CENTER FOR BIOLOGICS EVALUATION AND RESEARCH, FDA
THE NATIONAL HEART, LUNG, AND BLOOD INSTITUTE, NIH AND
OFFICE OF THE SECRETARY AND OFFICE OF PUBLIC HEALTH AND
SCIENCE, DHHS

HEMOGLOBIN BASED OXYGEN CARRIERS: CURRENT STATUS AND FUTURE DIRECTIONS

Natcher Conference Center, Building 45
Main Auditorium, NIH Campus
Bethesda, Maryland

Wednesday, April 30, 2008

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P R O C E E D I N G S

(08:30 A.M.)

SESSION III: CLINICAL FINDINGS AND MECHANISMS

OPENING REMARKS

MR. KLEIN: Good morning. Welcome back, can everybody hear me? You can't. Can everybody hear me now? Yes, all right, hearing nothing to the contrary, again, welcome, good morning, it's nice to have everybody back.

I'm Harvey Klein. I'm from the Department of Transfusion Medicine here at the clinical center, about 300 yards in that direction. I'm involved in blood transfusion, and have been since the early '70s, and have been interested and involved in substitutes for the red cell and the red cells function from the mid-'70s, along with Dr. Fratantoni, when we were both children, at the Heart, Lung and Blood Institute.

A few housekeeping issues, please turn off all of your cell phones, or least put them on mute, if you have them. In your folder should be all of the disclosures for all of the speakers today, all of the

conflict of interest statements. Those people who are speaking or are on panels, I encourage them, if there is an issue that relates to their disclosures, to disclose that orally. Otherwise you can look those up. Please fill out the evaluation forms that are in your packets as well. Those are helpful through to the organizers of the conference and we hope you'll do that.

Just to set the stage a little bit, yesterday we heard a lot about the unmet needs, and there certainly are unmet needs in the area of transfusion in the current clinical status and the way forward. We had an outstanding review of the physiology of oxygen delivery, and the role and the mechanisms of hypoxic vasodilation. We learned about the rational design of its HBOC molecules based on nitric oxide paradigm, and based on the facilitated diffusion paradigm of oxygen delivery.

We learned about hemoglobin oxidation and vasoconstriction, and how oxidation of hemoglobin can result in clinical toxicities. We need to know precisely how structure and function at the molecular level affect the in vivo function, and that we need to know if in vitro oxidative reactions predict in vivo events.

We've heard an awful lot about nitric oxide chemistry, and also about how and why animal models may or may not help us. Their species specificity and safety signals in one model might be difficult to understand in the human model. In the afternoon and late morning, we heard a bewildering amount of clinical data, and we learned that we don't have access to all of the clinical data. Some is proprietary; some is never reported, certainly not in the reviewed literature and not even to the FDA.

We also learned a little bit about the risks of over-analysis of severe adverse events, about the difficulties of adjudication, about the difficulties of analysis per-protocol. We learned that there may also be other explanations, and things that we consider severe adverse events for our molecules. Things like, perhaps, in appropriate dose during trials or fluid overload, or the rate of infusion, total dose administration, or perhaps we're just seeing misuse of these drugs. And maybe there's really very little toxicity. So all this underlines the importance of randomized controlled trials, or ethical trials as we've heard described by our ethicist

yesterday and what that means.

Today, we have two panels which are going to focus on the clinical findings and the mechanisms. The format is as follows. We asked each of the panel members to present, if they wish, for no more than 5 minutes and about three slides. And then we will have a panel discussion where there will be questions among the panel members, and I hope everyone will fit out their cards and send them up to the front, so that we can reflect your questions and have the panel members address them

This is the first panel. Dr. Stephen Cohn is professor of Surgery at the University of Texas in San Antonio. Dr. Demetrios Demetriades, professor of Surgery and Critical Care Medicine at the University of Southern California, Dr. Mitchell Fink, who's had a long experience in this area was professor and chair of Surgery at Beth Israel in Boston and at Pittsburgh. He is now with Logical Therapeutics in Waltham, Massachusetts.

Dr. Dan Freilich with the Navy, who is involved in the trials that have been proposed, Dr. John Holcomb from the U.S. Army Institute of Surgical Research at Fort Sam Houston; Dr. Charles Natanson, an anesthesiologist and

senior investigator in Critical Care Medicine here at the clinical center; Dr. Ed Norris, who is an associate professor of Anesthesiology at Johns Hopkins and has had experience with, I believe, at least three of these molecules in the clinic.

Dr. Ed Sloan, professor of Emergency Medicine at the University of Illinois, who is the principal investigator on the Sloan et al. slide you saw yesterday for the Baxter trial that was discontinued, and Dr. Gus Vlahakes from Harvard Medical School, professor of surgery, who has also had experience with these drugs. The four overarching questions for this panel are the following.

Can information about the safety and efficacy obtained from clinical trials in one clinical setting; for example, trauma, be used to inform a risk-benefit assessment in a different clinical setting. For example, orthopedic surgery, can you generalize? A second overarching question is given what we know about the biochemistry and pharmacology of the current and the previous HBOCs, can safety information obtained from the study of one HBOC be used to inform safety and risk

assessments for a different molecule, a different HBOC?

Third, are there toxicities or harmful interactions between these molecules in a patient's underlying disease -- hypertension, diabetes, or coronary artery disease that are common to all of these molecules, regardless of their structure and regardless of their modifications? And are there lessons for designing the next trial, that is those lessons that we've learned from what we've heard yesterday, rate of infusion, volume, oncotic pressure, et cetera.

So that's the nature of what we hope to address this morning in this first panel. And I believe the first speaker on this panel would be Dr. Demetriades.

FUNCTIONAL ASPECTS OF THE HBOCs AS A CLASS

MR. DEMETRIADES: Thank you, Dr. Klein. Thank you very much for this honor. I am a trauma surgeon and this means I'm going to make comments and recommendations from the trauma surgery point of view. Yesterday, we have heard some beautiful presentations from the NIH scientists, from the industry, from a biostatistician.

And from what you have heard and what you have read in the literature, where are we now?

Firstly, I want to say that I'm very excited. I was very excited. I still remain fairly excited about the products. It's promising, but we are still not there. We are very concerned about the reported complications, complications that increase mortality. We need to encourage the industry to go about and address these issues. We want to see clear statistics. We do not want the statistics to confess under torture, as the statistician said yesterday. We want these speakers to come out freely without any effort.

I believe that with the current status, we're not ready yet for quick trials. We might rush and broaden our inclusion criteria, but I think it will be counterproductive for everybody.

We also heard from a couple of industry speakers that it's not fair to group together all HBOCs. I think this is fair; it is appropriate to judge each product on its own merit. There are significant differences between all of them that you need it into account these differences.

In one area where I firmly believe that we are ready to move in is the compassionate use. We have patients; Jehovah's Witnesses or other groups of patients who are really, practically dying in front of our eyes without being able to do anything.

We know that for acute blood loss, if the hemoglobin goes below 5 -- acute blood loss, not chronic, the patients goes into cardiogenic shock, and you give basal pressors, and you give fluids, and you give whatever you want; they do not respond. This might be an excellent group for compassionate use. We know that for acute blood loss, if the hemoglobin is below 3, it's extremely unlikely that this patients will ever make it; an excellent candidate for the product.

Now, for future, what kind of clinical trials do we need? Well, we need to apply much restrictive criteria. Remember that on the one side we have patients; we have the products with significant complications. We need to use this product in patients who are at extremely high risk of dying. So in other words, the benefits should outweigh any possible disadvantages. I think it's a serious error, and I have seen these in the existing

standards. They include all mechanism, blunt trauma -- blunt and penetrating. It's a big mistake, and I'll tell you why. I have seen that one of the inclusion criteria was a blood pressure of 90 or less. I think it's a serious error, and it's unlikely that you're going to see any difference with this kind of criteria. Now, why do I say blunt and penetrating are different? They are very different.

Blunt trauma is extremely unlikely to cause hemorrhagic death within 1 hour -- very unlikely, unless a patient has a rupture of the aorta, a rupture of the heart; in these cases, there is no hope. He will be dead within a few minutes. The typical blunt trauma patient will bleed from the liver, the spleen, the pelvis, the long bones, and will die a few hours later.

The prognosis is very different. If you get patients with blunt trauma and hypertension lower than 90, excluding a trauma, the overall mortality from blood is 20 percent, is 33 percent for penetrating trauma.

And this is the temporal distribution of deaths, the time of deaths in blunt trauma and penetrating trauma. In penetrating trauma, as you can see, the vast majority

of deaths will occur within the first 1 hour. And this is the distribution of deaths in blunt trauma, very different. We shouldn't mix them if you really want the best possible scenario.

Now, let's come to the blood pressure of 90 or lower. A model concept in the management of trauma patients is permissive hypotension in penetrating trauma. We now teach and apply -- and this is in the military as well -- but if a patient with penetrating trauma has a systolic blood pressure of 80 or 90, don't give him fluids until you control the bleeding surgically. This applies in an abundant environment.

So it's inappropriate to get the patient of blood pressure of 90 and load him with HBOC or saline or whatever. On the other hand, we know that if the blood pressure is very low, extremely low -- blood pressure is about 40, about 50, there is a risk of cardiac arrest. This is a group which might benefit from aggressive fluids.

So what I would suggest for future trials include penetrating trauma, excluding head with a blood pressure of 80 or lower. And with the control fluids, you

might want to consider hypertonic saline, or maybe red cells, but fresh red cells younger than two weeks. And this concludes my presentation. Thank you very much Dr. Klein.

MR. KLEIN: Thank you very much. And we'll bring everybody up on the stage. You'll have an opportunity to send in your questions. Please write them down after each speaker, if you can, and we'll try to get to them.

The next speaker is Dr. Freilich, from the Navy.

MR. FREILICH: Good morning. Can everybody hear me in the back? So I'm not a trauma surgeon, and I'm an Infectious Disease doc, and as many of you know, in a hospital, the characteristic of ID docs is that they're somewhat compulsive, and annoyingly are willing to review all the pages in the record.

And I think one of the issues with HBOCS in general has been broad pressures. And I think that goes to phase 4 activity, trial designs, strategies, and even how to proceed forward. And I think that it can be broad pressures, and I think that's the most important point I want to make.

The Navy has been active since about 2002, with an approach for pre-hospital, where blood is not available, trauma resuscitation and we've been on clinical hold since 2005, and we remain on clinical hold despite a BPAC consensus recommendation that a phase 2 trial should proceed, about 16 months ago.

So we have had had quite a bit of experience thinking about how to potentially design a trial. And we may have made mistakes, and we may still be making mistakes, but at least we've thought about it, and I just wanted to transmit some of that information.

For the sake of disclosure, I should say that the Navy has material transfer agreements and CRADAs with Biopure, and there is a contract to purchase HBOC prototypes for research -- no transfer of funds ever to the Navy.

The final point is that we have no horse in the race. And in fact, I would propose now that any comments that I make right now with exceptions in general, I think, probably applied to most of the second generation HBOCs that are currently in the process of development, and there are certain exceptions to that.

What is the potential benefit? And I think this is an enormous problem in the way trials have been developed over the last 10 years or so, or longer. And this graph, in blue are controls, and red is HBOC 201, and again, I think that other HBOC could be superimposed in many ways in this graph.

On the left are studies with the mortality and controls was really low. And in fact, you can see many of them were 100 percent survival. On the right are high-mortality trials. And you can see, most of the control animals died. This is a summary of the data. What should be clear from the back of the room is that HBOCs don't, in preclinical studies, demonstrate a survival benefit in low mortality scenarios, and this makes sense.

And in clinical medicine potent drugs often are not necessary in low-severity design studies or in low-severity clinical settings. Nevertheless, the design of trials has relied on blood substitution in the hospital, or addition to standard care with the opportunity for benefit is extremely minimal. And if you look at studies or in other indications, for example, add heparin.

Heparin makes very little difference in

myocardial infarction when you add it to the whole armamentarium of PTCA and TPA and nitroglycerin, and all the other things. On the other hand, when you have very severe models repeatedly, whether you have associated traumatic brain injury or not, you see significant benefit.

Now, how does this affect your design of studies? I think this is where most of the studies have been done. We have never done a study like this, nor has there ever been a study that's truly against crystalloid or in general asanguineous controls. All of them have had, including even the coronary European style -- European study with DCLHb have always had some element of competition with blood, or as part of a competition with standard care, which included blood.

Now, this is a very busy slide, but I'm only going to reflect a few things. What I try to do is show that on the left these are studies that had low mortality in controls, and they increase. To get to the highest would be the RESUS trial that the Navy proposed, where we expect a mortality of about 62 percent. It is very difficult to hypothesize that you can extrapolate data

where the only potential significant benefit is transfusion avoidance to a study with 62 percent mortality, mostly within 24 hours. And obviously you're expecting a survival benefit.

Secondly, how do you look about -- how do you look at odd HBOCs? You really can't extrapolate one HBOC to another, and you have to be very careful with doing that. For example, if you look at the old DCLHb data, the in-hospital U.S. trial or if you look at the out-of-hospital Kerner trial, which was done in Europe; 95 percent of patients who enrolled in this trial would get excluded by the study, and 85 percent would get excluded in even the European higher-mortality study.

What the Navy tried to do is to exclude that bimodal distribution by using revised trauma score -- and by no means do I suggest that that's the only way to do that. But I don't think that information is static. And I think people learn from mistakes and/or from experience, and there are many ways to try to get that intermediate population. We think we've done it with the RTS of one to four, but there are other ways there to do it.

Finally, again, one learns from experience and

some prior mistakes, and one can optimize the trial. And I think that each specific optimization does not necessarily make an enormous difference in terms of the overall benefit ratio. But I think it's very reasonable to hypothesize, and I iterate the word "hypothesize," and therefore a clinical trial should be done to confirm the hypothesis that the totality of the changes are likely to shift way beyond equipoise.

I'm not going to go into all these because I am a little bit past my time. But firstly, I just want to reiterate what's in red. You should target a population with severe hemorrhagic shock and with severe -- with high likelihood of mortality, and you should target a population where blood transfusions are unavailable.

And if you look back at the animal studies, the animal studies have been criticized as potentially not predicting what happens in humans. But they do get vasoactive response, as you just don't see the cardiac side effects. And the reason you probably don't see the cardiac side effects is that they're young animals.

So pick a population that somewhat simulates the studies that you've done in preclinical studies. And I

think that young trauma patients probably are similar to that.

I think I'm going to stop here because I have run over time, and thank you for listening.

(Applause)

MR. KLEIN: Thank you very much, Dan. The next speaker is from the Army, John Holcomb. John, you want to give us your thoughts.

MR. HOLCOMB: Okay. Well, I am -- as opposed to the other discussions, I'm not going to talk about HBOCs very much. This first -- the first reference is actually Dr. Demetriades', and he's already showed the slides talking about deaths. Deaths occur very quickly, largely from truncal hemorrhage, they peak at 1 to 6 hours.

Fred Moore, Jean's brother published a paper in Journal of Trauma this month that actually shows this beautifully in somewhat greater detail, and actually that the mortality from hemorrhagic shock occurs within 1 to 3 hours of admission.

The point there is that anything we are going to do needs to be done very early. It can't be done with individual patient consent or LAR constant. And Rick

Dutton showed that in a paper in Journal of Trauma last month as well, along with John Hess's wife that basically LAR consent for hemorrhagic shock studies is a nonstarter, and will doom any study to failure.

Now, Usol (phonetic) and others have recently shown that we can predict massive transfusion within minutes of arrival, with easily available data that's in the emergency center with an ROC curve of 0.8. This is from Germany. There are other papers published from North America on trauma patients. So you have patients from both continents responding the same way physiologically. We can predict who's going to need massive transfusion in the first couple of minutes, and those patients are the ones who are going to die within 1 to 3 hours.

So this is getting at study design actually, so we -- both address the same thing. Rather than talking about HBOCs specifically, the study-designed questions are very important. And clinically, I think we get a lot of information about these kinds of patients in the last 4 to 5 years, previous to some of the designs that we've heard yesterday.

Now, Borgman (phonetic) showed -- this is combat

data -- that you can increase plasma by -- by increasing plasma use to red cell use, you can decrease mortality from 65 to around 20 percent. Seven civilian papers will be published this year that show exactly the same thing. Increased plasma platelet to red cell ratios improve survival. Now, why am I going to talk about plasma and red and platelets, instead of hemoglobin? I think that we have been too focused on oxygen consumption and oxygen delivery as a resuscitation endpoint. These are data from 466 massively transfused patients representing almost 40,000 admissions at 16 trauma centers from the last two years ago in the United States.

And as you can see, these patients are critically injured with an ISS of 32. They only have a 40 percent overall mortality. They are a young at age 39, get younger everyday, largely male, blunt injured, they come in moderately hypotensive, tachycardic, acidotic, and with an INR of 1.6; they're all coagulopathic. These are initial data upon arrival, and I point you the hemoglobin of 11. We were all taught that patients coming with hemoglobin of 14 to 15 after losing blood, these were all within 30 minutes of admission. That's not true.

Severely injured patients come in with hemoglobin of 11.

Now, that's plenty of red cells floating around to deliver oxygen. That's plenty of red cells, and yet, the focus of this meeting was on giving more oxygen-carrying capability to exactly this group of patients. They don't have an oxygen-carrying deficit, they have a bleeding problem, and they have a profusion problem, and if we fix that, they will do fine.

This is the Kaplan-Meier curves of 466 patients. You can see there's a 24-hour Kaplan-Meier and a 30-day Kaplan-Meier. These patients die very early, this goes right along with more data from the last month of Journal of Trauma, and by giving more platelets and plasma, you shift that curve from a 40 percent mortality up to almost a survival -- to a 90 percent survival.

You're giving same amount of red cells in each group. I think that the data from this is pretty instructive. I think the data coming out, it'll be able to predict massive transfusion, and physiology of what these patients have really going on, pre-hospital and in the ED is pretty instructive and informative for future trials in this area.

That's the end of my slide. I just want to make a couple of comments as well in the last minute. Many lessons have been learned from these studies; both this study and other studies. I think one of the major lessons that we've learned yesterday is that when you have 3,500 medics assigning our patients to a prospective randomized study that they're going to miss a sign about 20 percent of the patients. That is the real world, ladies and gentlemen. We don't have CROs and registered nurses assigning our patients in the emergency department. We have medics are doing a great job, doing as best as they can, and they will miss a sign. We need to track that and make sure that if there are medic units or systems who miss a sign at very high rate, we go educate them and if they don't respond to education, we kick them out of the study, that needs to happen. But it will happen.

Hence we got to figure out what is an acceptable rate of miss a sign. The other thing is that with 5024 and the discussion we've had yesterday about how the process works. The process actually makes us go right from preclinical studies to definitive phase 3 trials because mortality is the endpoint in the current 5024

paradigm of doing research in this area.

You don't get the benefit of phase 1 and phase 2 trials and learn from how best to treat these hemorrhagic shock patients, and what really is an inclusion and exclusion criteria. That I think is a problem. We need to be able to do smaller studies, the phase 1 and phase 2-types or sized studies, if you will, in this group of patients, so we can learn how to do the definitive trials in this group of population who stands to benefit. Thank you very much.

(Applause)

MR. KLEIN: Thank you, John. I hope we'll be asking questions about whether phase 1 and phase 2 trials in other settings would be applicable to trauma because I think that might be an important issue. Next speaker is Dr. Natanson from NIH.

MR. NATANSON: Good morning. I want to thank the organizers for allowing me to speak today. The charge that Harvey has given us, to see if we can find common properties to these hemoglobin-based blood substitutes as a class. They're all derived from red cells -- red blood cells, and then they have different biochemical

alterations. But they all interfere with normal nitric oxide functioning.

And therefore, they have a common mechanism of potential toxicity. The question we asked is, as a class, do -- are they associated with an increase in myocardial infarctions and deaths.

We did a meta-analysis; there were three sources for our trials. One was a standard literature search for which we found 13 trials. One was an FDA meeting which had a summary, one set of trials. And we also went through press releases, and we included two trials which had quantitative data from press releases for a total of 16 randomized controlled trials in this meta-analysis.

There are five products listed there in our meta-analysis. And I'm showing you on the left, mortality, on the right; the risk of myocardial infarction. This side favors control that there was an increased risk with the hemoglobin-based blood substitute, this side says there was benefit. Again here's the same for myocardial infarction.

And you can see here that overall, there was a statistically significant 30 percent increase in deaths

with these hemoglobin-based blood substitutes, and you can see there is almost a threefold increase in the risk of myocardial infarction with the hemoglobin blood substitutes.

And importantly, this is a test of heterogeneity. As you can see, there is no significant heterogeneity that is treatment effects were quite similar across these products.

This is an "I" square. An I squared of zero means the effects are very, very similar. An "I" squared of 100 per cent means they're very, very different. This says there is the minimum amount of heterogeneity across these studies -- zero.

We also did subgroup analysis in order to -- or sensitivity analysis to see if the effect is consistent across different patient populations. And as you can see here, in all the reported patients studied were -- they described myocardial infarctions, the effect is very consistent, and the mortality effect is very consistent, except in cardiac surgery.

The mortality difference is not statistically different compared to other forms of surgery, but it's

interesting (phonetic) to speculate that you do have an increased risk of myocardial infarction during cardiac surgery. But the vascularization -- the revascularization maybe protective to prevent death.

We also looked in studies to see if it made a difference if you had a blood product or a non-blood product as control. And these are the number of patients and these are the number of trials. As you can see here that the mortality in the myocardial infarction data is quite similar, whether you had a blood product control or a non-blood product.

We also sequentially removed each one of these products from the analysis to see if one product alone was responsible for this effect. And this shows you how many patients are left, and how many trials are left after we move the hemocyst. And you can see that no matter which one of these trials we move, or each one of these companies' products we moved, the treatment effect in terms of mortality and myocardial infarction is still on the wrong side. And there is no one trial responsible for this effect.

We did other analyses. We looked at published

versus unpublished, detected from your content, the P50, and none of these variables make any difference. Regardless of the product, the patient population study, the control, this was a very consistent and a very robust effect. We conclude, based on analysis of the available data from clinical trials, hemoglobin-based blood substitutes are associated with a significantly increased risk of death and myocardial infarction. Thank you.

MR. KLEIN: Thank you very much, Chuck. I'm sure we'll have a lot of -- a lot of comments about that. It's an important study. I think our next speaker is Dr. Norris, and I don't think -- Ed, I don't think we have slides. So he'll be speaking briefly.

MR. NORRIS: Again, I've no slides, so the lights can stay on, good morning everyone. I'm very glad to be here this morning, and I'm very honored to be able to participate in our panel discussion with such a distinguished group of individuals. My written disclosures did not make it to the printed materials, and therefore I wanted to make a brief oral disclosure.

I'm currently a consultant for Northfield Laboratories and participate as a member of the Data

Safety Monitoring Board and the related subcommittees for the phase 3 trauma trial. Since 1997, I've participated as the principal investigator in a number of phase 2 and phase 3 clinical trials with Biopure, Alliance Pharmaceutical, Northfield Laboratories, and most recently, Sangart.

I've also been the principal investigator for both compassionate use and treatment use protocols with the current generation HBOCs. I have received funding from Biopure, Northfield, and Sangart for work related to the interference of HBOCs with common laboratory tests, and lastly I've participated in several scientific advisory board meetings with Sangart over the last several years.

My bio sketch wasn't included in the printed materials either, and I wanted to make just a few quick comments. As mentioned, I am an associate professor in the Department of Anesthesiology and Critical Care Medicine at the Johns Hopkins University School of Medicine and a staff anesthesiologist at the Johns Hopkins Hospital. I'm a member of the Division of Cardiovascular, Thoracic, and Transplant Anesthesia, end I direct the

vascular and endovascular anesthesia programs.

I am also director of our Advanced Transfusion Practices Center and director of Perioperative Blood Conservation and Hemodilution Services. My day-to-day clinical activities involve the care of patients undergoing complex procedures that routinely require the transfusion of large amounts, and often massive amounts of donor blood and donor blood products.

And although I practiced just a few yards from one of the busiest transfusion services in the country, we were not able to be all things to all patients regarding the red cell requirements. One of my clinical interests over the last decade has been to develop ways to reduce patient exposure to donor blood and donor blood products. And as a result, we've attracted a large number of patients, often requesting complex and medical surgical without the use of donor blood and donor blood products.

Now, Jehovah Witness patients make up the largest percentage of this group. And I personally participated in the care of over 1000 of Witness patients. This clinical experience combined with HBOC clinical research experience involving nearly 100 patients,

receiving nearly 200 units of clinical trial material, I think, prompted the very kind invitation to participate in this panel discussion.

Regarding the topic of our discussion, I personally believe that the current generation HBOCs can indeed serve a critical unmet need in a variety of clinical settings, the common theme of which involves the temporary or permanent unavailability of red blood cells.

Further, I believe that our current understanding of the risk-benefit considerations for these products indeed favors the clinical use in these very select clinical circumstances. And I'm going to stop there and I look forward to a good discussion. Thank you.

(Applause)

MR. KLEIN: Thank you very much, Ed. I think you have as much experience with different HBOCs as perhaps anyone else on the panel. So thank you both for the disclosures and for the information.

Our next speaker is Dr. Ed Sloan from the University of Illinois, and Dr. Sloan, as I said earlier, was the principal investigator for the trial that we heard a great deal about yesterday with the Baxter hemosis

product, and he has kindly agreed to come and both speak and be on our panel.

MR. SLOAN: Thank you for the invitation to speak. It has been nice to see people with whom I worked for several years. As a matter of disclosure, the work with Baxter was done through a grant to UIC. I work now looking at this data at the request of the NMRC, through a grant from the Jackson foundation to UIC, and I have served on a data safety monitoring board for Biopure.

We are going to talk a little bit about the DCLHb trials, and share with you data that which you have not yet seen. I'm at the University of Illinois in Chicago. The goal of the development of HBOC is take a difficult clinical setting, and to improve clinical practice, and improve patient outcome. There were two studies, one an in-hospital emergency department study in the U.S. with DCLHb, and a paired pre-hospital study in Europe.

When you combine the information from those two studies, mortality was higher in those treated with DCLHb. Two observations. The first, in a perfect world, your desired mortality risk would be midrange, 40 to 60

percent; this would allow you to study optimally any new methods or therapeutics. In fact, our mortality, or our mortality risk was bimodal at the very low and very high extremes.

One other comment from those two studies; the use of an exception to informed consent was nearly universally accepted. The logistic side was manageable. It appeared appropriate and still does, and I think it remains a vital part of what we do in our emergency and trauma research.

Here are the list of the publications that were made regarding DCLHb, one to be added is the reuse study from the European experience, and the consent-related publications. We're now looking at this data second time at the request of the Naval Medical Research Center, and I'd like to just share with you five aspects of that.

Regarding blood pressure effects -- in summary, blood pressure did not differ with DCLHb use in the clinical trials. Those patients with markedly elevated blood pressures did not differ with the use of DCLHb. In fact, DCLHb with regression analysis only contributed 3 percent to the predictive -- prediction of blood pressures

over time. In other words, there was no clinically-consistent pressor effect.

Regarding base deficit and lactate; in the two studies, base deficit did not differ with DCLHb use even though expired patients had a greater base deficit than those who survived. In the U.S. study, where we only had data for lactate in the one study, lactate did not differ basically on DCLHb use, even though expired patients had a greater lactate than that did -- those who survived.

There was no clinically consistent poor perfusion effect as measured in these studies with lactate or base deficit. We also looked at the shock index. The shock index is a simple measure looking at clinically easily obtained markers; heart rate and systolic blood pressure. And in essence, when your heart rate is greater than your systolic blood pressure, it suggested you have uncompensated shock.

Conversely, when your systolic blood pressure is greater than your heart rate, you appear to have compensated adequately, and this is the permissive hypertensive setting in which we now don't over-fluid-resuscitate patients. So this is an easy measure of

shock.

And in summary, patients with a shock index greater than one are a clinically uncompensated population of shock patients who might benefit from infusion of an HBOC. And in fact, 120 minutes of shock index greater than one is associated with a two-and-a-half fold increased mortality risk; 40 versus 16 percent as compared to those with a shock index less than one.

Importantly, in these two studies, DCLHb use did not alter the ability of shock index to predict mortality, and the significance of this is in traumatic hemorrhagic shock studies, whether the use of an HBOC is planned, it doesn't appear as though these clinically important markers; systolic blood pressure and heart rate, are modified such that we can rely on our clinical acumen to determine whether patients still need to be resuscitated.

Regarding the study design, we looked at RTS entry criteria, and we found that patients with a low RTS 1 to 3.99 have a very low TRISS survival probability. This might be an optimal patient population for study, if you're looking at optimizing the risk-benefit profile. And we may need to exclude those with a GCS of three,

because it is greatly influences mortality and the RTS.

Lastly, we looked at traumatic brain injury. Traumatic brain injury in the U.S. study of DCLHb had a significant influence on 28-day mortality, and in fact, those TBI patients with a GCS of three increased study mortality by 63 percent. As such, I would recommend that the GCS of "three" patients not be included, or be excluded from any future traumatic hemorrhagic shock trials which attempt to look at HBOCs.

So in conclusion, this work continues to be critical. What's important is that the theoretical pressor effects of DCLHb could not be correlated with the most commonly utilized clinical variables that we use to assess patients, such as blood pressure, base deficit, lactate.

And so in order to maximize our studies ongoing, one thing might be to exclude GCS equals three patients, and be very clear as to who our entry criteria is and what the mortality is. So I recommend that we continue to look at these theoretical issues such as pressor effect, and see how they're playing out clinically, so that we can make good decisions as we look at future studies of HBOCs.

Thank you.

(Applause)

MR. KLEIN: Thank you very much, that was very helpful. And I think our final speaker is Dr. Vlahakes of Mass General Hospital. He has also had experience with these molecules.

MR. VLAHAKES: Thank you very much. I became initiated in this field when I joined the staff in 1986 because of the enormous pressure we were under from patients to avoid transfusions. This was in the heydays of HIV when the blood supply was in question, and we -- you can sit down with the patients and their family, and spend an hour discussing a complex heart operation, and all they really want to know was whether or not the patient was going to be transfused.

My interest in the field was in the context of these materials as potential blood substitutes, and I had high hopes for the field until the issue of auto-oxidation and rapid clearance came to light, and it's an area that we had worked on in association with Biopure that had provided us with some of the materials that we were working with.

Of note in this study is a potentially interesting hypothesis that awaits testing by someone, and that is that the most rapid clearance and the most rapid rate of auto-oxidation occurs in the early phase, when there are more low-molecular-weight entities present in the circulation. So one issue in the hypothesis for someone to test down the line is whether or not the auto-oxidation phenomenon is reduced by raising the average molecular weight profile of the materials.

We did conduct an interesting phase 2 trial in cardiac surgery, keeping in mind that we had this limited window and time of efficacy. In the first 12 to 24 hours following a heart operation, there is a need to expand the blood volume as patient is warm and dilated, and this results in a nadir in hematocrit, around which transfusion decisions occur.

So the concept was to temporarily support oxygen transport, until the patient was 2 or 3 days after surgery, at which time, they begin to hemoconcentrate by fluid mobilization.

This was a phase 2 study that involved 50 patients in each group. It was a true double-blind study.

The blind was tough to organize and maintain, but was successfully done. It involved wrapping the chest drains and the pleurovac with colored cellophane, so the blind would not be broken. It involved removing certain laboratory evaluation and clinical record that might give away the patient's treatment assignment.

And it was powered to determine efficacy with three infusions; two units followed by one unit, followed by another unit, and we used regular clinical transfusion guidelines that are in place at the institutions involved. Now, we also elected to do this in the ICU after the patients had been through their cardiac surgery.

And I think one of the things that you might want to get into the discussion is this is a brand new class of materials for hospitals and hospital personnel to be involved with. And how you introduce something that's brand new, this is not another antihypertensive, it's not a new antifibrinolytic or novel anticoagulant, it's a brand new class of materials that people have never seen before. And how you set up clinical trials has to keep that in mind.

One of the issues we found was that up to four

units of material resulted in saving only half a unit of blood, and with some other discussions around potential costs, et cetera. As the safety profile of the blood supply changed in the mid-'90s with donor self-deferral and testing, this took the wind out of this indication to a considerable extent.

Now, the study was not powered to look at safety, but there were a couple of points. There were no myocardial infarctions in the study, and one of the reasons maybe related to the fact that coronary disease was treated surgically before patients were randomized. Parenthetically, the vascular surgery trial which looked at major abdominal aortic reconstruction also did not see any myocardial infarctions, and those patients as a group tend to be very thoroughly screened for the presence of cardiovascular disease before they go through major surgery.

Now, although you've heard a lot about the nitric oxide binding and vasoconstriction, virtually all the HBOC preparations that have been studied do change systemic and potentially pulmonary vascular resistance. But despite these concerns, nitric oxide binding and

increased vascular resistance has never been shown to override metabolic autoregulation, and there are plenty in the studies -- preclinical studies and the literature to support this.

This could be a benefit in some clinical settings. In particular trauma and cardiac surgery, we are often dealing with low systemic vascular resistance from -- for a number of reasons in the postoperative setting. And one of the points I would make about using blood pressure as an endpoint -- and you might get it into this in some of the discussions -- vasoactive HBOCs may potentially result in under-resuscitation or under-volume repletion of patients.

And one of the reasons why we selected the ICU setting was the fact that the patients were all monitored, (inaudible) catheters and continuous monitoring of blood pressure. And the final issue is the vascular biologic problem. Besides vasoconstriction, do HBOCs do anything to vulnerable plaque? And one of the issues we're going to have to deal with is this potential risk posed by unrecognized coronary artery disease. Thank you.

(Applause)

MR. KLEIN: That was our last set of slides, but I think Dr. Steve Cohn wanted to make a couple of comments before we get the panel up on to the stage.

MR. COHN: Thank you. I'm honored to be on the panel. I have three comments, speaking as a clinician and surgeon, first in regard to the magnitude of the problem, and then a comment about the difficulty with clinical trials in the area of specifically trauma, and finally a lit bit about the risk-benefit considerations.

About recently, I had a patient that came in with a single gunshot wound right below her xiphoid. As she came down the elevator, she was talking to the paramedics and she arrested. Rather than going into the resuscitation room, we took immediately into the operating room, and there we did a thoracatomy and a laparotomy, in a very short period of time, while she was receiving the six units of blood that we kept down in that part of the trauma center, we fixed a hole in her vena cava, and she had -- the porta hepatis was divided.

So we removed her liver, and she stopped bleeding. She was stable, but because we had no more blood available, her heart gradually slowed down. We were

asking for more blood, is there more blood -- there was no more blood available. We used up to six units we have here. It will be another 10 minutes before we can get the blood down, and this 20-year-old girl died.

So this problem is the same as it was in 1999; the last time I was here at one of these meetings. We have patients who -- this is not some theoretical concern -- we have patients that are dying because they don't have blood. And this is at one of the busiest level 1 trauma centers in the United States that this occurred. This is not like some place in North Dakota that doesn't have a blood bank.

It turns out that less than 1 percent of trauma patients receiving greater than 75 percent of all the blood transfusions. So it's a fairly small population that gets most of the blood. And there has been a major cultural change since 1999, in that we as trauma surgeons and intensivists don't give blood very much anymore. We looked at our blood transfusion administration history, and we found that we had decreased the number of pack cells given to our trauma patients by 25 percent. Recognizing that trauma uses up about 25 percent of all

the blood transfusions in most major tertiary care hospitals, with another quarter being used by transplant, and then the other half is sort of like everybody else.

The other comment I would make is that 40 percent of Americans are greater than 1 hour from any trauma center. So if you're driving on a vacation, in all likelihood, you may well be if you are not in urban center, far away from a trauma center, possibly near hospitals that have no blood available. So if you are unfortunate enough to have a bad injury out in a rural area, you may not have access to a blood bank or the ability to get a massive transfusion. So that's item number one.

The second thing is on clinical trials feasibility. We recently completed a trial at seven of the busiest trauma centers in the United States over 18 months. These were all in severe hemorrhagic shock patients. To get entered into the trial, you had to receive a unit of blood within 6 hours. Okay, so in shock, receiving blood. In those seven centers which had thousands and thousands, and thousands of patients during those 18 months, we only had 382 patients who met entry

criteria into the study, and of those, about 90 got the massive transfusion -- that was Fred Moore's data -- but 90-93 got massively transfused to find these 10 units in 24 hours, and only 50 died.

So we're doing mortality studies. The fact is that from a care, the United States, pretty darn good. Not that many people are dying. Even in combat now, with Dr. Holcomb's help, the military has reduced mortality way down. So we're doing mortality studies, we're talking about large trials because not that many people are dying.

The third point is, you know, recently I had a family member who underwent chemotherapy for non-Hodgkin's lymphoma. The chemotherapy led him to have a white count of like zero; he went into septic shock, went to the hospital and almost died. Now, we didn't immediately go out and say, well, gee, we need to stop giving chemotherapeutic agents because we are treating his cancer, he is going to die from his cancer. These patients are going to die from the lack of blood, and we need to start thinking about it in a little different risk-benefit ratio, because this young woman, this 19-year-old -- no question. No question whatsoever. If we

had 10 units of a hemoglobin-based oxygen carrier, she would be alive today.

In fact, we had an airplane that was landing in Arkansas picking up a liver right then. Just serendipitously, the liver transplant team was coming in; we were going to put a new liver in this woman. She'd be alive today, 19-years-old, a member of our workforce, maybe she would be working for the FDA, you know -- if in fact we had an HBOC available.

So this is a very clinically relevant thing. It's not going to an easy thing, I realize, to approve, but I really think you need to start thinking about cost-benefit ratio similar to chemotherapeutic agents, rather than similar to a crystalloid or colloid. Thank you.

MR. KLEIN: Thank you very much. Would the speakers please come up to the podium? I thought you were going to say that it wasn't someone without a liver, but without a heart that was going to be working for the FDA.

(Laughter)

MR. KLEIN: And if there are -- if there are cards, someone is going to collect them and bring up there, and while people are getting settled, let me ask

the first question. Thank you.

Dr. Natanson, I will start with you since I know you so well. Your recently published meta-analysis showed increased mortality in most every category of trial, with most every HBOC that was available, and showed an increase in cardiac problems with virtually every setting and with virtually every drug. Now, we've just heard about situations where there are very high mortality and trauma in young people.

Is this a setting where one could think of using an HBOC despite the data that you've put together, because of the potential benefit outweighing the risk?

MR. NATANSON: Remember, there has been no meaningfully beneficial effect reported in any clinical trial of HBOCs. Yet, there has been a statistically significant overall increase in mortality, and almost threefold increase in myocardial infarction. So if you're going to study it in humans at this point, I think the only population that a justification could be made is with a 100 percent mortality. And you have to be assured of that.

MR. KLEIN: Would anyone comment on the panel,

we have a number of --

MR. DEMETRIADES: If we are ever going to show any difference, any efficacy, you need to select your groups very carefully. It's unlikely to show any benefit if you have a -- if you cast your net too broad. You need to get patients -- young patients with no associated diseases, with -- trauma, and a blood pressure, very low, and then overall mortality of 30 to 40 percent. You're choosing different groups; I think it's unlikely to show any difference.

MR. KLEIN: Any other comments, Mitch?

MR. FINK: So just had a couple of -- two introductory comments. First of all, I chose not to say anything during the formal presentations, because I really had very little to contribute. And secondly, although I've recently joined the dark side, and work in an industry setting, my current company has nothing to do with transfusion or blood products, or resuscitation, and so I'm unconflicted.

I've known Chuck Natanson for almost my entire adult life, and I have enormous respect for him, and I usually disagree with him. But in this case, I must say

that I think he makes perfect sense. I think his analysis is spot on, and this is -- he's exactly right. In order to show a difference on the positive side for HBOCs, given the current technology -- I'm not talking about the next generation HBOCs, which might be different and might solve the nitric oxide scavenging problem -- in order to show a benefit, you really have to study patients like the one Steve Cohn was talking about, where the probability of survival in the absence of the resuscitation fluid is exactly zero percent. There, there is a possibility for showing benefit. The problem of course is that finding those patients in meaningful numbers and being able to conduct a study in some kind of reasonable time is a extraordinarily difficult challenge.

Now, the other comment I would make is the following. Hemoglobin does in fact scavenge nitric oxide. There is a lot of controversy here, but there is no controversy about the fact that iron 2 in a heme moiety binds nitric oxide with high affinity. And the class effect is related to the binding of nitric oxide by hemoglobin. That's something that we can't get away from. I'd also point out that people have done a number of

trials by modulating the nitric oxide pathway. And when you modulate the nitric oxide-guanylyl cyclase pathway by activating it, you can turn things into useful drugs ranging from nitroglycerin to inhaled nitric oxide, to Viagra.

But when you turn off the nitric oxide-guanylyl cyclase pathway, you run into problems, and it doesn't matter whether it's septic shock, or resuscitation; that seems to be a problem. I think there is a lesson there, and before we would move on to study broad groups of patients, we need to solve the problem of nitric oxide scavenging related to hemoglobin-based oxygen carriers.

MR. KLEIN: Mitch, if I could just follow up on that. Are you suggesting that if you're still in Pittsburgh and I came to you with any of the current generation or the previous generation of HBOCs, for any clinical setting, you'd be reluctant to use any of them knowing what you know now?

MR. FINK: Absolutely. If you came to me in Pittsburgh, it's not in the middle of the plains in North Dakota as Steve pointed out, it's at a urban medical center where there is access to pack red blood cells, and

if you needed oxygen carrying capacity, which as Dr. Holcomb pointed out is usually not the case. But if you did in fact need additional oxygen carrying capacity, I would use pack red blood cells.

MR. KLEIN: This is for Dr. Vlahakes. Please expound on your statement that vasoconstrictors don't override metabolic autoregulation. And as a second part to that, do you believe that vasoconstriction is not a potentially adverse property of the current HBOCs?

MR. VLAHAKES: The first part, if you look at the studies that -- and again, we're talking about experimental studies -- shock preparations, et cetera, you do not get a deleterious effect on local and organ blood flow, including models of massive blood replacement that started out with -- that started out with preparations in shock.

Secondly, if you look at studies done on the coronary circulation, which is my personal interest, not only did the materials not override metabolic autoregulation, but they had oxygen carrying capacity, while decreasing viscosity. So if you hemodilute with an HBOC -- and this was published in Artificial Cells,

Biomaterials, George Hodacasceu (phonetic) is the first author -- you can actually increase maximum potential oxygen delivery in the coronary circulation. We've never seen an override, and this includes materials that contain substantial amounts of tetramer.

Your second question -- the second part of that?

MR. KLEIN: The second part was whether the vasoconstrictive effects of HBOCS are something that you're concerned about in your clinical work?

MR. VLAHAKES: Well, there is two related answers to that. In the cardiac surgery trial, we have complete control over the hemodynamics. We are measuring filling pressures; we are in an intensive care setting where blood pressure can be managed if it became an issue. But many of the patients have the problem of low SVR.

Recovering from a narcotic-based anesthetic, they may have been on vasodilators like ace inhibitors before surgery; there's a relative degree of surgically-induced anemia and its potential consequences on SVR. So in the cardiac surgery trial, any vasoactive effect was more likely to be a benefit rather than a detriment, and we were able to manage it again in the ICU setting.

We picked the ICU setting on purpose in order to have that degree of control and the ability to gather the data. Now, to carry that further, there was a study published -- an animal study that looked at resuscitation, with, I think, the diaspirin cross-linked, the Baxter material. And if you use blood pressure alone as a volume replacement endpoint, this particular study had instrumented the animals that were used, and you wind up with very low filling pressures. You wind up with the wedge pressures down in the low signal digits, if BP alone is used.

So one of the issues with the vasoactivity in clinical setting, where you might not have a lot of monitoring, particularly of preload, you can wind up under-resuscitating your patient, or in a laboratory setting, an animal.

So it is an issue, but it can be overcome with monitoring and patient management, and it's part of learning how to use a new class of -- as I pointed out, this is a new class of materials, and part of learning how to use a new class of materials is how to manage the issues associated with it.

For example, when aminoglycoside antibiotics were introduced to clinical practice, renal failure was potentially an issue with use of aminoglycosides, and what came into the practice to manage it well, the ability to measure peak and trough blood levels, which allowed you to control the risk of nephrotoxicity. Again, this is a new class of material and the vasoactivity, if it's going to persist as an issue with the class; it's something we're going to need to manage.

For those who haven't seen it, I would call your attention to a recent publication in circulation that came out of Warren's Air Force Laboratory, having to do with pretreatment with nitric oxide. It's fascinating, and it's an issue that's going to need some more follow-up in the laboratory. And potentially, if we wind up in further clinical trials with humans and the vasoactivity remains an issue, it's something that might need to be considered.

MR. KLEIN: Let me follow up on that question and ask you then, do you feel that any of the current generation of HBOCs would be usable in a trial of cardiac surgery if you think that that may have a benefit?

MR. VLAHAKES: Well, the only setting -- the

issue of course is the blood supply has changed and the safety of the blood supply has changed, and if you look at risk-benefit analysis, if you're able to use blood, if this is not a patient where there is an absolute religious issue -- religious issue for blood transfusion, it's hard to go up against pack red blood cells in patients that are having elective surgery; it's very hard to do.

MR. KLEIN: Dr. Cohn?

MR. COHN: Well, I just wanted to comment, I just had a relative that had an aortic valve put in, didn't require a pint of blood. My impression and that of many others is the use of blood in the hospital is dramatically dropping. We don't use blood hardly ever for general surgery, you know, aortic surgery it's -- basically most of it's gone, and has been replaced by endovascular, where they don't use blood.

The radical prostatectomy is used to be one of our high blood loss areas that's been replaced by robotics and a bunch of others. So there has been a progressive decrease in the use of blood. For sure, the redo, redo, spine and the redo, redo this and that still requires some blood, but overall blood use has really been reduced --

been limited, and I think part of it is because as intensivists, we don't transfuse people in the ICU like we used to; we let people be anemic. And I think that in cardiac surgery, I wonder, Gus, what percentage of your patients get transfused now?

MR. VLAHAKES: Well, it depends on your patient's substrate. So if you're dealing with people of advanced age, and people who have been -- who have had the surgery on the heels of a hospital stay, where they have been catheterized, they will come to surgery with a degree of iatrogenic anemia and the so-called "anemia of chronic investigation," as we call it. And those people will get transfused.

The elective -- the elective aortic valve replacement, such as you've mentioned, particularly with the techniques of autologous priming of cardiopulmonary bypass, now routinely use measures of blood conservation and scavenging all the red cells out of the profusion circuit, you can get by an elective surgery without transfusing people. But that's at least in our practice, that's less than half the patient population.

MR. KLEIN: This one is for Dr. Freilich, and

this is would there be a different perception of HBOCs, if the first clinical trial had been the one proposed by the Navy -- and I'm going to ask Dr. Sloan after you comment on that Dan, what he thinks after having seen two trials.

MR. FREILICH: The way I'd like to answer that is first, I'd like to address some of the comments that Dr. Natanson made. And I feel as though I know Dr. Natanson for decades also, since the Friday release of the do-not-distribute JAMA article.

And I just want to say, first of all, I commend the work that was done, and I think it's very important work. And I think the comment that it's an overall class effect, demonstrating potential -- or actually demonstrating statistically significant increased mortality and MIs with HBOCs in general -- and I think the key word is in general -- is really important. But I think it should go no further than the general comment that myelosuppression is a classic manifestation of most chemotherapeutic trials. Now, having made that comment, one could stop developing chemotherapeutic drugs, or one could figure out how to maximize the benefit and to work with the myelosuppression and still try to improve outcome

of your patients.

The second comment I'd like to make is that once again, I think we have to be very careful about broad brush strokes. And I think one of them is comparing the blood comparisons and the non-blood comparisons, because in fact, that article makes such a comparison, but there are no non-blood comparisons, there are no trails to date that have been done as such.

I just wanted to make a comment to Dr. Vlahakes about the potential for hyperperfusion due to hyperresuscitation. And I think that's definitely a concern. I must admit, I think in our institution we have now evaluated HBOCs in actually 200, maybe 300 pigs. And for what that's worth, what we have noticed is that if one does pressure-controlled circulation -- pressure controlled resuscitation, as was so often published in the 1990s and '80s before, that is high risk, and you see manifestations potentially including lactic acidosis.

And these have been published and have been noted by FDA numerous times. If you include a simple additional criterion such as heart rate -- and that's not surprising, in the stroke index -- I mean, the shock index

that was described by Dr. Sloan, that the addition of heart rate to mean arterial pressure narrows it down to a patient population that's really sick, and it allows you to continue to resuscitate despite the vasoactive effects.

So to answer your question, I think, there would be an enormous difference if one went and first looked at high mortality patients who have a potential for benefit. The potential -- last comment I want to make is that it is -- I find it ironic that in this science, one requires a zero adverse-effect potential.

When the regulations -- and everybody expects that it should be a reasonable risk, and to say that one should study only something where there is 100 percent mortality -- in other words, there is no risk -- I think, flies in contrast with what has been done with all other potent drugs. And I'm in complete agreement with all the comments that studies in 10 percent mortality, 20 percent are undesirable with the current generation of HBOCs, although with risk mitigation studies, such as the addition of nitroglycerin or inhaled nitric oxide or other proteins to get rid of vasoactivity; maybe that would be worthwhile.

But I think that to say that higher mortality studies should not be done unless they're 100 percent, does not seem to fly with current practice.

MR. KLEIN: John.

MR. HOLCOMB: I'd like to echo Dan's comments. It is interesting --

MR. KLEIN: He may be armed, so be very careful.

MR. HOLCOMB: Yeah, that's right. Well I'm going to speak to that. So the two guys in uniform here that are in dogmatic organizations are actually pleading for moderation from our civilian colleagues.

(Laughter)

MR. HOLCOMB: I find that an interesting phase to be in because, Chip, as a trauma critical care surgeon in uniform, it's not a normal place for me to position the whole -- 100 percent, do you really mean that?

MR. NORRIS: I absolutely mean that.

MR. HOLCOMB: That's really unfortunate. I would agree with Dan. Nothing is 100 percent. Standing in the emergency department, trying to figure out what cavity to operate in, what fluid to give, how much, when to start, when to stop; Sir, that is not a 100 percent

place to live, that's not reality. Your comments remind me of the statistician from yesterday. They don't live in reality. And so let's --

(Applause)

MR. HOLCOMB: Now, the flip side is, your article is fascinating, and I don't disagree with many other things you said. It causes us to pause, and have questions and to do further study. So my plea is actually, to do a series of iterative emergency research studies in this area. That's what we need, so we can have more data within which we make good decisions.

When you read the "shock" chapter in ATLS, I know all of you have taken ATLS because you're all are trauma experts -- when you read the ATLS chapter and go to the references, the guide for massive transfusion used in 2008 was a paper written in 1985 that has no control group, and has 11 patient centers. That's when we do massive transfusion today, in 2008. It was in a really poor paper from 1985.

The second paper is actually much better, it's from 1976, and recommended whole blood. That's the state-of-the-art in massive transfusion in the United States,

and around the world because ATLS guides early trauma care around the world.

And ladies and gentlemen, we need to do iterative studies. Nothing is 100 percent to get better than that. Thank you.

(Applause)

MR. KLEIN: I don't want to let Ed Sloan off the hook. Ed, you were the PI for the Baxter study, and I know that has been analyzed and re-analyzed, and re-re-analyzed. And we heard Tim Estep tell us that there may be lots of reasons aside from toxicity of the drug why the trial was stopped for excess mortality. We know about the trial that was stopped in Europe, even though there wasn't excess mortality.

You've looked at that so long, would you today be able to design a trial using one of the generation of current HBOCs, whether it was the now discontinued hemocyst or some other -- in a similar trial, knowing what you know?

MR. SLOAN: Yes, in reanalyzing this for 10 years, there are two things to consider. One, you need to have control over knowing what patients are being entered

to make sure that they are not call violations that sabotage the ability to study the effect of any therapeutic. The second would -- I would avoid inclusion of any patients with a GCS of three.

So with regard to the comments that have already been made, I would suggest the following. You cannot study a patient population in whom the mortality is likely to be 100 percent. You're pointing us in the direction of studying the most critically ill patients that we can study.

I would therefore look at patients with an extremely low RTS, who are physiologically ill, who likely have a great deal of injury as measured by the injury severity score. I would exclude the use of the GCS equals three patients, and then you might approach mortalities of 70, 80, 90 percent, which will allow you to study a very sick population of patients, and still understand whether or not there maybe benefit -- therapeutics.

Regarding Dr. Natanson's data, I'd like to just make two comments if I could. Much as we when doing any study, if the study ends up not putting as where we need to be, we try to look for subsets, in whom there might be

benefit in order to -- hypothesis generate for the next study. I would also take data and say, let's look at the patient populations for whom these class of drugs appear to impart the greatest harm.

In other words, if much of the mortality imbalance is related to a stroke study, and you don't include it -- include stroke patients in your future studies, you may have overcome some of the problem or some other hurdle that we now face, based on the aggregate meta-analysis.

The second comment I would make is we need to be very careful in looking at myocardial infarction -- because if many of the patients who are claimed to have myocardial infarction, it was on the basis of elevated cardiac enzymes. But ultimately, there was no left ventricular dysfunction and/or long-term mortality related to it.

I think most of us, if we believe that the therapeutic would improve outcomes in other ways, would settle for some elevations -- which may occur incidentally, just with the use of pressors or other agents, I state parenthetically. So there are some -- I

think that the work is important. I just think now what forces us to look closely at -- to find out how we can identify a patient population who is least likely to be harmed, given this aggregate meta-analysis look, and to consider things such as enzyme elevations, which may not be clinically relevant, if you have a gunshot wound and you no longer have a liver, and you're just in need of blood or some oxygen-carrying solution, or any type of therapeutic to get you over the hurdle.

MR. KLEIN: Thank you. I think I'm going to leave the definition of MI to the next panel, which is going to specifically look at organ toxicities, but that's an important point. I think Dr. Fink wanted to make a comment, and then Dr. Cohn. Mitch?

MR. FINK: So, just a couple of three quick responses. First of all, comparison has been made several times this morning, and I think even yesterday, to cytotoxic chemotherapy for cancer. As far as I'm aware, currently -- I'm not an oncologist, but as far as I'm aware, currently there is no alternative to cytotoxic chemotherapy for cancer.

But there is an alternative to HBOCs for

resuscitation, for the vast majority of patients who need additional oxygen-carrying capacity, and that's pack red blood cells. Although pack red blood cells carry their own risks, trolley (phonetic) being the biggest as far as I am aware, they do have a remarkable safety record.

So if you're going to study an HBOC in a high-risk population, it has to be in a population where the alternative, that is pack red blood cells is unavailable, I think, in order to conduct a study ethically. That is a reasonable study to do. It's just -- from a logistical standpoint, extraordinarily difficult one to do.

It's just an extraordinarily difficult study to do. The second point is I think there was a comment made this morning that because the blood-lactate concentrations in some of these subjects who received HBOCs were not significantly different than the control group, that there is no evidence of tissue ischemia. The problem with that assessment is that blood lactate concentration in trauma victims has nothing to do with blood flow to the tissues. It has to do with the circulating catecholamine levels in the patients. If you beta-block the patient, the blood lactate concentration drops.

It's a biochemical mechanism at the level of the skeletal muscle; it is not a reflection of local tissue perfusion. So it's not a useful measure of whether you are causing vasoconstriction in key vascular deaths.

MR. KLEIN: Dr. Cohn, and then Dr. Natanson.

MR. COHN: Okay. We are conducting a resuscitation trial and taking patients in profound shock including patients with severe brain injuries with Glasgow Coma Scale of 3, the highest we can get our mortality is about 40 percent. That's the people that come in that are herniated. And so, 100 percent population, probably not viable, unless they're actually in complete arrest, and now, you are talking about reanimation, it's completely different kind of bargain, that's number one. Okay, the Lazarus effect.

The second thing is in regards to Dr. Finks' comment. One of the problems with doing clinical trials is that the trauma patients were in urban centers, and as you've heard from the PolyHeme trial, they attempted to compare a blood substitute or an oxygen carrier with -- in a setting where there was not blood available, in the pre-hospital setting. But the pre-hospital time in both

groups is only 26 minutes. And it's hard to know how much you could get in 26 minutes, and also what kind of benefit there might be, which leads you to say, well, what about 3-hour transports.

Earlier in my time, and in my current position, I was on call in -- I got a MedCom Call that the helicopter was heading out to pick up someone 2 hours away -- 2-1/2 hours away in a place called Uvalde, who had been shot in the groin who had been shot in the groin and was hypotensive. And the hospital crew brought some blood with them to the small hospital, and en route back, he got four units of blood, and when he arrived he had no blood pressure and a barely palpable carotid pulse. He survived because they had brought their oxygen carrier out with them.

Doing the trial in that population, long transports, I think would be extremely logistically difficult to do. And one of the issues that Dr. Holcomb and I were talking about yesterday is that the crews typically break -- they go -- they're becoming noncompliant. They know that blood or a blood substitute is better than nothing in this person who is in shock and dying. They're going to go head and break the code and

give it if it's onboard.

They're just not going to comply with this. No one's going to let a patient die. The crew thinks that it's going to resuscitate him; they're going to give it. So it's very difficult to do this kind of trial, even though I agree that might be a good opportunity.

MR. KLEIN: Dr. Natanson?

MR. NATANSON: I want to state my case a little bit more clearly. I am fully supportive of HBOCs. I think this is a great idea in the area of research that needs to be fully, fully supported and -- move forward. It's a product that we desperately need. It's just at this point, if you compare the data that I provide and the data that Dr. Silverman provides, myocardial infarctions mortality are not the limit of toxicity.

The toxicity involves renal failure, stroke, pulmonary injury, liver function abnormalities, pancreatitis. If you look at these data sets, these are diffusely toxic. We need to return to the animal models. We need to get a new formulation, and in order to move this field forward -- which we need to do -- we need to come to that understanding. And that is only way I

believe, we are going to advance the field.

MR. KLEIN: Let me just follow up on that because I had a number of questions for you, some fairly inflammatory. But let me just ask this one, which is less so. Meta-analysis usually looks at outcomes from studies using identical drugs. How can you lump together so many different studies using different HBOC products?

MR. NATANSON: Are you asking me?

MR. KLEIN: Yes, Chuck, could you address that one?

MR. NATANSON: Say it again, I'm sorry. Say it again.

MR. KLEIN: Usually, when you're doing a meta-analysis, you're looking at a single drug. How can you lump together so many different studies using different HBOC products?

MR. NATANSON: There is no (inaudible) what you do in terms of meta-analysis. Meta-analysis begins with a question -- a scientific question. The scientific question we asked was is, these are all hemoglobin-based products, and they are all blocked -- or inhibit normal function of nitric oxide. But once you do a meta-

analysis, you then are required to not compare apples and oranges, you can only compare like effects. And so then, what you do is you do a test of heterogeneity. And the test of heterogeneity is the Breslow-day test we did, and we found that the treatment effect was very consistent across all of these clinical trials, regardless of the clinic indication that was used, regardless of the manufacturer, regardless of whether it was a published study or an unpublished study, and regardless of the chemical alteration.

MR. KLEIN: Dr. Demetriades?

MR. DEMETRIADES: In this panel, we've heard some real, hard, scientific facts, which unfortunately, we do not like. And then on the other hand, we've heard some one-liners and clever things, which we like. At the end of the day, there is a message for the industry. There is a major need for these products; we are still not there. You need to go about and improve these problems.

And I want to urge the FDA, at least, for compassionate use, to look into this again an allow us to go ahead with that. Thank you.

MR. KLEIN: Thank you. Dr Vlahakes?

MR. VLAHAKES: A lot of the questions that have been posed are only going to be answered by through clinical research. And you're not going to be able to -- there is only so much you can do with animal models, et cetera. And one of the decisions that's going to have to be made by the agency is whether or not it's going to be back to the laboratory and to the dreaded R. word "reformulate," in order to get us some clinical trials.

Secondly, I would emphasize the importance of piloting clinical studies in phase 2. And the agency has occasionally even suggested that -- to vendors that they should pilot their planned phase 3 clinical trails in phase 2. And on one occasion, that advice was not heeded to the detriment of the ultimate phase 3 trials.

So there is a lot -- there are a lot of bugs to be worked out, when you're using this in the clinical setting. And I'm not speaking now, so much of the kind of the fast-pace, fast-breaking trauma setting, but the setting in other surgical areas with inpatients.

The second thing is hospital care has changed a lot, and if you have a brand-new entity being put into clinical trial at an institution that has never used it

before, you really have to assess your clinical sites, and to find out about issues such as clinical areas that are covered versus not covered, other hospital list, how consistent is the postoperative care and the ability to get very good observations made, and potential problems, either evaluated properly and aborted.

The change in -- for example, how staff hours and the increasing number of services that may not be completely covered or cross covered in the off-hours can have a potential adverse impact on the conduct of a clinical trial. And some of that, you're going to find out through a well-analyzed phase 2 trial, before you get into phase 3.

MR. KLEIN: Thank you. We've reached the end of the hour. And I have a whole packet of questions. So I would just ask those who wrote them, attack our panelists in the dry coffee hour. I want to thank our panelists for taking the time to come here, for keeping to their time and for their opinions. Thank you very much.

(Recess)

ORGAN SPECIFIC ASPECTS OF SAFETY

MR. WEISKOPF: Please everybody take your seats so we can begin.

In the past day-and-a-half or day-and-a-quarter, we have heard much about toxicities and mechanisms and the planning group has put together and organized a group of experts regarding subject of organ toxicity and they will be examining the HBOCs in that light, in light of specific organ toxicity, trying to draw on various sources of information.

The names are as you see them before you and they are all professors at their home institutions, of course, with the exception of Mark Gladwin who is here at the NIH and that’s not to say, he isn’t as accomplished as the others, it is just it doesn’t offer that level of title.

In addition to professorships that you heard Mr. Mitch Fink point out, he is also the CEO of a bio-tech company and I also consult through a bio -- a pharmaceutical -- a company as well.

And these are the topics that these people will be making some presentations, followed by a panel

discussion. And here, I'm not going to read all this, this is just too much. I'll summarize it though. These are the conflicts of interests that go back much further than the government generally requires. Many of these are Paleolithic information, and the only thing that really is truly current is Mark Gladwin's disclosure that he has both a patent and a patent application regarding nitrites.

The format of this session is very similar to the one that we just finished, and that is, we will have very brief presentations by the panelists of five minutes each, followed by a question and again, questions from the audience are in the same written format.

But before getting to that, I'd like to make a few comments about the limitations of our discussion here. And I want to give you a little bit of my perspective before I even say -- talk about the limitations. That following a nearly 30-year academic career, which included not only consulting for industry, doing my own trials, doing trial designs, doing phase I trial in HBOCs, consulting for the FDA at times.

I then went to industry, a different industry unrelated to HBOCs, but worked with executive management

in a moderate-sized pharmaceutical company for two years, so -- and I understand industries' concerns very well, I think.

So I've come at this from a variety of perspectives and we have all heard those perspectives here in the past day-and-a-half. With that in mind though, I feel it necessary to point out the limitations that not only this panel will be discussing, but the limitations as we have heard other people discuss and perhaps even as we read the literature that the -- we are dealing with a limited amount of clinical information in the public domain.

The FDA of course has a database, which is much larger than the information we have been discussing and that is because much of that information is proprietary. Not all completed trial data even are available to the FDA, as we all know, some trials have been finished and data never submitted to the regulatory authority.

This raises issues not only of efficacy and safety that we have been talking about. But I think it also raises issues of ethics that I would hope that in the next panel will be addressed as well. In addition to

that, not all the public information we have been discussing or will discuss is peer-reviewed. Some of it, as you have heard, come from corporate announcements, some perhaps from abstracts that are not necessarily peer-reviewed.

In addition to that even the data -- all the data, whether it be public domain or not, peer-reviewed or not, much of it depends upon site-reported information as opposed to independently reviewed AEs and SAAs. Both of these types of data have their own problems, and make it difficult for us to have a full clear discussion.

And for those in the audience and elsewhere who believe that their data are not correctly interpreted by some, the only answer I can propose to them is that if you believe that, the answer is to be more transparent with a great deal of clarity.

In addition, what we have been talking -- a lot of what we've been talking about, have lumped things together with resulting heterogeneity, which has the potential disadvantage of diluting signals from individual study trials.

With those brief comments, I think, we'll move

on to the first speaker, and the first speaker is Professor Baines from the University of Toronto who will talk to us about renal issues.

MR. BAINES: Thank you very much. It's a pleasure to be here and very entertaining at times. My question is, why is acute renal failure so uncommon in these HBOC trials. If we look at Dr. Silverman's review of the available literature, it is only about one percent of the controlled patients and not significantly different proportion of the test subjects that have what is reported as acute renal failure.

There has been a recent review of careful analysis of renal injury, acute kidney injury, in various intensive care situations, the ones which are most relevant to, I think, our situations are those after elective cardiac and abdominal aortic surgery and what is found there is that the, the incidence of acute kidney injury and the word is different, is about 15 to 22 percent. That's without any use of HBOC. One wonders then, have we got a problem of definition here as to what is acute kidney injury and what is acute renal failure.

There has been a recent consensus conference,

which has modified the riffle criteria. The riffle criteria we developed about 2004, for evaluating kidney injury and classified it into risk, injury, failure, loss, and end-stage disease.

We now have stage 1, 2, 3, and they are classified on the basis of the serum creatinine changes and urine output changes. And if you use those criteria, and the data that's provided by Dr. Silverman, it seems that in the trials that we had information to go on that the prevalence or incidence, sorry, of stage 1 and stage 2, acute kidney injury in the HBOC trials was greater than 25 percent decrease in GFR in both the Hemosol and Baxter trials.

And interestingly, an apparent increase in GFR, in the Biopure and Somatogen trials, and there is only one trial where the goal standard was used for measuring glomerular filtration rate, and that was the Sangart trial which used iohexol clearance.

And one wonders whether, rare or uncommon predisposing factors account for the low incidence of acute kidney injury, which is still low by comparison with some of the reported ICU incidents, which can get up as

high as 70 or 80 percent of the patients in ICU having acute kidney injury by these new criteria.

There have been studies which suggest that polymorphisms and a variety of factors involved with processing reactive oxygen species and inflammatory reactions may account for the susceptibility of some patients to acute kidney injury where others will escape. It may be differences in drug therapy with ACE inhibitors and NSAIDS, and so forth, age, and diabetes.

There are limitations in picking up kidney injury by using just serum creatinine, because of the delayed response in the rise, the nonlinear relationship to glomerular filtration rate, and very often, almost always, I would think, the unknown initial glomerular filtration and serum creatinine.

This leads to an ascertainment bias, for example, you take the 0.38 milligram per deciliter increase that was reported in the Hemosol trials, and you put that increase on a base of 0 6 milligrams per deciliter, the lower reference range for a woman; that would be equivalent to a 40 percent decrease in GFR.

If you put it on top of the upper reference

range, 1.1, it’s only a 26 percent decrease in GFR, but it takes it out of the reference range, and that would lead to that individual being classified as having perhaps, acute renal failure, when in fact they had a modest decrease in GFR, and the other person would be classified as being within normal reference range and not abnormal.

What we need are better markers, sort of a troponin for the kidney and these are being now revived again in the Sangart trial NAG was used, N-acetylglucosamine, indices of inflammation and NGAL, IL, interleukins, and KIM-I which is a marker of proximal tubular changes.

Lastly, the one problem that we faced is that animal models don’t correlate well with human disease. It is very hard to reproduce acute renal injury that mimics the human disease.

That having been said, when you do look at what happens with acute kidney injury, it is primarily an apoptotic and necrotic condition in which there is a considerable component of tubular intestinal inflammation and the response seems to be triggered not by nitric oxide but by reactive oxygen species.

Changes in blood vessel permeability with gaps and leukocyte adhesion and activation of (inaudible) and so forth, must play a role. What we don't know anything about is what happens in the long term. There are studies in animals, which show that repeated hemoglobin injections or a single instance of ischemia or reperfusion will lead to long-term changes with tubular interstitial scarring.

So my conclusion is that some patients and some animals respond poorly to the stimuli or simulation of acute renal failure and we don't have any data in the older trials that -- to say how many had stage 1 and stage 2 acute kidney injury and we have no long-term follow-up on either HBOCs or red blood cells. Thank you.

MR. WEISKOFF: Thank you.

(Applause)

MR. WEISKOFF: Our next speaker is Mitch Fink who will be talking to us about GI system.

MR. FINK: So good morning, again, my appreciation to Dr.Weiskoff for inviting me to participate in this panel and to be able to attend this very entertaining and informative meeting. There is certainly no shortage of controversy in this field, and I think the

only thing that everyone really agrees on is that the medical need is enormous and we all really do have an interest in solving this important problem.

So my task was to spend a minute or two talking about GI complications. I interpreted the GI tract to mean the tube that goes from your mouth to your rectum and all the organs that are connected to it, and in her presentation yesterday, Dr. Silverman presented you with a lot of data related to organ system toxicities that have been associated with HBOCs and I simply extracted some of the data that she so carefully collected and presented, just to outline that.

Of the eight HBOC products, at least five of them have been associated with GI-related AEs and at least three of them have been associated with at least biochemical evidence of acute pancreatitis.

So although there are all kinds of GI complications that have been reported, really the three most consistent ones have been evidence of pancreatic injury, evidence of hepatocellular injury, and chest pain of a sort that’s consistent with esophageal spasm.

Pancreatic injury has been evidenced by

increased circulating concentrations of the pancreatic enzyme, lipase, increased circulating concentrations of amylase, and in much more rare instances, clinically apparent evidence of acute pancreatitis.

Hepatocellular injury has been evidenced almost exclusively by biochemical changes, specifically increased circulating levels of transaminases and the most consistent finding of esophageal spasm has been fairly classic chest pain findings.

I would point out that the biochemical changes associated with pancreatic injury and hepatocellular damage are likely the tip of the iceberg, and if HBOCs were used in a epidemiologically significant way, that is, hundred of thousands or millions of exposures per year, it is very likely that massive hepatocellular damage and massive acute necrotizing pancreatitis would turn up as rare, but clinically very important problems, just as been the case for when hepatocellular enzyme changes in initial phase studies have turned up later, once a drug is widely available in the market, as rare instances of acute hepatocellular necrosis.

So what are the mechanisms responsible for these

changes? I am not going to talk about all of them, but I am going to focus on acute pancreatitis. There is probably two non-mutually -- mutually compatible mechanisms. And the first, ENO scavenging is known to be able to cause spasm of the sphincter of Oddi and that would increase intraductal pressure in the pancreas, and in animal models increasing intraductal pressure is one of the ways that you can cause acute pancreatitis.

Secondly, ENO scavenging can diminish or impair pancreatic microvascular perfusion, and again, in animal models, causing pancreatic ischemia is one of the ways that you can induce acute pancreatitis. A combination of intraductal hypertension and pancreatitic ischemia is a really bad combination, and is very likely to be associated with the development of acinar cell damage and the induction of pancreatic inflammation and pancreatitis.

Additionally, as was pointed out yesterday, there is a possibility that a non -- or a mechanism that is not directly nitric oxide related or related to the scavenging of nitric oxide might be important.

And that’s, for example, the liberation of reactive oxygen species or hypervalent iron in the

pancreatic milieu causing redox-mediated damage to the pancreatic parenchyma. Thank you very much for your attention.

(Applause)

MR. WEISKOFF: Thank you very much. Each of these speakers really has a daunting task of trying to put together the myriad amount of sources of information and what we have heard over the past day-and-a-half, and perhaps the most difficult of this taskforce our next speaker, David Warltier, who is going to try and make sense out of the various pieces of cardiovascular information we have heard.

MR. WARLTIER: I thank Dr. Weiskoff and the organizing committee for the opportunity to participate today. The -- I thought, first, we'd take a look at some hemodynamics, and when I was deciding what subject matter we should take, and which of the many different HBOCs we should look at, I though maybe I'll just take one that we worked with in our research laboratory, and this is data from dogs, and it's with the recombinant human hemoglobin from Somatogen, the first generation product.

If you take a look at this data, it is change in

mean arterial pressure from baseline, with three different doses of this drug; plateau is around 35 millimeters of mercury, at a dose of between 1 and 2 grams per kilogram. So large increases in arterial pressure and the mechanism for this was an increase in peripheral vascular resistance.

In fact, this produced a decrease in cardiac output. The decrease in cardiac output was not related to ionotropic state. Here is left ventricular DPDT measured at 50 millimeters of mercury and there was no significant change in this.

Large impedance for left ventricular ejection produced by an increase in afterload is associated with an increase in left ventricular and diastolic pressure here at the high dose increasing to almost 10 millimeters of mercury; this increases, despite control of intravascular volume.

Now, just one last thing I would like to mention is there is a significant decrease in heart rate and one would think that this is probably related to baroreceptor reflex, but in fact, the decrease in heart rate actually occurs in isolated heart preparations.

Some more data with this Somatogen product, and this shows a vascular resistances in a number of different regional circulations, the data in rats using radioactive microspheres. And in almost all these beds, we can see an increase in vascular resistance.

This was especially true in the kidney. There were a couple of exceptions to this. A skeletal muscle, which has such low flow to begin with, it is really difficult to decrease it any further in the anesthetized rat, and -- but also in the left ventricular myocardium there was no visible change of vascular resistance, probably due to the importance of metabolic autoregulation in this preparation.

Now, interestingly enough, the second generation product, this is again recombinant human hemoglobin, but that’s been genetically modified so that it does not bind to nitric oxide. There were no changes in any vascular resistance with this compound.

Those were typical physiological changes. Although we would all agree that these HBOCs clearly are different chemicals, this is important I think anatomical, morphological data. Hemoglobin myocardial lesions were

first noted during dosed escalation studies of the diaspirin cross-linked hemoglobin. There were first seen in cynomolgus monkeys; it's species-specific, at least certain species are, primates are, more sensitive to this.

Now, what these lesions are, are punctate degenerative very diffuse lesions across the left ventricle, and they're very, very small. What you see is a formation of vacuoles in the lysis of nuclei in cardiomyocytes. It's actually very similar to chronic confusions of sympathomymedica means or even the chronic confusion of L-NAME a non-specific inhibitor of NOS.

These degenerative lesions are associated with only very small increases in creatinine and phosphokinase and it's only very minor changes in the T-wave, probably so, because only a very small amount of myocardium is involved. Finally, the recombinant hemoglobin does not bind nitric oxide, there is less lesions with this, nevertheless they're still present.

Now, this is the slide that everybody is using in a different format or another, these are different products of companies and HBOCs and over here lists adverse –- serious adverse events in –- that may be

related to the cardiovascular system.

I would just have you focus on this one line, again, that everyone’s been talking about, and this is myocardial infarction. The numbers here are treatment versus control. There may be different control numbers of patients, there certainly may be –- this slide is a snapshot of some studies and there may be ways to explain changes, post hoc analysis. But just let's just go through six MIs to one MI in the control; 14 MIs to 4, 14 to 7, 29 to 2 and 2 to 0.

This is a –- it's really a disturbing finding. I think what we have to do is understand before we move on with these agents or other new agents is the mechanism of how this occurs. It's certainly not due to the vasal constriction. There's other –- there's some other mechanism for this and that's what we have to understand.

Thanks.

(Applause)

MR. WEISKOPF: Thank you, David, I'm sure we'll hear more about that in the Powell (phonetic) discussion.

Our next speaker is going to address a topic that has been touched upon but only lightly in our

preceding sessions and that is central nervous system and Professor Raymond Regan will be talking about that.

MR. REGAN: Thank you, Dr. Weiskopf, and thank you for the invitation to speak here today and to revisit the HBOC field after several years of absence. There’s –- as Dr. Weiskopf alluded to, there's relatively little information about what happens when HBOCs enter the CNS –- if they do enter the CNS. I began studying HBOC neurotoxicity and hemoglobin neurotoxicity several years ago, back in the early ’90s at Letterman Army Institute of Research using a cell culture model –- and up here we see cell culture, cortical cell culture containing neurons and astrocytes. On the right is a sham-washed culture, just subjected to medicexchange, not injured and the neurons are identified by the immunostainings to a neuronal market called neuron-specific enolase, and this is a healthy-looking culture, you really can’t make out the astrocytes very well, because they're not stained. This is a culture treated for 24 hours with 25 micromolar hemoglobin, and all the neurons were just completely wiped out with a few sick-looking exceptions.

So we were really surprised to see this degree

of neurotoxicity from a relatively this low concentration of hemoglobin. We subsequently discovered that this toxicity was not due to the hemoglobin per se but due to its breakdown products, particularly iron. It could be blocked completely with deferoxamine and other iron chelators and also by reducing hemoxygenase activity in the neurons by knocking out HO2.

Looking at this toxicity and Hemoglobin A0 versus the Army's alpha-alpha cross-linked product we found that the neurotoxicity was very similar quantitatively and mechanistically. Cultures were exposed to hemoglobin in a constitution of heme 1 or 10 micromolar again for 24 hours and a alpha-alpha cross-linked hemoglobin and hemoglobin A0 had a similar release of LDH indicating a similar neuronal death, with about 75 percent with 10 micromolar, very toxic, both products.

Subsequently, about –- well, 5 years ago or so, we looked at Sangart's product in this model, NP4, comparing it with stroma-free hemoglobin in this experiment. And you'll see here that the concentrations used were much higher here than here. The reason for that is that this experiment was conducted in the presence of

serum, and we serum we have to increase the hemoglobin concentration about 40-fold to see a similar effect in a serum-free model.

The Sangart product was similarly cytotoxic, actually a little bit more than stroma-free hemoglobin in this Sanvitra (phonetic) model. Subsequent studies done by Vandergriff and colleagues at Sangart suggested that this may be related to an in vitro artifact of this product. It tends to autooxidize faster in vitro, but not in vivo. At any rate, it was neurotoxic in at least a similar fashion to stroma-free hemoglobin.

One other hemoglobin -- blood product has been tested in vitro and that’s Biopure’s product that was published in Journal of Trauma by Ortegon and colleagues back in 2002. This used a neural cell culture system, neuroprogeniter cells, not differentiated neurons and they looked at various concentrations of HBOC 201 versus human hemoglobin, and surprisingly HBOC 201 was relatively non-toxic in this model compared to human hemoglobin.

The reduction of proliferation which was the endpoint used in this particular experiment was observed with the HBOC 201, only to very high concentration. So

this would suggest that HBOC 201 may be less toxic. However, it's important to note that in this study, serum was present in the medium and also selenium and transferrin, both of which are strongly neuroprotective in neural models, against hemoglobin. So the absolute neurotoxic potential of HBOC 201 is difficult to determine based on this study.

The most –- the more important question, the more relevant question is, are these compounds neurotoxic in vivo. I think we can stipulate that it's very unlikely in the setting of an intact blood-brain barrier that a sufficient amount of these products get into the brain to cause neurotoxicity. That's based on a fairly limited amount of data that's available in the public domain.

So the question then becomes, in the setting of a disruptive blood-brain barrier, traumatic brain injury or stroke, are these compounds, or is this class of compound toxic? After many years, after reviewing all the data I could find in the public domain, I think this is still an open question.

Looking at recent studies, most of them have been done with HBOC 201 in a traumatic-brain-injury-with-

hemorrhage model, and I've highlighted three of those recent studies here. These are studies in swine, rat, and swine.

Various outcome measures were recorded, all of them are reasonable measures commonly used in traumatic brain injury research. The problem is they're not particularly sensitive to the neurotoxic effect of hemoglobin. Those of us who inject hemoglobin into the intact brain and look at injury have found that these tend to be the most sensitive markers, protein carbonyls, malonic dialdehyde and 8-hydroxy-2-deoxyguanosine, and they weren't measured in any of those models or in fact any models I've seen of traumatic brain injury or stroke when these products were given.

But what you look and what you measure is probably less important than when you measure it. Hemoglobin is a very slowly acting neurotoxin. If we injected hemoglobin into the mouse brain or the rat brain and look for injury 5 hours or 6-1/2 hours later, we invariably see nothing. It takes a while for the hemoglobin to oxidize, to release its heme and to be broken down to iron which is ultimately what's causing the

injury.

At 24 hours if you have a severe injury you might see something that the best time to look is not 5 hours, 6 hours or 24 hours, best time is 72 hours. So until those studies are done, I can't say with any certainty whether HBOC are neurotoxic if they get into the CNS.

How about in clinical trials? We heard Dr. Sloan's excellent summary earlier this morning about the traumatic brain injury DCLHb trial. I want to recap that this trial, however, focused on patients all of whom had a disruptive blood-brain barrier. Saxena et al controlled safety study of hemoglobin-based oxygen-carrier, DCLHb and acute ischemic stroke published in 1999 –- the trial was done back in '94 and '96 –- and the intervention, DCLHb 2550 or 100 milligrams to a kilogram every 6 hours, so they've got a total of 12 doses, within 18 hours of symptom onset or saline placebo, very small trial, total of 85 patients.

Now, we know now this trial had absolutely no chance of showing any benefit from this product, because of its faulty design. The therapeutic window is 18 hours.

The therapeutic window for ischemic stroke is 3 hrs. No matter what you do at 18 hours it's not going to work.

That said, it doesn't show any evidence of toxicity and the results, while not conclusive are not encouraging either. Eighty five percent of patients treated with DCLHb had and an unfavorable outcome, defined as a modified ranking score of 3 to 6 versus 51 percentage controls at 3 months. And there were 23 deaths in the treated group and only 9 in the placebo group.

There are some limitations to this trial, the randomization was not perfect, there were more severe strokes in the treated group than the placebo group. But that said, the trend tends to be that there was perhaps a deleterious effect.

So in summary there's pretty good evidence that HBOCs, at least some HBOCs are neurotoxic in vitro. Whether that's true in vivo remains an open question in my opinion. I think further pre-clinical trials, pre-clinical studies looking at relevant oxidative injury markers at relevant time points are important before TBI patients or stroke patients are involved in further clinical trials.

Thanks very much.

(Applause)

MR. WEISKOPF: Many of the trials that we heard about today, I mean much of the development is in our inpatients who are experiencing shock in one format or another, and Professor Parrillo will address this issue.

MR. PARRILLO: Good morning. I'd like to thank Harvey Klein and Richard Weiskopf and the committee for inviting me to be here today. By way of introduction I'm a cardiologist who's been interested in critical care medicine, somewhat uncommon combination for the last 30 years or so and specifically I've been interested in shock. I also will mention that I am the editor-in-chief of Critical Care Medicine, one of the journals in the field for the last 11 years, and as I look at –- on this audience a lot of you are reviewers for the journal. I want to say thank you for all of your help over the years; journals would be nothing without the great reviews that are necessary in order to make decisions.

In thinking about this topic I made the assumption that the cardiac manifestations and a lot of the other issues were going to be handled by other

speakers who have, I think, done a great job of telling you about all the different issues. So I decided to kind of take a broad view, considering the fact I had three slides and five minutes.

And so I'm going to give you kind of an overview of my thought about handling shock for these compounds, HBOCs, and really for any compound. Here we go. Okay, so this is the –- this is actually an adaptation of the Weil-Shubin classification of shock, hypervolemic cardiogeneric extracardiac obstructive and distributive shock, and I wanted to really make one major point which is that what we learned about all these different forms of shock is that the timing, the reversal of the form of shock is absolutely critical and our colleagues in trauma surgery area have done a beautiful job this morning of telling us how dramatic and important it is to stop the hemorrhage in hemorrhagic shock.

Cardiogenic shock, very important to get that vessel open. Getting the patient into the cath lab in 60 to 90 minutes is absolutely critical in cardiogenic shock. We all know that in a tension pneumathorax or pericardial tamponade or pulmonary embolus, you have to really lyse

the embolus, or you have to drain the pericardium in a matter of minutes if you're going to have a chance of making a difference. And it appeared for a number of years that septic shock might be an exception to this idea that being quick and being very, very urgent about doing your therapy in shock was not that important.

In fact in septic shock, an area I've been particularly interested in, there are a number of abnormalities that occur in the cardiovascular system and the thought was that maybe it didn’t make as much difference in terms of timing.

And I'm showing you an editorial I was asked to write about, in the New England Journal, asked to write about vasopressin norepinephrine; this appeared actually in the February issue of the New England Journal of Medicine and I'm showing it really because of the slide limitation in order to bring a number of concepts together.

And I made the point that clinicians don’t feel the same sense of urgency to initiate therapy in cases of septic shock as they do in cases of myocardial infarction or in cases of traumatic shock or in other cases of shock

such as cardiogenic shock.

Yet, there are studies now and there are a number of them, I'm going to just show you one in terms of time, that suggests that initiating therapy rapidly even in septic shock may play a critical role in reducing mortality associated with septic shock. And in septic shock it's known that it makes a difference, the antimicrobial that you choose; you have to choose an appropriate antimicrobial. And this is data from Nandakumar, a big observational trial done multicenter in which he looked at the time to giving the antimicrobial versus the odds-ratio of death in this particular trial, and "1" is obviously the baseline. And if you compared the first hour of giving antimicrobials to any hour subsequently, you found a statistically significant increase in mortality.

For instance, if you gave antimicrobials in the first hour of septic shock you had a survival rate of 80 percent. If you gave it at 6 hours it was down to 40 percent. If you gave it at 36 hours it was down to about 10 or 20 percent.

My point here is that depending upon where you

are in the sequence of shock, the HBOCs or any therapy may make a big difference if you do it in do it in the first hour, second hour of –- or it may make very little difference. If you're out at 36 hours –- in fact this study I was commenting on had many patients out at 24 hours, 30 hours after the onset of shock. I would argue that vasopressin or any agent would have made very little difference at that point in shock.

So, I wanted to kind of bring us back to one of the major concepts in handling shock, I believe, of any type and that is that the major therapy has to be homogenous and it has to be applied very early and that urgency is important in all forms of shock.

Thank you very much.

(Applause)

MR. WEISKOPF: Thank you for those insights. I'm sure those in the audience are taking home that message.

Our final panelist to speak in this session is Mark Gladwin here from the NIH and he will talk also about a subject that has been touched upon but only relatively lightly and that is pulmonary issues.

MR. GLADWIN: I'll review these potential conflicts in more detail prior to my next talk for which I think they're more relevant. I was asked to comment on potential pulmonary toxicity of these HBOCs, and as you know there's very little of data available to us. While some of these complications have been listed in the table that was provided to us by the FDA, for most of these complications there's an asterisk indicating we don't know or haven't measured the rates of these complications.

I'll point out that pulmonary hypertension could be a very important complication and could effect right heart function and contribute to arrest, but we really haven't measured this parameter in these clinical trials.

Pneumonia –- there appears to be a clear increase in risk of pneumonia as well as respiratory arrest. And considering the increased rates –- you've heard of a pancreatitis sepsis and multi-organ failure. One could imagine that there would be an increased risk of ARDS, but I think this has to be studied. And then there is a suggestion of a signal in terms of thrombotic complications that has to be considered.

So what I would have thought I would do is

briefly touch on some mechanisms and some principles that I think may be worthwhile considering. The first thing I'll mention in relation to pulmonary hypertension is this concept of NO scavenging versus premature oxygen delivery. So Winslow suggested that oxygen can vasoconstrict the arteriolar system which it clearly can. But what we have to consider then is how these HBOCs are constricted in the pulmonary circulation because oxygen in the pulmonary circulation is a vasodilator.

So I think exploring the vasoactivity of these systems in the pulmonary circulation will be informative about the relative importance of those two pathways.

The other thing I think we can learn from is the LNMA trials in septic patients which also –- there was a strong harm signal in the LNMA trials and we should probably study those trials when considering HBOCs that have NO scavenging properties.

And the last thing is we have been informed greatly by your research in HBOC field, in terms of extrapolating that data to hemolysis and hemolytic diseases. So I want to briefly do that in reverse now and share with you what we've learned over the last 3 years in

terms of hemolytic anemias and what does that tell us about NO biology when you're infusing higher concentrations of these molecules.

And I'll point out if you look at PNH, paroxysmal nocturnal hemoglobinuria, these patients suffer from many of the symptomatology that you see with HBOCs confusions: gastric dystonias, thrombosis, pulmonary hypertension, fatigue independent of total hemoglobin concentration –- that might be something we can discuss later.

But I'll move very quickly through this data jut to describe that NO is scavenged by hemoglobin, that hemoglobin in a red cell generates diffusional barriers that reduce the rate of that reaction so that when you hemalyze or when you infuse a stroma-free hemoglobin you disrupt the cell-free zone in this unstirred layer. So you increase the rate of reaction of NO coming from endothelium with the hemoglobin.

In the case of sickle-cell disease, they have hemoglobin in their plasma and it's very low, from undetectable to 20 micromolar. This is slightly less than 20 mgs per deciliter. During crisis it can go up to 20 to

40. But remember, all the data we looked at yesterday, that there was a constrictive property of the HBOCs that occurs at the lowest concentrations.

And you can imagine the ability of that plasma from a patient with hemolysis to consume NO, using basic NO assays, and the injection of plasma into a solution of NO destroys the NO instantaneously. And a patient with sickle cell with more plasma hemoglobin has more NO consumption, and NO consumption is proportional to the amount of heme and plasma. And if you take the hemoglobin out of the plasma you reduce that NO consumption.

And I think important to HBOCs biology, you can infuse NO donors into human patients with sickle cell and depending on how much hemoglobin they have in the plasma you'll impair that NO signaling. And just as shown in this experiment if you infuse sodium nitroprusside into the forearm of the patient with sickle cell, with low plasma hemoglobin, there's a normal response. Patients with higher levels of plasma hemoglobin, only 5 micromolar heme, and you have a near complete inhibition of NO signaling.

And this is the recapitulated and transgenic

hemolytic mouse models, so that a sickle cell mouse that hemolyzes and has a high plasma heme has almost a complete inhibition of NO-dependent signaling. And a hemizygote with less hemolysis and then the control has progressive increase in NO-dependent signaling.

So, very low levels of hemoglobin and plasma creates an NO-resistant syndrome. And I also want to point out that NO inhibition does not necessarily equate with blood pressure changes. In fact the sickle cell patient and sickle cell mouse is hypotensive, and what we found is they have to regulate COX-2 and COX-2 activity. So they're maintaining vasodilation secondary to their requirement for oxygen-delivery with critical anemia by COX-2 not by NO. But the NO-signaling pathway is inhibited and that can create other problems.

And then pulmonary hypertension is an increasingly recognized complication of every form of chronic hemolytic anemia. In patients with sickle cell, 33 percent develop pulmonary hypertension and it's associated with a dramatic risk of perspective death.

And finally, I’ll point out that if you look at the patients with higher levels of hemolysis, they have

multiple vasculopathy complications like pulmonary hypertension, leg ulceration and priapism, suggesting that this low-level hemolysis extended over decades can produce vascular harm.

And the last thing is that patients with higher levels of hemolysis have activation of platelets and as Loscalzo’s shown and others, hemoglobin will directly activate human platelets and blunt the ability of NO to inhibit those platelets.

So I think we need more study on the pulmonary-safety issues relevant to HBOCs and specifically looking at pulmonary hypertension in phase I, II, trials, right-heart function, thrombotic risk, endothelial dysfunction, immune modulation by knocking down that, the NO pathway and the effective heme in serous.

Thanks.

(Applause)

MR. WEISKOPF: Thank you. I'd like to invite all the panelists to come up and take a seat please, and while they're getting ready for the firing squad, please fill out those index cards and hand them to people collecting them.

(Pause)

MR. WEISKOPF: Okay. While we're waiting for some of the cards to show up –- there's been a great deal of discussion in the past day-plus about the issue of the heterogeneity of the compounds if they are in fact different chemicals and yet the apparent similarity of some of the serious adverse events that are seen with these compounds.

And I'd like to ask each of you whether you believe that we can look at these compounds with respect to SAEs as a single class, if there's a threat of that that runs through these SAEs or whether it is inappropriate to do so. And I'll just go down the line here and I'll ask Dr. Baines first about that.

MR. BAINES: I think the problem from the kidneys' point of view is that we don't know whether there is in fact kidney injury in many of these trials so I can't really answer the question. My supposition is that they do all have a common effect on the kidney in varying degrees and it does relate to the production of reactive oxygen species and the availability of iron and the breakdown products and to the susceptible parts of the

kidney.

MR. FINK: You know, it's an open question as it relates to GI complications but there does seem to be a fairly strong and consistent signal in terms of the biochemical abnormalities indicative of acute pancreatic injury. The lipase elevation seemed to be a common theme among all the products that at least where the information is publicly available and evidence of esophageal spasm seems also to be a class effect, at least again where information is publicly available.

MR. WARLTIER: Well, I think as far as the cardiovascular effects of these drugs, it is fascinating that they are similar. On the other hand I think the future and where to go from here is to treat the new compounds very differently, to think they are different, and we have to look for new solutions to avoid some of these cardiovascular effects such as myocardial infarction.

MR. REGAN: Well, related to the neurotoxic effect of these products I can only speak for alpha-alpha cross-linked hemoglobin because that's the only product I've really investigated mechanistically and that acts --

is very much like native hemoglobin, in that it's a heme-breakdown iron-mediated neurotoxicity. It would be very easy to test the other compounds, but that hasn’t been done so any speculation would be just that, speculation.

MR. PARRILLO: I would agree with the previous speakers, that I think the toxicity in particular is very similar with all the different compounds and the different compounds were manufactured, in part, to change time action, dose response, volume of distribution, and looking at efficacy, presumably, delivery of oxygen to tissues, I think that what needs to be done here is to look at the toxicities which appear to be, at least in part related to nitric oxide scavenging and see whether or not a product can be produced that doesn't do that or does it in a dose-response way that's very different than the previous compounds.

I notice no one has actually shown the slide of the treatment of septic shock within methylarginine which is a non specific nitric oxide inhibitor. It was used in relatively large trials focused in critical care medicine about 4 or 5 years ago; Lopez was the was to author it and it showed in fact an increase in mortality, an increase in

cardiovascular toxicity, but a reversal of shock that was relatively impressive, that is the blood pressure was brought up and the patient came out of what we would term shock as defined by blood pressure, but in fact the patient died more frequently.

So that –- and this has been seen in heart failure medications, also certain heart failure medications will produce an improvement in symptomatology; patient feels better but they die sooner.

And so these types of differences in toxicity needs to be I think understood better and the compounds need to be designed in such a way that they essentially blunt the toxicities.

MR. GLADWIN: So, I think, from a biochemical and physiologic standpoint the products are different, and I think you can look at the work by John Olsen in his study paper for nice figures of these effects and that is that size matters, for one. So because of the (audio break) release effect, larger molecules and cells orient to the center of a blood vessel and that will create a bigger cell-free zone.

In addition to that these molecules extravasate

so that the hemoglobin dimers, probably the most toxic in terms of small molecular weight, and as you decorate them you reduce the effect of mean arterial pressure. John Olsen has shown that nicely in graphs.

The second issue is that NO reaction rates will modulate the (audio break) effect and Winslow suggested that oxygen-release characteristics will affect the systemic responses and he has very compelling data on a number of systems, suggesting that's important.

So I think that we can change the effect on the NO system dramatically with different preparations so they should not all be considered the same. Now, having said that, this MI signal is concerning and it may be that that's the most sensitive indicator of low NO bioavailability and you've seen from the data I showed how little heme it takes to deplete NO.

And philosophically, perhaps we're dealing with a problem that for a therapeutic to be efficacious we have to have multiple mechanisms of benefit, but here we have a number of mechanisms at harm so we’ve got to shift from the harm to the positive. But I think that they're not the same and at some level for you to advance the field

we're going to have to look at them uniquely.

MR. WEISKOPF: As you might expect given the conversations that occurred in the previous session, a lot of the questions have to do with the myocardial infarction incidents and some of them center upon definition as well. And one thing that's relatively clear that it's unclear as to what some of the definitions that we use in the various trials are with respect to MI and they certainly were different.

And Dr. Warltier I'd like to ask you what impact do you think that has with respect to trial results and the information that we've been synthesizing here, the various definitions that have been used for Mississippi, and do we need –- does this industry, I don't mean the industrial people necessarily, I mean the field rather than industry, do we need to settle upon a recognized acceptable definition of myocardial infarction in the context of these trials rather than have –- each trial have its own separate definition.

MR. WARLTIER: This is actually a fairly complicated question. One would think that –-

MR. WEISKOPF: That's why you're here.

MR. WARLTIER: –- one would think that this would be a relatively straightforward problem to finding myocardial infarction. Usually, studies look at three different areas, one would be electrocardiographic changes, especially development of new Q-waves; a second area would be enzyme or protein leak from myocardium, and the third would be some kind of a physiological effect such as use of inotropes for a period or periods of time to support the circulation or a intra-aortic balloon –- a balloon bump; decreases an ejection fraction, new wall motion abnormalities, and ultimately of course one of the criteria would be on postmortem exam findings.

So –- and it’s just one more thing, but huge differences and some –- the importance of some of these for studies that are done, in cardiac surgical patients versus non cardiac surgery. And hopefully I've avoided your question completely, I think.

(Laughter)

MR. WEISKOPF: You did a fairly good job and I'm going to pass it onto the fellow to the right of you, he's a cardiologist and see if he could also directly address

this issue.

MR. PARRILLO: So troponin has been a big advantage to making the diagnosis of myocardial infarction from a sensitivity point of view. By that I mean troponin T and troponin I are first of all released very quickly, they stay up as opposed to some of the markers we've had in the last 30 years and they are very sensitive to very small amounts of myocardial necrosis. And so we can make the diagnosis of myocardial infarction in 2008 much better than 1978.

Now, having said that the truth is that troponin is probably too sensitive. By that I mean it brings in a whole group of patients who don't have an occlusion of their left anterior descending; they don't have a direct occlusion of one of the vessels that's big, that goes through the myocardium. Rather, there is some sort of low-flow state or some abnormality of the myocardial blood flow or maybe even an agent like a virus that has infected the heart and caused some mild or even severe myocarditis, and the troponin will go up in that situation.

The troponin will also go up in a lot of other situations. It goes up in septic shock, it goes up in big

pulmonary emboli probably because the right ventricle fails acutely in big pulmonary emboli, and it goes up in about 10 other diseases that we don’t have time to go through. But it requires some judgment on the part of the clinician. Number one, does the patient have coronary artery disease? Do they in fact have a syndrome of chest pain, EKG changes and the troponin elevation, which usually means they’ve got coronary arthrosclerosis versus, you know, a 25-year-old who comes in with bacterimia, usually has septic shock.

Now, usually, myocardial infarction –- due to coronary arthrosclerosis that is significant produces a big elevation of troponin. But unfortunately, it's not always true, so that there is a reasonable amount of judgment that is necessary, and I think, you know, I think in order to make that diagnosis now, you know, the definition is three pages long in terms of myocardial –- but it used to be simple. But now, it's a tougher diagnosis to make.

I would point out though that when you talk about myocardial infarction one of its big characteristics is that unfortunately it kills people. And so the fact

that the myocardial infarction rate is up and the mortality also appears to be up makes me worry that those two are associated and that one in fact is related to the other although we don’t know that for a fact.

MR. WEISKOPF: Which –- let me direct a similar question to you, they have sort of compiled perhaps several questions that have been thrown in your direction and maybe you've got a simpler answer than the heart and vessel guys, and that is are there reasonably consistent and accepted definitions for what constitutes pancreatitis, what constitutes massive pancreatic necrosis and liver injury? And then when you finish that I've got a second part to that question.

SPEAKER: (Off mike) Oh, no, I don’t think.

MR. FINK: Sorry. So –- the diagnosis of acute pancreatitis used to be fairly difficult; there are people in this room who remember something called Ransom’s Criteria which was a early severity-of-illness scoring system developed, I think, in New York City, when I was a surgical resident, some time during the Dark Ages, and it was based on things like the volume of food that was required during the first 24 or 48 hours of admission, how

high the LDH was and so on. In fact as a surgical resident I think you had to memorize the Ransom Criteria in order to get through the program.

In more recent years with the advent of computer tomography the diagnosis of acute pancreatitis is largely becoming an imaging-based diagnosis and there are now CT criteria based on the extent of the perfusion abnormality in the pancreas and the extent of pancreatic necrosis and the degree of fluid sequestration in the retroperineum that allow for fairly reasonable grading of the severity of acute pancreatitis and result in (audio break) prediction of mortality.

The biochemical diagnosis of acute pancreatitis hasn’t changed much over the years. The most sensitive measure is still the circulating lipase concentration and bumps in lipase concentration are prima facie evidence of acute pancreatic injury. And so there is a biochemical piece and an anatomic piece, and it's actually easier I would say to make the diagnosis of acute pancreatitis than it is myocardial infarction.

MR. WEISKOPF: All right, and based on that Mitch, and the information that's in the public domain,

you're asked to justify your statement that if these compounds were widely used, that there'd be recognizable incidents of massive necrosis.

MR. FINK: Yeah, so I can't really justify that at all, that's just my opinion, and it –- you know, it's worth exactly what you paid for it.

MR. WEISKOPF: That's government rates.

(Laughter)

MR. FINK: So there is, though, some historical basis for that. So for example there was an antibiotic a few years ago that some people in this room are familiar with, there was a sort of a third or fourth generation fluoroquinalone called Temafloxicin, and Temafloxicin was evaluated in phase III clinical trials and there was an incidence of paracellular enzyme elevations that was fairly low, but it was real, but the Agency, feeling a need for this antibiotic approved it and it was released into the market. And once an epidemiologically significant number of people were exposed to this antibiotic, cases started turning up of massive paracellular necrosis, and I think within 6 months the drug was withdrawn from the marketplace.

In other words the biochemical signal in small epidemiologically insignificant clinical trials translated into a clinically significant signal, once enough people were exposed to the drug. And it is my bet that the same thing would turn out to be the case for at least some of the HBOCs, and I suspect most of the current generation HBOCs that the life changes, which are of biochemical interests only and are of no significance to the patient would translate into episodes of acute necrotizing pancreatitis that would affect outcome once a sufficient number of patients were exposed to the compound.

There are a number of questions with respect to trying to get at the mechanism of toxicity, and I think there are perhaps a couple of schools of thought here. There maybe some that think that it can all be explained by nitric oxide scavenging, and there are others that as we have heard over the past day and plus that have proposed other mechanisms as well.

And so the question I am going to pose to the panel and again we can answer each in turn and perhaps start at the other end first with Dr. Gladwin and that is, is nitric oxide scavenging sufficient to explain all the

SAEs that people have noted in the past couple of days or is that insufficient and one needs to invoke at least one other potential mechanism?

MR. GLADWIN: That is not an easy question. Well, I think - to borrow a statement that Alan Sheppard (phonetic) uses frequently in my opinion looking at the data in both hemolytic diseases especially hemolytic diseases with a high rate of hemolysis like PNH, and then looking at the LMNA experience, looking at the Baxter data, looking at our own canine hemoglobin infusion experiments, a variety of knowing a lot about nitric oxide biology and the downstream effects of NO on platelets, tissue factor, adhesion molecules, chemotaxis, endothelial function, operation, ET-1 activation, ROS generation, Fenton peroxidase nitration chemistry, et cetera, I would say it is the 800-pound gorilla.

Now, I think there is a big debate in the sickle cell field where we are really focused on the mechanisms of plasma hemoglobin there is a big chicken-or-egg debate about reactive oxygen species generation from the heme and induction of oxidases like XO and NADPH oxidase and the direct effects of NO scavenging, but clearly hemoglobin

does both.

So I think in this field there is still a debate about how much of the hemoglobin is generating ROS and has deleterious ROS-dependent effects and how much is the NO inactivation. And of course those two things are yin and yang so if you decrease NO you increase ROS by availability and vice versa.

So it is not -- from a clinical standpoint I don't think that matters. So I think that my experience would suggest that that NO in addition to ROS generation is a primary mechanism. And again that is not the same thing as vasoconstriction because NO has pleotrophic effects on vessel homeostasis and vasoconstriction is just one.

In fact I like to tell people that if you infuse LMNA into your arm your blood flow only drops 25 percent, if you give acetylcholine only 40 percent of that response is NO, and if you exercise only 10 percent of metabolic vasodilatation is NO dependent. We know it is very important pathway but it is not you are not going to see all your vasodilatation and vasoconstriction when you -- even when you partly inhibit NO.

MR. WEISKOFF: I largely would agree with Mark that I think it is largely nitric oxide and I would just make the general point that clearly nitric oxide has a major effect on the vasculature and smooth muscle in the vasculature. It also has an impressive effect on the myocardium, something that I have been interested in because I believe it is one of the mechanisms of myocardial depression in septic shock. It is not the only one there is at least two others that we are able to uncover in humans but it clearly is one of the mechanisms and I think that is important.

If I had to estimate which of the nitric oxide inhibitions is the most important in terms of the toxicity here, my own judgment would be that it is the effect on the clotting system that is probably the most important. Just based on the idea that myocardial infarction and myocardial ischemia is probably an important consideration here, and probably platelet aggregation and things like that are important to myocardial infarction both with plaque rupture and without plaque rupture. And so I think that is probably one of the major areas that we would have to consider negating or advising in order to make the

compounds more efficacious less toxic.

MR. REGAN: Well in the CNS, CNS maybe the major exception to the NO hypothesis presuming that these products do get in, in the setting of a disruptive blood brain barrier. And the reason for that is that neurons are so exquisitely vulnerable to heme breakdown products particularly iron. And that is most likely because they just don't make any fair attempt to destroy the iron once it is broken down.

So of all the cell types we have looked at in our models neurons really stand out as being very vulnerable to hemoglobin and hemoglobin derivatives. That said certainly one can exclude NO reducing blood flow although I haven't seen much data portended here at this meeting to suggest that is a major effect in CNS.

So I would think that if HBOCs do create a neurotoxic effect in the CNS it is probably iron mediated not NO mediated. At least predominantly.

MR. WARLTIER: And so we spent a large amount of our time studying a couple different phenomena ischemic and pharmacological preconditioning and post-conditioning against re-perfusion -- complex signal transaction

pathways are involved in both these phenomenon and there is some overlap. But a key element in each is nitric oxide. I would speculate that the scavenging of nitric oxide inhibits these indigenous cardio protective hypothesis. In fact these hypothesis are just not limited to myocardium they exist in other tissues as well. And so I think this could be a one of the major mechanisms, certainly of myocardial toxicity.

And just to reinforce what is has been said already multiple time the vasoconstriction that is produced by scavenging nitric oxide certainly does not cause a myocardial infarction.

MR. FINK: So I have learned a lot at this meeting I guess one of the thing I have learned is that there is probably three mechanisms or potential mechanisms for toxicity for these oxygen carrying compounds; one is sort of the oxygen hypothesis that we heard about yesterday, the second is the redox hypothesis the hypervalent iron hypothesis and I guess the third is nitrous oxide scavenging.

I think the weight of evidence suggests that the third mechanism is clearly important and is probably, to

use Mark's term, the 800-pound gorilla in the room. When it comes to two of the GI problems one a esophageal spasm and second pancreatic injury.

I think the most parsimonious way to explain those problems is by proposing that the mechanism is scavenging of nitric oxide. We know that the way you treat esophageal spasm in the emergency room is by giving the patient some nitroglycerine to put under their tongue and it makes the pain go away, and we know that in animal models if you infuse a drug like LMNA or another NOS inhibitor. You can induce esophageal spasm and raise the pressure in the hepatobiliaries (phonetic) and pancreatic ductal system.

So I think that is prominently predominantly what is going on that doesn’t mean that other mechanisms aren't important but I think unless the nitric oxide problem is addressed in some way we -- our forward progress in this field is going to be hampered.

MR. BAINES: Well, most of the evidence that I was relying on for the pathological effects in the kidney comes not from studies with HBOCs but with ischemia reperfusion and similar models. And certainly there if

one interferes with nitric oxide it doesn’t do the kidney any good in the recovering stages.

So nitric oxide certainly is scavenging certainly is an important part. The evidence there is related to the effects of HBOCs really comes most of it from forms like native hemoglobins that are filtered reabsorbed and broken down and then produce their nasty effects through the ROS system and the activation of inflammation and so forth.

What hasn’t been investigated in the kidney to my knowledge is the effects of the leakage from the vessels in the -- with larger molecules and the longer-term effects. I think there maybe some analogies in the kidney with the brain although obviously there is no kidney/brain blood barrier. But the effects in the kidney maybe both acute and longer term and we aren't looking at the longer-term effects.

If I might just say one thing about the previous discussion about definition of the condition I think that once the new consensus of -- staging of acute kidney injury is applied to these trials you will find that the incidence of acute kidney injury is going to be at least

10 to 15 percent in the control groups in these trials probably higher in some of them.

And the question will be is it actually the same or higher in the HBOC group and were to add a substance like troponin which would be NAG or NGAL or IL to the analysis we might end up with a great number of these patients demonstrating that they had some kidney injury which is an independent predictor of outcome.

And even at the lower level the stage one relative risk of adverse event it goes up by two fold.

MR. WEISKOFF: Thank you, I am going to come back to that in a moment. But first I want to ask anybody who wants to answer this that in your answers to the question of what are the potential mechanisms of injury nobody commented upon the relatively rapid and extensive conversion to methemoglobin, does anybody want to pick up that they think this could be a potential mechanism of injury or is it just an irrelevancy.

MR. GLADWIN: I guess I can comment on that. I think that is a real important biological question right now, and there is Chris Coopers (phonetic) here and he came to speak at our lab and he and others have been

studying the role of fetal hemoglobin in kidney damage for example with myoglobinuria and there is a school of thought that methemoglobin will form fetal hemoglobin which will exert peroxidase chemistry and that this can be very toxic in terms of, you know, reactive oxygen species, mediated lipid peroxidation et cetera.

There is another sort of newer school of thought that we have been teaching, and that is that we think maybe methemoglobin is surprisingly less toxic because it is silent in terms of NO scavenging. If you infuse methemoglobin into an animal model you don't get any vasoconstriction beyond the colloidal effects, if you -- whereas hemoglobin you get NO scavenging.

We have considered that our approach, for example sickle cell, we are giving inhaled NO to oxidize the heme (inaudible). And we have considered many times that we may be doing something we call out of the frying pan into the fire. We may eliminate NO scavenging and then -- towards reactive oxygen species generation.

So in fairness these are diversion fields and both of them have potential toxicities and which is more important, we haven't worked on it. I will say one thing,

and that is that in hemolytic diseases, we have all thought that all that hemoglobin and plasma is met but in fact it is 82 percent ferrous and that plasma and red cells effectively reduce ferrokene (phonetic) back to ferrocene.

So most of the plasma hemoglobin in hemolytic conditions remains as the ferrous oxygen bound molecule.

MR. WEISKOFF: Thanks. Andrew I am going to come back to you now about some of the comments that you just made. Given that you might expect to see a greater incidence of renal injury than presupposed and I guess we in the field thought that this problem had been solved by cross linking hemoglobin, getting rid of the stroma and that solved the problem -- the early problems very early in the '40s of renal injury.

If you were consulting for one of the companies in this field and they said to you, okay we can go along with that what should we do to document this, what would you suggest for them to measure? Given the very difficult clinical circumstances that many of these trials take place, I have heard criticisms for example that NAG is too sensitive, that you get an increase in NAG but it is of no

clinical significance. I appreciate if you can comment upon those things.

MR. BAINES: This is a real problem. It would be lovely to do iohexol clearances, but that is clearly unrealistic except under very controlled circumstances. That means you are going to rely on creatinine, some people have used statin C as an alternative measure. But I think in the elderly and compromised patients that maybe a problem as well.

The urinary markers, I think we need to experiment with looking at some of the markers of information, no one to my knowledge that has looked at the NGAL in the blood substitute trial and I do agree that the NAG is highly sensitive and could be very non specific. It not only reflects injury, but it also changes when there is proteuria and as an increased lisosome will turn over (inaudible).

MR. WEISKOFF: So what is the answer?

MR. BAINES: The answer is all of the above I guess. Careful measurements of creatinine and careful documentation which -- and under the circumstances that one has to work in are going to be very difficult. Urine

output seems to be less useful but -- and going to be very difficult to measure. I would certainly like to see people using two or three urinary markers and over a more lengthy period. I think that one of the problems we have when you are looking at the literature everything whether it is an animal model or patients tends to be in the first few hours days and not enough longer term follow up.

MR. WEISKOFF: I would like to come to an issue that has been touched upon in several presentations but only in a very light way and has not engendered much in the way of discussion. And that has to do with the nitric oxide interaction with platelets and the thinking that perhaps that by scavenging one activates platelets causing thrombosis and some of the assays have been seen in the various trials.

How does this go along with some of the clinical trials in which patients who have a coagulopathy perhaps on the basis of trauma, perhaps on the basis of therapy following trauma that how does go along then with seeing potentially thrombotic lesions or thrombotic induced SAEs if platelets are in fact deactivated or activated. Mark, you want to -- or anybody -- but Mark you are

volunteering?

MR. GLADWIN: Sure well this is something we are very interested right now again looking at the case of hemolytic anemias such as PNH virtually all of the hemolytic anemias in these conditions -- there is very high rate of thrombosis, a confounding element as many of them have surgical or autosplenectomy which confounds that epidemiology.

But what we found, and this is really based on Les Causers (phonetic) work is that hemoglobin directly activates platelets, NO is a very potent inhibitor of platelet activation but if you have very small amounts of hemoglobin in the experiments I showed you 40 micro milliliter heme there is no effect of NO on those platelets because the NO I mean the hemoglobin intercepts that NO.

There is a paper out by A. Togg (phonetic) on the hematology literature now showing that almost multiple measures of hemostatic activation correlates significantly with measures of hemolytic anemia in patients with sickle cell and there is a growing literature now suggesting that acute hemolysis is associated with hemostatic activation

in multiple pathways.

And again the factor that clearly has been shown to touch on all these elements of hemostatic activation is NO in terms of platelet activation, in addition tissue factor release in other pathways. So I think that it is very likely that infusion of a potent NO scavenger would activate the thrombotic system.

MR. WEISKOFF: Thank you. Let me -- Prof. Regan, let me ask you a question related to your presentation. And that is you made the link between traumatic brain injury and potential leakage of compound into the brain and potentially causing direct neuro toxicity because the blood brain barrier is now been broken.

So given the -- depending on the population of course -- but many studies show a very high incidence of traumatic brain injury in the general trauma population perhaps as much as 30 or even 50 percent depending upon where that population is being gleaned from.

Would you advise those doing these studies to completely eliminate all traumatic brain injury from any further studies with these compounds in generalized

trauma?

MR. REGAN: Well, given the current state of knowledge about whether these compounds actually get into the brain in sufficient quantities to be neuro toxic I would have to say yes until that is further defined.

If there is a traumatic brain injury with -- I would put it the limit of GCH as much higher than three exactly where I am not really sure but certainly if there is substantial traumatic brain injury in a blood trauma setting I would exclude those patients from receiving the product, if this isolated trauma elsewhere with that -- especially penetrating trauma that wouldn't be a problem.

But in blood trauma setting where it is unclear whether this was loss of consciousness whether it is GCS of 10, 12 something like that I would recommend until further preclinical studies were done reassure me that these products really don't get in and cause any problems at least in animal models.

I would recommend excluding patients with traumatic brain injury a significant brain injury from the trials.

MR. WEISKOFF: I think we have just about run

out of time. I want to thank the panelists for their wise commentaries and their presentations and the audience for their participation. The audience did a lot of the hard work here by asking the incisive questions and we should be back for lunch -- from lunch at 1:00 o'clock.

(Whereupon, a luncheon recess was taken.)

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

SESSION IV: FINDING A WAY FORWARD

WHAT IS THE BEST WAY FORWARD SCIENTIFICALLY AND ETHICALLY

BIOCHEMICAL APPROACHES AND MITIGATION STRATEGIES FOR HBOCS

MR. BIRO: And where do we turn this on?

Ladies and gentlemen, if you would kindly gather and --

While the technical issues are being sorted out, welcome to this afternoon session. It looks a little mismatched set of topics and personalities but there is some method behind the madness. And while the technical issues are being sorted out, I would like to call your attention to the advert that was up before about the next ISBS meeting at the end of the summer in Parma, Italy.

Sorry, it's not the end of this summer, it's next summer, 2009. Thank you, Claire. So there is time to collect your travel funds.

There is one or two housekeeping announcements if I may make them. The first is that we have a fairly long agenda of very interesting topics and we would like

to keep to the timing since many of you will probably rush off to catch your flights. So if you'd -- we do keep to the timing, then -- and we can hold your attention, then you will have an interesting and challenging afternoon.

The way this session is going to work is that we will first have a series of 20-minutes talks. I would to like to ask the presenters to respect the timing. And this will be followed by the coffee break about 3:00 o'clock, and after the coffee break we will have one -- a bit extended presentation by Dr. Emanuel, the chair of the bioethics committee at the NIH.

And this will be followed by the panel discussion, who will have an opportunity to respond to challenging and interesting and hopefully profitable questions.

We're ready? Okay, so the first question is Dr. Mark Gladwin who was introduced to you in the session before lunch. And he will talk about nitrate reductase activity of the HBOCs.

THE WAY FORWARD: CAN NITRITE MODULATE HBOC TOXICITY?

MR. GLADWIN: Thank you. So first of all I'll state my bias. I think that it's very important that we don't stop working in this field because I think there have been some major paradigm shifts in our understanding of blood and nitric oxide biology.

I'm going to share with you some of these very recent, and I think, paradigm-shifting results. If you thought the last 2 days were controversial, I'm going to show what real controversy is.

But I would like to propose that the nitrite and the nitrite reductase activity of hemoglobin for the HBOCs could be a major way forward. And I'm going to show you both biochemical and preclinical studies that support this statement.

Now, I do have some conflicts. As a government scientist I don't receive any personal money, but I do have a collaborative research and development agreement of some duration with INO Therapeutics for inhaled NO in sickle-cell disease. I'm not doing any of the inhaled NO HBOC work. I'm also a co-inventor on an NIH government patent application for nitrite salts for cardiovascular

disease, and also have a nitrite methemoglobin NO generator patent that's relevant to the chemistry I'll show you.

We've initiated a collaboration with Dan at Navy to study nitrite in the swine model of HBOCs. I don't have that agreement with Biopure but they do receive their HBOC from Biopure. So those are relevant potential conflicts.

So the question I'm going to ask today is can nitrite offset the toxicity of HBOCs via the effects of nitrite on limiting NO inhibition, limiting vasoconstriction, and limiting myocardial infarction. I'm going to show a lot of data running through these questions but these are just going to be very basic central questions that I'm going to address.

As I showed you earlier, and as Alan showed you yesterday, when hemoglobin is released from the compartmentalization it disrupts diffusional barriers and you get excessive nitric oxide scavenging. There's also a very controversial and rapidly evolving data that nitric oxide may not just be a Perricone vasodilator (phonetic) but it could be stabilized in blood as an endocrine

species and carried in blood.

This work was launched by the pioneering work of Joseph Loscalzo, and Jonathan Stamler suggested an NO-modified albumin could carry or transduce this function, extended it to S-nitrosohemoglobin by some of the investigators in this room as well as Jonathan Stamler's group.

But while we believe this principle of hypoxic NO delivery from red cells we think that it may be transduced by the simple ubiquitous salt nitrite, and that's the data that I'd like to discuss today.

So first of all, is there endocrine NO transport in blood? This was very controversial, because NO, if you breathe it, despite all those red-cell diffusional barriers, it still has a half-life in blood of about 2 milliseconds. So there is no way you can breathe NO in your lung and there's no way it's going to get to your arm, which will take about 5 to 10 seconds.

So over the 10 years, with Richard Cannon and Alan Schechter we've done a number of human studies. In this case, we put catheters in the brachial artery in normal human volunteers and we block NO in the arms. So

we wanted to eliminate native NO in the arm, so we could see an endocrine signal of NO, and we had them exercised to create regional hypoxic stress and then we gave them 80 parts per million inhaled NO, which is the upper limit of what's approved by the FDA.

And we looked at blood flow at rest, during nitric oxide synthase inhibition, and with exercise. And by three different parameters of blood flow measurement, we consistently see a significant perfusion effect, and I'm just showing one of the most visually arresting examples of this.

This is hyperspectral imaging where we shine a bright light on the hand, it reflects off hemoglobin and we can deconvolute the amount of oxy- versus deoxyhemoglobin. Now, oxyhemoglobin in this image of the hand is white, deoxyhemoglobin is dark. And in a normal volunteer as you infuse a nitric oxide synthase inhibitor for 5 minutes, and what happens is the palm, the skin deoxygenates, because there is constriction, decreased perfusion, more oxygen extraction and deoxygenation. And you can see these dark regions of deoxyhemoglobin.

If you do this while breathing NO, you

completely prevent that deoxygenation; you maintain blood flow. And we also saw increased oxygen delivery to the issue by measurement of PO2 and we saw increased blood flow using string-gauge (phonetic) blood zymography.

And this is now accepted. In multiple animal models there is a confirmed endocrine vasodilator effect of inhaled NO. Now, when we looked what the putative mediators would be we measured SNO-hemoglobin, SNO-albumin, and nitrite. And we never saw AV gradients or increases in S-nitrosated protein in the human circulation, but we consistently saw increases in nitrite and AV gradients in nitrites.

So eventually we asked the question, is it possible that nitrite is this endocrine vasodilator?

And in another study with humans we cannulated the brachial artery again, we had exercise stress and then we had inhibited nitric oxide with exercise stress. We powered the study to see a very small effect right here, and we gave 200 micromolar nitrite.

Now, there is a group in Germany that had this experiment in three individuals and saw no vasodilator effect, and the field felt that nitrite had no intrinsic

vasodilator activity. So we were surprised when as soon as we infused nitrite, at 200 micromolar in the arm, every volunteer vasodilated. We had 170 percent vasodilation in 18 of 18 normal volunteers.

So we dropped our dose by 2 logs (phonetic), and now, the nitrite coming out of the arm was 2.5 micromolar, and 900 nanomolar at rest. And again, we saw dilation in 10 of 10 subjects. We saw dilation at rest, during nitric oxide synthase inhibition, and with exercise.

This is not controversial anymore. This has been reproduced in seven species, multiple human studies -- nitrite, in fact, is a potent vasodilator. How does it vasodilate?

Well, one of the hints is when we infused nitrite. This is the NO-hemoglobin formation. Within one half circulation left than -- less than 10 seconds from our artery to vein we were forming NO-modified hemoglobin, both iron-nitrosylated hemoglobin and on the heme -- NO on the heme, and to a lesser extent S-nitrosated hemoglobin where it's on the cysteine.

And importantly, if we looked at all experimental conditions in these 18 volunteers, under the

conditions where there is low oxygen, for example with LMNA and forearm exercise, the amount of NO-bound hemoglobin rose proportionately. So there seemed to be a relationship between the deoxygenation of hemoglobin and the formation of NO on hemoglobin.

And this led us to the work of Brooks from 1947. In fact Haldane really described this chemistry in 1901, and by Michael Doyle, who is now at the University of Maryland. And they described a nitrite reductase reaction. Nitrite plus deoxyhemoglobin plus a proton makes methemoglobin and NO. NO can then bind to another deoxy-heme to form iron nitrosyl hemoglobin which we hypothesize we are measuring as a dosimeter of this reaction.

Now, we were struck by the potential physiologic significance of this chemistry. It has proton sensing, or acidic sensing properties, and it requires deoxyhemoglobin, so it has hypoxic sensing properties. It makes methemoglobin, which won't capture the NO from that heme pocket. It will escape from that heme pocket, and it makes NO one of the most potent vasodilators known.

Now, we have to escape heme auto-capture, which

is another story that I can address later if anybody wants to discuss that.

So we hypothesize that hypoxia, nitrite, and red cells could constitute a three-component system that would regulate hypoxic vasodilation and hypoxic signaling. Now, further chemical work revealed that this was an allosterically regulated reaction. I'm only going to show this one chemistry slide because I think it would be important as we engineer HBOCs to maximize this chemistry.

And that is that nitrite has to bind to a deoxy-heme so that -- in the T-state or a deoxy-heme -- deoxygenated hemoglobin molecule you would more sites for a nitrite binding. In other words, you've got more reactant. But it turns out that nitrite is reduced faster by the R-state tetramer because the R-state tetramer has a lower heme-redox potential. The opportunity for electron transfer is greater. It's more reactive. It has a higher bimolecular rate for nitrite reduction.

So what happens in biology is your best nitrite reductase activity occurs when you start in an artery and you rapidly deoxygenate. Because you have R-state tetramer that then releases oxygen to exposed hemes. So

you-- essentially your R3 tetramer is your better nitrite reductase. So HBOCs with a low heme-redox potential would be better nitrite reductases.

And this is just shown here with a variety of mutants and Chien Ho who is in the audience gave us these mutants. As we increased the R-state character, we increased the intrinsic rate of nitrite reduction, and this is shown here as redox potential drops from 135 to 45; you get an exponential increase in rate.

Now, notice that many of our HBOCs have any one half around this point, but myoglobin, neuroglobin and cytoglobin have redox potentials at that point, which would suggest if deoxygenated, they would be effective nitrite reductases.

So the most controversial -- I think it's now well accepted that this hemoglobin reaction is allosteric, that it's driven by redox potential, that it maximizes at this 50 percent SAT (phonetic) point, but the idea that nitrite can interact with a heme globin to release an NO signal and that NO is not scavenged or auto-captured, that's the central controversy.

So the data I've showed you up to this point is

well-accepted. Now, I'm going to get into controversial areas and I'm going to suggest that there is indeed an interaction, and I'll show you the data.

So first of all, if you incubate -- this is a blood -- aortic ring from a rat, 25 millimeters of mercury oxygen, we've incubated with controlled red cells, nitrite alone, 2.5 micromolar nitrite. But when we do it with the red cells and nitrite, we accumulate cyclic GMP down the string of NO. It's inhibitable by PTIO and NO scavenger and it's inhibitable by oxygen.

One of the models we have used that I'm going to show you is a mitochondrial NO sensor experiment to look at the sensing of NO by mitochondria. And the reason is that NO binds to cytochrome c oxidase to inhibit respiration. This is a hypoxic signal that's of great interest to the NO field now. Moncada and others have been studying how this can regulate hypoxic oxygen consumption.

But so what we hypothesized, at low oxygen, deoxymyoglobin or deoxyhemoglobin could convert nitrite to NO. It would bind to cytochrome c oxidase and inhibit respiration. So what we do in this model is we put in rat

liver mitochondria, we let the mitochondria respire to zero oxygen, and then we take the lid off of the respirometer.

So oxygen can diffuse in, but the rate of oxygen diffusing into the system is slower than the rate that it's consumed by the mitochondria. So you see no increase in oxygen even though the lid's off. It's not until the mitochondria run out of substrate that oxygen accumulates into the system and you detect it by the Clark electrode.

So what happens if you inhibit the mitochondria with cyanide? Well, we see an inhibition. Mitochondria-inhibited oxygen accumulates earlier in the system. So this is our 100 percent inhibition control. What happens with authentic NO (inaudible) donor. We see inhibition about 80 percent of cyanide; this has been well-described in the literature that NO binds to cytochrome c oxidase and inhibits it.

So we use this system to look at the effect of deoxymyoglobin. Here is our control. They don't inhibit until they run out of substrates and stop respiring. Here is nitrite alone at 20 micromolar concentration. Below 50 micromolar we do not see a significant conversion of

nitrite to NO by the mitochondria alone. This is the effect of myoglobin, no effect on respiration.

What happens if you combine nitrite, and in this experiment, deoxymyoglobin? We see a highly significant interaction that inhibits respiration. And this again, controlled, nitrite myoglobin alone, the inhibition by myoglobin and nitrite is not inhibited by SOD or catalase to look at reactive oxygen species generation.

It's not inhibited by BHT, but if you have metmyoglobin, which can give that electron to nitrite, we don't see it.

This is hemoglobin. Hemoglobin exerts the same interaction. Nitrite alone, hemoglobin alone, nitrite and hemoglobin, and this just shows that the ratio of the hemoglobin to the nitrite in the system makes a difference. As you get to very high hemoglobins, you can start to overwhelm the NO generation with scavenging.

And this just shows the effect of the myoglobin, hemoglobin, and red cells in the system, to point out that the extent of inhibition is proportional to the intrinsic nitrite reductase reactivity as shown by the bimolecular rate constant. So as the bimolecular rate constant rises,

the extent of NO generation inhibition increases.

So we've now done studies with a group from Germany, Schrader, Kelm, and Rassaf, and they've given us myoglobin knockout and wild-type mice. And in these experiments we're looking at actual heart homogenates and their intrinsic consumption of oxygen. So these cells are now chewing up oxygen. These aren't isolated mitochondria, they are intact cardiomyocytes.

They're consuming oxygen to zero. We take it off -- this is what cyanide does, and this is a dose-dependent inhibition of respiration in the cardiomyocyte by nitrite. What happens with the myoglobin knockout? We see a dramatic right shift that nitrite is now not inhibiting respiration.

And this summarizes the effect in the wild-type with myoglobin. The -- there is a dose-dependent inhibition of respiration that's significantly reduced in the myoglobin knockout.

So is there an interaction of nitrite with heme globins that could modulate HBOC-induced vasoconstriction? And again, the idea would be we would be driving this chemistry with an HBOC. This is data from Warren Zapol's

lab and Dr. Yu. Both of them are in the audience. They were kind enough to loan me these slides from their recent publication in *Circulation*.

And first of all what they do in this model is they first infuse whole blood into the -- they use both mice and sheep, but this is the mouse data. And interestingly, whole blood doesn't increase -- cause significant vasoconstriction in this experiment. And this is systolic blood pressure. But look what happens if they infuse HBOC-201 or tetrameric hemoglobin. There is a sustained hypertensive effect that many of you see in your experiments.

Now, if they do the same experiment with the eNOS knockout mouse -- a very elegant experiment that I'm kind of surprised nobody's done before -- they don't see any difference between the three. Of course, the eNOS knockout mouse is more hypertensive, equivalent to this NO inhibition experiment. But they gave phenylephrine to prove that it could constrict more above that baseline, suggesting that this is dependent on NO scavenging from NO generated from eNOS.

So then what they did is remarkable. They pre-

treated with inhaled nitrite oxide gas for -- in this case 60 minutes, but they also did 30 minutes and 5 minutes -- and then they infused the murine tetrameric hemoglobin and it completely inhibits the constriction. So there is an interaction between something that the NO is generating before they give the HBOC and the HBOC that prevents vasoconstriction. And note, they stop the NO here. So it's not the oxidation of the HBOC to met.

And they show that here, there's no significant amount of met formed. So then they look at the same thing with nitrite, because we know that inhaled NO makes nitrite.

They give a single dose of nitrite, 50 nanomoles, which should get the blood level to about 11 micromolar, and then they infuse it. And again, the nitrite pretreatment inhibits vasoconstriction. In statistical terms this is a highly significant interaction. Nitrite alone at that dose didn't do anything. HBOCs alone had a major vasoconstriction. Together there's no vasoconstriction.

And there is an interaction that inhibits the vasoconstrictive effect. And again, it's not the

oxidation. They only had 10 percent met, so it's not that that 90 percent met can't scavenge NO.

So the other big toxicity we've talked about is MI. So the question is will nitrite, if given with an HBOC, also modulate, or would inhaled NO also modulate the risk of MI? And everything I'm going to show you has also been shown for inhaled NO, we think, via generation of nitrite in blood.

And again the idea that we looked at is nitrite is a reservoir of NO. At low oxygen, low pH, regenerates NO, and this could affect critical organ function. So in the first with Lefer -- David Lefer, he did the left coronary occlusion and gave nitrite right before reperfusion and in the controlled animals -- and in this case we used nitrate as a control, there were very large infarcts, as shown by the size of the lack of TTC staining, which is white, and consistently with nitrite there is a very small infarct.

This just summarizes the data -- and this is 2.4 nanomoles of nitrite and 48 nanomoles of nitrite. This is the dose that they gave in their HBOC experiment, and when you inhaled NO, you get about this does of a nitrite.

Dietary nitrite levels from eating a leafy spinach salad is on the order of this level and multiple laboratories are now showing that if we deplete the dietary levels of nitrate and nitrite you worsen cardiac infarct size. Now, this data again has been reproduced in about nine laboratories in seven species, and canine studies have been completed now, and this is going to human trial phase 2, VNH OBI (phonetic) but there does appear to be a robust across-species effect at very low diseases of nitrite and limiting MI.

This is in press from -- in PNS right now and this again the Schrader group and Rassaf and this looking at the wild-type mice and the myoglobin knockout mouse. And if you look up here, this is infarct size, relative area at risk, and in red is the wild type that has myoglobin. There is a reduction in infarct. But look if you have a myoglobin knockout, there is no reduction in infarct, again supporting an interaction that is transducing an NO signal.

So will this translate to humans in the last few seconds? This is data from Rakesh Patel published in the JCI last year. They gave inhaled nitric oxide. It has

direct pulmonary effects which could be of benefit in HBOC therapy but it also generates nitrite and nitrate, and this nitrite could have effects on NO deficiency or ischemia as you've seen from the Zapol work. And they looked at this in the context of orthotopic liver transplant.

And I'll just show you very quickly, it's a very small double-blind, placebo-controlled trial. They gave inhaled NO, starting here when the liver was taken out, and during the insertion of the new liver. And when they looked at species there was no formation of plasma SNO, but there was an increase in nitrite. And the nitrite level went up to 800 nanomolar, which is about that second dose on the dose response from the mouse studies.

And nitrite AV gradients formed consistent with extraction of nitrite across the circulation. They saw an increase in methemoglobin but less than 2.5 percent. They saw an increase in cyclic G levels, and they saw a reduction in the platelet requirement that was significant, a reduction in liver enzyme release consistent with the parasite (phonetic) protection, and a weak effect in these 10 patients on reduction of length of

stay, but after adjustment for gender and cold ischemic time. So I think this is a very preliminary result in terms of length of stay.

And they saw significant -- there's apoptotic cells in the animal -- in the human that did not get the NO, and you prevent the formation of apoptotic cells in the liver from people that get the NO. And we see that in these animal models as well.

So in conclusion, our data suggest that nitrite's a major stable intravascular endocrine reservoir of NO, that hemoglobin is an allosterically regulated nitrite reduction with maximum NO generating enzymatic activity at the R to T transition, that myoglobin is a nitrite reductase that generates NO at low oxygen to modulate cellular respiration, that nitrite potently mediates cytoprotection after ischemia reperfusion injury, and that nitrite interacts with HBOC to inhibit vasoconstriction while maintaining oxygen delivery.

Thanks.

(Applause)

MR. BIRO: May I remind you to get out your index cards and please write down your questions? Dr.

Gladwin will have to be -- leave early to catch his early flight, but this was a beautiful presentation.

The next presentation is by Dr. John Olson. I don't think he needs introduction, but it is worth it to note that in addition to his enormous contributions to hemoglobin chemistry, he has also contributed very valuable human resources, many of whom are employed in the industry and academy. Thank you.

STRATEGIES FOR ENGINEERING SAFER, MORE EFFICIENT AND MORE STABLE RECOMBINANT HEMOGLOBINS FOR USE AS O2 DELIVERY PHARMACEUTICALS

MR. OLSON: I'd like to thank Abdu for inviting me to talk about recombinant technology. In the way of disclosures, I really officially have no conflicts of interest because I was on the scientific advisory board at Somatogen and then later Baxter but those projects have been dropped.

So as of now I have no official things, but as he said I know a lot of the people in the industry.

Before I get started I'd like to make a value

judgment based on what I've heard so far. Seems to me the way forward right now is with the current products. And so I think we're going to have to figure out how to -- or not me, but the FDA and the companies are going to figure out how to work together to see if they can make progress.

What I'm going to talk about is what I would say IS the future, 5 to 10 years where I'm going to try to convince you that the potential for using genetically cross-linked and engineered recombinant hemoglobin is really the way to go as a starting material but the question is how to package that material.

And that is what is being learned by the current states of -- the current HBOCs.

So let me get going here. By way of acknowledgements, a lot of what I'm going to talk about today was done with some very bright scientists at Baxter Hemoglobin Therapeutics and Somatogen, Doug Lemon, Mike Doyle, Tony Matthews, Eric Brooker (phonetic), a lot of my work is related to studies with George Phillips and Quentin Gibson who is now retired, and then I'm going to talk about some newer work with Chien Ho, Mitch Weiss, Doug Henderson and other people, and you'll see that in

just a minute.

So with recombinant technology we can actually sit down and say, how can we optimize these parameters? These are parameters that I've heard lots of people talk about over the last 2 days. We can argue about whether it should be moderate oxygen affinity or high affinity, but in the recombinant we can make whatever you think is the correct P50, and we can drive multiple kinds.

And my approach is structural. We take the active site of alpha chains and effect in the computer, rotate it around here into the distal pocket, here into the heme pocket of beta chains and myoglobin, and they looked very similar and roughly like this. And by using a library of myoglobin mutants, over 300 or so, I can tell you what regulates oxygen affinity, quantitatively, and then reconstruct the active site to give the desired affinity and rate constants.

So let's just go through this quickly. First there's hydrogen bonding from the distal histidine to the bound oxygen. This electrostatic interaction is preferential for oxygen. This is what causes the discrimination in favor of oxygen despite sometimes what

you hear about steric hindrance. It's electrostatic, but steric hindrance can play a role. I could put an isoleucine there to push on the bound oxygen and raise P50.

There is a cavity that we're going to talk a lot about here that's more related to kinetics. And then down here is another way to regulate oxygen affinity over several thousand folds. This is the classic changes that are involved in the R to T transition. It's multiple things. The histidine has an orientation with respect to the pyrrole nitrogens. If it's eclipsed so that it bumps into those nitrogens, it can't move up and it can't bind oxygen. If it's rotated and staggered, it can. There's also a pressure holding the distal histidine away from the plane of the heme by the F-helix and it is these manipulations that are allosteric that is away from this active site that can regulate affinity.

So we can make mutations in the distal pocket, or in mutations away from the active site that regulate at the proximal site. So if you want to look at a schematic diagram, here is the alpha 1-beta 1 interface. That's the proximal imidazole here; the proximal imidazole on the

beta chain is here. Mutations in this area can affect that tension or that proximal geometry.

There's arguments that mutations in this interface may do the same thing. I'm not going to get into those arguments, but we can change things at that interface as well.

So I'm going to give you an example here from Chien Ho's work who is in the audience and I need to plug his work, he -- his group made a mutation based on some of our work for reducing NO scavenging and auto-oxidation and put a phenylalanine at the B10 position in alpha subunits.

And as you can see that makes a very high affinity hemoglobin. Well, in some circles you'd say, well, that's not going to transport oxygen. Well, you can fix that and you can fix it not by changing things at the distal pocket but by creating mutations that's unique that Chien invented really, V96W in the alpha 1-beta 1 interface. He used the naturally existing one, hemoglobin Presbyterian and you can move the P50. We can move the curve anyway you'd like if you tell us what the P50 is that you need.

So oxygen affinity can be regulated by site-

directed mutagenesis. And we have a multiplicity of ways of doing it. What about NO scavenging? You've heard a lot about that today and I guess I'm partly responsible for some of that historically because we started worked on this in 1994.

And here is the one of the classic experiments that came from one of the papers that Mark Gladwin referred to by Dan Doherty and Mike Doyle. This is a 10 percent topload with genetically cross-linked tetramers. This is the rHb 1.1, it's equivalent really to D -- to the initial hemasis (phonetic) Baxter product. And here is another cross-linked tetramer with high-affinity low P50.

When you do this 10 percent topload in the right you get about 30, 35 millimeter rise in mean arterial blood pressure. You've heard about some of the associated side effects. So what's the cause of this? And what is NO scavenging? It's not NO binding. Everybody uses the term NO binding but it's really an oxidative reaction in arterials where -- and it's a highly conserved activity, it's not really a side effect of hemoglobin.

Hemoglobin does this on purpose and so does myoglobin because NO, as Mark alluded to, is toxic. It

shuts down respiration, irreversibly damages aconitase so the TCA cycle doesn't work, inhibits cytochrome oxidase.

Our hemoglobin and myoglobin is designed to bind that or capture that NO in a distal pocket while oxygen is bound. When that happens, the NO is a radical, the oxygen is almost a free radical, so as soon as it's captured, boom, it makes this cis-peroxynitrite intermediate that than rapidly isomerizes to nitrite. It's NO dioxygenation because both atoms of oxygen end up in the nitrate.

And we've proven that with Paul Gardner, the fidelity is 99.5 percent. So it's an NO dioxygenase.

Hemoglobin has actually evolved to do this I think. Here is the structure of what I would call a transition analog of this reaction. It's a methylase to cyanide complex. When you put these four atoms in, which are roughly the same conformation as the cis-peroxynitrite, it fits and none of these residues really move out of the way. And so this cavity is designed to carry out this reaction, at least that's one of my premises, and you'll find this in many, many globins, including myoglobin.

Now, there's a lot of arguments about this cis-

peroxynitrite intermediate at high pH and we've done a whole bunch of fancy biophysics. For the physiology at room temperature, this reaction is bimolecular and fast and almost diffusion controlled. As soon as the NO is captured in the protein, boom, you make nitrate.

So I'm arguing, and many people are now, that a secondary function of myoglobin and hemoglobin is to get rid of NO. In the case of myoglobin, to protect the mitochondria from inhibition through either sepsis or uncontrolled signaling; in the case of hemoglobin encapsulated in red cells from inhaled NO or from sepsis again.

And the red cells, as you've heard already, don't -- and several times, don't appear to interfere with NO signaling because -- due to the Farius (phonetic) effect, they're streaming down the center, so there is a cell-free layer that acts as the diffusion resistance.

In addition they have their own unstirred layer. The extracellular blood substitutes, particularly the tetramers do mess up this gradient and could skew it even if they don't get into the interstitial spaces, but almost certainly they get here and intercept the NO, and that's

the basis of the idea of the hypertensive effect with the extracellular hemoglobins.

So what's the strategy to inhibit this? Well, one strategy that was -- has already been adopted with the latest products is to polymerize the hemoglobin to minimize extravasation or to pegylate them; same reason.

And in my view, after listening to all of this, these products, I think, work -- we can debate that -- and are relatively safe. And one of the reasons is that -- this is another article that Gladwin was talking about. It's not my work, so this is other people's work, but I plotted either percent mean arterial blood pressure effect or TPR, total peripheral resistance versus size or molecular weight. And the tetramers are up here, and they have a large effect.

And the products that are on the market right now or being developed are somewhere in this range and you see they have roughly a third of what was in the tetramers. So comparing DCLHb data to the current products is not really fair. The vasoconstrictive effect is much, much less. NO scavenging is less, presumably because of extravasation.

So what was our strategy with -- in the 19 -- in the late 1990s? And that was to say okay, if we know a lot about hemoglobin biochemistry, we ought to be able to reduce the rate of NO scavenging. And could we reduce it low enough to get rid of the vasoconstrictive effect?

So that's the strategy and we have to use recombinant technology and we have to know how ligands are captured. And when I first used this analogy in 2001, it was based solely on mutagenesis and that's that distal histidine is like the thumb of a baseball glove, and there is a pocket. And that's how you catch -- or myoglobin or hemoglobin catches oxygen.

And now it's actually based more in fact and that's from time-resolved crystallography. And so we kind of know that the distal histidine opens and closes. If we're looking at bimolecular ligand capture, every once in a while that histidine is open, the ligand comes in, and it doesn't immediately bind but is captured in the space.

And those pictures that you see there are actually based on electron density and time-resolved crystallography, looking at the reverse reaction. So there is the binding process.

If we go to dissociation, when it dissociates -- we can do it with a laser -- you can watch it rattle around and then escape.

So if we want to inhibit NO capture what's the best thing to do, is to fill that space so the NO is reflected back out before it has a chance to react with the bound oxygen. So simple things, this is -- surrounds the cavity at the B10 position, put in tryptophans or phenylalanines.

If we do that we sometimes will mess up affinity and/or dissociation rate constants, but we know how to fix the P50 and sometimes the rates changes to a glutamine, weakened hydrogen bonding, so it can go out. Go down here, and make allosteric mutations. So we have all the tools to fix this.

Now, one question that I get a lot is, all right, if you're going to slow down NO capture you should slow down oxygen binding. And this is a complicated slide. Here is the bimolecular rate of NO dioxygenation versus the bimolecular rate of reversible NO binding --- or I could have plotted oxygen bonding. This is just a nicer experiment. In one case I react NO with

deoxyprotein in there, and then I do the experiment over again, expose it to oxygen and do NO dioxygenation.

And we -- this is with myoglobin. We're slowly building up a library with hemoglobin. But it's interesting. There is a linearity between simple reversible binding and NO dioxygenation, but multiple mutants lie off the line.

The error in these individual parameters is roughly the size of the dot, because this is a log scale. And some of these guys have actually fast rates of bimolecular binding, but low rates of NO dioxygenation, and from this work with Somatogen we constructed -- or they constructed an alpha chain which had a triptophine in the B-10 position, a glutamine and then a triptophine at the E-11 position in batus (phonetic).

They're slightly different, but the idea is the same. You fill this pocket to slow down NO capture. And the rate of NO dioxygenation is 2 compared to 70 in the simple tetramer.

And this is TPR, total peripheral resistance and in this case, this particular tetramer in the rat 10 percent top-load has no total peripheral resistance

effect. That's pretty interesting.

So -- and this is just an aside. We actually -- they actually with us constructed a bunch of molecules that either had high oxygen affinity or low oxygen affinity and if we plotted K-Prime of NO dioxygenation versus the blood pressure or TPR effect, we have this linear relationship.

So it didn't matter what the P50 was and it shouldn't in a top-load experiment like this, because there isn't enough free hemoglobin to do much oxygen delivery.

Okay, so that strategy works. So we can manipulate O2 affinity. Relatively independent of NO scavenging we can reduce that. What about oxidation and heme loss, which are other processes that you've heard about today. What do we know and what can we do?

Well, hemoglobin or myoglobin, when they're auto-oxidized, release superoxide. You have this happen twice, the superoxide can dismute to hydrogen peroxide and oxygen. This is the killer and not superoxide. Hydrogen peroxide then can re-react with the oxyhemoglobin or the ferric to make radicals.

And the other bad thing that can happen -- in the ferric state the heme can come out much more readily. And when the heme comes out, the globin is usually -- and hemoglobin is unstable at room temperature and will precipitate leaving the heme to go into membranes.

The heme itself can react with oxygen to generate radicals. So even in the presence of catalase if you have net precipitation in heme, you're going to generate radicals. So it's really the loss of heme that's the key problem in the oxidative stress that comes with these processes.

But before this can happen it auto-oxidises, so we'd like to be able to figure out ways of inhibiting those processes. Well, here's a picture of autoxidation. The way it works at high oxygen tensions when the oxygen is bound, is that this oxygen very weakly and infrequently becomes proteinated and dissociates as the neutral superoxide.

The negative superoxide anion is never going to leave, because it will immediately re-react with the ferric iron which has a net plus 1 charge.

And when the distal histamine is there, it's

inhibiting proteination, because it's taking the non-bond of electrons and forming a hydrogen bond here. So in wild type myoglobin -- hemoglobin actually auto-oxidises even more slowly than myoglobin, but the same curve -- out at high oxygen tensions.

This is the mechanism of autoxidation, and it's very slow, so slow that when you get down to the P50, a second mechanism kicks in. That's when the oxygen is dissociated, water has come on and sometimes weakly binds to the iron and then there's a bimolecular outer-sphere reaction which gives you this bell-shaped curve, something many of you have noticed, if you store hemoglobin at low oxygen tensions, roughly at the P50, it auto-oxidises faster, so.

If you then look at various manipulations in the distal pocket which we've done, if you take out the distal histidine, autoxidation jumps up several thousand fold and that's because it's easier to proteinate the oxygen and it's very PH dependent. Low pH, it auto-oxidises more because it's proteinated.

R to T transition facilitates the dissociation of both oxygen and the superoxide radical. Dimerization,

monomerization, speed up these processes. So already, polymerization, PEGylation, or stabilization in the tetramer already is helping you out.

And then the question is can we construct mutants to slow down autoxidation. One way is to prevent water from getting in here into this pocket. Then the same kind of mutations that inhibit NO dioxygenation slow down autoxidation.

And this also brings up the conundrum, if you want a high P50, you usually have to put up with a high rate of autoxidation, and that's kind of true, and here's the linear regression. But as in the NO dioxygenation case, with a Fe (phonetic) at this position, we can get a wide range of P50s at very low rates of autoxidation.

And in fact this combination is found in Asian elephant myoglobin and probably to prevent oxidative stress in the pachyderm, and it has -- a P50, these are all things that -- at 37 degrees, it has a P50 that's about the same as sperm whale myoglobin, but it auto-oxidises much more slowly.

What about heme loss? Met-hemoglobin -- met-myoglobin is very stable, compared to hemoglobin. These

are idealized time courses. I just took the data out to make this clear. These are the fits to the observed data.

So hemoglobin is much less stable with respect to heme loss than myoglobin. Why is that? Well, again, structural biology tells you what's going on.

There's an arginine at the CD-3 position that forms a strong electrostatic interaction with this propionate. There's a histamine down here at FG-4 which forms a electrostatic interaction with the heme 7 propionate.

There's a hydrogen bond from serine F-7 to the proximal emitasol, and in both oxy and the ferric form, this histidine forms a strong hydrogen bond. In the ferric form with water it rotates down.

What about hemoglobin? Hemoglobin loses heme much, much more rapidly. Why is that? Well, again you just look at the structure. Down here, where they were polar groups in myoglobin, there's nothing but lysines. There's nothing holding on to the propionate down here -- either propionate.

Alphachains lose heme much more slowly than betachains. Why? Because they have histamine at CD-3

that forms an electrostatic interaction. There's only a weak interaction, at least in the crystal structure, between this lysine and the propionate and a weaker hydrogen bond either with water or oxygen.

Betachains lose heme much faster. Just look at the structure. There's lysines down here. The serine's too far away to interact with this propionate, and there's only this weak interaction, and weak hydrogen bonding.

And if you dimerize the hemoglobin it loses heme faster, particularly betas. In the isolated sub-units, you can't really even keep in the ferric state. They just plop out of solution. So again polymerization, cross-linking helps you out immediately by at least getting you to hemoglobin tetramers.

And we have patented, actually Baxter let us keep this patent. They didn't want it anymore, putting in histamine, lysine, or arginine here -- and in fact that will stabilize betachains and we could do similar things in alphachains.

So we know how to fix by recombinant technology many of these problems. If they can be identified as being important, we can probably mitigate some of these

properties for oxidative degradation, NO scavenging, or just P50.

Why isn't the recombinant stuff -- why aren't you hearing more about it? And here's the dilemma. This is my take on sources.

Potentially recombinant hemoglobin production is what you want. It's unlimited. You just need corn syrup, minerals and a stab of E. coli. The problem is it's very hard to produce.

And so what is -- how are we going to solve this problem? This is really what I've been working on hard for the last few years and I just have two more slides. Here's basically erythropoiesis in a bacteria.

We've worked pretty hard to see that what happens is the alpha and beta chains are made. They fold up, we think they make a molten globular intermediate where the alpha-1, beta-1 interface is formed and then heme is added to make the tetramer.

The dilemma is apohemoglobin is incredibly unstable. You take the heme out and it precipitates at room temperature, it won't last at 37.

That's a dilemma, but if you get the heme into

it, it'll last for almost ever. And you know, the products that are being developed now, you can store in your refrigerator for -- or on the bench.

So how do we address this problem? And this is what we're working on. First thing, increase the stability of globin by comparative mutagenesis. It turns out deep-diving whales have more stable hemoglobins and myoglobins because they go acidotic after an hour of diving.

So we look at mutations and put them in and most of them are in this interface. Fetal hemoglobin -- this is something we should have done right away. Fetal hemoglobin is more stable, so we compare and make a more stable interface.

A second approach that we're trying and I can't -- this works, we've done this. We're not sure whether this works. Mitch Weiss discovered this alpha hemoglobin stabilizing protein. He thinks maybe it's a chaperon.

The third and the best thing is instead of just adding heme and hoping it gets in, we have been co-expressing heme transport genes from -- this one from (inaudible), a lot of the hemolytic bacteria, and that

actually works, we can get the heme in really fast.

So our technology is really pretty sloppy and this is where I wish I had a better education in microbiology. We use high copy number plasmid for hemoglobin, a low copy number plasmid for the helper genes.

What we really need to do is adopt this strategy and redesign the whole E. coli chromosome and that's really the next generation, is to put the helper genes and maybe even the hemoglobin and streamline the bacteria to be able to produce large quantities.

So it's my guess or hope that recombinant hemoglobin will be the source. We can do the protein engineering and now we have to do the cellular engineering.

Thank you.

(Applause)

ROLE OF MICROVASCULAR REACTIONS IN THE DESIGN OF HEMOGLOBIN BASED OXYGEN CARRYING PLASMA EXPANDERS

MR. BIRO: We've been privileged to hear and see

the new dawn. The next speaker is Dr. Marcos Intaglietta, professor of bioengineering at University of California, San Diego and he will talk about HBOCs and the microcirculation.

MR. INTAGLIETTA: I will discuss today experimental findings and studies in the microcirculation. I am professor of bioengineering and applied mechanics at University of California, San Diego.

I have collaborative agreement with Waseda University of Tokyo. I have financial interest in Sangart Inc. that was founded by Bob Winslow.

We were both professors at the University of California at the same time when the idea of making artificial blood came about and I have innumerable collaborative agreements and grants with Albert Einstein University, mostly directly by Joel Friedman, who is here in the audience.

The point of view that I'm going to take is to make a comparison of three fundamentally different hemoglobin based oxygen carriers.

One is already accepted abroad for clinical use, one is in process, and the third one is still at a

clinical stage -- at a preclinical condition.

What I'm going to discuss is variables that are associated with the reuse, like dosage, viscosity, P50 and finally what happens with nitric oxide.

Now, while taking a look at the microcirculation, the point is that when you change even the simplest, the most elementary physical characteristic of blood, you affect all of the systems here. They all become affected. They all interact with each other and well, it is theoretically possible to intellectually come up with what should be the result of changing one property.

It turns out that this is a problem with many variables and it is more practical to make an experiment. Now we make experiments in microcirculation, we make experiments in the hamster and if there is one virtue to doing this, it is that we were able to make this comparison always using exactly the same model, the same protocol.

Now, we're fully aware, particularly of the point that was raised by Professor Biro. We look at healthy animals, we don't look at disease, but we look at

mechanisms.

Now we look at very simple things. The simplest thing that we look at is functional capillary density. This for us is the meter that determines whether the microcirculation, the organ and finally the whole system functions. This is the number of capillaries through which there is passage of red blood cells. If there is a capillary through which red blood cells do not pass, functional capillary density has decreased.

Now this is determined by many factors, but the -- one of the most important ones is diameter, the diameter of the blood vessels. So we don't measure blood pressure. We don't even talk about this activity, we talk about vessel constriction and vessel dilation.

And for us it means whether the arterial constricts or dilates and this is a fundamental determinant of functional capillary density but it is not the only one, but it is a fundamental one.

But it is definitely what we all have been discussing here in the past day or so, when we have used the term the vaso-activity and this is the origin of that, the change of diameter and we do this using our own eye,

the experimenter's eye and we do it electronically through a optical trick.

Our model of study is extreme hemodilution or acute anemia and basically what we do is we take the organism to a condition in which the remaining red blood cells are barely sufficient to sustain metabolism, and at that point we introduce the plasma expander, oxygen carrier and if it is something that functions it goes one way and if it does not function, it goes the other way.

Now, as the organism is poised at a very critical point, this is the critical hematocrit for this species. This is 11 percent, we reach this by hemodilution with dextran 70,000 molecular weight and it is an isovolemic process.

So this is where we get to and finally we get to this point here and that is where we make the test of the material. Okay, which materials I am going to use? I'm going to use MP4, which is the material that was developed by Bob Winslow, but it is really representative of PEG-hemoglobins -- hemoglobins conjugated there with polyethylene glycol.

I'm going to use the hemoglobin vesicles

developed by Professor Tsuchida and his team at Waseda University in Keio and I'm going to use the veterinary version of polymerized bovine hemoglobin produced by Biopure which we have purchased commercially in the market.

And I have here two plasma expanders that -- one I use perforce to do the hemodilution and the other one, you will see that there is a method to the idea, and I will come back to that in a minute.

So what I present has all been the result of an arduous battle of publications, because some of the ideas are a little bit outside of what we have normally thought about doing, which maybe is in part the consequence if you let engineers do work in biology.

At any rate, this is part of the publications and it is -- reflects the contribution of three individuals, Amy Tsai, Pedro Cabrales, and Hiromi Sakai.

So let us go to work. This is a comparison. When we look at the diameter and the functional capillary density of PEG-hemoglobin, hemoglobin vesicles and polymerized bovine hemoglobin -- this is diameter and this functional capillary density.

Okay, there is an interesting message here. The vessel constriction appears to be a function of concentration of hemoglobin independently of how this is packaged.

This is the concentration of the hemoglobin in plasma, in this extreme hemodilution experiment. This is also the viscosity of the plasma, right?

And this is probably not very different but this one here is significantly different from this. So, vessel constriction and functional capillary density track each other.

But how about this concept of dosage? Okay, this is same experiment done only using polymerized bovine hemoglobin, the veterinary version. And as we increase the dosage, we lower the diameter.

So interestingly at the dosage of one gram per deciliter in plasma, it's perfectly okay. There is no problem with this. In fact, the functional capillary density is 75 percent. It's not really a vessel constrictor, it preserves as well as or better than dextran, functional capillary density, but the problem appears when the dosage goes up.

We should note -- we should entry that by chance or happenstance or unnaturally physiological restrictions, for instance, PEG-hemoglobin can more or less only be used at one percent, maximum two percent concentration in plasma, because it is limited by the very high oncotic effect.

So there seems to be a sort of a natural limit to where molecular hemoglobin can be used without causing vessel constriction. Okay, now is this really what can be done if the name of the game is vessel dilation and preserving functional capillary density? And the answer is, no.

If I -- instead of using hemoglobin, I use a high viscosity plasma expander, I get vessel dilation and I get significantly sustained functional capillary density. So what is going on here?

Well, what is going on is that all of this is at very low plasma viscosity but if I use dextran 500, a large molecule, everything -- all those at 11 percent hematocrit, I get vessel dilation.

So viscosity is a player in this and the idea comes, what happens if we were to increase the viscosity

of this materials. But the thing before doing that, why? What's going on here? Why is viscosity at play here?

Well, let us first of all take a look at what happens systemically, because it is a very valid critique, it is fine, you look at the capillaries in the skin of the hamster, what does that have to do with anything? Is this any central correlate that tracks what happens in the skin of the hamster?

And this is functional capillary density, this is the data that I presented before and this is base excess. Now, Peter Keipert with Bob Winslow's presentation gave lactate, but here is base excess which is a similar but different global measurement of what is going on.

Clearly, the dextran is -- it leaves you to make up this base excess. How wonderful the high viscosity is, apparently not carrying oxygen; doesn't do wonders for base excess. While on the other hand carrying just a little bit of oxygen with this material, the PEGylated hemoglobin does do in fact restore base excess to basically normal values and the vesicles also -- at that fairly high oxygen concentration, you can package a lot of

hemoglobin inside the vesicle, do give you a reasonably good base excess.

And we have advanced many times in discussions with the Waseda group, (inaudible) that maybe the formulation of this material is not sufficiently viscous, if it were more viscous they would get the desired result.

Well, now, why? What is going on here? We propose that what is going on is basically mechanotransduction. The shear stress acting on the endothelium produces vasodilators.

I use the plural, we're all fixated with nitric oxide, but the original paper by John Frangos (phonetic) put -- shear stress produced was prostacyclin, another vasodilator, and who knows what else might be being done there, but we are fixated with nitric oxide and there is good reason for that.

But I indicated this is not the only vasodilator that is being produced, but we can measure. So, Amy Tsai designed a system of measurement for this with microelectrodes. We go next to the wall of the arterials, venials (phonetic), we can go into tissue, we can go anywhere, and measure the concentration of nitric oxide

under this different conditions.

And if shear stress has anything to do with this, we will determine it, and if viscosity has anything to do with this, we will determine it and in fact this is what comes out. This is the circulation normally. There is a concentration of nitric oxide measure next to the wall.

This is what happens -- animals hemodiluted with dextran. This is what happens animals hemodiluted and added polyethylene glycol hemoglobin. This is PBH and this is dextran 500. Now hematocrit in all of these animals, these three here, is the same, 11 percent.

So viscosity is a player. Viscosity is a phenomenal player because here we cannot argue about extravasations, here we cannot argue about scavenging.

All of these materials apparently are scavenging, of taking out nitric oxide equally and the point is that this one here, if it is not a vessel constrictor, is definitely something that maintains the circulation exactly at the same level as it is under normal conditions. You saw the diameter does not change, so there must be something else going on here.

And the -- now the search is what is this. Before arriving to the final point, oxygen obviously is the major player here. All of this centers around oxygen. And doing PEG lowers P50, increases significantly the affinity of the hemoglobin to oxygen and the question is, is that okay, how that modifies then subsequently everything that depends on oxygen, and I -- in this particular case, having available the vesicles and with the goodwill of Hiromi Sakai and the group of Waseda and Keio, we were able to test simultaneously vesicles that have low and high P50.

This is ideal experiment because everything is identical other than the P50 of the hemoglobin within the vesicle. And this is the result, this is P50 of eight and this is P50 of 28.

This is a tissue PO2 and this is the oxygen delivery and it is clear an advantage, by high oxygen affinity in this extreme hemodilution condition.

So that -- we're okay with that. So oxygen is not really being put in jeopardy by the conditions of polyethylene glycol conjugated hemoglobin.

So what is it? Pedro Cabrales organized the

following experiment. This is a normal animal and we give L-NAME. So we wipe out a good portion of the ability of the endothelium to produce nitric oxide.

And correspondingly, the peripheral diameter decreases and now we make a infusion -- top-load infusion of either a saline PEG albumin. Alpha-alpha hemoglobin, PEG-hemoglobin, and the veterinary version of polymerized bovine hemoglobin -- top-load infusion.

Nothing happens, stays vessel constricted. And now we give nitrate and bingo, the PEG-hemoglobin causes vessel dilation. Nothing happens to the other materials. Nothing happens to Peg-albumin. It's here, you see, but PEG-hemoglobin has apparently nitrite reductase activity and in this sense we are in high agreement with Professor Dr. Gladwin about the potential for nitrate as being a critical parameter, particularly with regards the management of the circulation by PEG-hemoglobin.

So to conclude, we do have a microvascular blood substitute. We have a blood substitute in process -- we have two actually. We have PEG-hemoglobin and we have vesicles that are able to maintain the microcirculation.

But we are poised to actually make something

that is actually better than blood. If we realize first of all that hemoglobin based oxygen carriers are all fundamentally different, that there is a viscosity effect here, that in principle could be used to counteract this activity, we propose that there is a mechanism by which PEG-hemoglobin overcomes NO scavenging, but this is just the beginning.

We have to introduce the glyco-chelics (phonetic) if viscosity is a player in this game. If PEG-hemoglobin will carry the day, we have to -- we have one hemoglobin and we have an infinite variety of PEGs. We have to find the right combination.

Joel Friedman asked me the other day, do we really need the heme, and I have an NIH grant trying to prove that we don't, and obviously toxicity is still the major reason to really be concerned about all of this and in all probability to have this meeting and having received the invitation from Abdu.

Thank you very much.

(Applause)

ENDOGENOUS HB SCAVENGERS AND HBOC TOXICITY

MR. BIRO: I hope some of you are furiously writing your questions. The next speaker is Dr. Schaer. He comes from the University Hospital in Zurich, Switzerland and he will talk about endogenous scavengers of nitric oxide.

MR. SCHAER: Thank you very much. As he already said, my name is Dominik Schaer. I'm from the internal medicine department in Zurich and that's actually a very nice view from our department across the campus, over the lake and to the Swiss Alps.

What I will do within the next 20 minutes or so is to give you a very brief overview on our current picture of endogenous hemoglobin scavenger and detoxification systems.

I will mainly talk on haptoglobin, and scavenger receptor CD163. I will then go into one example. I will show you how haptoglobin prevents the hemoglobin-induced hypertensive response.

I will then switch to HBOCs and introduce a simple kind of a structure-function relation module, which might help to -- might help us to understand how and why

some HBOCs do interact with haptoglobin and CD163 and others do not, and I will show you some examples how an HBOCs interacts with haptoglobin, or CD163 can fundamentally alter the biologic profile of an HBOC.

So this is a very simplified scheme of the hemoglobin scavenger system we have and hemoglobin is released into the circulation or in the interstitial space.

Consider any wounded tissue, then it usually binds very rapidly to haptoglobin, which is an ubiquitous protein. For many years, haptoglobin was thought to be exclusively synthesized by the liver. This picture changes more and more. We know that haptoglobin can be expressed in such cells as adipocytes. It can be expressed in macrophages.

Haptoglobin is one of the major proteins of neutrophil granules. So as long as the hemoglobin, haptoglobin complex is within the circulation, it is very rapidly cleared by the liver.

We don't know exactly by which mechanisms macrophages and Kupffer cells within the liver, which express the hemoglobins scavenger receptor CD163 might

play a role, but very likely there are other low-affinity transporters or receptors which mediate the clearance of the hemoglobin, haptoglobin complex by the liver.

What we believe is, as soon as the hemoglobin-haptoglobin complex is out of the vascular space into interstitium, that the only specific pathway which can clean hemoglobin from the interstitial space is the macrophage, with it's scavenger receptor CD163.

Once the hemoglobin-haptoglobin complex is endocytosed by the macrophage, the heme is released and induces a very specific gene-expression profile in the macrophage.

One of the most highly induced genes is the hemoxygenase 1 and the increased -- as the result of an increased heme breakdown, the macrophage release is the heme breakdown products, bilirubin, ferritin, and carbon monoxide.

And one very interesting aspect on this is that these three substances all have very potent anti-inflammatory, anti-oxidative and antiapoptotic properties. And by doing so, CD163 links actually, potentially toxic extracellular hemoglobin exposure to a very protective

gene expression in the macrophage.

So this is just one example to show you human (phonetic) macrophage altering the cytosis of hemoglobin, just to show you that the hemoglobin really ends up in the green-stained glycosomes here.

This picture should show you that the CD163 hemoxygenase system is really expressed in important sites within the master system, that's atheroscleritic (phonetic) black.

You can see that this is a complex -- a complex black with multiple neovessels, with multiple intra plaque hemorrhages, and you can see that there are multiple CD163 positive macrophages which do also express hemoxygenase 1 and the interesting thing actually is that all these cells which have high level of hemoxygenase 1 expression, do also have an expression of CD163, so there seems to be a direct link of CD163 expression and hemoxygenase expression.

So when you talk about protection by haptoglobin and CD163, I could go through older studies which show that haptoglobin has the potential to protect environment from oxidative stress and oxidative damage.

I could also show you current data from our research that haptoglobin extremely effectively protects the hemoglobin itself from oxidative damage. But today and yesterday, everybody was talking about hypertension, about base activity. I will therefore show you how haptoglobin prevents the hemoglobin-induced hypertensive response.

These slides actually summarizes three independent studies. Two studies were performed in dogs, the third study in guinea pigs. In the dog studies, we had one (inaudible) study which was performed in conscious dogs with non-invasive blood -- non-invasive blood pressure measurements, we infused stroma-free hemoglobin over 8 hours.

The second study was done with anesthetized dogs, with a full hemodynamic measurement of invasive blood pressure, pulmonary artery catheter and everything. We infused stroma-free hemoglobin over 2 hours. In most experiments, the haptoglobin concentration in the plasma was about 200 micromolar. The important thing with these tools that is -- was that we had two groups of dogs. We had one group, that's the black dogs, which had low or

normal plasma haptoglobin concentration, normal plasma haptoglobin concentration in dog means about one to two milligrams per ml.

This is quite interesting since the dog is one of the very, very few animals, which has plasma haptoglobin concentrations about in the range of humans. Then we had the second groups of dogs with a highly induced haptoglobin concentration. The haptoglobin concentration in these dogs was about 5, up to 10 milligrams per ml. As you can easily see is that we had pronounced and sustained the increase in the mean -- arterial blood pressure in the dogs with the low haptoglobin concentration, but we had absolutely no response in the dogs with the high haptoglobin concentration.

To prove that this difference in the blood pressure response is really the result of haptoglobin and not any other cause, we went to the guinea pig. We performed the 10 percent top-load study where we injected either a very, very low dose of hemoglobin alone or the same amount of hemoglobin together with a slight excess concentration of haptoglobin to make sure that all the

hemoglobin is bound in the hemoglobin-haptoglobin complex.

You can very easily recognize that the blood pressure response through the hemoglobin-haptoglobin complex is much more lower than to hemoglobin alone. What goes on in these animals? That's just hemodynamic measurements for the second talk study, but we had this (inaudible) catheter and we're able to calculate the systemic vascular resistance. Do you concede that in the low haptoglobin concentration animals we hadn’t about 30 percent increase in the systemic vascular resistance, which is very well compatible with about 30 percent increase in the mean arterial pressure, which I have shown you before?

In the animals with the high -- with the induced haptoglobin concentration, we had only very low, with four percent non-significant increase in vascular resistance. So what's going on in these animals? These are actually very reasonable experiment if we look at the urine of those animals after infusion of the hemoglobin. These four tubes represents urine from animals after infusion of hemoglobin of the low haptoglobin group.

These four samples of urine are also taken after

the hemoglobin infusion, but they come from animals with the high haptoglobin plasma levels. And you can easily see by mass spectrometry that these dark color, of course, is the secreted hemoglobin. We cannot detect any hemoglobin in the high haptoglobin plasma concentration animals.

If we go now to the plasma side and analyzed the plasma by high pressure liquid chromatographically, also after infusion of the hemoglobin, this is an example -- an elution profile of one of the animals with the low haptoglobin concentration. We see that the heme level about 25 minutes. That's exactly where we expect the hemoglobin tetramer to elude. What happens in the animals with high haptoglobin concentration? We have a huge left shift of the heme (inaudible) what means that the heme is now in a large molecular complex.

This complex is even bigger than haptoglobin alone. Haptoglobin alone has been -- acted as a control. So this complex is much bigger than hemoglobin alone and it is also considerably bigger than haptoglobin. And all that together is very compatible with the fact that hemoglobin really circulates within this about 150

kilodalton hemoglobin and haptoglobin complex. So what haptoglobin actually does in these experiments is the key to the hemoglobin within the circulation.

So we then switch to the HBOC question, and asked whether chemically modified hemoglobin could -- also interact with haptoglobin or CD-163 or both or neither. Would it be possible to predict from simple structural characteristics of HBOCs, would it be possible to predict which HBOC would interact with haptoglobin or CD-163?

And the most important question, does an interaction of an HBOC with haptoglobin or CD-163 does that modify the biologic profile of an HBOC? What we did, we started with a very heterogeneous group of available HOBCs and classified them according to two simple structural characteristics namely the predominant type of intra-molecular cross-linking. We determined the molecular size of each of the HBOCs, and for each of them we measured haptoglobin binding affinity and CD-163 binding affinity.

It was very easy by mass spectrometry to find two distinct groups of HBOCs. One of those -- one of these -- one group was exclusively of HBOCs cross-linked

via there alpha-globin genes. The other group of HBOCs was exclusively stabilized via covalent cross-linking of the beta genes. From a molecular perspective -- from a molecular size perspective, it was possible to find HBOCs, which were small, mainly tetrameric. We found other HBOCs, which were more or less homogenous but big, and the third group was very heterogeneous group which contained tetramerous (inaudible) of different sizes.

This figure in the right upper-corner here represents the typical BIAcore measurement of haptoglobin binding affinity. That is a very elegant and easy method of how we can determine the affinity between haptoglobin and hemoglobin or an HBOC. Haptoglobin is bound to a tip and we have a flow of hemoglobin or an HBOC over the tip. And the higher the curve -- if the two molecules interact, we get the signal, the higher the curve goes, the higher is the signal and the higher is the affinity of the HBOC or hemoglobin to haptoglobin.

You can easily see that non-modified haptoglobin has the highest affinity. Then it starts to become interesting. We have a very distinct path and with the red lines which represent these beta-beta cross-linked

hemoglobins having a very or a relatively higher affinity. And the blue lines, which represent the alpha-alpha cross-linked hemoglobins, these HBOCs have a very low, almost absent affinity to haptoglobin.

Alpha-alpha cross-linking or beta-beta cross-linking has nothing to do with the binding to CD-163. The binding to CD-163 goes with the molecular size, as I can show you in this figure where you can see the very strong inverse relationship between molecular size and CD-163 binding.

We have small molecule, small HBOCs tetramers, which have a very high affinity to CD-163. These molecules are created by CD-163. These molecules, they do induces an heme oxygenase-1 response in the macrophage. Then we have the very big molecules, which have no affinity with CD-163. These molecules are not created by a CD-163. And finally we have some intermediate sized molecules, which have also an intermediate affinity to CD-163.

Taking all these together, if we tried to group all the HBOCs in two classification systems, this represent the structural classification system, here we

have the functional classification system. Just to give you a few examples what we can do with this, we can group each of the HBOCs in one of those classes. Here we had the beta-beta cross-linked molecules which are small. In this group, we find a beta-DBBF cross-linked hemoglobin.

Then we have large molecules which are beta-beta cross-linked. One example is the BTC and dextran polymerized hemoglobin. We have alpha cross-linked hemoglobin which are small. One example on alpha-DBBF cross-linked, tetrameric hemoglobin. And on the other side we have the functional classification. We have strong CD-163 binders, which have weak binding to haptoglobin like this alpha-alpha DBBF cross-linked hemoglobin. Then we have weak CD-163 binders with strong haptoglobin binding affinity like this BTC, dextran polymerized hemoglobin.

Now, since the cross-linking pattern directly determines the haptoglobin interaction and since the molecular size determines the CD-163 interaction, it was possible to fuse or combine these two classification system and we end up with a kind of structure-function relationship model which helps us to find out, based on

these simple structural parameters, to find out whether an HBOC can interact with haptoglobin or CD-163 or both or none of them.

I'll give you just again one example, this beta cross-linked and dextran polymerized hemoglobin is a large molecule. Because it's large, it's a weak CD-163 binder. It is better cross-linked and because it's better cross-linked, it binds strongly to haptoglobin. So all this is interesting, but it's only important if binding of an HBOC to any of those scavengers would also modify the biologic profile of an HBOC, and it actually does.

I go back to the -- to our dog infusion model, and you have seen in the first slide, the stroma free hemoglobin infusion in dogs with a normal or low haptoglobin concentration induces quite a considerable and sustained blood pressure response. You also recall that if infused the same amount of stroma free hemoglobin in dogs with a high haptoglobin concentration, that these dogs are protected, they do not show any blood pressure response.

But now what happens if we infuse these same dogs with a high haptoglobin concentration and alpha-alpha

cross-linked hemoglobin which should not interact with haptoglobin? We have the same increase in blood pressure as we have with the stroma free hemoglobin into dog's with a low haptoglobin concentration. So what's happened -- what happens in these animals like first show we have the physiologic concentration.

We have haptoglobin -- two hemoglobin dimers bound to that haptoglobin, and if we analyze this mixture in a special high-mass MALDI mass spectrometry, we can see that there is a high mass complex. We don't see any haptoglobin and we don't see any free hemoglobin. Everything is bound within this complex. If we make the same analysis with a mixture of haptoglobin and alpha-alpha, this alpha-alpha cross-linked hemoglobin, we don't see any complexes. What we see is free haptoglobin and free hemoglobin detriment.

So these experiment very nicely shows that for the protective activity of haptoglobin, the physical and very high affinity interaction between the two proteins is really important. So from this you could consider that haptoglobin interaction should be preferable or could be a good quality of an HBOC, but there is also enough space

severe troubles with beta cross-linked hemoglobins and haptoglobin.

This child in dispute is the result of simply mixing haptoglobin -- fluid solution of haptoglobin with another fluid solution of a beta cross-linked hemoglobin. What's going on here? I go back again to the physiologic situation we have. Haptoglobin. Each haptoglobin molecule has two binding sides for hemoglobin at each -- to each binding sides binds one hemoglobin dimer. Each dimer has one binding site for haptoglobin so it is a kind of closed (inaudible).

This is even simpler when we talk about alpha-alpha cross-linked hemoglobins because there is no interaction between the two proteins or mixtures of alpha-alpha cross-linked hemoglobins, and haptoglobin remain fluid.

But now consider a beta-beta cross-linked hemoglobin which is polymerized to be a large molecule. We have big molecules now with multiple binding sides for haptoglobin and each -- to each of these binding sides can one haptoglobin bind. And this haptoglobin can link into other big beta-beta cross-linked polymerized molecule,

which again has multiple binding sides for haptoglobin and so on and so on, and we really have the possibility of infinite binding events within one single complex and the formation of these mega complexes.

We can of course look at this phenomenon with more sophisticated technologies. I can show you here shear rate is (inaudible). All these lines represent mixtures of haptoglobin with different beta-beta cross-linked HBOCs. These lines represent mixtures of haptoglobin with different alpha-alpha cross-linked HBOCs. And you can easily see that even at very high shear rate rates there is a significant and highly increased viscosity with the beta-beta cross-linked hemoglobins.

And when we slowed down the shear stress, this has caused increases and increases and increases. It's called the process of gelation until we end up with something we want to have in our blood vessels. This does not happen with the alpha-alpha cross-linked hemoglobins.

So to conclude, we have very effective endogenous hemoglobin scavenger in detoxification systems, which I have shown you -- I did show you one example which can, for example, completely separate the waste activity

of hemoglobin. I did also show you that some HBOCs can interact and others do not interact with haptoglobin or CD-163, and I did also show you that interact of an HBOC with haptoglobin or CD-163 can fundamentally alter the biologic and maybe also the clinical profile of an HBOC.

And the last question which I cannot answer now is, of course, whether we -- whether it will be possible to take advantage of these extremely successful endogenous detoxification systems to limit HBOC's toxicity. Thank you.

(Applause)

UTILITY OF ANIMAL MODELS IN HBOC EVALUATION

MR. BIRO: For a change in pace now we are shifting to something that is generated some discussion during yesterday, and Dr. Joy Cavagnaro is going to talk about the utility of animal models in HBOC evaluation.

MS. CAVAGNARO: Thank you, George. I thought he was going to say we're finally getting another female to present (inaudible) compliment.

(Laughter)

MS. CAVAGNARO: Interesting field. In the interest of full disclosure, I will state that my company in the past has consulted with Northfield, Biopure and Hemasol. I suspect that I have been invited today by the organizers, thank you, I think, because of my role. And I refer to myself these days as a recovering regulator, so is my colleague Joe Fred Anthony (phonetic).

So as a recovering regulator then, the slides -- I don't have to make the disclaimer that the slides represents my own opinion and you probably have to take fewer notes in that regard. But I will now give you my experience based -- in the whole area of preclinical evaluation and its relevance to HBOCs.

And I'll start even though this is a preclinical discussion, I'll start with what I referred to as a clinical dilemma, and that is with novel therapies by definition are potential high risk due to their uniqueness and novelty. And I would submit to you the thought of administering hemoglobin outside a red cell was at the time and perhaps still isn't a novel therapy or idea.

The initial first in human subjects are often with disease with our normal therapies versus volunteers.

Although unlike the standard paradigm with small molecular weight drugs where a single dose in the animals will allow you to go single dose in healthies (phonetic) and -- at least in the United States with certain other additional tests. But in principle, with novel therapies we tend to go into subjects with disease.

In large part there's an extreme interest of developers of novel therapies, which tend to be smaller companies, to actually see some activity. There are almost kind of encouraged through various milestones to see activity in that first trial. And so those initial first inhuman trials are in subjects with disease to assess not only safety but some activity.

And in some therapies, the proposed patient population based on the risk benefit is actually the least likely to show activity. And it's interesting the discussions that we had over the past couple of days that which patient population should we go into.

When you go to the extreme, they are least likely to show activity. They are also most likely to show toxicity, but I think we heard today the suggestion that perhaps even in the hemoglobin that a population

where we should go into is a very dire population, which would have the most opportunity to see activity but, of course, then we will then minimize our ability to now address the safety of these products.

In defining risk versus benefit has been discussed, of course, and how do we justify clinical development. We heard two suggestions made in terms of the current status of hemoglobins in the U.S., and having in a previous position -- latest position at the agency, having been the chair of the clinical whole committee and then exit in the agency into a biotech company, which was on the cusp of discovery development, the concept of clinical hold is really incompatible with gaining venture capital funds. Okay, this is very difficult.

We have heard about compassionate use. And as a way forward, a potential way forward, are encouraged compassionate use, but then again, as best as I understand, the use of these products is best in its immediacy. And compassionate use doesn't quite allow that because you know if you're transporting the drug from a developer, then you get clearance through the agency, which is quite quickly, but then you have that transport

issue.

And so perhaps maybe continuing development, another thing to think about is the concept of instead of compassionate use or single subject use, treatment INDs. Again, going forward treatment INDs offer some point of recovery but, again, allow this continuation of development, cost recovery in that regard.

So I hope we've quite understood now this fundamental statement, the first statement is that no drug is a 100 percent safe.

And that is, again, inherent in the definition that drugs are approved based upon outweighing foreseeable risks in a specific indication, in a specific population which, of course, means not -- and this benefit risk assessment isn't based on off-label use. And a drug is less safe it's used in a way that decreases foreseeable benefit or in a way that increases risk or if the actual risks are greater than the predictive risk.

And, again, so here are -- is example again of presumably all of these trials were approved and made their statistical endpoints and were considered safe and effective at the time of approval as they were. And with

the exception of cardiomyopathy, which was seen in the clinical trials, the point of this trial is not that -- based upon now various accreditations of serious and life threatening chronic diseases. The point of this trial is these black box warnings were not part of the approval in the approval, that these black box warnings were introduced after the phase III clinical trials, after approvals, during phase IV. And we heard yesterday about EPO et cetera which has happened after a large experimental base.

And this slide for me is my justification because I'm constantly trying to justify the relevance of animal models. And so just a reminder that humans may not even predict humans, at least in how we assess them in our current clinical trial designs. And again, some of these seriousness include deaths, or some of these box warnings. And in addition, there is now way even retrospectively that we could access the mechanism.

Again, some of these are human proteins. So that's quite different in itself, but mechanistically in an animal model. So there is no way that we could today go back and mechanistically perhaps even create a modified

Tysabri that may not result in PML, not can we modify a Xolair in terms of preclinical that would relieve us of this potential black box warning.

So predicting, estimating potential human risks, free the clinical to support clinical decisions. And we do that in vitro and in vivo and its scale up. So we can find a lot out in vitro. And what validates our in vitro is the in vivo, right? So, again, we heard today about the sensitivity in models. And we can make in vitro studies very sensitive, but then how do they relate in vivo?

So do we have relevant models and I'll speak to that in quite length. Are they available and are they feasible, technically feasible? And we learned in this area that we had to develop assays entry agents even to help us avoid some of the interferences with these products, and then how best can we distinguish real from theoretical risk.

So fundamentally the objective of what we do in animal studies is to answer the question, is this new product safe in humans and how best to recommend initial safe starting dose and dose escalation scheme, identify

potential target or organs of toxicity, what do we monitor, can they be monitored in the clinic, are they delayed, are they reversible, and how best to identify risk populations.

And we recommend the at-risk populations in the inclusion/exclusion criteria. They gets translated into the clinical trial design. And, you know, interestingly -- and we can do that. I mean, we modify based upon renal toxicity or hepatic toxicity we can -- we make that recommendation and it gets assumed in the clinical trial design. But interesting for many of the indications that have been discussed, the recommended exclusion criteria, based upon toxicology studies, often times for many of these studies is actually the inclusion criteria to enroll in the study.

Clearly what we do is iterative. Even though it's pre-clinical, I would submit that it's -- we're always pre-clinical, even carcinogenicity studies prior to lifetime exposure. So it's iterative. And what I have learned at least over these last couple of days is that while the products are not on the shelf today, we have learned much over the years in terms of mechanisms.

And in this last presentation, I mean, it's even important that we have actually had many different hemoglobins to evaluate this. And whether or not hemoglobins are alike or different et cetera. I think we have, at least in this last presentation, shown that there are attributes that maybe more amenable to modification in some aspect and others. And -- but it's because we've had a number to look at.

And we have done, I think, advances not only from industry base, but academia and the agency. And their research efforts have, for me, at least over these last couple of days, have really advanced I think much in the area of mechanism based toxicity with these agents. I think that's a positive thing.

In terms of preclinical safety evaluation, what we need to know is we need to understand the product attributes and their characteristics. And again this has been a hallmark of what we -- for a biologicals or biopharmaceuticals in terms of the process related to the product. But it's important that we understand related products as well, and I think that's the benefit that you're referring to at the agency, that these products are

related and they cannot be dismissed in terms of what you know.

The principle mechanism of action, I think, we have an idea what that is, for products that are rationally designed. Efficacy models, and I'll speak more to that, but they -- not only do we need to know them, but we need to know their limitations, those exposure information, identifying again the target organs of toxicity and whether any of this toxicity is reversible.

And now I'll propose the pre-clinical dilemma, and that's the schizophrenic use of pre-clinical data. We generally believe the efficacy and we question the toxicity. And that's often in suggesting that the species maybe too sensitive. So the animal species. So we've heard a couple of days that some species are more sensitive, but are they -- so are the least sensitive most like humans or the more sensitive? And this is what we always struggle with.

The inefficient use of "proof of concept" studies models. Again, these are very important and I'll get into this in terms of speaking about animal models of disease, but often these studies are designed to look at

an active dose but not always it does responds, not always it define a minimal effective dose. And they really include high dose or safety end points. So we do a study at a dose, it works and we feel very good and we believe because it's efficacious.

We are concerned about seeing toxicity in the toxicity study. And I'll go back, and again, in terms of discussing what's an acceptable toxicity. But the whole point of toxic studies in the animals is to see toxicity. So when we see toxicity in the animals, what does that mean? We are more conservative in our dose extrapolation?

Again, many of these toxicology studies -- most toxicology is done in normal animals. And so when we now in our first inhuman trials in patients with disease, we are asking them not only to extrapolate cross species, but cross physiological states. So it's not really giving them a chance to actually be predictive.

Oftentimes these studies are designed to satisfy a discipline in rather than providing answers to questions for clinical decision making. And my experience in that is, is that at least once a week somebody calls and asks me what's the least amount I have to do to get into the

clinic. And so, you know, clearly that's not -- what are the questions that we've been asked, what is needed. What are the -- and I think what we've learned, at least in this area, is that we know now what questions to ask better than we did perhaps 5 or 10 years ago.

Current practices. Again, most of the tools used for toxicology and human safety testing are decades old and this was identified in the critical path initiative, and that not very useful in terms of predicting safety and often times halts development.

So some of the key issues include selection of the relevant model, which includes understanding species, relevant species, and the physiological state, again, root and how high do we go and whether or not we're going to take advantage of not only looking at safety activity, but safety in our studies.

Again, this is just how traditionally we have looked at species usually on an empirical basis rodents and non-rodents. And in a retrospective look at studies in 2000, it was suggested that we might be doing better to look at you know, PD-physiology et cetera. And clearly, what was stated with biopharmaceuticals in 1997 is this

non-relevance species may be misleading or discourage.

So we have opportunity for normal animals and animals with disease and again the basis is -- should be based on anatomy, physiology, disease under study. We consider the availability of these species and the size, housing requirements, the costs and whether or not we have had experience with these models.

Now the real reason why many of these animals models of disease are not used to assess safety in the traditional sense in most pharmaceutical, biopharmaceutical is the concerns, these concerns. And these are highlighted here, the advantages again are that they parallel the target population and maybe have a direct estimate of therapeutic index and they have a potential for increased sensitivity.

And the disadvantage is and why people don't use them is that they are variable, they may not mimic all aspects of the disease, there might be a paucity of background pathology to interpret. And then again, of course, again, that the sensitivity may not be relevant.

So what has happened over the evolution of HBOCs? Again, in their late 1980s, I was the principle

toxicologist and the only card carrying toxicologist, walking my way down to Dr. Fred Anthony's office one afternoon and saying, "How do we compare these HOBCs that are coming, they may be different, they may not be different but how can we assess them because nobody is testing them the same?"

And that was a discussion one late afternoon and, you know, is it volume overload, is it replacement et cetera, and I think it was quite clear that volume overload does not make a whole lot of sense.

Again this was in the 1990s, again recommendation now came as in terms of looking at sensitive species in 2004, using recommendation to use animal models to mimic the intended clinical use, to monitor specific toxicity end points and so where are we now in 2008?

So I would like to propose a categorization of toxicology. Again are all hemoglobins the same, and may be not exactly but is there a way to categorize them. And I would -- and thinking about this is how in 1987 Professor Disbindin (phonetic) introduced his concept for biopharmaceuticals in general to distinguish them for new

chemical entities and had a classified toxicity in terms of areas of concern contaminants, biological toxicity, toxicity related to pharmacodynamic effects in intrinsic toxicity.

And again after listening to a couple of the presentations of the last couple of days I might put these in different boxes so I won't focus that but it is the idea of -- is that the contaminants we probably have a good idea about except when we start scaling up, this is going to be a bigger issue.

Biological toxicity, perhaps we have addressed this in terms of reducing the unmodified hemoglobins in that respect, but whether or not we can distinguish now between a pharmacodynamic effects, exaggerated pharmacology which are inextricably linked to the product, and we can mitigate that through dosing, we can mitigate that through inclusion/exclusion criteria, what the subjects -- or these modifications that we just heard about. So -- but here is an --

But intrinsic toxicity is a little bit difficult because in principle intrinsic toxicity are never predicted by the animal studies, it is only in the clinic,

and then we go back and that is iterative right there.

So again it maybe in different buckets but there perhaps we can look at the product class as a class where the commonalities are related to its exaggerated, its intended effect and then it is -- again it is intrinsic toxicity.

So again to close, no drug is a 100 percent safe. Animals are not a 100 percent predictors of the human effect and neither are humans. More than one animal model might be needed to assess safety but then it is based upon the question, so a specific question in terms of the animal model and -- but then they may not be available to assess all concerns, use of the animal models encourage and mimic the clinical indication, definition of the use of standardized models and I will just highlight these and perhaps this is what we will talk about in the panel.

Development of new, do we need them. Use of positive controls or other comparators, and agreed definition in terms of what is acceptable versus unacceptable toxicity in an animal study that is specifically designed to see toxicity. And with that I

will end.

(Applause)

ALTERNATIVE FOCUSED CLINICAL DESIGNS

MR. BIRO: The last presentation in this series is Dr. Jeffery Carson who is going to talk about focused clinical designs.

MR. CARSON: All right, anyone awake? I want to thank the organizing committee for giving me the pleasure to be the last speaker after two full days. Thank you all George, it is very kind of you to give me that, that pleasure.

Well, it really is fun to be here and the committee also was very, very kind in saying well, you know, these guys have spent a gazillion dollars, they got all these really incredibly smart people so you give 20-minute talk and tell them how to fix all this stuff up, I mean I really appreciate that as well.

So in any case what I will try to do in 20 minutes -- and I will stay to 20 minutes -- is briefly touch on what I view as sort of the current knowledge

related to red cells. So that is two or three slides.

We will then go to some design principles that I think we should think about in planning clinical trials here. I have three studies I am going to propose, none of them work completely, they are going to need modification, they are ideas to think about, they all have problems.

But that is the nature of how you design clinical trials you start with ideas and you refine them, and you refine, and refine them maybe none of them are any good but at least it is some ideas that I thought about.

I am going to talk about trial performance, which I think it has been an issue in this field and then try to summarize for you.

So I have the same usual stuff that everyone else has, we have all worked for many of these companies and it has been a pleasure to do so. So I want to begin with some preliminary thoughts and the first is I have not done a trial like this and, you know, until you have done them you don't really understand what it takes.

And I think I have been at a big disadvantage, I think it also really easy to second guess what has been done here and -- but these trials are incredibly hard, are

incredibly hard, and I actually really admire what I have seen done here.

The commitment of the companies, the commitment of the FDA to help the companies, the commitment of the NIH to try to contribute to this whole process, to see if we can move the field forward, I think all of them deserve tremendous credit and each of your recognition of what it has taken to get where we are.

So I think when you think about these drugs you need to that about them in the context of allogeneic red cells. What do we know about allogeneic red cells? First, what are the indications for red cells? Second, what are the adverse effects of these -- of blood? And third finally, what is some issues related to blood supply, which turns out to be quite relevant I think in the way that you think about this.

So there is one trial that has been published in the world's literature that is adequately powered looking at the indications for blood transfusion, this is called the trick trial. I am sure most of you know this. This was done in an intensive care unit, patients, about 800 patients were randomized to a 10 gram threshold and a 7

gram threshold, so called liberal and restrictive.

Okay, and you can see here the 30-day mortality was the primary outcome. Overall there was no significant difference between mortality, but you can see that -- that in general the restrictive group if anything did slightly better although not statistically significant.

What is interesting though if you look at MI and pulmonary edema, in fact there were statistically significant differences in the group that got less blood than who got more blood, and also ARDS was marginally significant.

So the point here is that this is the only published trial which begins to evaluate what we are trying to quote unquote "substitute for," this is what we know about the indications for red cell transfusion.

Now there is another trial coming, this is a study that I am the study chairman that of that has been funded by the NHLBI, it is called the focus trial or transfusion trigger trial, and this is a trial in -- we are trying to get 2,000 hip fracture patients with evidence for cardiovascular disease a risk factor.

Risk factors, we are randomizing them to a 10

gram threshold or a symptomatic threshold or 8 grams. We have a functional outcome as a primary outcome myocardial infarction as our most important secondary outcome and lots of other outcomes as well.

This trial has enrolled almost 1,600 patients, where we should have results in about a year, maybe 14 months. So this should also contribute to our understanding of indications for red cell transfusion.

Now, what about side effects of blood? Well this group certainly knows this story this is a table that the Harvey Klien put together for a paper that we wrote in the Lancet recently, and you know these risks. My point of view is that in general the risks of allogeneic blood appear to be pretty low.

So to briefly summarize, we actually know very little about the indications for red cell transfusion in that control group that we might use in studies here. The risks of blood, in my point of view, are relative -- are pretty low.

And what I didn’t touch on but I think is reasonably consistent with the general opinion is that the blood supply in general is adequate in most situations.

But these kinds of issues influence the way I think about how you would study and evaluate these drugs.

So what kind of principles and baseline assumptions should you consider in planning a trial? Well, there is two issues that come up immediately and that is what is the right control group and whether you are doing a superiority or a non-inferiority trial.

Well, the choice of a control group clearly depends on the study population in the question that you are looking at. If you are comparing the drug to a crystalloid then what you need to do is you need to demonstrate that the drug is superior that you demonstrate a statistically improved outcome, if your comparison group is saline or lactated ringers.

In comparison if you are comparing the use of this drug to red cells then what you are trying to do is trying to prove that it is as good or by some definition not inferior to red cells that, is the so-called non-inferiority trial.

Now, there are many end points that are relevant in these trials, mortality obviously, is a big one myocardial infarction is one that I think has come up and

clearly deserves to be in all these trials .

There should be systematic evaluations of this outcome with central classification and systematic that requires -- requiring of EKGs and troponins.

Heart failure, stroke, and other things that are obvious that are on this slide should all be part of potential outcomes in a trial of this sort. But my point of view is that we should not be focusing on a reduction in transfusion.

You know, as some of the early studies had we are looking at reducing red cell transfusion but the differences were small -- a unit or two. And if you buy that blood in general isn't particularly risky and that we have enough of it for the most part, then if you are going to do a trial here and you want to look at blood reduction then there needs to be a lot of blood reduction. It can't be a little bit it needs to be a lot.

Okay, now three -- choose what number you wish but it can't be a little bit. Now, what clinical settings should you be studying these drugs in? Well there are a lot of clinical settings that have been described in the last couple of days I am going to focus my discussions on

issues related to blood replacement or in situations when blood is not available.

So what are the potential populations that you might include in such a trial? Well, I think they include trauma patients, injured soldiers and patients who decline blood transfusion. And I am going to touch on these populations in the studies that I am going to propose for you -- for discussion.

So let us begin with the first trial that I think perhaps is the doable, perhaps not. This is a trial in Jehovah Witnesses where patients who decline blood for whatever reason they choose to do so.

Now, this is a graph that comes from a paper that comes from a paper Steve Gould published in the Journal of American College of Surgery in 2002, in which he compared the experience with polyHeme here with a historical cohort of Jehovah Witnesses that I had published a number of years ago and work that had been funded by the NIH.

And what you can see here is that the differences between these two groups begins to emerge around 6 grams per deciliter, and really becomes rather

large at the lower hemoglobin levels. So one option to consider is to do a clinical trial where we take consecutive patients with hemoglobins less than 6 grams per deciliter, which stratified by age and cardiovascular disease.

We try to do a double blind trial maybe not realistic but it would really be important if we could blind these trials, and I will come back to why in a moment. Perhaps you can find enough of these bloodless surgery centers that have been all over the country today and perhaps other parts of the world as well, there are a large number of these centers that potentially could enroll patients.

What would our outcomes be? Well you need a composite outcome to have the power to be able to answer this question. But clearly it includes mortality, clearly it must include myocardial infarction, and there is a whole bunch of other things it might include as well.

Now, these are -- this is a description of a paper we published in transfusion in which we looked -- took patients from our Jehovah Witness cohort who had post-op hemoglobins less than 8 and this is just the lower

end of this group, but you can see the number of patients that we had in each of these hemoglobin groups and the proportion of those who died at 30 days or had some significant mortality/morbidity event.

And what you see is below 6 grams mortality wasn't too bad but they sure were having a whole lot of other problems; 28.6 percent morbidity events. And below 5 grams mortality really begins to take off.

Now, I know this is not a 100 percent mortality as was proposed in earlier discussions today, but this is sure is a darn high mortality and a whole lot of complications. So we are looking at a very high risk group of potential subjects.

Now, if you go and do some sample size calculations this is one that I kind of put together myself so this hasn't been checked by statisticians, so I apologize to any of the statisticians in the audience, but in any case if you have event rates around 40 percent you can pick up about 50 percent difference in outcomes in these patients with about 200 patients or so.

That becomes a number that perhaps is achievable. Well, is it or is it not? Can you really

find 200 patients within a several-year time period who have hemoglobins less than 6 grams per deciliter, who are willing to sign consent and enroll in a trial? Maybe you can, maybe you can't I don't know. You sure as hell there would be a lot of centers, okay, to be able to get these patients and to be able to then implement a trial you would have to develop some creative ways of sending research teams around to help these centers do the study well so that we maintain adherence.

Is this ethical? I think that would be open for a lot of discussions. Could you consent these patients? I think the main issue in a trial like this is when the control group gets into trouble, there is going to be tremendous pressure to cross them over. Okay.

And so could you design a study that said if the patient develops a complication quote unquote, "gets into trouble" they have the option to cross over if they are indeed control group and that would be -- that would count as one of the events. Maybe, maybe not I am sure.

Of course if you look at some of the studies that were presented maybe you don't want to be crossed over but the bottom line here is that it would be an

option and maybe you could develop an approach to this. If this trial was blinded you would have a better chance at being able to maintain adherence to the protocol.

Now let us present another idea, perhaps just as bad as the last one, but let us see. Short-term trauma trial. So we propose doing the clinical trial either in civilians or military, now, I will tell you I think the military is the ideal population to do this sort of study if you could possibly do this and I know there is tremendous difficulties to overcome related to ethical issues. But never the less that is the population those are our kids they are in harms way who might be benefited most by such an agent like we have been talking about the last few days.

You randomize these folks to either HBOC or saline and you do a superiority trial, your control group is saline therefore you have to prove that this stuff is better than saline. Okay, you switch to allogeneic blood when they get to the hospital and blood is available you have a composite outcome of mortality and morbidity.

And this should be short term because you are studying from the time they get injured to the time they

have access to blood and then you can follow them a bit longer looking for events that may emerge after the first day or so I mean this of course would require a community consent.

Now, there are lots of issues with a trial like this, you know, and I think it is one of the things that we saw in some of the work that is already been done here is that our system of trauma is really good, you know, patients get to the hospital quick and that is wonderful, but it is not good for a trial like this right? If this drug have a much greater chance of working if there is a prolonged period of time between the time they are injured and they have access to blood then there is a much greater opportunity that the drugs might help those patients.

So for example, if you study this in a typical large urban community in the United States, Steve Gould was able to show that they could get patients on average to the hospital around 25 minutes or so right?

And if you were to do the same thing in Iraq John Holcomb tells me that they do about as well in getting our injured soldiers to a hospital in a battlefield quite remarkable. But he also told me for

example if you tried to do the same transport in Afghanistan because of the very difficult terrain it actually takes often an hour to get our soldiers to a hospital where blood is available that might be the group to try to study, for example, because then these drugs have a shot of actually helping those patients. Whereas if the time is too short you might not be able to do anything.

Issues are do really want to delay to stick a line in to get these patients up that is an issue that you have to manage. You want a roll of patients who have a chance to impact, so you don't want them too sick and you don't want them too well, sort of obvious.

Short term follow up, if you follow these people for relatively short period of time it makes the study a little easier to do, the logistics are easier. But when you are doing trials out in the field you don't have the control that you would like to have in a clinical trial, so that makes it harder.

Do some power calculations here (inaudible) depends on the event rates here. and I don't really understand what these event rates are but you can see if

you got a 20 percent event rate here you are looking at trying to get 2,400 patients, my goodness is that a difficult number to reach?

If you have higher event rates then you need quite a few less. But figuring out the power of figuring the right population to make this work is critically important.

Now, those are two trials to think about in the context of the principles that blood supply is adequate, reducing a little bit of blood uses isn't the critical thing. We are trying to establish that these drugs work. We are trying to use it as a replacement in situations where blood is not working.

Now, what if the scenario of our blood supply changed. This is a paper, a graph that was published from the New England Journal just a month or two ago which was out of the Cleveland Clinic which compared this as an observational study comparing patients who got new blood versus older blood, and what this showed is that the group that got older blood died more frequently than those who got newer blood.

It is not randomized it has issues of course but

what if this is true, what if this is true? If this is true, this would have a huge impact on the blood supply wouldn't it? Because if people said, "Well, hell I don't want the old stuff, you know, send them to a nursing -- or I will -- you know, I want the new stuff, okay."

And what is that going to do to our blood supply that may torture you our FDA friends, yes that should torture you guys pretty good. Anyway what if there is, you know, God forbid an emergency an epidemic you can make those stories up, what about if the risk of blood rises because remember we are talking risk benefit here, a new pathogen, there is a lot of observational studies out there that say blood kills people, blood leads to infection.

Now those studies I think are fatally flawed but maybe if further studies made sure that they are correct maybe blood doesn’t work as well so this would change the risk benefit. I think the only thing that is particularly likely that could happen more likely than not here is maybe this age of blood story is real. And this could change the whole dynamics here.

So what would you do under those circumstances?

Well then it becomes more important to find something that really substitutes for blood because you are going to have a blood supply issues. So another idea for a trial is to randomize patients in an intensive care unit to HBOC or allogeneic blood and basically replicate the trick trial.

You randomize when the hemoglobin is less than 7 to allogeneic blood or the HBOC and keep in mind that in the restrictive group in the trick trial those patients on average got 2.6 units of blood, so you could possibly say that on average in this population of patients you follow these people you only give them a drug or give them blood up to some time period 30 days maybe that is too long practically 14 days maybe more practical.

Primary outcome is once again a mortality and morbidity outcome and there is lots of other outcomes. This would be a non-inferiority trial, you are trying to show that the drug is as good within a certain definition as allogeneic blood.

And so what are the problems with a trial like this? There are problems at every single trial. Couldn’t get enough of the drug? You could be in situations where you are mixing patients who get a small amount of the

blood the HBOC, a moderate amount, or a very large part. Maybe those risk benefits differ depending on how much they get.

Short half life of our current products could be a problem in accomplishing such a trial, and if you unable to blind the trial it is really going to be pressure to cross people over. And it becomes then much more difficult.

Now, when you do a non-inferiority designs what you are trying to do is define a difference between your standard and this drug and you calculate (inaudible) what is the difference you would accept?

Well what you see here is you get into a really big numbers really quickly. And that of course has tremendous impacts on the feasibility and the expense of doing such a trial.

All right the last concept that I want to entertain on -- I am going to be about a minute late so George excuse me -- is trial performance issues. So I have left the design issues lets talk about trial performance. These trials are really, really hard -- really, really hard I -- when I think about what the

Northfield folks were able to pull of in that trial is unbelievable.

I know how hard my -- the focus trial is and I don't even think it is on the same page as what the Northfield folks were able to do. These trials are really, really hard there is some principles to think about as you plan them and consider options here, you really would like to do them where you have control of the patients.

So the problem with being in the field is you don't have them this much. It really would help if you could figure out how to blind these trials at least maybe in the beginning if you are out in the field where you got all these paramedics if they really didn’t know which one they were giving they might much more likely to do adhere to the protocol.

Can you figure that out I have been told that it is almost impossible, but maybe we could get beyond the almost part. A critical issue here is trials must be piloted, they have not been piloted enough, doing small numbers of patients 25, 50 patients you really figure out where your problems are.

And you get in the head of who is doing the studies and understand where they the Achilles Heels are and how you might have to change your design, and how you have to change your monitoring. You can't pilot trials enough. You can't pilot them enough because adherence was a problem in a few of the studies that were presented to us in the last couple of days.

Adherence was a problem, and it really compromise the interpretation of those results. I think you have to do intention-to-treat analysis and that is what you need to solve the adherence issue as best as you possibly can.

So to summarize -- I am done - these very difficult trials to design and execute superiority trials should be done by comparing to crystalloid non-inferiority trials when comparing to allogeneic blood, you got to do intention to treat analysis. The primary end point should be clinical outcomes not reduction in blood use.

Have I said pilot trials yet? Pilot, pilot, pilot them so you will improve the adherence and the performance. And it is possible that the clinical landscape maybe changing related to blood supply, and if

so it is going to make these drugs even that much more important for the future.

Thank you very much.

(Applause)

MR. BIRO: Ladies and gentlemen, I thank the presenters for a very interesting afternoon and we will have a coffee break now but if you will return by 3:45, 15 minute if it is possible to reconvene. Thank you.

(Recess)

MR. BIRO: Ladies and gentlemen in the interest of having sufficient discussion time and also so that people can catch their flights could we reconvene please? Would you please take your seats and if we can reconvene. Thank you, whoever that was.

Ladies and gentlemen, we do have a number of questions for the panel which would create interesting discussion, but before we do that there is a slight alteration in the program.

Dr. Ezekiel Emmanuel is going to make some comments about the ethical issues. Dr. Gould Klien (phonetic) yesterday has placed for us the ethics-based regulatory framework. And Dr. Emmanuel is going to expand

on the ethical framework, and I think he is uniquely qualified to do so, especially at this session, this meeting.

He completed a Ph. D at the Philosophy Department at Harvard Medical School, where the department was dominated by the -- probably two greatest philosophers of the second half of the 20th century. He is a practicing oncologist. He is the Chairman of the Bioethics Department at the NIH Clinical Center and he has a particular familiar relationship to hemoglobin, because he is related, I'm told, to Max Perutz.

MR. EMANUEL: I thought that's why they invited me, because I probably had Max sleep on my floor and served him more overripe bananas than anyone else in the world other than his wife.

But I'm not a 100 percent sure why I'm here, expect that they wanted someone who knew nothing about the field, who is willing to tolerate a lot of risk in research, who is very bullish on research and who has a lot of experience of looking at trials where there's a high mortality rate because I do a lot of oncology as you heard and look at a lot of oncology trials, but I really

have no expertise in this area.

Let me begin by just saying I have no financial interests of anything in this area, I have no non-financial interests of anything in this area, although I do work now with a couple of drug companies. I'm trying to improve informed consent related to research and to try to study something about the European healthcare system with -- learn something about that.

You heard I'm a government employee. I haven't given these slides to anyone in the hierarchy, so no one's responsible for them.

(Laughter)

MR. EMANUEL: I can see it's after lunch, people are a little tired, okay.

(Laughter)

MR. EMANUEL: If you have to use that disclaimer every time, you have to make fun of it. So, a number of years ago, we argued that for a clinical research trial to be ethical, it had to fulfill eight criteria, and our eight ethical principles, these are the principles, and that you have to begin at the start and work your down.

You don't worry about informed consent until

you've got the stuff at the top done. Now, some of these are in purple, some of them are in yellow. Let me say -- I'm not going to comment about all of them because I don't have a lot of time.

I'm going to focus on social value, scientific validity and risk-benefit ratio. But if you're really going to evaluate the ethics of these trials, you have to deal with all of them.

Collaborative partnership is particularly important in these trials where you can't get individual informed consent in the trauma setting, but I'm not going to mention that because I don't think it's unique to this trial.

Let's talk about social value. Social value asks the question of why do we need these things. Why do we need something else? What is it going to add to improving human health? So there are probably lots of answers.

Here are some of the answers from my quick perusal of the literature. Avoid the complications of red cell transfusions, the availability of oxygen carrying capacity when there's urgent life-threatening blood loss

and no red blood cell supply -- save money at no higher risk level.

And you can imagine that these things are needed in the developed world and in the developing world, but I'm not going to be able to go into that in any detail. We can, I think, safely ignore the developing world because there's no way they're going to be cheap enough, at least in our lifetimes.

I have a little problem with those social values. You saw a part of this slide in the previous presentation, which I thought was informative, but red cells are just very, very safe, probably the safest intervention that the whole healthcare system uses.

You know, some of you may know about the six sigmas in production, that if you can get the six sigmas, you know, you're very, very safe. The airline industry gets there, other -- your cell phone gets there and it turns out that, as best as I can see, red cells are probably the only thing we do in the healthcare system that passes the six sigma rate.

The six sigma rate is 0.4 defects in a million episodes. It's just really tremendously safe. Just to

give you a comparison, here's hospital acquired infections.

We had about 35 million hospitalizations in 2005, the last year -- the CDC. Five in every thousand admissions had any type of infections. 0.5 in a thousand admissions had bloodstream infections and the CDC estimates are 26,000 deaths from hospital acquired infection. We have nowhere near that in red cell transfusion, just nowhere near.

So the whole issue of do we need something better than red cells is a big, big question, it seems to me, that is not clearly, "yes."

Maybe HBOCs will prevent emerging blood infections like HIV. It's hard to quantify this risk and therefore the value of having something sitting on the shelf for maybe the possibility that it will.

But the infection could affect HBOCs also if they're made from human blood and it turns out that whatever the infectious agent is difficult to sterilize or could be just as problematic.

So this is not just straightforwardly obvious, and HBOCs and other things made from cows and swines

create other problems, which again are hard to quantify.

Blood sparing, in developing countries, as I said, it's probably not realistic because they're not going to be cheap enough to be used there, at least in our lifetime. The availability of oxygen carrying capacity is something we would need.

We could use it in excess demands when there's disasters, we don't have enough blood, or insufficient supply, trauma in the field, prolonged transportation -- I mean, the question is how big a problem really is this.

You heard in the presentation that even in Iraq this isn't a sufficiently big enough problem. So the question is what is the social need?

Now, I apologize, I wasn't here yesterday, but I actually haven't heard anything today, which I have been here pretty conscientiously, that's changed my mind on that.

So I'm not a 100 percent convinced that that has to be a compelling social value to HBOCs. Maybe there is and I have missed it, and that's perfectly fine.

As I started out, I don't know anything about the field and I'm willing to be educated, but at least

reading what I've read on the literature and listening through today, I didn't hear it overwhelmingly. It's certainly not like we need another drug to cure lung cancer.

What about scientific validity, superiority versus equivalence or non-inferiority designs? You heard in the last presentation that if you tested against colloid, you have to have superiority, if you tested against red cells you have to have non-inferiority.

I don't actually agree with that. If you're going to use it for blood sparing, given the safety of red blood cell transfusions, I think the design requires it to be a superiority design, if you have adequate supply and -- you get it tested against red cells.

Now if you don't have adequate supply and something bad is happening to people because there's not adequate supply, for example, in the case of the sort of young versus old blood and suddenly we don't have enough blood, then maybe non-inferiority will be acceptable.

But in the current circumstance where we have an adequate supply, I think you need to superiority design. Equivalence could only be justified it seems to me if

HBOCs are substantially cheaper than red cells.

Given the amount of investments, my intuition says they're never going to be substantially cheaper than red cells. What about O2 carrying capacity?

In that situation, I think you have to have your HBOCs superior to colloid in the field and when they -- you -- everyone gets the same treatment in the hospital.

Equivalence again only could only be justified if HBOCs are cheaper than colloid and I just can't imagine that happening. I actually think here, and again, I could be wrong and I'm willing to be persuaded because I admit that I'm completely ignorant, that I can't imagine why a non-inferiority design would be acceptable in the current circumstance.

I'm also skeptical, given what I know about conducting large-scale clinical trials in cancer, that there's a way to conduct the superiority trial which is most desirable, given what we know, and given what you saw in that last presentation, again a fabulous presentation, about the size you need for these trials.

Two thousand people, 2,400 people, I don't even think that Jehovah's Witness 216 is a reasonable estimate,

but 500, 700, 1,500 people, just way too big, I think.

Risk-benefit ratio: Well, if you're sitting on an IRB looking at a trial like here, you have to be aware that there's repeated failures of HBOCs, terminations of trials previously and I would say that even the ones which are claimed to be successful are really failures.

And I went to look at the Northfield's PolyHeme trial to look at the most recent case that was released and you've seen these slides, right?

PolyHeme had worst 30-day mortality, although not statistically significant, certainly not better. Adverse events were worse, serious adverse events were also different.

If you look at their breakdown, there's not one category here, not one single category, in which PolyHeme is better than control. So I can't even point to one thing I would say well, it does better here, when the conclusion of these slides is acceptable benefit-to-risk profile. I'm a guy who looks at cancer trials all the time. We make people vomit, we make people lose their hair, we put people in the hospital for infections, we destroy their hearts, we destroy their lungs, we sometimes

kill them with our chemotherapeutic drugs. I don't think you can say this risk-benefit ratio is acceptable.

It's not better in any circumstance and blood is very, very safe. So I don't see why you would even think about saying it's better. So I don't think it's an -- oops, I don't think it's an acceptable ratio. It's no survival benefit, adds adverse events, and special myocardial infarctions, and probably at a higher cost.

So where exactly is the benefit here? So, as I said, acceptable trial of HBOCs must fulfill these eight criteria.

I have said, at least to me on a first pass, sitting on an IRB, it's unclear that there's a compelling social value to HBOCs for either the developed or developing countries, and I think you have to have a superiority trial design and it's unclear that a superiority trial design is feasible given the enormous numbers that would be necessary to complete it, and I think there's an unfavorable risk-benefit ratio here.

There's no benefit in terms of increased survival or reduced complications and there are added risks in the latest results.

So I don't see that when you compare it to blood that you really have a compelling case here to go forward, but again as I said, I'm ignorant and I'm open to be persuaded here.

Thank you.

(Applause)

MR. BIRO: Okay, we'll start with Dr. Olson. I have so far two questions for Dr. Olson. The first one asks, please clarify what you meant by this generation of products in the way -- is the way forward? This generation -- ?

MR. OLSON: Well, I can say that in a couple of ways, that the -- this is personal -- the recombinant technology that I work on is unlikely to be developed unless the current products proceed forward, further trials, for a variety of reasons.

We just heard, you know, a bunch of negative comments now but you know, there's -- and I'm not a physician, I don't treat patients, but if I'm someplace without access to blood and I'm bleeding to death and they can't save me and I need oxygen carrying capacity, then there's definitely a need for this, and what I've seen is

that I would certainly take the material myself.

You know, I can't judge all of this, but I can say from looking at the day -- the things I look at as a biochemist, the products that are out there now have minimal or small hypertensive effects.

They're actually, whether they were by design or not, are larger molecules that don't appear to get into the endothelium and I mean that's why I feel that you know, somehow we need to keep -- proceed further with these.

To develop the recombinant technology is 7 to 10 years. I'm not convinced that Baxter dropped the recombinant technology because of safety concerns.

It's most likely due to financial concerns and another dilemma with recombinant technology, if I make a new molecule, then how do I proceed?

Do I have to go all the way back and do all the trials and every time I make a mutation, and which I can't stop myself from doing, because you want to make the ideal molecule.

So these simpler formulations are really the -- what we have to deal with over the next few years.

MR. BIRO: I think that probably satisfies the question about definition; and one about the yield of the E. coli expression system -- what is the current yield?

MR. OLSON: Well, I can tell you roughly what the yield was and maybe Tim Estep can help me out here, of roughly what -- it's really not so much yield but cost, right?

So the cost has to be on the order of $10 a gram, liposome -- lipopolysaccharide-free. That's pretty tough.

Somatogen and Baxter, I gather -- or at least that's what they told me -- were on the order of 20 to $30 a gram, and that was an optimistic view.

In terms of total soluble protein, it -- I believe they were 20 percent was hollow protein with the heme in it that could be purified, and there's always about 10 percent that didn't have heme that was in a precipitate.

And so that's roughly where we stand now. And that's the idea of getting the heme in faster and making it more stable.

I haven't had the lot of courage to try to do

the calculation in my own laboratory what it cost me at this moment in time, because I don't have the scale of capacity, but all I can say is that they were estimating 20 to $25 a gram at Somatogen when -- or at Baxter when they quit.

Now their rHb 2.0 was not just a tetramer. That was also in some formulation that -- I don't know what it is, because it's top-secret and wasn't published -- but my -- I guess, or Tim can speak to this, or maybe he's not allowed to, it was polymerized and PEGylated in some way.

The basic molecule is the one I showed you up there that was tested and published with the distal pocket mutations, but that tetramer by itself was not studied. They went further.

MR. BIRO: A supplementary to this is a specific question about whether you add extra iron to the system to increase yield, and do you use complex or minimal media?

MR. OLSON: Well, we try everything. Minimal medium sometimes is used when considering a glucose feed, but what we're trying -- we do add iron sulfate if we need to and supplement with L-broth and things like that, but what we'd really like to do is get that heme in ourselves,

and that's why we're trying to use the heme transporter, which is -- so then I don't need -- I need the iron really to get the bacteria to grow up to like 25 ODs, or 20 ODs, which is hard for us to do, but Somatogen was able to do it easily and then we induce, and then I want to add heme, and I want the heme transporter to -- I have to calculate and guess, one hemoglobin, because excess heme causes trouble, because the bacteria always take the heme out, it will make porphyrin -- porphyrin will get into your product, it's photoactive and all sorts of problems.

And Somatogen had actually figured out a way of getting rid of the porphyrin by pasteurizing the sample and heating it. And that also helps to get rid of the pro-polysaccharide. That's probably more than you want.

MR. BIRO: Thank you. We'll pass to somebody else so that you get a rest and the next question is to Dr. Schaer. The question is, what is the mechanism for haptoglobin-binding and the lack of hypertension, is it a decrease in oxidation, decrease in extravasations, or NO-binding?

MR. SCHAER: That's difficult question. What we measured was the NO-binding kinetics. We did measure the

NO-binding kinetics and there is no difference between free hemoglobin and hemoglobin and haptoglobin.

We also did measure the autooxidation. That's also the same for hemoglobin, and -- hemoglobin-haptoglobin.

One mechanism can be related to the size of the molecule, which is about 150 kilodaltons, which would prevent dissociation -- this diffusion of the protein out of the endothelial layer. That's our current hypothesis.

MR. BIRO: To Dr. Carson, so you don't rest too easy. The question is, for a clinical trial in trauma, how would you manage patient consent?

MR. CARSON: You have to have community consent to be able to do these trials, because you know, as was really I think illustrated by our surgeons as well as Joe Parrilo, you know, it's all about getting the intervention in very early, and so you can't do consent.

It's not practical, it's not achievable. So it has to be a community consent process, otherwise you'll -- you don't have any chance of succeeding.

MR. EMANUEL: I agree with him, and I don't think that's a barrier here. That is not an ethical

barrier here.

Community consent is something that's acceptable. It's acceptable in an emergency situation where people are facing serious life-threatening problems and you have a reasonable alternative.

So I don't view that as a serious barrier to these kind of trials at all, and I completely agree.

MR. BIRO: Then we'll go back to Dr. Carson again, and the question now is a little bit more specific. Would trauma development or development in the trauma setting lead to a commercially viable indication? The subtext is --

MR. CARSON: Was this answered by the business people of this community -- by the --

MR. BIRO: The subtext is, it seems so difficult --

MR. CARSON: By the Wall Street Journal?

MR. BIRO: -- to find the patients.

MR. CARSON: Try the --

MR. BIRO: It seems so difficult to find the patients.

MR. CARSON: Yeah, I'm not really the right

person to answer this question. I mean, it seems to me that the standard in which this country seems to provide trauma care is -- and even in Iraq, which is quite remarkable, another tribute to our own forces -- is that you can get people who are really critically ill to an institution within 20-25 minutes, 30 minutes or so and so you know, will these drugs make a difference in such a short period of time?

I don't know. I think, you know, if you were to plot success by time to hospital, my guess is the longer -- you probability of success is going to go up the longer it takes to get to an institution where you can provide care.

If you have a really good drug and maybe it would make a difference in some people, but you know, I'd like to able to help with this question, but I don't really know the answer.

MR. BIRO: Then we'll pass to Dr. Cavagnaro. The question is fairly long. Bueller (phonetic) and Alayash have published a recent paper claiming that rats are ideal for toxicity studies, due to the ascorbate level in plasma, and they suggest using -- excuse me -- guinea

pigs. Do you agree?

Do guinea pigs have other features that are good or bad vis-à-vis, mimicking human physiology?

MS. CAVAGNARO: They are difficult to bleed. No, I think that again it's -- we looked for a species that's relevant and we assessed it in that context and I think the data in that publication suggests that in fact rats are not -- and why some of the toxicities may not have been predicted because the rats weren't appropriate.

And so when we find that appropriate species -- now of course, we can test and we can validate, but we need the reagents to validate, and when we have a clinical toxicity that provides us the reagent, actually, to validate our preclinical model.

Those reagents need to be forthcoming so then to distinguish and you know, we've heard a lot about all HBOCs are not alike, which of course may be true, but actually to support that, you'll need to test them to ensure that you are different.

And I think when you have a sensitive model and you have a reagent that has -- that again can be used to technically validate it, because you've seen something in

the clinic and I think that's quite useful and then you use that as a comparative, so I think that -- again, guinea pigs are not ideal for every thing, it's not a standard tox (phonetic) model and as I said, again they're difficult to bleed.

But you know, short of that, I think if the pharmacology is there, then I think that it makes sense to do that.

MR. BIRO: Well, here's one to challenge you. How much would you estimate the cost of a full preclinical development for a new HBOC species?

MS. CAVAGNARO: So you know, the toxicology testing again, is mainly for first in human trials, as again we're into single-use conditions or technically a single-dose, defining -- we may need a few species to address -- species that has been defined as sensitive, that we currently as sensitive, to address some of the potential toxicities.

So that maybe monkey, that might be guinea pig in this regard. And then I don't know how much animal models of disease are, but -- so I would say that single-dose in a species would probably would be about -- in a

rodent species is close to 50,000 and in a non-human -- in a non-rodent species and you're up into -- closer to a 100,000, 200,000 if you do specific physiological measurements et cetera, so you can get up to that.

You know, I think one of the challenges -- and then I would propose that for each of the indications that then we use the animal model that most closely mimics the disease, so you're looking at general toxicities and then you're looking at the intended disease as we've talked about earlier and that those are very difficult to conceptualize cost, because many of those, as you pointed out, are done in academic settings.

But if we looked at like that study as a phase zero study, then perhaps it may cost half a million dollars to enter the clinic.

MR. BIRO: If we can go back to Dr. Schaer, there is a very specific question. Does the ability of haptoglobin to blunt the hemoglobin depend on vasoconstriction, depend on the hemoglobin phenotype?

MR. SCHAER: We didn't direct address this question yet. The purified haptoglobin which we used for the guinea pig studies was mostly phenotype 22 -- the dog

has phenotype 11. So we have some evidence that both phenotypes have some protective activity. Whether there is some differences between, I cannot answer that.

MR. BIRO: Thank you. Dr. Intaglietta, there is a bunch that I'm trying to sort of synthesize, but it's not easy.

The first question is quite specific. Why was the plasma hemoglobin higher in the experiments with oxyglobin, when -- than with MP4?

The plasma hemoglobin with oxyglobin was higher than in the experiments with MP4; why?

MR. INTAGLIETTA: The way that the experiments were conducted is we took the material as it is formulated by the producer, so -- which is probably how the material is intended to be used clinically.

So for instance, the PEG-hemoglobin is formulated at four-and-a-half percent, the Biopure product is formulated at 13 percent, and the vesicles were formulated at 10 percent.

MR. BIRO: Back to you again. There is a question about the tissue PO2 estimates. The questioner is saying that some have criticized this measurement and

asks to disclose to the audience the disagreement with the methods.

MR. INTAGLIETTA: Yeah, there is tremendous disagreement in the literature as to how to do that. Our position is that it is always good to make comparisons and in this particular case, the best message to be drawn from our result is that low P50 produces a higher tissue PO2 than high P50.

Similarly, this is further corroborated by the fact that we measure the oxygen being delivered to the tissue. It is more oxygen being delivered to the tissue, with the low P50 material than with the high P50 material.

The -- what there's a question is the actual values, particularly of the tissue PO2. We claim that our technique uses very little oxygen from the tissue in obtaining the measurement and there is a significant disagreement between different laboratories as to what is the actual oxygen consumption of the method.

MR. BIRO: Thank you, and another one. The question is really related to what is the significance of the functional capillary density when you're looking at the (inaudible.)

If there's a decrease in functional capillary density and there is constriction of the arterials or small vessels, the question is does that really translate into something more tangible about tissue survivability or something as concrete as reflecting the whole body and resulting in death at (inaudible)?

MR. INTAGLIETTA: Yes, we have a substantial background on the experiments on hemorrhagic shock, treated and untreated, where we have demonstrated repeatedly that animals -- experimental animals that are able to sustain a threshold of functional capillary density survive, while those that do not do succumb, and this appears to the only visible -- an objective parameter that we can identify in the microcirculation as being the determinant of survival.

The actual mechanistic reason behind this is that you need functional capillary density to extract from the tissue the products of metabolism, as well as to ensure that the little oxygen that there is, is evenly distributed and that there are no hypoxic pockets, if we -- whose probability increases as the functional capillary density goes down.

MR. BIRO: Thank you. Now we're going to try and generate a little controversy. There's a question for Dr. Carson and I hope that others will chime in.

The question is as follows. Do you think that conducting a non-IND clinical trial in India to support the U.S. IND will make sense?

MR. CARSON: Glad such a straightforward question. Thank you so much, George.

MR. BIRO: I didn't -- I'm just reading them.

MR. CARSON: You know, I've actually thought a fair amount about wanting to do trials elsewhere, and -- because you know, one of the big limitations of putting together the focus trial that I briefly told you about earlier today was that I had to work within the construct of how clinical practice and how transfusion is, what standard of care is, or what usual care is in the U.S.

And you know, in that trial, I actually wanted to look at the 7-gram threshold like the trick trial, but I couldn't get clinicians to agree to do that because they weren't comfortable with it.

And the point of that comment is that I don't understand how blood is used in other parts of the world

to be able to clearly give you answers to it, but there's little doubt that the risk-benefit and the standard of care is completely different than what comes -- goes on in this country, in North America and probably Europe.

And therefore the way you would design the trial, what your comparison groups would be, and what the ethics of it would be, might be completely different than in this country.

And you know, if you go to an environment where the risks, you know, of HIV are really high, the blood's not tested in the way that it's carefully done here, that donors are not screened in the same way and things like that, then the risk side becomes a completely different issue.

The cost issue becomes completely different as well and so all the parameters are different. I think you have to look at them, completely drop your biases from North America and take the setting that your -- you want to do a study to in that country and decide whether what you can do and what you can't do.

Now, the question was if you find something can you bring it back here and well, you know, you have to ask

your FDA buddies there about the legality of that.

You know, the -- you know, as a physician and just asking the physiology of it, I mean, I don't know that -- why that it would necessarily be different, other than there's -- you know, clinical care is completely different in many of these societies so that you know, the relative importance of this particular intervention may be very different in other parts of the world than it is here. And so I think it would be very complicated actually. You have to understand it and how –- where it's done. There are hospitals in India that are very sophisticated, that are probably starting some of our radiology studies, and we have patients going there now. And I'm told, I haven't been to them, but that they're equivalent to a North America institution.

So maybe if it's done there and the quality of care is similar and the technologies applied there is similar, but that the way the patients are cared for and the relative style of care and how much blood is used and everything else is different maybe it would be more suitable. So in that case, if really care is comparable then maybe it would be reasonable to generalize to this

country.

MR. FLEMING: There are really two key issues here, and I think Dr. Carson has appropriately focused on one of them and that is the generalisability with the results in fact applied here. There's a second key issue and I'm sitting next to an anesthetist's chair, but the second key issue was what we would offer for a distributed justice.

If you're going to do a trial in one population for purposes of benefiting entirely a separate population, that would violate the distributed justice principle. So if we were studying a population in India it's certainly appropriate for those results to be relevant to the U.S., but is –- if that study shows a favorable result, is there a viable plan for the implementation of that intervention in that population in India and we've heard about whether that would in fact be cost-feasible.

So I would think this issue of distributed justice also would have to be addressed if you're going to go forward. Is it –- is there a viable plan if the study is positive to be able to implement this intervention in the population in which you're studying it?

MR. EMANUEL: That's a whole another conference, come here for tomorrow or another two days. The short answer is that there are at least some people in the audience who say under circumstances would that be acceptable. I am not one of those people; I don’t think that's true. The question you'd have to ask is, if you do a trial what are the overall benefits to that –- in that country and what are the –- compared to the risks –- and so you'd have to ask yourself what are the benefits that are going to be accrued, one of which would be do they have access to the agent.

But that's only one of which –- one of the questions, and I don’t think that –- even if they don’t have access to the agent, that settles the matter. I think there would be a longer discussion. I remain skeptical about that at least in my view, because this is more on the he generalisability side.

If you go to another country, where one of the reasons you're doing the trial is because they don’t have the (audio break) the risks from their blood are high and you find out well, they do better with the HBOCs, I'm absolutely not clear that that has any applicability to

the United States where the scenario is completely different. You know, it's much different if you're testing a straightforward bill for HIV, and even there you do have to have a different risk/benefit ratio and take into consideration other things.

So I do think justice is a major concern, but it doesn't absolutely –- even if they can afford it, I don’t think it absolutely rules or makes it unethical. I still remain quite skeptical that that's the way to go.

And if you end up in India at one of these hospitals that are just comparable to the United States, you're back in the same box. How are you going to show that it's really better if you know, they're doing the same kind of care we have and they have the same kind of screening et cetera?

MR. BIRO: Anybody else would like to contribute to this? There is a definite recent trend for big pharma to move clinical studies to China and India, but this is big pharma and their conditions may be more amenable to studying drugs. Dr Vlahakes?

MR. VLAHAKES: I wouldn't necessarily give up on the Jehovah’s Witness population and just share with you a

couple of insights in having managed a number of those patients over the 22 years I've been in the specialty, our group along with our colleagues in cardiac anesthesia have had a number of these people referred through their own network. This is for those of you who've not had the opportunity to work with Jehovah's Witness patients. They have a medical liaison network in every city in the U.S. and the headquarters of the religion is the Watchtower Society in Brooklyn. And we have had the senior members of that organization come to our administration and there have been meetings about creating a referral relationship, and the patients who are willing to travel.

And so you could conceivably design a surgical trial and you could pick a handful of surgical specialties where there is a reasonable likelihood of needing a transfusion, and design it regionally at several major centers in the U.S., engage the Church and the medical liaison people that are part of the Church and to reach out into the community to bring those patients into the system, and then carefully design the clinical trial and even potentially have a crossover option if it comes down to life-threatening anemia which we're going to encounter

in some of those patients as you start to do larger and larger surgeries.

So that's not necessarily an impossible task, and given the issues with doing a trauma trial, and the variability and the clinical substrate and the variables you can’t control, give that some thought. I think you could –- particularly a vendor that’s got –- that has done some clinical work I think could pull it off very well.

Second comment I would make is if you do studies overseas, make sure you have very good control over what takes place. And if it means having a CRO base there, really have very tight control over the rate at which the patients are enrolled and the quality of the dataset that's generated, really audit it on an ongoing basis, and make sure the protocols are adhered to and that's based on having been to India and China and a few other places in South America, seeing how medical care is conducted. We never see that problem hazard a couple of potential interpretations.

MR. BIRO: The –- what you mention is clearly the elective surgery setting where people will choose the center they will go to. And is there going to be a likely

large enough population that will get down to a critically low hematocrit that will practically mandate improvement or resuscitation with an age bar?

MR. VLAHAKES: I would organize like trial 75 in cardiac surgery where you use clinician-specified transfusion triggers, and the other arm would be, you know, what else you have available, you need in this Jehovah’s Witness, which is crystalloid or non-heme –- a non-heme derived colloid.

MR. CAVAGNARO: I asked this question to Dr. Shander who runs the Englewood program which is one of the biggest programs in the country, and he said, he went on line, he said they had about 50 patients that would have been our criteria, which actually surprised me.

MR. BIRO: In a single center?

MR. CAVAGNARO: In a single center, but I don’t think that's typical. He says there's a number of places that really are kind of regionalized as well. But, I think you know as –- when I proposed that design, I think that's one of the big questions, is could we really get enough cases and how could we do it, because a lot –- as you said patients who get acutely ill, they don’t know –-

they don’t go to the centers and a lot of times they're going to be too sick to be transferred and so it would be –- you know, whether you can actually generate enough patients. And just having the patients there doesn't mean they're going to consent and that they're going to be suitable and for –- you know, you don’t –- the fact there are 50 patients doesn't mean you're going to have 50 patients into a trial. I can assure you that it's –- won't even be close to that.

MR. BIRO: Now, just to generate a little more spark, we were witness to a polarization of opinion this morning. Would anyone care to comment further about the desirability of very high risk of death, practically 100 percent? At the same time, the practicing surgeons will regale us with anecdotal –- anecdotes and stories in which there is a huge personal benefit. In cases where blood was not available, the issue of risk is not only transfusion; the issue is also risk that blood for transfusion is not available.

MR. CARSON: I'm of the view that the Witness population if you use, there are really low blood counts there. May be not quite a 100 percent but really they

have lots and lots of complications and their mortalities are really, really high, that that's a model that would be reasonable to study.

MR. BIRO: Dr. Emanuel?

MR. VLAKAHES: I think the issue of the population that has near 100 percent mortality the argument would be most valid when blood is an alternative and you do an EchoPlus (phonetic) analysis. But again, Jehovah’s Witness, it's not you're going to either add morbidity from surgically-created or medically-created anemia versus avoiding the morbidity of surgically-created anemia.

Oh, is this on?

MR. BIRO: No, it isn't.

MR. VLAHAKES: I think the issue of only offering this to patients where there's a near 100 percent mortality might be in a situation where you have blood as an alternative. If you did the risk/benefit analysis of using blood versus using a HBOC in the clinical trial as was pointed out in the presentation here, that's a lot different. We're talking about patients who don’t have that option, and you're talking about either somebody who

will die from anemia or will have potentially a long and unpleasant postoperative course because of profound anemia from their medical care.

So I think the thinking in that particular patient population is different. The other issue that –- and I don’t know if anybody at the federal level has thought about this, but has any thought been given to what would happen if we faced a national crisis with a problem in an urban center like a nuclear explosion, and will we wish, down the line if that ever happens that we had some kind of alternative support, short-term oxygen transport unless help was mustered from other intact urban centers? Has anybody at the federal level talked or planned that or tested that scenario?

MR. EMANUEL: This goes back to the social value of this entity and whether we really need it. So let's us roll the Jehovah’s Witness trial here for a second. So the Jehovah’s Witness trial is HBOC versus colloid in very low hemoglobin, right. That does not generalize to the non Jehovah’s Witness group because for us who aren't Jehovah’s Witness, the choice –- that isn't the choice we're confronted with.

So if you prove HBOC is better than colloid in that circumstance you still haven’t proven that HBOC is better than blood which is what I would need at less than six, seven hemoglobin. So I'm not sure that trial generalizes. What that trial does is it says for the companies we can get license and then have doctors use it more widely than what's actually been shown beneficial. That's not a good way to go in my humble opinion. That's a ruse to get approval and have wider use off-label. So I am very skeptical that that's actually a trial we ought to embark on. It might show us that these are better at low hemoglobin than colloid.

So the real trial that we have to be concerned about is the trial for oxygen-carrying capacity. Now, I agree with you, if we have a disaster we may need this. The question is can we do a trial to show that if all we have are these two choices, HBOC versus colloid, because we don’t have any blood, can we get that trial up and running?

Now, if we're having a lot of difficulty thinking about that trial, now, it suggests to me going back to the question that was asked of Dr. Carson before,

maybe there's not such a need. Now, I'm very skeptical and you know, I know that this is not what's necessarily wanted by everyone in the audience, but you have to ask that question, if you're having difficulty figuring out how to enroll 2,500 people because we might not have that many in 2 years, you know.

MR. BIRO: There's another possible scenario other than a nuclear disaster. In Denver, a pandemic influenza would debilitate a very large portion of the population; blood collection would clearly suffer diffusely across the continent. Would that be a different scenario?

MR. EMANUEL: Look all of those disaster scenarios where we don’t have a sufficient blood supply are very important and we do need to think about them. The question for us at this point is how do we design a trial that would give us something to use in that circumstance, right? We can all think about disaster scenarios where we don’t have a sufficient blood supply and Dr. Carson’s given us another one, old blood is no longer useful and we’re not going to permit its transfusion. But the question for us is can we design a

trial before that scenario hits where we can actually test whether the –- having the HBOCs on hand is sufficient.

MR. BIRO: That was –- sorry, that was the easy question. The answer is difficult. Dr. Vlahakes?

MR. VLAHAKES: I think the issue that, you know, you design the trial in Jehovah’s Witnesses and get approved, and can you really use it there for another settings, that's the way of life in the pharmaceutical industry and in clinical practice.

So for example if you look at what's required in the way of pharmacology and anesthesia and postoperative care, to take an infant through a heart operation, none of that stuff’s ever been tested and approved in a pediatric system. It's a way of life. If you have an HBOC that has been through the regulatory process and the carefully controlled clinical trial, patient screened for coronary disease and all that stuff in the Jehovah’s Witness population, and it's out there and it's market-approved, and you have a practitioner who wants to use it in trauma patients, well, they're going to do it. I mean –- and you're probably better off having it used in that setting in young trauma patients, an agent that has been through a

carefully-controlled clinical trial rather than one where you had all the consent issues and the nature of the patient population that you have to deal with in trauma.

So that extension of indications is, it's an old story in the field and I don’t think it's –- I don’t think you can be that much of a purist based on the long history we have of doing it in other areas and with other agents.

MR. CARSON: I think it would be good to show that the stuff works. We did drill into basic stuff. I mean wouldn't everyone feel a whole lot more comfortable if we could show that it works? And in a clinical setting that –- with clinical endpoints –- and maybe it's not the final road to approval but it sure would help the whole process to know that it works.

MR. EMANUEL: That it works in a situation that is not the normal situation in which we're going to be confronted, right. That it works in a situation where you're comparing HBOC against colloid, not HBOC against blood, and it seems to me –- where blood is available.

MR. CARSON: So you've just described the second study, okay, but the first study is to have a control group that's not getting anything, and if you can’t show

it works there then you wouldn't go and do another study. So it's a proof of concept, and it would benefit that small group of people and there are more and more people, you know, who are not from a religious point of view, but have other reasons why they would prefer not to have blood.

So I think, to know that we –- you know, we haven't –- you know, there's no trial on red cells that shows that it works, okay, our control group, there’s no trial in red cells against the placebo that shows that it works, and there’s no trial with these drugs that shows that it improves outcome.

I think you know, I don’t know what the regulatory part of this thing is, but it sure would contribute to the field to know that, you know, they actually do what we want them to do and they might save some lives. That –- I think that has value and whether or not it gets to the point whether it's widely used or not and approved, that's not –- I think that's a simple question.

MR. BIRO: Any further comment? If not –-

MR. EMANUEL: I mean I agree with you, it's a

concept and from that standpoint it would be valuable, but it would only be commercial –- it would only interest the commercial companies in this room if there was a widespread off-label use. There is no market and no one would sink a dime into it if the market were Jehovah’s Witnesses.

MR. BIRO: Dr. Vlahakes?

MR. VLAHAKES: Just to sort of stay on, with respect to doing a trial like this with what's been presented the last couple of days and the concerns, I think you probably have to meaningfully screen patients for coronary disease, and there are all kinds of ways to do it. Again, look at the cardiac surgery trial, look at the vascular surgery trial that was done and, you know, really drill down into those studies that did and didn’t see myocardial infarction.

And you have all kinds of ways of doing this, but you will have to pick an age threshold for men and an age threshold for women, a family history threshold, analyze the risk factors, and if needed, you could image people if you had to, with high-resolution gated-CT or put people through stress tests, and I think if you were going

to do –- with the current generation of materials and the concerns that have been raised, if you want to do a clinical trial like that I would include detailed screening for coronary disease and probably at some point perhaps piloting in phase 2, take a look at platelet activation, just in terms of some really concrete things you might want to think about if you were to going to design that kind of trial.

MR. BIRO: Okay. Unfortunately the time is passing and people are beginning to leave for their flights. If we can terminate this discussion and just give a few minutes to Dr. Fleming, and then we'll close the session with the thanks of the audience and the panel members.

MR. FLEMING: Thanks, Dr. Biro. I was asked to –- in lieu of a formal presentation take some time to talk through the fact that we now have these safety overviews by Dr. Silverman and Dr. Natanson, and take some time to talk through what are the pros and cons of these meta-analyses and when are they interpretable, are they interpretable.

So I'd like to do that and spend a little bit of

time also talking about issues regarding mechanism of action which are so important to understanding when you can pool across various agents and then lead into some closing comments on benefit to risk, in fact getting right at your question about, at least my own views about what is the way forward for where we can test.

So what are the pros and cons of meta-analyses, and obviously, the particular "pro" is to be able to address benefit-to-risk in an adequately sized cohort of patients.

What's "adequately sized"? And I think Dr. Carson has nicely laid out what those challenges are. If you're talking about detecting or having an event rate or ruling out or doubling an event rate, it takes 88 events. If you're talking about even just a 50 percent increase in event rate, it takes 250 events; if you're talking about ruling out a one-third increase, it takes 500 events.

And so if you're talking about a population where events are death, everybody dies that's how many people you need. But if only 10 percent have those events then you don’t need 100 to 500 events, you need 1,000 to 5,000 people. And so that's the nature of what's

motivating meta-analyses, is to give us enough evidence to be able to –- to be reliable. The concern is, are we pooling apples and oranges and there's a lot of discussion about that in the context of the meta-analyses that we've seen. Are we pulling apples and oranges in terms of different agents, different doses and schedules, different clinical indications, different endpoints? Even if survival is used to cross to all studies is it different durations of survival, different durations of follow-up and as we heard today, even if it's MI what was the definition of the MI as your adjudication?

So all of these things make it difficult to interpret when you're not pooling apples and apples. And one of the most important aspects of this are the agents. Do they have common mechanism? Well the complication with this is we can get some sense based on the commonality of the intended mechanism, but all agents have intended and unintended mechanisms and unintended mechanisms are often unrecognized.

So what I'd like to do –- I think we can often learn from other settings, so that we're not recreating wheels. There's a lot of insight and there's been

discussion about other settings to guide our way forward in this setting. So I'd just like to quickly touch on settings of three major meta-analyses done in other diseases. The first is in COX-2, it's the COX-2 inhibitors which have been a multi-billion market in this country for pain relief and improvement in reduction of GI ulceration relative to non-selective NSAIDs.

But a meta-analyses of 50,000 people that had 500 patients having cardiovascular (inaudible) stroke and MI showed a relative 50 percent increase. But how do you interpret this? It was heterogeneous. There were rheumatoid-arthritis patients, osteoarthritis patients, Alzheimer’s patients; the agents were different, Vioxx, Bextra, Celebrex, but with 50,000 patients it was in fact possible to make some discernment and Vioxx and Bextra were taken off the market.

Celecoxib was left on the market, because the signal was less clear there. But for celecoxib to go it alone, they’ve now had to mount a 20,000-person multiyear trial to target 500 events, 500 cardiovascular strokes and MIs, to rule out there's a one-third increase. And that's the burden when you're going it alone, is we can’t pool

the data across to all of the class; then celecoxib distanced themselves from Vioxx and Vioxx and Bextra stayed on the market but then have to do a 20,000-person trial to rule out that they in fact also have an unacceptable increase.

In antihypertensives we have long approved these agents based on blood pressure lowering but with a lot of uncertainty about what the clinical benefit is, and on the Cardio-Renal Advisory Committee several years ago, FDA presented its data from 500,000 patients from randomized trials.

And with that many patients we were able to look at the overall true benefit-to-risk for many different classes, for low-dose diuretics, Beta blockers, ACE inhibitors, calcium channel blockers, ARBs, relative to many different endpoints, and the answer is different for different endpoints.

Yes, in fact with all these data we data we did conclude that reductions in blood pressure were reliably telling us about effects on stroke, but it was much less reliable for overall mortality and very unreliable for heart failure hospitalization. And the third example is

the one that I referred to yesterday which is erythropoietin-stimulating agents, so ESAs. And there was just –- and they, as you know they’ve been very frequently used in renal disease and in chemo-induced anemia. And there was just a major meta-analysis done in this past year in the CIA (phonetic) setting, using dozens of trials, 6,000 patients showing now evidence of a 5 to 20 percent relative increase in mortality, 67 percent relative increase in venous thrombotic events. Yet in those settings the conclusions weren’t specific to whether it was Aranasp, Epogen, Procrit, the data weren't sufficiently rich even with 5,000 people to make that discernment.

So if you really want to get to the point where we can do meta-analyses that allow us to understand whether the results apply uniformly across stages it takes large numbers of patients. If we try to group these agents by mechanism –- we’ve heard a lot of you about how difficult it is to know whether or not the agent has the key mechanisms that we expect. And just to return again to Epogen –-

MR. BIRO: I'm sorry, but if you could wind up

in the next minute.

MR. FLEMING: I need another five more minutes. I think I was asked to take –- instead of a presentation to take 10 to 15 minutes just to present this. So I'm –- maybe about four to five more minutes?

So in understanding mechanism with Epogen and end stage renal disease, the issue here was it was known that the lower the hematocrit the higher the death rate and so the intention was to do a trial of 1,300 people randomizing standard Epogen to high-dose Epogen. And when that trial was –- when that trial was complete, it was shown that in both the standard and the high-dose Epogen arms, the lower the hematocrit the higher the death rate. And standard did normalize hematocrit.

But the study was targeting a one-quarter reduction in death rate. And so when we had halfway through, there were 160 deaths, in the standard arm you would have expected 40 less, 120 in the high-dose arm. There were actually 40 more, and the bottom line wasn’t that lowering hematocrit wasn’t a good thing, but in so doing vascular access thrombosis was occurring, there were unintended negative effects and this was only apparent by

looking at the totality of the data.

And so in essence what does this tell us about the interpretation of the meta-analyses that we’ve seen here regarding the HBOCs? Do we pool, do we not pool? Well, the first point is the knowledge of the HBOC mechanisms is still emerging and this makes it very difficult to knowledgeably group these into what would be subsets. Secondly we have very limited data available and hence it makes pooling much more in essence unavoidable. And finally as many have noted, there is in fact apparent heterogeneity of MI effects here that we're seeing as well as other effects.

So it is difficult to not –- in looking at these data to not accept that the signal in fact applies to all the agents that we have. So what's the way forward, in conclusion? What's the way forward if there is such a signal? And it all comes down to benefit-to-risk.

And an example was given this morning in the cancer setting, saying even though cancer agents induce myelin suppression we still use them. And I would argue that's really not a particularly good example, because myelin suppression is largely manageable in clinical care.

It doesn't induce irreversible morbidity and mortality. A better example would be that the CISAMAP (phonetic) which is a widely used agent now used in lung cancer and in squamous cell lung cancer, it induces two disks for 100 people treated.

But the overall clinical trials have shown the net effect on survival is positive. And so it shows in particular that it's advantageous to be able to do trials that are incorporating the negative effects and the positive effects, where the endpoints are then looking at what the overall net effect is. Now in –- so in essence, the agent is used in spite of this negative effect.

What about returning then to ESAs and CIA? Well the actions that have been taken on these erythropoietin stimulating agent is that –- by the Oncology Drugs Advisory Committee last month or two –- is that in low-risk patients these are no longer in the label. So if you're in the adjuvant setting, this has been removed from the label because reduction in the RBCs is not viewed to be an adequate benefit in the context of people who have a long prognosis whereas in the advance disease setting, they're still on the market, but the sponsors have been

required to do a 5,000-advent trial to rule out that there would be an unacceptable increase in that setting.

So relevance to us in terms of benefit-to-risk? My own sense about this is the arguments that have been given to move forward in a high-risk population, makes sense, To move forward with –- to pursue compassionate use and to pursue clinical trials where there is a high-mortality risk, discussions have been acute blood loss, severe heme shock, high mortality et cetera.

Settings where there is not an RBC option available, where the comparison would be against crystalloid, in that setting even though there is the risk the potential benefit would be sufficiently substantial that the plausibility of febrile benefit-to-risk could be real. And then the –- and then as has been stated the endpoints in that trial should be in an endpoint such as death and MI, because you would then be able to show whether or not the overall net benefit-to-risk is positive. By the way, last comment, I agree very much with Dr. Carson’s arguments for phase 2 studies as in our intermediate steps.

MR. BIRO: Thank you. I'm afraid we do have to

wind up. I would just like to express the organizers' thanks to the audience, to the panelists, for two extremely interesting days and Dr. Jay Epstein, the director of Blood Products Division of CBER, is going to say a few words.

CLOSING REMARKS

MR. EPSTEIN: Thank you, Dr. Biro. First, let met just say that on behalf of the FDA we thank our cosponsors from NIH and the Department for their support of this workshop. I'd like especially to thank our moderators and our speakers and our panelists who provided us with a very edifying meeting.

Though we leave with many unanswered questions, and really a very wide range of opinions on critical issues, I think we can all agree that these last two days have been very highly informative in multiple areas including vascular biology, the physiology of HBOCs, the potential underlying mechanism of the toxicity of HBOCs and insights into the experience in pre-clinical and clinical trials with a variety of HBOCs.

I think that Dr. Emanuel has challenged us to reconsider the social value. Perhaps up to the time of this last panel there's been a broad agreement that there are important unmet medical needs and that advancing the science and development of HBOCs and newer strategies remains important, and I think we each have to reflect upon that point.

I can assure everyone that FDA will reflect very carefully on what we have heard, both with respect to the science and the ethics, either of continuing HBOC trials with the current products, and importantly the prospects for novel products and novel approaches.

So in closing, I just want to first give a special thanks to the organizing committee, whose members met quite often over the last 6 months and most especially to Dr. Jonathan Goldsmith who invested countless hours to ensure a highly successful meeting.

Again, I want to thank Jennifer Sharp (phonetic) and Rhonda Dawson (phonetic) for their outstanding logistical support of this meeting and I just want to remind the attendees that transcripts will be available in about 3 to 4 weeks. You each had in your meeting packet a

flyer that gives you the website and/or phone number where you can call in to request or identify the transcript, and lastly to request that you submit evaluations. Please just drop off your forms at the registration desk.

So again it's been a great pleasure to host this meeting and we, I hope, all leave better informed, and I look forward to further discussions of ways forward for HBOCs. Thank you very much, everyone.

(Applause)

(Whereupon, the PROCEEDINGS were adjourned.)

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