of toxicity. Any exposure to the current generations of HBOCs is associated with unreasonable and unavoidable risk. Therefore, development of HBOCs is the way forward.

Having spent over 35 years in the field I take umbrage with that particular approach. As we have heard, all HBOCs are not created equal. They differ in many characteristics on many dimensions in a particular purity and purification processes in the manufacture, which we have just heard a little bit about, the hemoglobin concentration, the critical oxygen carrying capacity, which is vital to tissue survival and reproducibility of the product and manufacture, and various physical chemical properties and other traits and parameters that are noted her

A particular importance is that there have been no head to head trials for comparison, common mechanisms may not exist. Moreover, the different compositions invoke a principle of heterogeneity that would argue against any comparisons made from mumping of data. Homogeneity is necessary for those comparisons.

The current HBOCs do share one common critical property. A low concentration of hemoglobin ranging from 4 to 13 grams per deciliter, and cannot provide the same

increase to oxygen-carrying capacity as packed red blood cells in unit to unit comparison.

Recall that oxygen delivery is complex physiology, it is a complex physiologic function, the product of cardiac output and oxygen content or hemoglobin concentration is pointed out earlier by Dr. Bunn. In my view it is too early to discount the current generation of HBOCs.

We need to understand the origin of adverse events, and safety signals that have emerged in these trials, product by product from the perspective of composition and clinical applications.

Biopure's safety profile Dr. Silverman's recently presented tables was to derive from documentation provided for the December 2006 BPAC meeting which rejected the United States Navy proposal for the recess trial by a vote of 11 to 8 practicing conditions voting for it.

But it did conclude that a phase II trial should be allowed to go forward. Approval for such a trial has not yet been forthcoming. We have reservations about the construction of these tables, and here expressed concerns

that over estimates -- that it over-estimates and exaggerates the safety signals seen with HBOC-201 use.

We do not deny the emergence of safety signals in our trials. We do however challenge the theories underlying their origin. With respect to Biopure's data there are mistakes in arithmetic and some flaws in construction.

With examples of the pooling of data from heterogeneous trials, the multiple counting of patients and signals, the misrepresentation and exaggeration of signals by either arbitrary grouping of events or/and failure of, to present incidents data which would be the most reflective

At Biopure we have an alternative view on how this table should be constructed. Only trials HEM-114 and HEM-115 are sufficiently comparable to permit pooling. Data from HEM-115, represents all of the safety signals seen in the pooled database. The list of observed imbalance is identical and not exaggerated.

Moreover HEM-115 is a population of 688 patients. Relatively, homogenous selective surgery

patients, represents 47 percent of all the trial patients and 68 percent of those in the RBC controlled trials, and it contains an incredibly extensive data collection, the 688 patients that qualifies, for an appropriate safety and efficacy analysis.

Concerns over the safety of HBOC-201 arose, from the interpretation of results from this pivotal trial. Let's try to understand the increase in safety signals and the associated problems. This was a randomized, controlled trial comparing red blood cells to the HBOC-201, in elective orthopedic surgery, 688 patient enrolled.

Randomization was at the first transfusion decision. And about 60 percent of the patients in the HBOC-201 arm avoided any red blood cells through six weeks follow-up, 90 percent clearly in the first 24 hours.

Forty percent of the HBOC-201 patients required as a decision by their physicians treatment with red blood cells to meet their needs for increased oxygen carrying capacity.

Unfortunately, the intent to treat safety profile was not favorable. It is in the proscribe

subgroup that efficacy, avoidance of red blood cells, was not attained. More importantly it is in this group that the majority of the serious adverse events, the safety signals emerged. The results of this trial, and all of the necessary analysis have been accepted for publication and will appear shortly in the *Journal of Trauma*.

A significant incidence of the adverse events and serious adverse events both absolute and have a per patient incidence basis were noted in the HBOC-201 arm. From the study and these data the FDA concluded the profile shows, "unreasonable and significant risk of injury" and "most of the SAEs observed in the orthopedic study HEM-0115 are consistent with the hypothesis that they result from the vasoactive properties of HBOC-201, with emphasis on the term "hypothesis."

I intend to demonstrate that there is a more reasonable and more likely explanation for HBOC-201 safety signals than toxicity. And moreover there is no evidence of a causal relationship between vasoactivity and vital organ toxicity with HBOC-201.

This difference may well be reflected in the difference in the ability to increase oxygen-carrying capacity, overall tissue perfusion and oxygenation especially in critical organs. I will address two hypotheses regarding the emergence of safety signals seen in the HEM-115 trial.

Now, I'll begin with an exploration of alternative hypothesis the relative advocacy of HBOC-201 could not provide sufficient oxygen-carrying capacity, and this contributed to the emergence of serious adverse events when the comparator was packed blood cells.

Concentration difference, 13 versus 20 grams per deciliter of infused product could result in an excess of serious adverse events especially those associated with ischemia. This slide is a graphic representation of the difference in the relative efficacy of the two solutions to increase the oxygen-carrying capacity with a single unit, HBOC-201 and red blood cells.

The efficiency of HBOC-201 is not as good, and I think that's clear. Here, we have modeled the increase in total hemoglobin concentration attainable across the range

of patients starting reference hemoglobin levels for fat red blood cells, whole blood, HBOC-201, and hetastarch.

This model is based on many assumptions and there is insufficient time for a full and complete description, which is not possible here today. This was presented at the Clinical Pharmacology Society meeting in Orlando earlier this month in explicit detail.

Whole blood, and HBOC-201 are similar about pack red blood cells clearly have a greater relative efficacy. A rather dramatic difference actually. Given the range of ability to increase total hemoglobin is there a correlation of anemia, a low hemoglobin or a hemoglobin deficit with the emergence of adverse effects in particular cardiac ischemic events, I think we should explore that possibility.

Here I show the ability of the model to actually predict the increase in total hemoglobin concentration, the oxygen-carrying capacity with data from the HEM-115 trial. There is a very good approximation of a model to reality.

How does the -- how does this translate to the HEM-115 trial data? Patient population mean total hemoglobin levels in the HBOC-201 arm are in red, packed red blood cells in black both show as a function of infusion number. Clearly, the HBOC-201 arm demonstrates a population with lower total hemoglobin over time.

This could reflect either inadequate efficacy and/or unsuccessful patient management. The higher incidence of serious AEs 0.34 versus 0.25 could be due -- sorry about that. Patient population here is the total -- it shows the total hemoglobin levels in HBOC-201 population. Clearly the HBOC-201 arm demonstrates the population with lower total hemoglobin over time, reflecting as I just said, efficacy and/or unsuccessful patient management. A higher incidence of serious AEs per patient 0.34 versus 0.25 could be due to failure to increase oxygen carrying capacity, insufficient treatment.

Recall that by design the HBOC-201 arm of this study had two groups. The brown line represents the 60 percent of patients, who avoided any red blood cell treatment, the red line the 40 percent of patients, who

required red blood cells in addition. The serious adverse event rate per patient in the 60 percent of those who remanded successfully was 0.14. Strikingly different from the number you have seen in the packed red blood cells and the HBOC group as a whole.

The second group for whom HBOC-201 treatment was not sufficient as determined by the treating physicians were exposed to the risk of ischemia from ongoing anemia had an SAE rate per patient of 0.63 over a four times higher incidence. The total hemoglobin as a group over the course of study was clearly less than those treated successfully, and led below the treatment threshold sought for this trial.

Again in this group the bulk of the serious adverse events for this trial emerged. There was clearly a hemoglobin deficit under treatment, and we all know that mortality and morbidity are related to hemoglobin concentration, in my experience across all diseases across all levels of hemoglobin concentration in almost all patient populations.

How can hemoglobin deficit be expressed, quantified to make it a useful tool for understanding whether or not this may have been a predictor of outcome. In the schematic representation of a hypothetical patient is shown, for two clinically critical relevant elements of hemoglobin deficit, the magnitude and the duration of the anemia.

These elements were operationalized by calculating the area under the curve to use the data in assessment. The emergence of an adverse event was the time stopping point for the calculations. The area below the clinically defined significant low acceptable hemoglobin level, transfusion trigger, if you will, was calculated and reflects the magnitude of the defect.

For each and every patient in the HEM-115 trial the results were used for further analysis. This was also presented earlier this month in Orlando.

The area under the curve representation of the hemoglobin deficit was entered into a logistic model with data from all of the 115 patients, using covariates of

age, history of cardiac disease and whether or not they received HBOC-201.

Selection of these variables is consistent with known and accepted data concerning the impact of age, history of cardiac disease in the presence of low-levels of hemoglobin to the emergence of cardiovascular and CNS complications in most studies of surgical patients. Indeed modern transfusion guidelines consider these important variables.

And are elements used to define transfusion triggers. In this model age, history of cardiac disease and the previously noted hemoglobin deficit were more significant predictors of cardiac ischemic events, being in HBOC-201 group was not a predictor in this model.

This table represents a concordance of the measures of anemia in both therapeutic groups. Numbers in the middle column represent the 40 percent of patients, who also receive red blood cells. Both measures the time the duration of anemia and magnitude the area under the curve, are greater for the HBOC-201 group compared to red blood cells. And further exaggerated in the patient

population they received HBOC-201 and red blood cells the middle column.

There is an increase in adverse events and serious adverse events associated with both measures of hemoglobin deficit. Then there are the clinical aspects of any hard analysis, the additional of clinical contextualization to understanding the basis of the difference seen.

The results of an independent clinical root cause analysis of the serious adverse events observed in the 40 percent of patients, receiving both treatments is shown. The major factors identified by this analysis were volume overload, and issues of volume management, under treatment and age. Being old is not particularly good.

Once more constraints of time do not permit a full and complete explanation of this analysis, much of it is in the accepted 115 paper, and a paper presented last year in Beijing.

The red numbers in the left column represent the number of crossed-over patients with these root causes for their CNS and cardiovascular events. And the numbers in

parenthesis are the numbers of patients over 80 years of age, with these causes, those most sensitive to significant changes in oxygen carrying capacity, the patients over 80, dues to a decreased hemoglobin concentration are shown.

Inadequate treatment of a deficit in oxygen-carrying capacity and volume overload in this older patient population account for the entire difference in the groups and clearly supports the results obtained from the logistic model just presented.

I believe, I have demonstrated the fact that the preexisting imbalance in solution or efficacy produced contributed to a deficit in hemoglobin concentration as measured in both duration, and magnitude, and the decrease in oxygen-carrying capacity, which results in two forms of patient management issues, under treatment and volume overload.

HBOC-201, while effective at providing an alternative to red blood cells to a reasonable degree was less effective at achieving the goal in a population at risk, those with high needs, those who are elderly, those

with cardiovascular disease, patients in need of adequate tissue perfusion.

The fact of an efficacy mismatch may have been overlooked by those who designed and those who approved the HEM-115 trial. Indeed in my opinion the assessment of relative efficacy as part of HBOC trial design is a general principle to be applied to all red blood cell controlled trials. Competition with packed red blood cells or blood less than 14 days old is probably not justified.

I believe I have demonstrated a simple and compelling explanation for the emergence of CNS and cardiac serious adverse events in the HEM-115 trial based on the hemoglobin differences of the solutions. I will now turn attention to addressing the question of vasoactivity as the putative basis for HBOC-201 toxicity, is it reality or is it a myth.

As a reminder, following submission of the Biopure BLA in 2002 as subsequent discussions in communication with the Food and Drug Administration, Biopure was sent to stated hypothesis. And the FDA

requested additional studies to address these particular issues.

And so we undertook the requested studies with their approval and assistance in protocol design. Once more the allotted time does not allow me, or permit me to do justice to complete data sets and only a few specifics will be presented.

The first request study examined blood flow in individual organs of swine undergoing isovolemic exchange of 10, 30 and 50 percent blood volume. HBOC-201 is in red, colloids in blue, and black is a time control.

If the vasoactivity hypothesis were operative increases in plasma total hemoglobin concentration would be associated with decreases in organ blood flow. This was not the case for heart, brain, kidney, or pancreas which is not shown. Only skeletal muscle demonstrated a significant decrease in flow associated with increasing degrees of hemodilution.

In the prescribed model, there does not appear to be generalized vasoconstriction associated with HBOC-

201. The same hemodilution protocol, which used in the second proscribe study in swine shown here.

Here EPR imaging was a technique for measurement of tissue PO2, if the theory of vasoconstriction is the underlying mechanism for serious AEs is true. Reduced blood flow with reduced tissue for fusion would lead to ischemic events in criminal organs.

We just saw no change in flow, and here we failed to demonstrate changes in oxygen tissue in tissue oxygen and oxygenation with hemodilution. The hypothesis proposed is once again not supported by the data obtained from the requested studies.

Encouraged by these observations verification of the observation of vasoconstriction in the skeletal muscle was the next step. This microcirculatory study in rats shows an increasing blood pressure with increasing clinically relevant doses of HBOC-201 the upper graph.

Changes in vessel diameter are shown at the bottom two graphs decreases vasoconstriction in the vessels of the skeletal muscles on the left, no change in

the diameter of the mesenteric vessel the absence of vasoconstriction on the right.

Key findings from these requested preclinical studies in two species with two methods of evaluation no vasoconstrictions in organs of concern measured only in the skeletal muscle.

A question. These studies do not support the stated hypothesis. The results from these requested studies have been rejected or significantly discounted and Biopure and the United States Navy remain on clinical hold in the United States despite the BPAC recommendation for a phase II trial, with the exception of the individual approvals for compassionate use of INDs.

Could we find directive in some vasoconstriction, cardiac toxicity in patients; especially, the vital organ heart?

In the next few slides, I will show you data from swine and human studies performed in one of the premier clinical cardiovascular sites in the European Union that support the following points. All of these

studies have been accepted and/or published in peer reviewed journals.

First intracoronary HBOC-201 dose dependently corrects LV dysfunction induced by total interruption of coronary flow. Secondly, HBOC-201 does not vasoconstrict coronary arteries in patients with coronary artery disease. Thirdly, intracoronary infusion of oxygenated HBOC-201 protects against myocardial ischemia in coronary artery disease patients experiencing complete coronary occlusion.

Some times, as alluded to by Dr. Biro earlier, humans are good models of our animal experiments. Clinical trial core 0001 intravenous infusion of two doses of HBOC-201 in to patients with coronary artery disease. Upper left panel shows increase in mean arterial pressure, about 23 millimeters of mercury, but coronary flow an indicator of coronary micro vascular tone was unchanged.

The upper right panel shows the same mean arterial pressure data. And the absence of change in the diameter of an epicardial conduit coronary artery has

determined using quantitative coronary angiography as depicted in the right lower angiogram.

Increases in systemic blood pressure, a surrogate for vasoconstriction does not predict vascular tone changes in the coronary artery in high-risk patient with sensitive coronary arteries. Here, we show that I've HBOC-201 compared to hydroxy-ethyl starch do not alter coronary flow.

Two protocols to evaluate the effects of intracoronary oxygenated HBOC-201 and left ventricular function in the absence of coronary blood flow. For the animal studies on the left function of a wall segment was measured with sonomicrometry. For the patients on the right left ventricular function was assessed from pressure loop recordings obtained from a conductance catheter.

The swine were anaesthetized and the patients slightly sedated. They were undergoing a coronary artery intervention before the study commenced. In both studies a Helios (phonetic) style low pressure balloon catheter was used to occlude the proximal left anterior descending

artery for three minutes or less stopping criteria were met.

During this occlusion oxygenated HBOC-201 was infused distilled to the instruction. Shown here is the swine data of left ventricular function near the end of the 3-minute occlusion without HBOC-201 on the left in blue, we see dysfunction indicative of ventricular dilatation and loss of ventricular wall function, essentially loss of contractility.

With oxygenated HBOC-201, we see a dose-dependant response with full protection of function at 50 milliliters per minute. It is hard to consider this as a particular toxic material to the heart.

In this representative patient of the five, studied with 3 minutes of total coronary occlusion, no native coronary profusion a dry occlusion the blue PB loop on the left shifted to the right and up on narrowing of this loop compared to base line in black represents a smaller ejection fraction into a higher diastolic pressure, signs of a dilated heart, just what was seen in the swine.

Intracoronary infusion of oxygenated HBOC-201, the red loop on the right, maintains pump fit function with little change from baseline. The left panel shows left ventricular endiastolic pressure during occlusion, the blue line dry occlusion indicates a rising pressure.

With HBOC-201 the pressure is maintained near baseline. Dry occlusion results in a fall in cardiac output as it would be expected, while coronary profusion with HBOC-201 permits it to remain very near the baseline.

The asterisk indicates that this patient, like in all in the study, terminated the dry occlusion before the 180-second point for symptoms. With HBOC-201, all patients went 3 minutes per protocol.

Mean ST, segment changes on the electrocardiogram for baseline are shown here. Red is an intracoronary electrocardiographic lead, the green lines are the surface leads. During dry occlusion ST segments increase significantly, an indication of intra -- of transmural myocardial ischemia.

Perfusion with oxygenated HBOC-201 prevents shift in the ST segment, the segment that represents

ischemia. Summarizing the preclinical data HBOC-201 is without the fact our material tone in vital organs, skeletal muscle bits do show vasoconstriction, the primary cause for the increased blood pressure. Direct exposure of the heart to oxygen in HBOC preserves myocardial in function in the absence of blood. It's unlikely the HBOC-201 has intrinsic cardio toxicity.

Summarizing the clinical toxicity -- excuse me summarizing the clinical data, HBOC-201 induces a modest increase in blood pressure, sealing the property of the class. This increase is for the most part modest and transient and once seen in clinical trials or in patient granted compassionate use, manageable with standard interventions.

HBOC-201 does not appear to vasoconstrict coronary vessels, nor does it have an effect on coronary function. HBOC-201 is an oxygen therapeutic, devoid of cardiac toxicity as it clearly maintains myocardial and function as out -- ECG evidence of ischemia.

I believe that we have shown on the left that there is no evidence in these models to support theory of vasoconstriction.

I'd like a moment to take a brief launch into something on efficacy. Last night University of Maryland hosted a symposium where first-time users of HBOC-201 presented their experience in the treatment of severely anemic patients under FDA supported compassionate use IND. I selected a case where there is a clear and under critical evidence of efficacy, without evidence of toxicity at levels of native hemoglobin concentrations that would normally be considered lethal is Dr. Gould showed us.

Yet this supposedly toxic substance sustained life for 18 days until she succumbed from her underlying disease. Dr. Thompson is here, and would be happy to discuss the case with you. The young woman with hemophagocytic lymphohistiocytosis, a rather advanced form and rare form of autoimmune hemolytic anemia was treated. A 25 year-old 50 kg woman was first treated when here hemoglobin was below 2 grams per deciliter.

Over the next 18 to 19 days, she received 53 units of HBOC-201, 13.5 liters providing a total hemoglobin load of 1.73 kilograms. We -- her native hemoglobin over that time of course was barely greater than 1 gram per deciliter. In the course of treatment, she was switched from bolus infusions to constant infusions, and note the upward trend in the hemoglobin.

During this time she underwent a splenectomy, she had normal kidney function, normal cardiac function, normal cerebral function, and mentation. Ischemic issues were not a problem, and the nurses who cared for her said she had higher brain function, when they allowed the sedation to ease.

At autopsy the ultimate clinical test and examination, there were no lesions of toxicity in the liver, brain, kidney, or heart. And additional clinical perspective, if I may take another moment, Biopure has treated more than 17,000 patients to date in clinical trials, in South African market in compassionate use.

We have learned a great deal from these experiences including a clear awareness of a side effect

profile. However, inappropriate indications in patient populations, side effects can be effectively managed. I believe that what you see on this slide is the true side effect profile the HBOC-201. This should be considered side effects as all agents have side effects.

In situations where blood is neither an option nor available excepting these treatable side effects while saving a life should be recognized as beneficial. The benefit outweighs the risk in my opinion.

The way forward, HBOCs are not blood substitutes, they were about that well, over a score plus 10 years ago. Their oxygen therapeutics and can be useful when there is a need to increase tissue oxygenation as either a rescue therapy for stroke or MIs we have heard was an adjunct to radiation and chemotherapy as we have heard and many, many other possibilities.

Because they vary in composition on many characteristics they must be evaluated individually and not assumed to have a common mechanism of action or toxicity to explain the emergence of adverse events. The same event could arise from different mechanisms.

HBOC-201 is neither toxic nor illegal, and it can be used to save lives now. HBOC should be evaluated in trials where blood is not an option or immediately accessible. The efficacy of an oxygen carrying solution over colloids or crystalloids is all too obviously especially when oxygen delivery to tissues is essential for life.

We are developing additional new generation of HBOCs and for conjunction with U.S. Navy and the NIH investigators. The development cycle to bring any of these products to fruition and online is 7 to 10 years when we seem to need something now. Who among us is willing to let the mother of a young Jehovah's Witness patient with menometrorrhagia and an native hemoglobin of 1.8 wait that long for treatment?

I want to acknowledge and thank the efforts of my team to fly up here for their help and support in putting this together and my time there. I want to thank you for your attention and I want to leave you with this thought. Thank you.

(Applause)

MS. ALVING: Thank you Dr. Greenburg. Okay. We are going to now hear from Dr. Tim Estep. I don't know how he went from receiving a Ph.D. in biophysics to the wonderful of hemoglobin-based oxygen carriers, but he has certainly has had the great experience in this. He was at Baxter at the time that they really I think joined up with Letterman to develop the product hemoglobin-based oxygen carriers at that time.

He was involved in the very initial clinical trials that were launched in the United States, he then went over to Somatogen, and he is now a -- has his consulting firm in Colorado.

What I would like to say and probably most of you know that as I understand it, currently there are no clinical trials in the United States that utilize hemoglobin-based oxygen carriers. I believe that it is available in compassionate use case-by-case basis as approved through the FDA. Perhaps Dr. Estep can tell us some of these adventures and thoughts as he described the clinical trials.

MR. ESTEP: But enough about me. Thanks a lot Barbara I appreciate it.

One of the things that I want to start off by mentioning is as Barbara alluded to that I am no longer a Baxter employee, therefore I am not acting as an official Baxter representative. However, I do retain financial interest in the company and even though Baxter is not going forward with development of these kinds of products, I thought I should mention it, so that the audience could appropriate discount what I have to say.

I am primarily going to be talking about the data generated from the diasprin crosslinked hemoglobin, because that is where by far we have the most clinical data, which for those of you, who may not be familiar is human-based crosslinked hemoglobin.

And in a particular given the focus of this workshop I am going to be -- I tried to pick out basically those properties, and topics that were, we think of most

relevance to interpreting the clinical results. And the way I structured this is talk a little bit about some of the preclinical data at first.

And then spend most of the rest of the talk talking about the phase III clinical trial results in particular the U.S. and European trauma studies.

Well, in contrast to some recent assertions we in fact did do a tremendous amount of preclinical testing on DCLHb. Somewhere between 100 and 200 studies was done internally and with collaborators to assess various aspects of this kind of product. We looked at a variety of indications, and we saw some degree of indications of efficacy in the indications shown on the left, like one of collaborators only half of these actually said it looks like we had all the depth covered.

But we decided to focus on the top two blood replacement and hemorrhagic shock ultimately as our lead indications. Although, we did do some -- a lot of preclinical work and the angioplasty indication much as Dr. Greenburg was talking about earlier. And we also turned up some safety concerns, the primary ones being the

top two heart lesions and vasoactivity.

I'm just going to talk briefly about those. Now, there were a couple of others that showed up that we thought were of lesser concern; jaundice, which is mostly a cosmetic issue and consequence of hemoglobin metabolism, a transient centrilobular necrosis in the liver and some GI effects.

So I want to -- first, I want to talk about the -- just summarize the myocardial lesions. Now, this was something that we found in the early '90s as part of our systematic toxicity testing. And frankly, it gave us a great deal of pause. We delayed filing of our R&D for about 2 years while we systematically evaluated what was going on here. This testing has been summarized in a review article that Don Gordon, Tim Berop (phonetic), and I published a few years ago. I did bring a few reprints with me if someone missed the initial article and has an interest in it.

What I'm going to be doing today is just summarizing the highlights, or I guess perhaps low lights depending on your perspective, of this phenomena because I know it has been of substantial concern to the Agency and

some others as well. In the nomenclature of pathologists, it's described in this way. And basically, in the observation microscopically are foci of cells that are either sick or in some cases dying.

There is a lot of species variation as was mentioned, I think, earlier this morning in the incidence and severity of this, the most sensitive species amongst those we evaluated was the rhesus. Swine was next. Cynos (phonetic) had a different dose response curve. And rabbits showed a somewhat similar lesion, although had more of an inflammatory component. And there was actually a fairly high background in rabbits, so it didn't turn out to be a very good model.

It's most evident 1 to 2 days after infusion. And the lesions do resolve with time, the sick cells recover, the dying ones are removed, and it actually becomes progressively more difficult to detect whether the lesion has been there if you look later on. There was a very definite dose response character, which I'll show you in the next slide and a morphometry study which basically means we took a lot more slides than usual so one can get an estimate of the actual volume of cells involved.

That was performed in rhesus, getting a dose sufficient to elicit the maximum lesion types showed that there was about 1 percent on average of the cells that were involved although there were some individual variability ranging from about two-tenths of a percent to 3 percent in individual animals.

And this just shows the dose response curves and how they vary. The blue diamonds represent the average severity score for rhesus. And this is based on the typical pathology classification of minimal, mild, moderate, and severe. And I think, roughly speaking, you should probably think of this as a logarithmic scale in terms of the percentage of tissue involvement.

Again, with the rhesus you see -- you start seeing some appearance of this at a few hundred milligrams per kilogram. And then it maximizes out at doses a little bit below one gram per kilogram. And then no matter how much more you give, there are no more cells that are involved. So that's only a subset of the cells that are susceptible to this.

The pig dose response curve looks very similar except it's somewhat right-shifted. Cynos have a much

flatter curve and then we did not detect this kind of lesion in our tox testing in rats or dogs.

Now what are the consequences? Well, one thing that we observed was although in swine there is -- appears to be a transient elevation of CK and LDH, the myocardial specific isoenzymes were not elevated. Moreover, we could not detect a functional deficit. We did blinded electrocardiogram studies and could find no difference between animals treated with DCLHb or oncotically matched albumin solution.

In fact, we looked at pigs as a model for angioplasty, which I already mentioned. And typically -- and this was infusing the hemoglobin solution down an aluminum catheter. And the hemoglobin was actually able to preserve normal function when the balloon was inflated.

And in fact, we also did some studies where we induced a myocardial infarction and then treated with DCLHb. And in that case, both short-term and long-term functionality was better preserved than in untreated controls. Moreover, and this was the study that also was alluded to earlier in Conrad Messer's (phonetic) laboratory where they introduced a critical coronary

stenosis and then hemorrhaged the pigs. Again, treatment of DCLHb actually reduced mortality compared to the control groups.

So that gave us a greater deal of comfort that, in fact, this -- whatever heart lesion was occurring and they were present in those animals in those studies that it did not have a functional consequence. We then did a lot of studies looking at various interventions to try to ascertain the mechanism of development of this lesion and also potentially identify co-medicaments that might help ameliorate it.

We looked at mode of infusion. We looked at antihypertensive, anticoagulants, antiinflammatories, antioxidants, iron chelation with desferrioxamine, catecholamine depletion because the lesion is actually identical to that observed after pressor agents are infused into animals' manipulation fluid volume. And none of these had a significant effect on the development of the lesion nor did the gender of the pigs, monectomy, hydration state, or whether the pigs were anaesthetized or not.

These lesions were observed after the infusion

of human or swine stroma-free hemoglobin. And most of the products, of course, were human-based that we were looking at. There were two hemoglobin alterations that consistently tended to mitigate the incidence and severity of the lesion formation. One was polymerization, but I would maybe generalize that to making it larger.

That did appear to help in reducing the incidence and severity. And the other, once we acquired the tools, after Baxter acquired some antigen to manipulate the hemoglobin on the molecular level, we found that reducing the rate of interaction with NO in and of itself had a substantial effect on reducing the incidence and severity of these lesions.

Subsequently, we read reports in the literature that (inaudible) caused heart lesions. And when we repeated our typical experiment, indeed we found that we could generate the lesion that was identical to that that we observed with hemoglobin. So the NO involvement in the mechanism seems to be substantiated by that observation.

Well, we eventually decided to go into human testing and the rationale was the fact that, again, there was no functional effect that we could detect. It appears

to be that only a small part of the cells even in the most sensitive species are sensitive to this particular effect. In fact, we were required to do a repeat dose study to enter into a clinical trial -- some clinical trials in Europe. And even at accumulate doses up to 112 grams per kilogram; we saw no greater tissue involvement than we did with the 2 gram per kilogram dose.

And another thing that helped set the context, at least for me, was a discussion that I had with Michael van Essen at the LDS Hospital. At the time, he was head of the department of internal medicine but a practicing cardiologist by trade. I took him through all of these data in great detail. And he mentioned that, well, typically they cause more damage than that when they're doing an angioplasty procedure. So given the fact that that occurs, the fact that you see it with pressors, we thought that the risk was worthwhile and acceptable.

Now, the question, of course, is what's going on in a man. And unfortunately, it's still unclear. One study that we did in cardiac bypass patients, we were looking at DCLHb in lieu of red cell transfusion, suggested there was no difference in the myocardial

specific enzymes between the two groups. They were both elevated because this -- these were cardiac patients subsequent to cardiac surgery. But basically, the investigators did not feel that the hemoglobin caused issues. And of course, we were sensitive of that in light of the preclinical observations and also the concerns about the vasoactivity effects.

One question that's come up is whether autopsies would be useful and it's something we discussed and thought about. And we concluded probably not for a couple of reasons. One is from a practical standpoint, understand that it is often difficult to get permission on a consistent basis to perform autopsies on patients. And the other reason is that in some of the patient populations, there would be a high background anyway.

And thinking about what we could do to elucidate this because these data now are anywhere from 10 to 15 years old is whether later generation assays might be more sensitive. I believe there are subsequent generation component assays. There's now a company that has human cardiac tissue assays although I'm personally leery about extrapolating from cell culture results.

Are there some newer in vitro scanning methods that could gives us a better idea of what's going on with regard to this particular finding? If so, I would suggest that, among other things, we should probably perform on a -- some population, either volunteers or patients who have a lower background pathology so that we'd have a greater sensitivity to detect whether something's going on there.

I've just got two slides on basal activities since this has been discussed probably now to the point of nausea today. But I did want to mention a couple of things that, I think, might be relevant to the discussion of the clinical results. One is that, again, this is something we started seeing around 1990 overtly manifested as an increase in systemic blood pressure.

I believe that it's highly correlated with the extravasation of hemoglobin into the interstitial space and NO scavenging. I believe the preponderance of data supports that as the hypothesis not to say that there aren't other things going on because it's mitigated by slowing down hemoglobin extravasation or interaction with NO.

This is something that tends to manifest and

maximize at relatively low doses for the HBOC world. And there are a number of things that can counteract this effect with drugs, anesthetics, fluid manipulations, and free (phonetic) treatments, which can also confound, if you will, the observation of this clinically.

And again, this varies in species, tissue, and vessels. And I want to emphasize this because a lot of times I've heard and continue to hear vasoactivity mentioned as if it is a global phenomenon. But the fact is it occurs differently even in the same vessels within the same tissues.

We did isolated vessel work. And to maybe diverge a little bit to answer the question that was raised this morning, I don't think that vasoactivity is exactly the same thing as hypertension because you could have local vasoactivity going on that's not going to be manifested as an overall increase in mean arterial pressure and which after all is sort of a weighted average of everything that's going on.

So those things aren't exactly the same. But one manifestation of it occurring is an overall increase in blood pressure. So it's one thing we need to keep in

mind.

And this is just one data set amongst dozens that have been generated. And it's qualitatively typical although quantitatively more extreme than most. This was work that we did with Anil Gulati (phonetic). This happened to be an anaesthetized rat model with a 400 milligram per kilogram dose.

In this particular model, there was a 78 percent increase in mean arterial pressure. And on most rat studies we did, especially conscious rats, it's more like 30 to 40 percent. And this was associated with an increase in total peripheral resistance. But in this case, the cardiac output actually went up. There was no significant change in heart rate.

In other models, sometimes the cardiac output does go down a bit but the real issue that we were worried about was what happens to blood flow, especially in vital organs. And this is just a subset of the data that was published in this paper and there was a whole string of papers. And the bottom line, and this is very similar to what Dr. Greenburg was just presenting with regard to the Biopure product, is blood flow to major critical organs is

preserved.

One exception in this model was the heart, where it actually went up by several fold. That's a more -- a larger increase than we typically see. But usually, we do see an increase in blood flow to the heart. And that was important because there are papers in the literature with isolated heart models which clearly show vasoconstriction. So in the whole animal, the response in this case is different than that observed with some of the isolated organ models.

Animals -- one of the other things that gave us confidence to go forward into people, because of the fact that it appeared that in fact the blood flow was preserved where it should be preserved. So now I'm wanting to change over into discussing a little bit about the clinical experience.

After going through phase 1 and phase 2 studies. We initiated several phase 3 studies at the same time. And two of these were in the trauma indication, the third was in elective surgery. There were two studies, one in the U.S., one in Europe, some similarities in the protocols but also important differences.

In the U.S., the treatment was initiated in the emergency room, and in Europe, the treatment was initiated on scene. Now, these trials, as is well-known, were stopped after an interim analysis; the first interim analysis of the U.S. trauma study, which revealed that there was significantly higher mortality in the treated group. And this is the 28-day mortality for the treated group; a 46 percent versus 17 percent in the control group.

So the question that has been asked and reasked on many occasions is, "what happened?" Well, first of all, neither of the independent safety monitoring committee, or our internal review was able to identify a specific cause and effect relationship. It's also notable that in the European study, that there was not a statistically significant difference between the treatment and control groups; in the mortality it was 42 percent, treatment group, 38 percent in the standard treatment.

Also, there were only 98 patients, total accumulated by this point in time in the U.S. study and the patient population was quite heterogeneous. Literally, ranged in age from 19 to 90; a variety of both

genders, and a variety of background. So there were a number of confounding factors present that make it difficult to define a cause and effect relationship.

One thing that's been noted, and here I'm basically -- throughout these quoting and deriving results from the studies that have been published, there are -- I think, about 8 to 10 papers are abstracts on these two studies. There was a difference in the baseline mortality risk to a certain extent. There were eight patients who had pre-hospital traumatic arrests and seven wound up with a treatment group. And patients in the treatment group also tended to have lower diastolic pressure and higher base deficits.

There was a lengthy post-op mortality analysis that was published by Ed Sloan and the collaborators. And it is actually here today and tomorrow. And basically what they found was, of the 32 patients who died across the study, the deaths were expected in 30 of the 32, and of the two remaining ones, one was in each group. Another important observation, and I think one that emphasizes the difficulty of doing studies in this kind of environment is that although the objective of the DCLHb clinical trial

was to identify patients with intermediate mortality risk, so that one could have a reasonable sized study that would detect the difference. In fact, the distribution of mortality was bimodal; with a large number of patients at each end.

And this was not because of lack of effort. Our clinical staff and our collaborators spent about 2 years defining inclusion and exclusion criteria to try to carve out that reasonable middle ground, but we still wound up with this kind of distribution. And I think it's a challenge that we still have in defining those criteria for this kind of patient population.

And I think Ed's going to comment more about that tomorrow, about maybe some ways that that can be adjusted to make these more reasonable studies. Another peculiarity of this study was that the mortality rate changed with time. If you look at the mortality rates among the first -- the very first patients that were enrolled, at the clinical sites, it was 62 percent in the treated group and 0 percent in the control group. Subsequently, these were much more equal and actually not significantly different.

So there is a question. What was going on here in the initial patients and I'm -- I have one hypothesis later on, that may be addresses that. It's also worth noting that the patients were expected to have a 40 percent mortality on the basis of prior experience of the investigators. So if you think about it, amongst the two treated and two control groups in this -- these two studies, three of those four had a mortality that was similar to that -- the control group in the U.S. study was much lower.

Another point I want to make is that we were testing the hypothesis that addition of DCLHb to standard of care would improve survival. That did not appear to be the case, and there was a subsequent study, which has not been published that suggested that actually the combination of DCLHb and large volumes of fluid gave you an adverse outcome.

Most of the studies we did were hemorrhage resuscitate and have moderate amounts of other fluids. One study that was published further to this point was out of George Kramer's lab, it was specifically looking at a sheet model at the volume expansion effects of -- in this

case DCLHb solution versus an oncotically matched albumin solution.

And what George found was surprisingly the DCLHb had twice the volume expansion effect of albumin. Now, we expected DCLHb to be a volume expander, because it has a significant amount of colloid osmotic pressure, but not that much. So I think these observations are interesting in light of a difference between the European trial and the U.S. trial.

One of the exclusion criteria in the U.S. trial was patients resuscitated with more than 1 liter of fluids, that was not an exclusion criteria in the U.S. trial, and in fact the patients average about (inaudible) pre-hospital and got an additional 4 liters in the emergency room. So I would like to suggest that one issue of this trial and perhaps others may -- is that there may have been an adverse interaction between DCLHb and large volumes of other fluids.

Another point I want to make is that HBOC solutions have multiple properties that are important to tissue perfusion and oxygenation. Of course, oxygen transport, which is why we're doing all this, vasoactivity

has been discussed. Oncotic pressure, I think, actually is very important in affecting perfusion, viscosity and even just the -- even in the absence of oncotic pressure, the amount of fluid volume in a number of these patients is substantial.

These properties have very different dose response functions. And I've tried to illustrate that on this slide. And I've actually used rodent data, because we don't have human data as far as the response out here, but the human data that we did accumulate suggests that it follows the same kind of dose response behavior if you look at the maximum change in blood pressure versus dose. And you see it's again manifested at low concentrations and then it maxes out at what is -- and this has been converted to blood volume equivalents at about one unit worth.

If you look at the augmentation of oxygen transport, and this was just a calculated value assuming additivity, and it's probably not that simple. But the point here is that this is a very different curve, and if you were to plot the effect of oncotic pressure, you would probably get yet a third function, which would be

different from these two, and I should note that in both of our trauma studies, the dosage range was between one and two blood unit equivalents.

So we were operating at this range, which as you can see from those curves maximized the effects of vasoactivity whether they're good, bad or indifferent. But the effects of some of the other characteristics, additional fluid volume oncotic pressure, et cetera were not maximized. And I was very interested by Dr. Greenberg's comments, because I think this may be another manifestation of the interesting analysis that they've performed about hemoglobin dosing and whether we in fact have been at the optimal place.

So it's also interesting that, in their published papers, both groups of physicians that were involved in these studies suggested that inadequate dosing of DCLHb possibly contributed to the lack of a positive effect. I think there are other possible interactions that need to be contemplated perhaps not so much -- excuse me -- in the trauma study, but in surgical studies is the interaction HBOCs with anesthetics.

We did some isolated vessel studies. Actually, they were done at Uniformed Services University with whom we are collaborating. It showed that halothane actually tends to blunt the vasoactivity of DCLHb, isoflurane does and Propofol is kind of in between. The question may be what about the interaction with other anesthetics. I don't think these have been systematically explored. What about shock factors?

As already have been mentioned, there were some literature that HBOCs under some circumstances in rodent models can enhance the lethality of endotoxin and there are some valid criticisms of those studies as to whether they're clinically relevant. But the fact is that it does occur, HBOCs tend to bind endotoxin, and that's an issue we all wrestled with during manufacturing.

What about the interaction with other cytokines, hormones, stress factors, that are running around? I'm kind of lapsing into my NIH advisory committee mode here, but I think these are several areas of additional research that might be of benefit. And I just put these data out, because there may also be an interaction with the type of trauma, both in the DCLHb study and I noticed in the

PolyHeme study, there was higher mortality in patients that endured blunt trauma versus penetrating trauma. So there may be some differences in those two states that affect the response of the patients to HBOCs.

Another topic is product learning curve. One of the anecdotal comments that our investigators made was that patients tended to improve immediately after receiving DCLHb, improvements in vital signs, skin color, mental status. And this is something we saw in a lot of our animal studies. It was -- it's rather amazing if you haven't seen it to see a hemorrhaged animal respond literally within minutes to resuscitation with an HBOC.

However, another comment after that was that they thought the patients were doing well, they came back a few hours later or maybe the next night and the patient had crashed. In that regard, it's interesting to note, among the patients who died within 24 hours of infusion, the DCLHb patients received less blood fluid.

So it raises a whole bunch of interrelated questions as to whether the patients in fact looked better than they were or were they in fact better, but not for a long enough period of time, or as long as the investigator

expected, or is there a delayed adverse effect. Would in fact treated patients have done better with more DCLHb and/or some additional intervention?

And this is one thing that I think might have affected the initial patient results versus the subsequent patient results. I suspect that once this occurred with the physician, they were probably much more attuned to monitoring the patients more often or more closely. Again, that's a hypothesis, but perhaps a learning could be taken away from these studies. One thing I want to mention is what didn't happen, because I've heard several versions of this hypothesis bandied about, and it's basically that okay, you gave this vasoactive substance to these patients and they bled out and that's why they had a problem.

The fact is that's not supported by the data. And this is a direct quote from Ed's paper that in fact "bleeding nor higher blood pressures were systematically observed in patients who received DCLHb." And I wanted to mention this, because actually in this study and in the phase 2 study, even though we know the DCLHb is

vasoactive, the average blood pressure in treated patients was within a few millimeters of the control patients.

So there's something different about these patients, it's not to say vasoactivity isn't going on. But it's not manifested as the increase in overall mean arterial pressure. And perhaps because the trauma patients are in shock or the effect is blunted by some of the other factors or just the ongoing volume depletion. So just kind of summarizing why did things not go well in that particular study. Well, there's multiple reasons and they're not mutually exclusive.

Now, there's certainly evidence that sicker patients were interviewed in the treatment group. I think, it's possible there were adverse interactions with concomitant therapy, with the wisdom of hindsight. I believe, the patients may have gotten too much other fluids, not enough HBOC, and there may have been a lack of appreciation for the duration of response to DCLHb.

And this then gets us down to, well, the possible adverse side effects of DCLHb. So I think to be fair and balanced, I wanted to throw out three possible adverse effects that might be worth exploring further.

There was a study published in 2005 by this person whose name I can't -- I don't know exactly how to pronounce, but he was looking at the effect of pre-treatment of rats with actually two different HBOCs, one of which was the Baxter product and he emarginated to see whether it primed them to survive a subsequent hemorrhage better. And he found -- he emarginated, that was the case, but actually with the two HBOCs, they did worse, the time to decompensation was less, and at some doses the mortality was higher.

So I found this a bit disturbing, and these authors looked at a number of different things. The one thing that caught my eye most was the fact they looked at ATP levels in the liver. Of course, shock depletes them, but they found it was depleted more in the animals that were treated with the HBOC.

Now, liver is a major organ for DCLHb metabolism. We know that from the pharmacokinetic studies, and I mentioned that we observed the transient centrilobular necrosis in the liver in our tox study. So livers get real busy when DCLHbs is onboard, and perhaps that stress combined with a subsequent hemorrhage, causes problems.

Another issue is pancreatitis. It was observed in a couple of our studies, and it was noted in the FDA summary and that imbalance is true. The thing that's puzzling is, this was not a target organ that we turned up in any of our preclinical studies. We did not see pathology. We did not see adverse effects of blood flow to the pancreas.

And yet, something happened in some of our patients. Now, six of those occurred in the European trauma study and four of those patients had trauma to that area. So it's a little bit hard to assign causality there.

The other five were in the -- actually in the phase 3 U.S. surgical study, and one of the things, it's not in the paper, but I recall from my reviews was that, upon further review, a couple of those patients had previously undiagnosed gallstones, which is also a risk factor for pancreatitis.

Nevertheless, the fact that in a broader range of patients, we see elevation in pancreatic enzymes suggest that maybe there is something going on that is different in human pancreas as compared to the animals.

A third observation is that, in the literature there is a suggestion that if you infuse inhibitors of NO synthesis, the overall oxygen consumption goes up. And this has been ascribed to a degree of decoupling at the mitochondrial level.

Don't know whether that occurs with HBOCs, but if it does, it might be a little bit counter-productive in situations where we're in fact trying to enhance oxygen delivery to tissues. So these are perhaps three areas we are exploring further.

So as far as where we would go from here, kind of, presaging tomorrow's discussion, I think, it would be very useful to have a physiological map of human response. I'm not sure we need another rodent study until we understand what's going on in humans.

And I know that's easier said than done, but again, perhaps this could be an area of emphasis for NIH funding. Wouldn't it be nice to know what the blood flow was like in human critical organs, human pancreas, human heart, and how that affects the organ pathology.

So I don't know whether the techniques have been developed and not applied or need to be developed, but I

think better, minimally invasive monitoring of some of these questions would really help to inform our further development and understanding of HBOCs, and for that matter blood transfusion.

I think we need a better understanding of HBOC interactions. I've already talked about fluids, anesthetics, shock factors, drugs of abuse is another area. Actually, Anil Gulati published one rat study which suggested ethanol can inhibit or alter the vasoactive response of rats to DCLHb.

Again I'm not sure whether those are clinically relevant concentrations, but it does raise the question, what about other drugs of abuse? And I don't think this has been systematically investigated as well. So in conclusion, I would just state I haven't talked about blood sparing, but I think there is in fact evidence that HBOCs can reduce the need for blood transfusion.

And it is my sense, that in fact HBOCs have benefited some patients, admittedly a number of these observations are anecdotal. However, some patients are not benefited, and in fact may be harmed. So it seems to me that one of our issues that we need to get at, is how

we identify better of those two patient populations.

I think the field still struggles in comparing efficacy and safety to a product that after all has never gone through the kind of approval process that's required for HBOCs. I personally believe that the adverse effects of blood are generally under-appreciated and efficacy has been surprisingly hard to demonstrate, as individuals have started to do clinical trials with blood in a controlled randomized types of situations.

And I think new clinical paradigms will be very helpful, especially in emergency area where we are fighting this very unfortunate distribution of patients. Despite this, I personally believe that HBOCs still have enormous promise, and that ultimately we will get there; I hope sooner rather than later. So that's it from me. Thank you.

(Applause)

MS. ALVING: Thank you very much, Tim, for a very fine overview. Our last speaker for this session is going to be Joseph De Angelo. And he is the chief development officer at Apex, and he also has had undergrad and grad training at MIT, and has a very strong scientific

interest in NO.

And after his talk, we will collect your cards, and maybe give you about a 10-minute break. They can take 10, don't you think? And then we are going to have a panel, but let's try to get down to maybe some of the core issues. So we look forward to your talk. Thank you.

DEVELOPMENT OF PHP AS AN NO SCAVENGER IN THE TREATMENT OF DISTRIBUTIVE SHOCK

MR. DE ANGELO: Okay, great. Can everybody hear me okay? So I'm going to be talking about the development of PHP as a nitric oxide scavenger in the treatment of distributive shock. And I want to point out that distributive shock; I'm talking about, really a high cardiac output, a low systemic vascular resistance form of shock, most common form being septic shock.

And this is fundamentally different than what the other speakers have been talking about in terms of treating hypovolemia. So not only our HBOC is different, but we are not an HBOC, we are treating a different patient population, we have a different mechanism of

action, and in particular, we use a much lower dose rate and dosing regiment.

I want to point out the SOFA scoring system just so that you can get an idea of our patient population. Basically we define cardiovascular dysfunction or hypotension -- hypotension by the requirement for catecholamines to maintain a blood pressure of 70 millimeters of mercury in patients that are adequately fluid resuscitated.

And if you look at Grade III, Grade IV cardiovascular failure based on the SOFA analysis, it's about 15 percent of the ICU admissions, which is a relatively large population in the United States and in Europe. PHP is pyridoxalated hemoglobin polyoxyethylene conjugate. It's a chemically modified human hemoglobin that's pyridoxalated and then conjugated with polyoxyethylene.

It has an average molecular weight of about 120,000. The polyoxyethylene is bifunctional, and therefore it can form ditetramers and tritetramers, in addition to the monotetramers. This has been discussed before, people have explained about the extravasation of

nitric -- of the hemoglobin and the scavenging of nitric oxide.

But what I want to point out in addition is that the hemoglobin remains extracellular. So the scavenging of nitric oxide is extracellular. And that means that the tendency will be to interfere with paracrine effects, but not with autocrine effects. And many of the effects of nitric oxide are in fact intracrine. So a lot of the nice -- NOS isoforms are actually coupled with their signaling target.

And therefore, not readily accessible to a extracellular nitric oxide scavenging. Normal levels of nitric oxide play important physiological roles. There is absolutely no denial of that. However, when there is excess nitric oxide, a lot of things can go wrong, the most obvious being vasodilation, but excess nitric oxide pathological levels are also associated with adrenergic receptor desensitization, vascular leak syndrome, mitochondrial dysfunction, myocardial depression.

Platelet activation, now of course we know that normal levels actually prevent platelet activation, but excess nitric oxide can also cause platelet activation.

So we're not trying to get rid of all of the good effects of nitric oxide, but we are trying to reduce the excess of toxic levels that cause the pathophysiological effects.

It's important to recognize that in nitric oxide induced shock or distributive shock it is a final kind of mediator, which is independent of etiology, independent of redundant cytokine pathways. It does have direct toxic and pathophysiological actions, and you can see an immediate effect when you use it on a patient. So you know you are dosing a patient that has excess NO, and you know how much to give them and how long to give it for them?

We know that catecholamine save lives, but of course, they like blood, were really never tested in a randomized phase III trial to demonstrate to improved mortality. But we also know that they have undesirable side effects, and probably two of the most important ones would be the increased myocardial work, where you could have somebody in a hyperdynamic state for days, which is like running a marathon for days.

The other being, of course, adrenergic desensitization, so a lot of these patients actually

become hyporesponsive to the adrenergic agents, both alpha and beta adrenergic agents. This was the first study we did with PHP in healthy volunteers, and this was a 30-minute infusion of either the control 50, 100, or 200 milligrams per kilogram per hour.

So I want you to put that in perspective in terms of dosing of what dose rates that were using versus dose rates that are being used in the other indications for HBOCs. There was no effect on mean arterial pressure in these patients. However, there was an effect on heart rate.

Now, there has been discussion about the decrease cardiac output. We didn't have Swan-Ganz in the healthy volunteers, so we don't know what happened to cardiac output, but if you assume that there was no change in stroke index, it means that they had a drop in cardiac output, which was essentially, if you look at the graph, you could see it's essentially dose-dependant decrease in heart rate.

The most -- the simplest explanation of this is that it's a baroreceptor response. So even though you see no effect on mean arterial pressure, the drop in heart

rate suggests that systemic vascular resistance is increasing and that there is a baroreceptor response in normals to compensate for that.

However, when we did the exact same dose rates in shock patients, and these were presumed septic shock patients, which meant that they had a systemic inflammatory response syndrome, and they had catecholamine dependant -- fluid-resuscitated catecholamine dependant shock.

In this case, the three doses, there is really no difference at 5 minutes; you can see between the 100 and 200 milligram per kilogram per hour doses, so the dose rate has already really plateaued at a 100 milligram per kilogram per hour in terms of its vasoactivity.

However, at the 200 milligram per kilogram per hour dose, one patient experienced a 40 millimeter increase in blood pressure within the first few minutes, which resulted in a serious adverse event of pulmonary hypertension and right heart failure.

So we concluded right then and there that this exceeded a maximum tolerated dose for this indication, and we did future studies below 100 milligram per kilogram per

hour dose rates. The next dose ranging study we did, which was a uncontrolled open label ascending dose study; it was at 20, 40, and 80, or 80 milligram per kilogram per hour. And here you see that really at this dose rate -- at these dose rates, the change in mean arterial pressure is much more modest, and only reaches a few millimeters of mercury at the 8 hour point.

However, if you look at the effect on catecholamine dose, you can see that the 40 and 80 millimeter -- 40 and 80 milligram per kilogram per hour dose rate groups, all had very similar reductions and fairly rapid reductions in catecholamine doses. The 20 milligram per kilogram per hour dose was effective, that was in reducing catecholamine slowly, and it was also effective in increasing mean arterial pressure slightly.

So it was a vasoactive dose that we wanted to work at for future studies. In animal studies, we had shown that this dose was effective in maintaining systemic hemodynamics without affecting adversely the pulmonary hemodynamics, and so we chose to work with that dose in future studies.

This is the study I'm going to spend most of the

time talking about, and I will just say that this isn't pressed now in critical care medicine, which is why where the big blank column in everybody's tables that have been presented so far on results.

This was a placebo-controlled, randomized, open label study at 15 sites. It was PHP for standard of care versus placebo plus standard of care. It was only a study of 62 patients because we terminated it early because of protocol design issue. So we had a requirement for pulmonary artery catheters, and at the time we started the study, pulmonary artery catheters were in common use and they fell into very rapid disuse in the United States.

And we had about 800 screen failures for NO PAC. So we decided to terminate the study and redesign the protocol. But we analyzed the data, and I'll present that now. So this is the inclusion criteria for SIRS, which was one of the two inclusion criteria. And this is the inclusion criteria for shock. Again it was basically fluid resuscitated and a requirement for a catecholamine to maintain a blood pressure.

This is the dosing regimen, again, 20 milligram per kilogram per hour, continuous infusions. We had a

maximum dose weight of a 100 kilo and this is to minimize the dosing. We had a maximum duration of infusion for 100 hours. We had a standardized fluid resuscitation protocol, a standardized vasopressor weaning protocol, a standardized PHP weaning protocol, and a standardized vent weaning protocol.

The first thing I'll show you is that the patients with distributive shock were clearly a patient population that had elevated NO levels, the way NO is generally measured in humans is to look at plasma nitrite/nitrate which is the end product, a metabolic end product of nitric oxide.

The normal range is about -- it averages about 20 micromolar, and you could see that essentially all of the patients exceeded this baseline level. Getting -- starting to get into some of the data then, the mean arterial pressure was significantly increased within 30 minutes of the beginning of the PHP infusion. So these patients are on catecholamines, at baseline, and so this is on top of the baseline catecholamines.

The heart rate also decreased within 30 minutes of PHP infusion and continued out throughout the study.

In terms of vasopressor use in these patients, we looked at when the first conventional vasopressor was withdrawn. And here I'm just looking at the survivor population, because it's difficult to integrate patients who die rapidly in the study in terms of how long they were on a catecholamine.

And if you look at this, the survivors were on for about 14 hours versus 26 hours on the placebo, which had a p-value of 0.07. One of the problems in small studies and in many studies is the baseline imbalances, and this was the baseline APACHE score for these two different groups, and you can see that the PHP group was -- were severely ill at baseline based on the APACHE scores, and that that was consistent for the total group as well as the subgroup of survivors, and the subgroup of non-survivors.

Predominantly, one of the major factors in the baseline imbalance was renal function, and here you could see that -- again this is using SOFA scores, and not APACHE, but the -- which is based on creatinine levels. There were more Grade II and above SOFA renal scores in the PHP survivor group, and there were more Grade II and

Grade III in the non-survivors as well compared to the placebo groups.

So this is a factor that has to be taken into consideration when you are actually looking at the data, because the two groups have very different predicted outcomes. This is the Kaplan-Meier survival curve, there is a divergence that begins early, but out by day 16, the lines converge, and remain converged, and one thing I will point out about the survival is that if PHP is in fact treating shock, you expect it to have an early effect on survival.

Because that's when patients are in shock and are dying of shock. So shock generally results within a few days or patients do not survive. When you break down the data, there was only a one percent difference at day 28; the maximum mortality difference on day 10, however, reached 18 percent favoring PHP.

The unadjusted risk ratio using a Cox proportional hazards model was 0.9, favoring PHP, with a very large 95 percent confidence interval, and when we did the adjusted risk ratio based on prospectively defined covariates, the adjusted risk ratio was 0.79 favoring PHP,

again with a very large 95 percent confidence interval.

And in a study of this size, it's -- I think it's pretty obvious that we would not expect to see a significant difference in mortality, but at least we did not see a numerically negative trend. One of the endpoints that we used in this study was based on organ function, and the way we evaluated organ function was based on medical interventions.

So the medical intervention for cardiovascular dysfunction was vasopressor utilization, the requirement for vasopressor utilization. And the intervention for pulmonary function was mechanical ventilation, and the use of these and the weaning of these was protocol defined, so we attempted to standardize them.

There was a -- and this was basically one of the primary endpoints, but -- that was originally proposed in this study. There was a two-day difference in cardiovascular dysfunction favoring PHP, and a 7-day difference in vent use, favoring PHP in the survivors, the combination of those only attained a p-value of 0.2.

We also looked at other medical interventions, not from the point of efficacy, but from the point of

safety. So we did not expect PHP to worsen medical interventions for liver, kidney coagulation, or CNS function. And just for example, liver dysfunction was considered, but the medical intervention for liver function was considered to be fresh frozen plasma. For kidney dysfunction, it was considered to be renal cell replacement therapy, and for coagulation it was considered to be platelets.

So there were really no differences, PHP was never worse, numerically worse than any of those, there is no difference obviously in the p-values. If you look at the days in the ICU for the survivors, there was also a 4-day difference favoring PHP with a p-value of 0.2, did not attain significance, but this would be an important outcome for future studies.

One of the criticisms of Xigris by the FDA advisory panel was that there was an improvement in survival rate at day 28. However, the surviving patients remained hospitalized. And if you added the deaths plus hospitalized, the two groups were roughly the same for Xigris. And the FDA advisory board criticized that saying basically that the drug wasn't effective because the

patients didn't get better, they just lingered in the hospital.

So one of the things we looked at was discharges. Again, we are talking about small numbers, nothing significant, but this -- again numerically, favored PHP in the study and will be an important thing to look at in future studies. This is just a Kaplan-Meier of the day -- the survivors in the ICU and you could see the divergence that occurs beginning right around day 9 in terms of survivors getting out of the ICU.

This is what survivors on catecholamines look like, you know, they obviously look like they are totally convergent lines, but if you really look at day 2, there is a 25 percent difference. There are -- 75 percent of the placebo patients are still on catecholamine compared to 50 percent in the PHP group.

And again that would be really one day less in shock or two days less in shock really could have significant clinical benefit to patients. So it's something that we intend to look at carefully in future studies. The Kaplan-Meier for survivors on vent is similar in many ways to the ICU, Kaplan-Meier, because

this is one of the things that keeps patients in intensive care units and this divergence also begins at about day 8.

In terms of safety, if you first look at treatment emergent non-serious adverse events, there is no difference with a slight positive trend favoring PHP. If you look at treatment emergent serious adverse events, there is no difference with a slight numerical trend favoring PHP.

The one area where we had some imbalances was in cardiac events, and I'll show you three sources of different looks at that data. There is an unblinded investigator -- this one actually summarizes two of the different ones, the unblinded investigator that did not have prospective definitions, a blinded investigator that had prospective definitions and you could see what emerged was that the investigators called three SAEs for myocardial infarcts compared to zero in the control group. The investigators also called two SAEs for a myocardial ischemia compared to zero in the control group.

If you go to the blinded reviewer however, it was the opposite. There was one versus five. One thing that's evident in this is that none of these values are

really significant in terms of statistics, because of the small sample size, but there is definitely a difference of opinion between the blinded reviewers and the unblinded reviewers, and that's something we'll have to deal with in future trials.

This was something that we published in the critical care medicine article and the reviewers were somewhat critical of this, because our blinded reviewer was a single blinded reviewer. So we repeated this exercise just to satisfy ourselves. We used blinded adjudicated review using the current international consensus definition of myocardial infarcts.

And they -- the blinded adjudicated reviewers basically asked all these different questions. The first question of course is, is there a change in cardiac biomarkers, preferably troponin, and what you can see first of all is that there are a lot of troponin abnormalities in this patient population.

The ECG changes were two in PHP versus four in placebo, and this is based on the consensus definition. So this involves ST-T changes. So this is looking for STEMI basically. In terms of ECG showing development of

pathological Q waves, it was zero versus two. We had no imaging evidence, so there was none. There were no sudden unexpected cardiac deaths in either group. There was no PCI.

There were no pathological findings, because we didn't do pathology in this study. Another thing to note was that there was evidence of pretreatment myocardial infarction in five versus three of the patients in terms of pathological Q waves on pretreatment ECGs.

And finally, when they did the assessment of whether MIs occurred or not based on whether there was a abnormal cardiac biomarker and an abnormal ECG finding, and again it was two versus four, PHP versus placebo. So what can we really say? Well, you know, the unblinded investigator -- none of these can be ignored, and I'm not trying to ignore any of them, but the reality is the unblinded investigator found an excess of MIs, myocardial ischemia.

The two unblinded found it to be the other way, and I think what we can really conclude is that we can't make a firm conclusion based on this data set, because it is too small and because we didn't use a prospective

definition of an MI that would be uniform throughout the study.

So that was a deficiency in protocol design, so future studies will require a blinded adjudicated review of all cardiac events using a prospective protocol defined definition. In terms of selected treatment emergent SAEs, I threw these in after a lot of publication started coming out citing certain areas of concern.

There was no imbalance in renal and urinary disorders. There was no imbalance in respiratory thoracic and mediastinal disorders. In terms of treatment emergent adverse events, there was no imbalance in thrombocytopenias, no imbalance in afibs, some maybe a trend in bradycardias four versus one, one pancreatitis versus zero, I don't think we could say much about that.

And two, decreased cardiac indexes versus zero in the PHP compared to the placebo. Again, these are adverse events though and not serious adverse events. So they are not life-threatening or potentially causing any long-term morbidity in these patients.

Continuing with more of the selected treatment emergent AEs there was no apparent imbalance in the renal

and urinary disorders, or in the respiratory, thoracic, and mediastinal disorders. And in terms of vascular disorders, there were possibly more hypertensive-type events versus more hypotensive type of events, which again is what you might expect considering we are using a vasoactive substance to treat hypotension.

One thing about the severity of pulmonary treatment emergent AEs is that there were equal numbers, but there actually were more severe ones in the placebo group, not serious, but severe versus moderate or mild. So that pretty much covers everything I can tell you about the adverse event profile and the study.

If you look at the hemodynamics based on the pulmonary artery catheter data, this is mean pulmonary artery pressure, there was a slight increase. However, what I did also will show you the paired data, there is a lot of missing data in these studies. It's difficult with these patients in their critical state to collect all the samples all the time. And pulmonary artery catheters are particularly difficult to use in these patients.

So there is missing data. So I'm also showing a paired data here for mean pulmonary artery pressure and

there is no meaningful difference there. A cardiac index does drop, this is the paired data. You could see the drop in cardiac index that occurs very quickly within the first hour. However, there is no change in stroke index.

This is the paired data in stroke index. And that's important if you are talking about cardiotoxicity, because a drop in heart rate without any effect on myocardial contractility, which would be reflected in stroke index, would not be suggested I think of a cardiotoxic event.

In terms of Phase III design criteria, what we're looking for is a population that has excess nitric oxide, distributive shock defines this population, but the excess nitric oxide should also be related to the outcome and in this case, we are going to look at mortality. And not all mortality and distributive shock is due to excess nitric oxide or even to shock.

So the patient population we're looking for should be a subset of distributive shock, where mortality is attributable to shock unresponsive to standard of care, which is catecholamines. I'll go through this very quickly just to give you an idea of our thinking on this.

This was a study done by Bruno Levy (phonetic) and collaborators in France, at ten sites in France with a 110 patients. And they did a dopamine challenge of patients that were going into shock. And if these patients responded to the dopamine challenge, they were put into the dopamine, the dopamine responder group, and if they were non-responsive, they were put into the other group.

The difference in Kaplan-Meier is rather staggering. The overall 28-day mortality in this group that responded to dopamine challenge was about 16 percent compared to 78 percent mortality in the group that did not respond. And we felt that this was potentially a very good way of finding the patient population that we are looking for, which is one that has a high mortality due to failure of catecholamine therapy.

We wanted to confirm this in a larger database, in a different database, so we were actually very fortunate that Dr. Nandakumar (phonetic) is a collaborator in CATSS, which is the Cooperative Antimicrobial Therapy in Septic Shock study group. It's a database of about 5,715 -- exactly, as a matter of fact, 5,715 septic shock patients

from 26 sites in the U.S., Canada, and Saudi Arabia. There are some abstracts published, but the (inaudible) was a personal communication.

We first of all looked at validating the Dopamine challenge that was observed in the Bruno Levy study. And you could see the Kaplan-Meiers of the two different groups. The Kaplan-Meier for the non-responder group is almost identical to the one reported by Bruno Levy; very rapid mortality within the first few days, and overall, about a 75 percent mortality -- close to 75 to 80 percent mortality by day 28.

The difference between the two groups here is that the Dopamine responders also had a higher mortality in this particular group than in the Bruno Levy study. One of the things that happens to us all the time is that standard of care changes while we're planning our study. And one of the things that's happening right now is that Dopamine is falling into disuse as first-line presser in treating distributive shock and norepinephrine is becoming the favored first-line presser.

There is no good evidence that norepinephrine is superior to Dopamine in terms of mortality effects, but

it's a stronger presser, and fewer patients fail to respond to norepinephrine. And for that reason, there is a tendency for clinicians to use norepinephrine as first line.

So what we did then was look at the exact same database, and this time look at norepinephrine first-line use. And in this case, there was about 3,285 patients in the database that received norepinephrine in the first 24 hours of treatment. I should point out that we're only looking at first 24 hours of treatment and not beyond, because we want to get patients as early as possible.

And this is what the Kaplan-Meier looks like for this population. So this population is almost -- this Kaplan-Meier is essentially super-imposable on the Dopamine groups that I showed earlier. So it suggests that in fact we could use a norepinephrine-resistant distributive shock subset to define our patient population. And that's the direction that we're moving in, in our clinical trial.

Our proposal for a Phase III, then, is to do a placebo-controlled, randomized, open-labeled, (inaudible) multinational study, PHP plus standard of care versus placebo plus standard of care. We will predict the control

mortality of about 70 percent, and have powered it to CA (phonetic) what we consider to be a clinically significant effect on mortality.

This will be -- a data-monitoring board will provide safety oversight. There will be informed consent required in this study, and there will be blind and adjudicator review of cardiac events by a independent panel. The objectives are to compare mortality at day 28 and to compare safety in terms of adverse event and serious adverse events.

The inclusion criteria are SIRS and shock which will be defined as adequate fluid resuscitation which is protocol defined, and a catecholamine-resistant shock which will be as I showed you in the slide from the CATSS database.

The dosing again is .25 milliliters per kilogram per hour, which is equivalent to 20 milligrams of hemoglobin per kilogram per hour continuous infusion. Standard fluid resuscitation protocol, standard vasopressor weaning protocol, and a standard PHP weaning protocol and a standard vent weaning protocol. All protocol defined and followed by investigators.

So I just want to conclude by saying that I think that the potential benefit of reducing mortality in a -- essentially a population where standard of care fails, and there is no treatment. That reduction of mortality compared to any risk observed to date in completed studies of PHP is favorable.

And so far, our protocol has been reviewed, our study was reviewed by our data-monitoring board, and they recommended Phase III study. And the current protocol has been reviewed by a study advisory board of international critical care experts as well as our data -- a new data-monitoring board. And so that is where we are at right now. Thank you.

(Applause)

MS. ALVING: I think it's very brave of you to pursue shock. That's been a challenge for multiple companies that's -- over the years. Let's now take about a 5-minute break. You could even just stand up and stay in this room. I'd like the speakers to come up here. We are getting the cards. Please deliver your cards.

Those of you who work at the FDA, please be available. You may be called on for a little bit of advice

lest we give out misleading information about what the FDA can do. And we'll resume in about 5 minutes.

(Recess)

MS. ALVING: Can we please have the panel come up and can we take our seats, because there are lots of little white cards waiting for the panel members. And we also are going to have a very short discourse on biostatistics, and so we're going to get a lot of questions just out of the way right off.

(Pause)

MS. ALVING: Okay. Good. Let's just start out. There are multiple cards for everybody. And what I thought we'd start out with is just ask Tim -- Tim has about five or six cards, but we'd like him to answer the most -- well, maybe some of the most interesting ones. If you have written Dr. Estep a card and you don't get your answer, please tackle him at the end of this session.

But basically, Tim, why don't you answer, you know, why exactly did the Baxter study stop and what went on with the recombinant hemoglobin of Somatogen?

PANEL DISCUSSION

MR. ESTEP: Thank you for inviting people to attack me. I appreciate that.

SPEAKER: (Off mike)?

MR. ESTEP: (Off mike). I'm getting signals -- the microphone. So that's unfortunate.

(Laughter)

MR. ESTEP: Is this better?

SPEAKER: (Off mike).

MR. ESTEP: I'm going to try this -- how about this one? How is this one?

(Applause)

MR. ESTEP: Let me begin again. Why did Baxter stop? Well, as I was saying, with the HemAssist if that was what it was being directed to, it was because we would have had to go back -- even if we continued development of that product, we would have to go back, restart a different clinical trial because the futility analysis that was done by the independent data safety monitoring committee suggested there was a less than one in two thousand chance we would be successful with that study.

And we became less enthusiastic about the effects

of vasoactivity. We actually thought it was an advantage on the basis of a lot of our preclinical studies with regard to the redirection of blood flow to critical organs, and actually some of the things Dr. Greenburg mentioned that they found with their HBOC and -- so that was one factor.

The other factor was we were -- this was all occurring when we were acquiring Somatogen and the ability to modify hemoglobin with recombinant techniques, which opens up a lot of other possibilities. And specifically they had been working on a technology with input from Dr. Olson who is here, to inherently modulate and reduce the rate of interaction with NO which we --

So we basically decided to start with a white sheet of paper knowing what we had learned and other people learned, and develop a second generation recombinant-based product. Now, with regard to the recombinant-based products, the Somatogen sort of equivalent to HemAssist was Optro, and we terminated development of that because it basically had the same kinds of characteristics as HemAssist.

It was also a stabilized tetrameric molecule. So

we spent the next several years developing a recombinant product which was polymerized and mutated to inherently reduce the rate of interaction with NO, specifically to address cardiac lesions, vasoactivity, GI effects. I was actually amazed at how many things were made better by inherently reducing the rate of interaction with NO at least in our preclinical models.

Unfortunately, somewhere along the way, we created a problem that we didn't have with first generation product. We did a couple of what turned out to be a very small Phase I clinical trials with the second gen recombinant product, and we saw a complement activation. We saw it at very low doses. It was evident clinically, it was evident biochemically.

So we think what happened was that we inadvertently created an epitope that kicked off that system. And that was particularly frustrating, because we worried about that. We did -- and this is going to be a familiar refrain, but we did a lot of in vitro and in vivo preclinical testing to see whether that would be an issue. We did not see a signal in those models, but we saw a signal in man.

So that was also about the time that Baxter stopped hitting its quarterly numbers. It was not a very good time to be flunking out of clinical trials with the project. And the company underwent a major reorganization, but they decided not to do that anymore. So that's why that was stopped.

Another question why HBOCs would produce vasoconstriction in some vessels but not in others, my simplistic opinion -- and actually, I think there is some evidence for that -- is that different vessels use the constitutive production of NO to different degrees to regulate homeostasis. I believe this has been observed in a variety of different tissues.

So if what you're doing primarily is getting rid of NO, then you would expect the response to vary from vessel to vessel, tissue to tissue, different species. And I think that's at least to a first approximation what we saw and why you get different responses. There may be other mechanisms, but the facts are that we looked at, that the responses are different in different organs and different vessels.

So -- and I think that's important to keep in

mind. This is not a universal effect across all those different tissues.

MS. ALVING: (Off mike) I'm going to give -- I'm going to let you rest for a little while --. We want to give everybody equal opportunity here to get a little -- if you have not had your questions answered, again, please see the panel members at the end.

Okay, Dr. Keipert, I want to toss a couple of questions your way. Do you have any evidence from your clinical trials that Hemospan is acting to deliver oxygen rather than just acting as a volume expander? Is this an expensive volume expander?

MR. KEIPERT: Well, at the moment, you know, from the way the trials are designed, I mean clearly the primary mechanism, if you will, that we're going after, is looking at hypotension as a surrogate marker of Hypovolemia.

MS. ALVING: (Off mike.)

MR. KEIPERT: All right. No, we're using hypotension as a surrogate marker of the hemodynamic effect and the volume expansion of the product. So at the moment, it's correct that the trials were focused on -- the primary endpoint is focused more on the plasma-expanding capability

of the product.

In discussions with EMEA, one of the points they made is that they wanted to show -- we have to obviously show some kind of clinical benefit for approval. And because of that, we developed secondary endpoints, two of which are composite endpoints for organ dysfunction and organ failure.

And the reason they're composite endpoints is because these patients tend to be fairly healthy, so the incidence rate of serious complications tends to be fairly low. And composite endpoints looking at organ function or organ dysfunction, there we believe that if we have profusion of these organs and good oxygenation in the face or in the absence of hypotension, that with a large enough study we would hopefully be able to show some benefits, some decrease in morbidity or perioperative complication.

So that's the part of the trial that demonstrates additional benefit beyond just imply some expansion capability.

MS. ALVING: Why is this being -- is this being done in United States as well, or have you thought about it?

MR. KEIPERT: The reason most of the trials are much further ahead in Europe is just the sort of the history of the development of the product. A lot of the work was originally done in Sweden at the Karolinska, the preclinical work. And that naturally evolved into discussions with the MPA and encouraged us to get into Phase I trials. So before we knew it, we were doing Phase I trials and Phase II trials at the Karolinska.

Meanwhile, an IND was filed in the U.S. which led to the radical prostatectomy trial at Hopkins that took a much longer period of time both initially to get the trial started and also to run the trial. So in the time it's taken us to run one single center Phase II study in the U.S., we've essentially run Phase II and Phase III in Europe.

That's partly, you know, the regulatory environment, it's partly just the clinical development that we had done over there that allows to go further. But it's certainly our intent to continue doing these studies both in Europe and in the U.S.

MS. ALVING: What's the status of the trial that was run at Hopkins?

MR. KEIPERT: That trial has completed enrolment of patients. We are close to database log; we haven't actually seen all the data yet, although we certainly know about the safety findings. But it's not published yet because we haven't unlocked the database yet.

MS. ALVING: Okay. Let's give you a rest for a while. Let's go to Biopure and Dr. Greenburg. Could you comment on the commercial use of your product in South Africa? And also, let's just say do you see any problems with safety, efficacy as you continue your trial or your studies in South Africa?

MR. GREENBURG: We are currently running two trials in South Africa and it is in commercial use. And I would prefer Dr. Levian (phonetic) to get up here and talk about his experience and the experience that he reported last evening. With over 480 patients treated, no issues with management of vasoactivities reflected in blood pressure, no SAEs reported, no myocardial infarctions, none of those things.

The two working trials are a trauma trial HEM-125. It's an in-hospital trauma trial. Results of the first 22 patients were presented at BPAC in December of

2006. That trial is still recruiting, and they are running a trial in cardiac surgery preload -- before going on the pump, a cardiopulmonary bypass. And we do not have much data from that.

We did finish a study, a closer study there in limb amputation and diabetics. We're analyzing some of that data which we find interesting. That's our clinical experience in South Africa at the moment.

MS. ALVING: So you're not seeing excessive reports of MI or -- in some part of the --

MR. GREENBURG: I think if we were seeing -- if they were happening, we would be seeing. The product is being used in the larger cities. We understand it's being used in the smaller towns, in the bigger hospitals. It's being used for the first time by many people. Sales are going up. It -- they like it. I mean I get reports and we can tell you what the social aura if you like.

Weekly, there are reports in the newsletter that says the patient was -- this was done or that was done and it's all very, very good. We also have some interesting anecdotes that come out, but not necessarily related to the patients, but certainly things we should consider. And

they're using it in a wide variety of patients.

We understand there is one surgeon who is so impressed with its use that he tends to prefer it over blood at this point. In many of these hospitals there is no blood available readily, no blood available within a few hours, and it's being used. The product is there.

MS. ALVING: Thank you very much. You may have noticed an extra panelist down at the end of the table there. This is Dr. Tom Fleming who's actually going to be on a panel tomorrow. But he is topnotch biostatistician from the University of Washington. And he's been listening to this entire session very carefully.

And I thought that he could perhaps give us some of his impression of what he's heard from the point of view of biostatistics and looking at clinical trial data. I mean I'm hearing words like "protocol" and how there's been some adjudication and was sort of wondering how is this all done within the realm of clinical trials. And maybe Tom can give us some framework with which to evaluate some of the talks we've heard.

MR. FLEMING: Thank you. What I'd like to do is just take a few minutes. We were talking after the break,

and I was mentioning that we've had a lot of discussion today about design of trials, conduct of trials, and analysis. And there are a lot of complexities that we need to keep in mind when we are interpreting data.

And I was indicating what would have been great is if we would have not only had the presentations that we did, but had critiques of these presentations that were set up, after people had a chance to really go through the protocols, the SAPs, and the clinical study reports in depth and to hear the reports from people that essentially are looking at data in an independent way.

I always say when I read a protocol and the objective of the protocol is to show HBOC is effective, I always say, well, the goal of clinical research isn't to show it's effective, it's to evaluate whether it's effective. And that distinction is critical to objectivity.

And so my disclaimer is I didn't review all these materials in advance, although I did serve on the HBOC-201 Blood Products Advisory Committee in December. So I did get a chance to see those data in more depth. But I would like to just take a couple minutes.

And I'll try to be objective here and comment on each of the presentations but just very briefly to bring out some of the issues I think we do need to keep in mind as we try to understand the interpretability and reliability of the results. So starting with the Sangart/Biopure, we saw in principle two major clinical trials that had 830 patients for prevention and treatment of hypotension.

Earlier in the day, Goldkind had presented, and specifically in section 50.24 the quote that risks are -- need to be reasonable in relationship to the anticipated benefit. Everything is benefit to risk. So if you're showing that you're preventing mortality or reverse morbidity, you have a much higher bar for what's acceptable for risk.

If you're showing as important as prevention of hypotension is, it's not the same as establishing beneficial effects on morbidity and mortality. And so when the totality of the HBOC data do provide at least a clear signal of major morbidity and mortality risks, it seems that if you're going to do a trial that shows an effect on hypotension, it's important to not simply look in that

trial and find out whether there is evidence of excess harm in morbidity and mortality.

The old issue is absence of evidence isn't evidence of absence. If there is a signal for excess risk, what one needs to do is to have sufficient data to rule out major morbidity and mortality effects which if real, would offset the beneficial effects on reducing hypotension.

And an example of this, most of you I'm sure are familiar with this. Erythropoetins that have been given 10 million doses in renal disease and anemia and chemotherapy-induced anemia. And they do reduce red blood cell transfusions. But the evidence now indicates that there is potentially a 5 to 15 percent increase in mortality and an established 45 percent relative increase in thrombotic events.

So that sponsor is conducting a trial of 7,000 people for 5,000 deaths to rule out -- to determine whether they can rule out a 10 to 15 percent increase in mortality. That's evidence. It's ruling out an excess. Going on to the Northfield PolyHeme discussion, the 171-patient trial with historical controls needs to be credibly cautiously interpreted in the absence of randomization.

Fortunately, there is a randomized trial. It's PolyHeme plus red blood cells against crystalloid plus red blood cells. And this is correctly identified to be a superiority trial and a non-inferiority trial. You can do both at the same time. But to do non-inferiority, you've got to justify the margin. Essentially in this case, to do a valid non-inferiority trial essentially is PolyHeme not inferior to crystalloid.

To justify that, you have to know that crystalloid provides a major benefit, and then you have to argue what level of benefit could you allow to be lost with PolyHeme before it's clinically meaningful. Because all non-inferiority does, is it establishes that you're not unacceptably worse than crystalloid.

And so typically to do that, your agent PolyHeme has to be established to have a better safety profile, better convenience, better cost structure than crystalloid to justify that. And I didn't hear that nature of that justification. But there was also an argument when the ITT analysis was in the wrong direction -- 47 deaths against 35 -- that we could do a per-protocol analysis.

And there is a big debate about those two types

of analyses. But the reason that there is a big debate is that in a non-inferiority trial, if there is noise in adherence between the active comparator and the experimental, it can dilute true differences.

So the concern with ITT is if PolyHeme is really worse than crystalloid, but the PolyHeme patients have cross-in's from crystalloid, or the crystalloid patients are an underadherent, then that noise could make PolyHeme look the same as crystalloid when its really worse. For that reason, people have gone to per protocol analyses as backup analyses.

It's because the worry is the ITT analysis will make you look better than you are. In this case, the ITT analysis is worse. And the argument that you can drop these patients out and do a per-protocol analysis is the exact reversal of what it is that justifies deviating from an ITT analysis. The patients that were dropped out had a 23 percent death rate on PolyHeme and 11 percent death rate on crystalloid.

Not a neutral result that was deluding it, that's where the signal was. So it makes no sense in this case to do a per-protocol analysis. Very quickly in the Apex -- I

was on the (inaudible) advisory board. And in fact, it's correct to note that we were concerned that there was a 6 percent difference in mortality but only a 1 percent difference in being alive out of the hospital.

So the essence of the goal here was to get through the acute risk to show that you're improving mortality through the acute risk. And for that reason, a time-to-event analysis showing that even though there is no difference in 28-day mortality, there was a difference of 10 days would be viewed simply irrelevant.

The issue isn't can you keep someone in intensive care a few more days before they die, it's can you get them through the acute risk. So that (inaudible) regression analysis really wouldn't be the proper analysis for that dataset. We heard about the -- as we had seen back in 2006 on the FDA advisory committee, we heard today from Dr. Greenburg again about the HBOC-201 red blood cell comparison in the HEM-0115 trial which does show a signal.

It does show a signal for excess risk and in particular the SAEs are relatively 50 percent more frequent. And the issue is as these analyses, exploratory analyses were done to try to see if we could explain why,

there was a focus on the 40 percent that required red blood cell transfusion, the 60 percent that didn't.

And when you break it out that way, the 60 percent that didn't only had a .14 SAE rate per patient. But you can't compare those patients with that rate to the control arm rate, because essentially those patients that didn't need transfusions are inherently different, probably inherently better, and their controls would have been inherently better.

So it's an interesting suggestion, but one has to interpret that really with great caution. And there was a further analysis that defined a hemoglobin deficit -- the area under a line for anemia. And then there was analysis that analyzed whether or not HBOC-201 was still a predictor of ischemic -- cardiac ischemic AEs even after adjusting for this negative effect on hemoglobin deficit.

So it's important to remember that that analysis doesn't lead you to conclude whether hemoglobin 201 is neutral. It's whether it has additional adverse events -- effects in addition to those mediated through its negative effects on hemoglobin deficit. And the hemoglobin deficit doesn't necessarily represent only a mechanism of

underdosing.

It could be that those patients are in fact also inherently different, and there could be other causal factors for those patients doing badly. There were subgroup analyses by age. And I guess the bottom line to this is all of these analyses are interesting hypothesis-generating analyses. They have to be viewed with great caution.

It's the -- ITT analysis is truly the one that gives you the most reliable sense about causality. Einstein was quoted to say not everything that can be counted counts. But I suspect that even a physicist would know that death, MI, cardiac arrest, and CVA, count. Those are the events that do count. I was on the FDA Blood Products Advisory Committee.

I didn't vote for this Phase III trial to be done. Now I think I did vote for the Phase II, because I did find these hypothesis-generating analyses to be of interest. But they do need to be interpreted with great caution. And I think the statement that it's unlikely that HBOC-201 has intrinsic cardiotoxicity is a strong statement based on these post-hoc analyses.

My last comment is in the Baxter hemorrhagic shock trials that were done where there was quite a signal for mortality. The trial was stopped 45 percent against 15 percent. To state that it was 40 percent mortality historically doesn't weigh very much. The reason I do a randomized comparative trial is that historical estimates don't necessarily apply to the context of the trial.

There was a statement made that's undoubtedly true, and that is HBOC appears to benefit some patients and maybe harm others. Of course the challenge is which are which. And it's very easy to do post-hoc analyses and identify who those people are that seemingly weren't -- identify -- benefited who those or that were benefited, but you're fitting noise.

And so it's extremely important to distinguish a post-hoc analysis versus one that was truly prospectively established. And I guess the last statement I would make is if you torture the data, it will confess.

MS. ALVING: Are there any questions? Are there any answers? I think -- thank you very much, Tom, really, and it -- I mean I think it just shows the complexity of what we all face.

And I think it's fair to ask the FDA that the data come in, the analyses come in from the manufacturers and then you at the FDA -- and I'm looking at Jay, he could be the spokesperson if you will -- you look at the data, you look at the analyses, but you often will repeat the analyses, you'll ask for more data, so it's really an iterative process. Am I correct in saying this?

MR. EPSTEIN: Well, basically yes. What the FDA will do is first of all challenge the dataset, (off mike) valid dataset. We will resolve differences of interpretation between ourselves and sponsors and more often than not, we will also do our own statistical analyses sometimes with the same model, sometimes with other models. This was not working? Can I be heard? Raise your hands at the back.

MS. ALVING: No -- okay.

MR. EPSTEIN: So again, yes, FDA will invariably seek to validate the dataset to assure that discrepancies, omissions, inconsistencies are resolved. We will typically do our own statistical analysis. Usually we will attempt to reproduce the methodology that was agreed upon in the study plan, and sometimes we will do additional analyses

that appear to be important.

And we will try to resolve disparities in our findings and interpretations with those of the sponsors. So, you know, that's the long answer but the short answer is yes, we do reanalyze the data.

MS. ALVING: Thank you very much --

MR. GREENBURG: Can I make a comment to Dr. Fleming's comment? I think I have to. Dr. Fleming is a brilliant biostatistician, and I appreciate all he's done in the field. And I certainly was present when he proposed that we do -- go forward with a Phase II trial for Resus in the Navy, and that was very nice of you to do that.

I come from a different world. I come from a clinical world. And when say that there is things that could be counted, I have no problem counting them. I do have a problem associating counting those events with the infusion of 500 ccs of the Biopure product and the evolution of a myocardial infarction that is a papillary muscle rupture in an 86-year-old patient who has received 20 liters of fluid.

I don't think my product did that from a clinical perspective. And I think I'm entitled to explain

clinically why things happen. And I know that's not the rules of the intent-to-treat analysis. But in other worlds of analysis, quality measures, clinical evaluation, malpractice suits, these things come up.

And I think I would like to say that I've looked at this analysis, I've looked at it very hard, I've looked at these patients, and there are clinical things that happened in these patients that I see everyday. Your intent-to-treat analysis doesn't necessarily take into account the risk that if you're 85 years old and having your hip or knee replaced, that there are lots of other things that happen, there are baseline references that should come out.

We all don't have the luxury of doing trials that include 7 to 10,000 patients which is what we all would like to seek and all we can do is what we've done.

MS. ALVING: Rebuttal.

MR. FLEMING: Just a quick comment. It is almost certainly the case that in, for example, the HEM-0115 trial or any other trials of HBOC-201 that when deaths occur, they weren't all due to or exacerbated by HBOC-201. And the same is true for MIs. There are, in fact, multiple

mechanisms of the disease process that influence risk for outcomes.

There are also multiple mechanisms of intervention that influence its induced outcome. Some of them are the intended mechanisms; some of them were unintended mechanisms. And to ultimately be able to determine which of that multiplicity of disease mechanisms and treatment mechanisms actually caused a given patient to die or to have an MI, is usually beyond the level of science as we currently know it.

And so a randomized ITT analysis is the most direct way to understand the totality of those mechanisms in a causal fashion. So what I can say is when I've randomized and I have persuasive evidence of differences that this in fact reflects a causal relationship -- if those differences are in the right direction it's causal -- it's evidence of causal benefit, in the wrong direction, evidence of causal harm.

And I can do my best to try to assign cause, was this treatment related or not. But I've been on 200 data-monitoring committees, and I can tell you repeatedly on data-monitoring committees we look at large-scale trials

and we're looking at the safety profile and you look at events that are in fact called "drug related."

You frequently see many drug-related events in the placebo arm. And you also see excess events that are clearly an excess of the treatment arm that aren't being called "drug related" because the mechanism through which it was induced was not understood. And so I completely endorse all of the in-depth attempts that you made to go beyond the ITT analysis to try to understand in a hypothesis-generating mode.

But it's that ITT analysis that provides us the most reliable way to determine what is causally treatment-induced versus what's due to the disease process.

MS. ALVING: I think I would like to say maybe one word to Dr. Biro about animal models. I would like to take -- well, some degree of exception, I think, saying that we haven't really had animal models that have looked at safety. I think many of us have done that, and sometimes we've had animal models where we wanted to look at one type of interaction with hemoglobin-based oxygen carriers, and found that because of the adverse event or side effect, we had to study that instead.

And I think one of the problems -- one of the challenges with animal models is do we want to believe what they are telling us. And I think this is even true in our Phase I studies with normal volunteers. I mean -- so a normal volunteer who gets an HBOC gets a little bit of dyspepsia. So is that a big deal? Is that just a side effect? Or is that an ominous sign of things to come?

And I think there's no one answer for this. It's a matter of judgment. And often looking back, we can say, well, that's -- we were getting those messages. So I think it will be interesting as we go forward, to say what are some ways that we can really approach these issues, and they're not really -- I think they're generic issues. How can the FDA help with this?

In other words, perhaps set up a level playing field for really good animal models with access to the materials that manufacturers can provide, and then study in a neutral fashion with neutral feedback. And we're going to continue, I think, to have to go from animal models to clinical trials or clinical studies back to animal models again. It's going to be a constantly iterative process.

So having said my piece on that, we've got all

these other pieces of paper. Maybe we could just start with you, Peter, and if you want to just answer maybe your favorite question, and then we'll go on down the line here. And then we could probably do the rest of this out in the hallway, unless you would like us to continue longer. Which would you like?

MR. KEIPERT: All right. Well, there's obviously way too many here to answer in the panel. But there is -- there are several that relate to the PEG, and since the PEG is going to be a unique component of this material, I'll make a comment on PEG and PEG metabolism and antibodies to PEG. I mean we've done studies when the hemoglobin is taken up within the RES and metabolized.

The PEG is then excreted through the urine. So there are other PEGylated compounds that are FDA-approved drugs, or there have been studies on PEG metabolism and PEG in fact does get excreted from the body. The -- we've also done a talk study injecting extremely high doses of the (inaudible) activated PEG the active reagent that we use so that if there were to be any free residual activated PEG in the formulation, we could look at the toxicity.

So we injected huge doses of the free PEG and saw

no evidence of toxicity. So I thought I would just encapsulate those two questions quickly to answer those and any other questions I'll be happy to answer during coffee breaks and lunch.

MR. GOULD: Well, I have no questions there, so --

MS. ALVING: Well, that wasn't (off mike).

MR. ABUCHOWSKI: Okay. I have a question about the hemorrhagic shocks, that is the species, the dose, endpoints, and to define oxygen debt. This was a PEG of 45 -- approximately 45-kilo PEG that is very highly instrumented; this is a very severe model. The animals are, of course, anesthetized. They are shocked with a captive bolt into the head of the femur and both of them four times.

And then they are bled very rapidly to reach an oxygen debt of 80 ccs per kg. They are bled -- probably within about 10-minute period they are bled out. And so this mimics what might happen to a soldier in the field. The dose that we give these animals is a 500 cc dose. They lose, in actuality, about 1.2 or 1.4 liters of blood. They just get back the 500 ccs.

We've also done over 250 ccs with fairly similar results. The endpoint is repayment of oxygen debt. What is the oxygen debt? As I said, these animals are very highly instrumented, and the animals are fitted over their mouth so that all of the oxygen that goes in and all the oxygen that comes out is measured.

So they're not open to breathe air; so oxygen that is merely the difference between the resting oxygen rate and the amount of oxygen that is utilized by the animal during the Hypovolemic period. So if the animal breathes in, you know, ten molecules of oxygen during the normal rate, and then during the oxygen debt period it only utilizes five molecules, it has a negative utilization of five molecules, but that's presented positively in oxygen debt. So it's a positive number.

So basically, when the animal uses 80 ccs of oxygen per kg less than they utilize during a normal resting state, that's the point when we give them the PEG-hemoglobin. And the endpoint is restoration of the -- repayment of the oxygen debt since that's the most important thing in the case of these animals is to get that oxygen debt repaid, because if you don't repay the oxygen

debt, the animals will die.

So at least that's the model that is being utilized down at Virginia Commonwealth University. But in addition to oxygen debt, as I said, there is probably another 100 parameters that are captured. All the organs are monitored for oxygen utilization, and all the -- we look at venous oxygen and arterial oxygen, and all the blood chemistries are monitored in real-time.

So there's an immense amount of data that is captured. But because the main concern was oxygen debt, that was just what I presented.

MS. ALVING: (Off mike).

MR. GOULD: Sure. I'm ready. There's one quick question. Have we analyzed the age of the red cells transfused and controls versus the PolyHeme group because of the interest and the literature about age of blood and we haven't done them. There are a number of questions here related to the protocol violations. And let me kind of lump them together.

And I'm actually going to thank Dr. Fleming. I'm not going to challenge his comments, because he's the expert and you'll find his name all over our protocol. I

do want to make one comment to clarify, Tom; part of the issue in an unblinded trial for us to try to again deal with a clinical issue. So one of the major errors that led to what we're calling the "protocol violations" are patients who got the wrong therapy.

And so I'm going to answer this question here. So there were a total of 41 patients in the trial who actually received the inappropriate therapy. There were 21 patients randomized to the PolyHeme® who never received a drop, and there were 20 patients signed under control, who did get PolyHeme.

So those are the types of things -- and I actually left out the data on the as treated -- another analysis group which is also defined in the protocol and the SAP. So our -- I guess, I comment just like (inaudible). I am a clinician and we're trying to understand the truth here and those are my comments.

So I actually look forward to in the reception to talk on -- Tom more to understand that. I appreciate his input. He's the expert in them, but we're simply trying to look at the data and get accurate information to understand what's going on here. I think all (inaudible) the other

person's variations on them.

MR. GREENBURG: Switching gears -- there are a couple of questions here that I think I can answer quickly. One is could a trial be performed in compassionate use? Just briefly, since the BPAC in 2006, we had requested 38 times from the FDA compassionate use protocol. Single patient IND has been granted 37 times, maybe 36 times, and we've treated 24 of these patients.

We did provide to them a outline -- rather sophisticated outline of a protocol for compassionate use. We had a meeting about that. And we're revising that protocol, and very much hope to bring it back to them for a discussion.

There is an absolute need for this kind of material to be out there for the religious group that cannot -- will not take transfusion, and those patients with autoimmune hemolytic anemia who have run their gamut of least incompatible blood, and they need something to get them through their acute phase.

Of these 24 patients and the previous patients done for Biopure compassionate use from the year 1999 to 2001, we have a total of 54 patients who are -- for whom we

have sufficient data to talk about elements that may contribute to their survival or factors that may apply as to how we should select these patients for use.

I presented probably the most spectacular patient of my career ever, 53 units, 1.73 kilograms of hemoglobin, 18-day survival, and an autopsy, as I said, the ultimate clinical test, there was no evidence of toxicity to any of the major organs -- brain, heart, kidney, liver, or pancreas as far as we know.

So I think -- yes, I think there should be a rule for this. And given that if we have the protocol then we can position the material away from Boston, I can regale you during the reception or the discussion upstairs with tales of how our airlines and FedEx and everyone else can't do what they're supposed to do and things like that.

But the reality is there's a delay, and that delay probably cost these patients' lives. And that delay is related to the hemoglobin deficit, that relay is -- delay is related to oxygen debt. They were just not able to care for these patients fast enough, that's the issue.

The other question that I thought I would address, if I may, comes from your insufficient hemoglobin

theory flies in the face of the heart (phonetic) trial. *New England Journal*, please explain. Briefly, they list a series of complications associated with blood greater than 14 days of age. They call them complications.

If that were a clinical trial, we would probably call them adverse events. We have adverse events in the HEM-115 trial. If we call them complications and then run them up against that list, I would offer that the lists are extremely similar. That's my response to that question.

MS. ALVING: Would you like to say anything about APEX -- anything further?

MR. ESTEP: No.

(Laughter)

MS. ALVING: Okay. Do any of the panel members want to add any more comments -- just free flowing?

(No audible response)

ADJOURNMENT

MS. ALVING: Oh, okay. Well, I would like to thank all of you for presenting your data for the audience, for providing all the questions. As I've said, there are

more to be answered. But I think it's actually we worked very well. And I think it's time now to do those hallway discussions and relax. So thank you very much. And thank you, Tom, for your comment.

(Applause)

(Whereupon, the PROCEEDINGS were adjourned.)

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