# FOOD AND DRUG ADMINISTRATION (FDA)
# Center for Biologics Evaluation and Research (CBER)
# 120th Meeting of the Blood Products Advisory Committee

## OPEN PUBLIC MEETING

## TOPIC II: Review of the Research Programs in the Laboratory of Biochemistry and Vascular Biology, Division of Blood Components and Devices, OBRR

**FDA White Oak Campus**
**10903 New Hampshire Avenue**
**Great Room, Building 31**
**Silver Spring, MD 20903**

**March 20, 2019**

*This transcript appears as received from the commercial transcribing service after inclusion of minor corrections to typographical and factual errors as recommended by the DFO.*

# ATTENDEES

| | |
|---|---|
| Richard M. Kaufman, M.D. | Brigham and Women's Hospital |
| Prabhakara Atreya, Ph.D. | Food and Drug Administration (FDA) |
| Judith Baker, Dr.PH, MHSA | UCLA- Division of Pediatric Hematology/Oncology |
| Sridhar V. Basavaraju, M.D. | Centers for Disease Control & Prevention |
| Evan Bloch, M.D., M.S. | Johns Hopkins University School of Medicine |
| Barbara Bryant, M.D. | University of Texas Medical Branch |
| Meera B. Chitlur, M.D. | Wayne State University |
| Alfred DeMaria, M.D. | Bureau of Infectious Disease and Laboratory Science |
| Michael DeVan, M.D. | Walter Reed National Military Medical Center |
| Roger Lewis, M.D., Ph.D. | Harbor-UCLA Medical Center |
| Thomas Ortel, M.D., Ph.D. | Duke University Medical Center |
| Martin Schreiber, M.D. | Oregon Health & Sciences University |
| Amy Shapiro, M.D. | Indiana Hemophilia and Thrombosis Center |
| Jack Stapleton, M.D. | University of Iowa |
| F. Blaine Hollinger, M.D. | Baylor College of Medicine |
| Andrei Kindzelski L., M.D., Ph.D. | National Institutes of Health |

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**DR. KAUFMAN:** All right. We'll get started. So, I'd like to thank the committee for a really interesting discussion from this morning. We're going to be moving on to topic two for this afternoon. So, this is a review of the research programs in the Laboratory of Biochemistry and Vascular Biology, Division of Blood Components and Devices, OBRR. I'd like to ask Dr. Atreya to please read the Conflict of Interest statement.

**CONFLICT OF INTEREST STATEMENT**

**DR. ATREYA**: Okay, thank you, Dr. Kaufman. Again, for the record, public record, the FDA is convening today, March 20, 2019, for the 120th meeting of the Blood Products Advisory Committee, under the authority of the Federal Advisory Committee Act of 1972. This afternoon for topic two, the BPAC committee will meet in open session to hear all your presentations on the intramural laboratory research programs of the Laboratory of Biochemistry and Vascular Biology in the Division of Blood Components and Devices

in the Office of Research and Review.

Per agency guidance, this session is determined to be of non-particular matter, which will have no impact on outside financial interests. Hence, no affected forums are identified, and members are not screened for this topic.

Later this afternoon, after the open session is concluded, the meeting will be closed to the public to permit discussions where discussion would constitute a clearly unwarranted invasion of personal privacy according to 5 U.S.C. 552 (b)(c)(6).

With the exception of the industry representative, all participants of the committee are special government employees or regular government employees from other agencies and are subjected to federal Conflict of Interest laws and regulations. This Conflict of Interest statement will be available for public viewing at the registration table.

Dr. Sue Stramer, Susan Stramer, is currently serving as the acting industry representative on the committee; however, Dr. Stramer is not participating

during the topic two this afternoon.

Dr. Meera Chitlur is a voting member of the committee, and she's participating by phone. Dr. Judith Baker is serving as a consumer representative for this committee. As a consumer representative, she's an SGE, Special Government Employee, and is screened and cleared prior to her participation in the meeting.

They are voting members of the committee and, hence, they do have voting privileges and are authorized to participate in the closed sessions.

Dr. Blaine Hollinger is an SGE and is participating as a temporary voting member during topic two for this meeting.

This concludes the reading of my Conflict of Interest statement for the public record. And at this time, I would like to hand over the meeting to Dr. Kaufman. Thank you.

**DR. KAUFMAN:** Okay. So, I'd like to introduce the first speaker for this afternoon, Dr. Monica Young from CBER, and she's going to be presenting for Carolyn

Wilson.

## OVERVIEW OF CBER RESEARCH PROGRAMS

**DR. YOUNG:** Okay, in the next few minutes, I'm going to give you an overview of the CBER research program, how it's managed, and how the site visit reports are used.

CBER regulates a number of complex biologics including blood and blood products, cell and gene therapies, tissues, vaccines, therapeutic probiotics, and over 1400 allergenic products.

This schematic demonstrates how CBER's research program fills gaps in scientific knowledge and helps to overcome obstacles in product development. As a public health need arises, a novel product is developed, and with that comes regulatory challenges introducing questions such as how best to characterize complex products, how best to design non-clinical trials, and how to overcome the potential of contamination. This is where we apply science to developing the new tools and standards and approaches to access the safety, efficacy, quality, and

performance of FDA regulated products.

The discovery of new tools assists in the regulatory policy and decision making and overcomes the regulatory science to improve data and assess benefit/risk ratios of the products, in many cases, leading to a licensure of novel products. It's also important to note that research plays a large role in the post-market surveillance.

There are many benefits to the research program which include -- this allows our scientists to prepare for future innovative products in public health challenges, as well as develop tools and data that are available to stakeholders and support development of product classes. The research program also attracts and maintains highly trained scientists with the necessary expertise to review these regulatory submissions.

CBER has scientists with broad areas of expertise covering a wide variety of topics and challenges that arise in regulating biologics, as you can see listed on the slide.

Currently, CBER has core research facilities that include flow cytometry, confocal and electron microscopy, as well as biotechnology core facility with state-of-the-art instrumentation, as well as the bioinformatics support for next generation sequencing analysis. The center also has a vivarium and BSL-3 facilities.

A few years ago, an informal peer mentoring group was initiated where a senior principal investigator leads informal discussions with various PIs on various topics. This is a monthly meeting and it opens all the PIs in the center to fulfill a mentoring need.

The next few graphs show our heavy investment in collaborations. As you can see that CBER is involved with collaborations nationally, internationally, and across multiple sectors.

The research management process includes the CBER Regulatory Science Council, which is responsible for developing the research goals and objectives, also, for the research evaluation framework that's used to

measure the scientific and regulatory impact of the research programs.

The Regulatory Science Council conducts a portfolio review of the research program as well. Evaluation of the research program was conducted annually by the Office and Division Management, as well as every four years through internal peer review and every four years through external peer review via the site visit.

The CBER research goals are to advance the scientific basis for regulation of biologics, human tissues and blood by developing and evaluating technology, reagents, and standards to inform and improve chemistry, manufacturing and controls; developing and assessing non-clinical models and methods predictive of clinical performance with respect to toxicity and effectiveness; improving clinical evaluation, pre- and post-licensure through use of big data, innovative designs, and statistical, analytical, and modeling approaches; and finally, preparing for future and regulatory public health challenges.

The CBER research framework includes four areas: First, the mission relevance, which looks at the alignment of the goals and objectives, the scientific and review capability; dissemination, which involves presentations and publication; impact on the scientific community and regulated stakeholders; and what's unique to our program is the unique contribution of the regulatory practice. This is where we look at how the scientific outcomes of the research program enhances our regulatory mission. We now have the tools to track the components that make up this evaluation framework.

Now let's take a closer look at how the management and peer review evaluates the research program. So, the management review includes annual review of the research program at the project and program level. In addition to horizon scanning, which is done by the Offices and the Regulatory Science Council. Every four years, there's an internal peer review at the project level, and there's also a site visit to review the program as a whole every four

years. In addition, every four years the Committee for Promotion and Evaluation of Researcher Reviewers reviews the progress of principal investigators.

So, researcher reviewers who undergo external peer review include fellows who are a part of the service fellowship program, both senior staff fellows and visiting scientists, staff fellows and visiting associates, as well as permanent staff, including principal investigators and staff scientists.

The site visits are a subcommittee to the advisory committee, and I want to thank the chair for his leadership on this committee. The draft report of the site visit is distributed to the advisory committee and the advisory committee will accept, amend, or reject the site visit report. Once the report is approved, this final report is very, very valuable and used in many ways.

The report is used by the committee for promotion and evaluation of researcher reviewers for internal peer review, for personnel actions, as well as by principal investigators to improve research programs

and by the management where resource allocation decisions may be impacted by the report.

So, on behalf of Carolyn Wilson, I would like to thank everyone on the committee for writing the report as well as each of you for reviewing it. Thank you.

**DR. KAUFMAN:** Thank you. I'd like to introduce our next speaker Dr. C.D. Atreya from FDA.

Oh, and actually, C.D., we're just going to wait for just a minute for Dr. Chitlur to call back in.

(Connecting with Dr. Chitlur)

**DR. KAUFMAN:** Dr. Chitlur, can you hear us?

**DR. CHITLUR:** Yes, I can hear you now. Thank you very much.

**DR. KAUFMAN:** Okay, great. Thank you.

## OVERVIEW OF OBRR RESEARCH PROGRAMS

**DR. C.D. ATREYA:** Good afternoon, everybody. Thank you for being here to review the lab site visit report. My name is C.D. Atreya. I am the Associate Director for Research for the Office of Blood Research and Review.

I will give you -- thanks, Monica, for giving an overview at the center level, and now I'll take you down to one notch down at the office level. I will give you a brief introduction to our office.

The office has an immediate office director and then under that office, we have two divisions: One is the Division of Emerging and Transfusion Transmitted Diseases. Under which, we have one product review branch and the three laboratories. One is the Laboratory of Molecular Virology, the other one is a Laboratory of Emerging Pathogens, and the third one is Laboratory of Bacterial and TSE agents.

On the other side, we have another division. That is the Division of Blood Components and Devices. Under that, we have two branches, full-time review branches, which is the Devices Review Branch and Blood and Plasma Branch.

Then we have two laboratories. One is the Laboratory of Cellular Hematology, and the other one is a Laboratory of Biochemistry and Vascular Biology, which you have been now reviewing the site visit

report.

Our office mission is to ensure the safety, efficacy, and availability of blood and blood products through regulation of blood and blood components for transfusion, and plasma for fractionation; devices used in the manufacture of blood and blood components like automated cell separators, blood grouping and cross-matching reagents and devices, and HLA reagents and tests; and the blood collection containers and additive solutions; and then plasma volume expanders; oxygen-carrying solutions; serological and nucleic acid test for blood donor screening and confirmation for transmission-transmissible agents; and diagnostic tests for human retroviruses; and also pathogen reduction devices.

To fulfill our office mission, we engage in establishing policies and standards to assure donor safety and efficacy of blood products and blood; review of applications for investigational and commercial use of blood products, related devices, and retroviral diagnostics. We also perform establishment inspections

and assess the agency in regulatory compliance actions; perform health hazard evaluations and risk assessments of broad and blood products.

Where feasible, we also engage in emergency preparedness; and the global outreach cooperation; organize scientific workshops of timely importance, such as we did a couple of months ago about the pathogen reduction technologies. We also conduct a focus of research to facilitate the development, manufacture, and evaluation of blood products and retroviral diagnostics.

To support the FDA's initiatives in regulatory science, including medical countermeasures to facilitate product development through focus on scientific questions critical to effective regulation; concentration in areas where our unique role as regulators is most contributory. We have a provision of an infrastructure for investigation of product limitations and failures. We also do the advanced innovations in research areas that can enrich FDA's regulatory science base.

With that, we have different subject expertise for research programs. Right now, we have virology, retrovirology, bacteriology, parasitology, prions, cell biology, immunology, biochemistry, and physiology expertise.

We have 16 investigator-initiated programs, which we call them researcher reviewers. They are located in the two divisions under five laboratories as I mentioned to you in the second slide. The programs are mostly funded by both intramural and external sources such as NIH, NIAID, NHLBI, NCI, Department of Defense, and through CRADAs.

Our office research goals, there are two. Goal one is to assess and promote safety and effectiveness of transfusion products and related devices and technologies.

Under that goal, we have several objectives. One is the evaluation of ex vivo stored platelets and red blood cells for safety and efficacy; for example, programs on toxicokinetic, development of biomarkers for product quality, microparticles-associated

toxicities, et cetera; and evaluation of the safety and effectiveness of oxygen-carrying solutions, platelet-like products and related biologics; and the development and evaluation of reference panels for molecular typing methods for blood groups and HLA antigens; and facilitating development of pathogen reduction technologies, mostly applicable to whole blood and blood components.

The goal two is to assess and promote safety and effectiveness of transfusion-transmitted infectious disease agents donor screening, and supplemental tests and retroviral diagnostics.

Under that goal, we have three objectives. One is the evaluation of screening and confirmatory technologies for detection of TTID agents for assurance and enhancement of blood safety; development and evaluation of reference panels for screening and confirmatory tests for TTID agents and retroviral diagnostics; and to facilitate preparedness for blood safety from emerging infectious agents and other pathogens of global significance through investigations

of mechanisms of transmission and pathogenesis.

OBRR staff also participates in many international outreach programs. One of them is the WHO initiatives; for example, like collaborating with Center for Biological Standardization, Expert Committee on Biological Standardization, Blood Regulators Network, Prequalification Program for Diagnostics.

And also, we're in many European Directorate for the Quality of Medicines and Healthcare, Blood Transfusion Sector; International Society of Blood Transfusion Working Groups on Transfusion-Transmitted Diseases, Hemovigilance, and the Global Blood Safety; and FDA/EMA/Health Canada Blood Cluster participation in those meetings.

So, in conclusion, we believe research is integral to the mission of OBRR, CBER and FDA. Our office programs, research programs facilitate product evaluation and development and plays an important role in enhancing the regulatory science mission of CBER and FDA. Thank you.

**DR. KAUFMAN:** Thanks very much. Our next

speaker is Dr. Orieji Illoh from FDA.

## OVERVIEW OF THE DIVISION OF BLOOD COMPONENTS AND DEVICES RESEARCH PROGRAMS

**DR. ILLOH:** Good afternoon. So, my name is Orieji Illoh. I'm the Director of the Division of Blood Components and Devices. So, my job here today is to kind of give an overview of our division. You will see a lot of repetition from Dr. Atreya's presentation since we're basically within the office.

Our division has approximately 60 staff, and we have four branches and also a team called a clinical review staff. Each branch has a branch chief. So, I'll go over them. We have the Device Review Branch. We have the clinical review staff that has a team lead, the Blood and Plasma Branch, the Laboratory of Biochemistry and Vascular Biology, and the Laboratory of Cellular Hematology.

Now, the two labs also have -- they do combined work. They do regulatory work and also research work. So, again, those groups, they are principal investigators, and I have their names listed

here. And, of course, the group who we're focusing on today is the Laboratory of Biochemistry and Vascular Biology, which is led by Dr. Alayash.

So, our mission is to assure the safety, efficacy, and availability of blood and blood components and the related biologic products. So, as you know, blood transfusion involves multiple processes, including donor screening, testing, collection, storage, and compatibility testing.

So, our group is involved in all these aspects of blood transfusion, blood collection, whether you're looking at immunohematology reagents are looking at apheresis devices or pathogen reduction. In addition, we also look at biological products which include hemoglobin-based oxygen carriers and volume expanders such as albumin, dextrin, and other products.

So, in terms of our review activities, I have some of the things that are done within DBCD. We review applications and perform inspections related to the manufacture of blood and blood components for transfusion and plasma for further manufacture into

derivatives such as IVIg.

We look at devices used in the manufacture of blood and blood components, such as pathogen reduction devices, blood bags, apheresis collection devices. We look at immunohematology reagents for blood compatibility testing, plasma volume expanders, and hemoglobin-based oxygen solutions, and also therapeutics that are fluorocarbon-based oxygen solutions.

In addition, we look at a wide variety of investigational new drug and investigational device IDE or also called IDE reviews, and other pre-marketing activities such as pre-submission meetings from sponsors.

So, this slide just gives a really high-level overview of how we deal with regulatory review processes here. So mainly our regulatory decisions are based on scientific data showing safety, efficacy, and purity. A lot of our reviews involve internal review by the reviewers/research review staff and their supervisors; and basically, the decisions are typically

made at this level.

If necessary, facility inspections will be performed as appropriate. Then on occasion, we might have presentations to the advisory committee, for example, for a novel blood product or a novel device. On occasion, we'll also have public workshops to discuss topics of interest.

So additional activities within our division include the development of regulations and guidance governing practices related to blood donor eligibility and product manufacturing. We have a lot of liaisons with industry, government agencies -- for example, the NIH, CDC -- and other regulatory agencies of foreign governments, and international bodies that we constantly work with.

But here today, we're here to talk about their research. So, in addition, our folks conduct mission-relevant research to facilitate the development, manufacture, and evaluation of products DBCD regulates.

So, in terms of our goals and objectives,

they're similar to what Dr. Atreya presented. So, our goals and objectives are to assess and promote safety and effectiveness of transfusion products and related devices and technologies.

Some of the work that is done within our division include evaluation of the safety and effectiveness of oxygen-carrying solutions, platelet-derived products, and related biologics.

We also evaluate pathogen reduction technologies applicable to whole blood and blood components, and development of animal models to evaluate RBC quality. There's much more work that is done within this division, but these are examples.

So, in summary, our research compliments the regulatory mission. It enhances our ability to promote the review and development of safe blood products and related devices. The work contributes to the development of regulatory policies for product development and review. Our staff have made significant contributions to the field and are recognized internationally for their work.

So, Dr. Alayash will now give a broader overview about the Lab for Biochemistry and Vascular Biology. Thank you.

**DR. KAUFMAN:** Thank you. So, the next speaker is Dr. Abdu Alayash.

## RESEARCH PROGRAMS IN THE LABORATORY OF BIOCHEMISTRY AND VASCULAR BIOLOGY

**DR. ALAYASH:** Okay. Thank you. I'm Abdu Alayah, Chief of the Laboratory of Biochemistry and Vascular Biology.

I'm going to give you some highlights of some of the presentations that we made at the site visit in October a few months ago. This presentation is presented by principal investigators and their associates. The lab generally focuses on the interactions of blood-derived products with the vascular system. The vascular musculature is being the point of contact with some of these materials that we deal with, blood-derived or non-biological molecule.

So, some of the products that we regulate both in terms of CMC chemistry and in terms of pharm tox

reviews are listed here. Transfusion-based products that include hemoglobin and fluorocarbon-based products, dextrans and starches, albumin. And we also deal with red cell-derived heme from hemoglobin, red cell preservation technologies. And we do quite a fair amount of inter-collaborative center consultation outside CBER in some instances in areas such as the RBC processing technology, stem-cell derived technology, and plasma-derived product. We also do some consultation with other offices in terms of gene therapy and related products and medical devices.

So, this is the chart of our lab, the organizational chart. I'm assisted by Yiping, who is the team lead. Both of us, we actually overview the regulatory component of our responsibilities, assigning responsibilities, following up with any other function, training and oversight of the overall review process.

There are three sections in the lab: My section, which primarily deals with hemoglobin, biochemistry, and physiology. This section I started, we initiated in 1989, almost 30 years ago. The primary

focus in my lab is on the biochemistry and physiology of hemoglobin and hemoglobin-based oxygen carriers. I'm assisted by the number of the good people listed there.

The second section is the vascular biology section which was started in 2004. This section is headed by Felice D'Agnillo. The primary focus in this section is to really deal with the interactions of blood-derived products, biologics, and in some instances pathogens with the vascular system. More recently, we started another section which primarily deals with red cell and toxicology of storage lesion and emphasis on hemolytic anemias as well.

I have sort of another section within the lab which is under my supervision house this particular lab, house very specialized equipment and technology such as mass spec, circular dichroism, and highly specialized HPLC. This unit primarily focuses on characterization of products. It works internally and, of course, it has also other responsibilities in terms of collaboration with other labs within the center.

So, I'm going to start with my section. Again, this is a highlight of my presentation and also of that of Dr. Michael Strader who is a staffer in my lab. The section of the program, like I said, primarily deal with hemoglobin, chemistry, physiology, and the main interest since the inception of this lab really was on understanding with the oxidative toxicity of hemoglobin, particularly free hemoglobin and ways and means to control these toxicities.

So, for those not familiar with these products, these are high-profile products (inaudible) sometimes back. And we have basically two classes of products, either hemoglobin-derived products or fluorocarbons which are synthetic oxygen carriers. I'm going to just focus on the hemoglobin-based product.

These are products that are derived from outdated blood or, in some instances, from animals or even a genetically engineered product. The proteins are purified, isolated and purified, and they are chemically modified. The modification either cross-linking of the tetramer, the hemoglobin tetramer,

cross-linking and decorating the surface of the protein, or polarizing the protein in a lone polymer, or in some cases encapsulating the hemoglobin.

The primary reason for the chemical modification is really to stabilize the hemoglobin in the tetrameric form or polymeric form, because otherwise the hemoglobin, if you infuse it, it will be cleared by the kidney and will be largely very toxic. And again, remember these are not really blood substitutes because they do not perform a function of blood. All they do is provide the oxygen bridge to the patient until the proper care would be provided to those individuals.

So again, these products were -- we have at some stage about eight, nine of them at the time. The problem with the hurdles that face both the research community and the regulatory community is we do not really have a historical precedent that we can compare to. This is a new environment. The hemoglobin never worked in a free environment and that will also require a lot of additional work from our part of the industry.

So, in recent years, we were able to actually finally have all the products in our hand; and we've done an extensive investigation of these products under one roof and the work was published recently. And we were able to come up with a simple conclusion which is, of course, we knew before that I had with this project is really these are very diverse products. And they are not really created equally as they function differently. And that one could look closely at these individual hemoglobin and can design against the bad properties and for good properties.

Based on that, we were able in recent years to really come up with formula as to what is the best way to go about producing an HBOC or blood substitute, which is oxidatively stable, are relatively safe, and importantly it still functions.

One of the major side effects reported in human clinical trials when these products are infused is the cardiac effect and the reported event in humans and in animals. So, we decided in recent years to look at these events in a simpler system in an isolated

heart, and we use a variety of the hemoglobins that we have. And also, we introduce another element, a reducing agent that in recent clinical trials in Jehovah's Witness, the patients were given really megadoses of ascorbic acid with the HBOC with the aim at controlling oxidative pathway of the hemoglobin.

So, we thought we'd do this experiment in a simple model system which is the Langendorff isolated heart system. And what we did basically, we used a variety of hemoglobin in the ferrous form, the functional form, and the ferric form which is not the functional form was living without hypoxia and with and without ascorbate. We focused on the left ventricle. We measured the size of the developed pressure, several other parameters that are not really listed here.

The bottom line, we saw both the ferrous, the functional form, and the ferric form have some serious impact on the functionality of the heart and other hemodynamic parameters would profuse the heart with ferrous hemoglobin or with ferric hemoglobin, more so with the ferric hemoglobin.

The conclusion here is obviously these products do impact the physiology and the function of the heart. In a setting where red cells are available, these issues will be minimized because the red cell luckily -- the red cells has its own reductive capability when you have ascorbate on the membrane of the RBC.

Ascorbate is a reducing agent. It gives off electrons and, of course, it gets oxidized and you need to get rid of that. Normally the red cells will do that for us, but in the extravascular space where red cells are not there, what we have found that ferrous and ferric, more importantly, are very damaging indeed, and one has to keep that in mind when you perfuse these materials.

We went further and we looked at the mitochondrial function in the heart and we looked again at the oxygen consumption rate and again using the different perfusate which is a different form of hemoglobin, plus/minus hypoxia. And we isolated the mitochondria and we provided the mitochondria with the

intermediate to start the growth cycle on the mitochondrial respiration.

And again, what we found that the ferrous hemoglobin, generally, if you can keep it through, the ferrous will provide a decent amount of oxygen; but the ferric, obviously, it will have a detrimental effect on the biologistics.

On the right scheme, what we're showing here is the impact of ferric hemoglobin on the cytochrome oxidase. This is the last terminal protein in the mitochondria, which is a very critical position where electrons are transferred to the oxygen, and we saw some serious impact when we perfused the heart with a ferric form of hemoglobin.

This is the part that was presented by Dr. Strader, and Brad is an expert. And the focus of Brad is to look at the proteomic of the heart tissue or some other tissue. And more importantly, in recent years, we were interested in the post postulation effect on the hemoglobin in particular when hemoglobin is exposed to oxidative stress. And this is just a schematic

presentation as to how he goes about digesting the material, running the initial mass spec. Then he will either do bottom up, which means looking at the peptide and any changes in the peptide that could be picked up.

More recently, actually, he developed a method that we can quantify the oxidation of pseudo amino acid among many amino acids. And we use this as a reporter to tell us the state of hemoglobin, hemoglobin-infused, whether it's the heart of an animal or in simple system.

I'm going to share with you just two examples from his latest work where we actually designed an oxidatively stable HBOC. And we collaborated with our colleagues at Rice University where we generated recombinant hemoglobin. We crossed the hemoglobin by the two-alpha chain with an amino acid. And then we added a mutation that we discovered a few years back which is a mutant hemoglobin called Providence. The change here is lysine 82 is changed to aspartic acid, only one amino acid on only one chart. To our surprise when we looked more carefully with this hemoglobin, we

find it to be extremely stable, oxidatively stable.

So, we incorporated this mutation in a cross-linked hemoglobin and the result is -- just to give an example on the right table, when you look at the oxidation of cysteine 93, Brad was looking at, you can see regardless how much you add oxygen such as hydrogen peroxide onto the protein, there's very little oxidation.

So obviously, clearly the hemoglobin maintains decent oxygen work and overall a decent functionality. So, what we're saying is that we indeed we could fix the problem if we knew how to go about it. And we are in a process of actually scaling up this particular protein and studying it a little bit further.

The second project that he presented was a slightly different model here. We use sickle cell hemoglobin simply because it has an oxidational profile which is much worse than obviously HBOC, hence, we can look at the reactions very rapidly and we can study them more carefully.

Again, what we did here, we incorporated

Providence and mutation, which is aspartic acid 82, and then we added another mutation tyrosine. We put it close to the heme and tyrosine 41. Tyrosine is going to give electron to the iron and reduce the iron. And, again, to our surprise, the sickle cell hemoglobin, known to be very oxidatively unstable, we were able to stabilize the protein. And to our surprise, we also found that this mutation, these two amino acids actually promote elevation of the delay time. And this is very critical for sickle cell hemoglobin because if you can delay the polymerization of sickle cell hemoglobin, then you can actually clear up the microcirculation within additional seconds.

So, in summary, as far as my section, we provided first-time side-by-side comparison of (inaudible) in our hand. Then we also developed a number of biological assays such as the mitochondrial assay that's really useful and only looking with red cells and hemoglobin but other products.

We have a better understanding of oxidative toxicity of hemoglobin. We were able based on that

actually either intervene with a reductant or re-engineering the hemoglobin to produce more oxidatively stable hemoglobin.

The second section is headed by Felice D'Agnillo. This section primarily deals with the interaction of products with a vascular system, biological product, or in some cases, pathogens.

The two areas of research that Felice and his team were engaged with in recent years is, again, on HBOC and the safety, developing of biomarkers for studying of hemoglobin-based oxygen carriers. And also, he worked on some medical countermeasure initiatives dealing with toxin interaction with the vascular system.

The challenges with HBOC, like I said earlier, and particularly from the regulatory point of view is how do you strike a balance between the safety profile, which is a terrible safety profile, and the potential therapeutic benefit? And as you can see, this is some of it on the right. Some of the adverse events that were associated whatever we and humans particularly

when hemoglobins, double hemoglobins, actually all hemoglobins share these commonalities.

But these HBOCs, if they are approved and made available, they can still fulfill much unmet medical needs. In fact, in trauma and surgery and emergency medicine, and particularly the military would be interested, of course, and in a situation where the red cells are indicated but not available, you know, because it's incompatible or because of religious objection.

So, Felice and his group developed several animal models. This is one of them where the animal is exchange transfused with an HBOC, and normally they exchange animal blood 25, 50 percent with the HBOC. The animal's blood is out, 50 percent of the HBOC in, and we used well-known HBOC PolyHeme. This is a polymerized hemoglobin that has literally no tetrameric form. And that's a very much desired property among the HBOCs as the glutaraldehyde-polymerized hemoglobin. And this is the average molecular weight, very little oxidized hemoglobin in it.

So, one of the interests of this particular program is looked at the interactions of hemoglobin within the vascular system, and here it's looking at the myocardial endothelial glycocalyx. These are embedded proteins in the inner lining of the blood vessels. And these proteins are basically decorated by a variety of carbohydrates and non-carbohydrates. And what happens they are important in permeability and in adhesion, neutrophils, and what have you; and also important, apparently, in the nitric oxide bioavailability.

But what happens here when you're stress the system, when the inner lining is exposed to oxidative stress, they sheer off these proteins and fragments are produced into circulation and then they go as measure of these fragments in the circulation.

This is an example of one of these proteins that have been shed from the vascular system and they're looking at the western blot. And you can see here at the bottom that the amount of this protein was found in animals that were infused with PolyHeme

three-, four-fold increase in present circulation.

They also used a number of imagery techniques where they looked at the interaction between the two, the hemoglobin and the glycocalyx, and they stained the glycocalyx with the lecithin and hemoglobin will stain red and the glycocalyx green. And here you're looking at several panels in which the interactions between the two, the hemoglobin and the glycocalyx, are very clear. And, of course, on the right, he also uses fluorescence to detect the degree of interactions between the two entities.

So again, in summary for the vascular biology program, again they developed several animal models. The emphasis is on the vascular responses to HBOC and other related biologics. Also, they are in the process, and in fact, they have developed several animal models with a compromised vascular system. With these events, they have described it more profound and more meaningful.

The third and the last section is the red cell toxicology section. This is again headed by Paul

Buehler and assisted by several people, including Jin who is a staff scientist, both of them are pharmacologists. And again, they focus here on studying including the quality of RBCs. And, of course, there's the need for donated blood is there as ever. And in particular, in recent years, a number of novel techniques were introduced in terms of the storage systems, in terms of pathogen reductions, and in terms of by bioengineering, particularly the use of stem cell-derived RBC. So, the primary really interest of this group is to look at the impact of these technologies on red cells in terms of quality and, of course, safety.

Again, the primary interest here is really about post-transfusion safety of processed blood and attempted successfully, I would say, measure -- an accurate measurement of oxygen homeostasis in the tissue in animals or any specific simpler model.

This is a very complicated cartoon, but what really it conveys is the number of pathways that they have established in recent years. The emphasis here is

on RBC safety post-transfusion. Of course, the primary event that they are interested in his hemolysis. And as you know, hemolysis can occur in circulation immediately after infusion or an organ such as the spleen.

The main focus here really is to follow the hemoglobin. From based on our experience with the free hemoglobin, we have already built an expertise to deal with free hemoglobin. They looked at a number of endogenous clearing mechanism, and this is particularly so within plasma such as haptoglobin, and the complexes formed with -- and how the complex hemoglobin haptoglobin is picked up by the macrophages.

They also looked at the role of endogenous hemopexin which is the heme scavenger. And, again, they also looked at the hemolysis in these macrophages and the degradation of finally the heme and release of iron. They looked at several pathways in terms of iron storage and clearance.

All in all, they have actually published several fundamental mechanisms describing for the first

time the effectiveness of endogenous pathways and how one could actually enhance these pathways in case of HBOC or in case of transfusion of RBCs.

Also, as I said there earlier, they made several serious attempts to actually have a very good look at tissue oxygenation and losing -- this is one recent experiment, they use EPR in collaboration with colleagues and Dartmouth University. They used a probe, oxygen probe. The probe is implanted in the animal and the EPR signal is -- the width of the EPR signal on the right is widened in proportion to oxygen. So, this is a very direct and very precise method of measuring tissue oxygenation.

This is a typical animal model where they applied these studies in which the probe was implanted in the muscle of the hind leg of the animal. Blood is exchanged with either fresh blood RBCs 1 day old, 7 days, and 14 days old, and also with albumin and then the oxygen-carrying solution.

The message here, the system is very responsive, whether it's one day or 14 days. Very

little difference really was seen, simply because the RBCs after 14 days in this animal, the rat, rejuvenate and produces its own 2/3-DPG. But the model is sensitive enough that in the future, they can obviously exaggerate the situation and follow up on other side effects in the tissue and the level of the red cells.

More recently, they looked at more endogenous oxygen sensing system. And this is primarily the hypoxia and usable factor that has been discovered in recent years. This is when the tissue was exposed to normoxia, this particular transcriptional factor is degraded. But when the tissue is exposed to hypoxia, the HIF is ubiquitinated and translocated to the nucleus trigger genesis and production of specific genes, target genes. Primarily one of these genes is erythropoietin, which make up for the loss of oxygen. They also use chemical probes to correlate the two experiments using endogenous oxygen sending and a probe that, again, be sensitive enough when it reacts with the hemoglobin, with the protein.

This is one experiment that was done where

they infuse the animal with these HRBCs and specifically look at the HIF expression in the stored and the fresh and compare the expression; and you can see quite a difference between fresh and stored RBCs, which means there is some hypoxic event.

And the lower panel, they looked at the level of erythropoietin, the gene expression, and they also looked at the erythropoietin in the plasma of those animals that were infused with these materials. And again, couple this with the EPR, the group has a really, very good comprehensive look at the responses in the tissue when the tissue in animals is exposed be it hemoglobin, RBCs, and other solutions.

So, in summary, again what Paul and Jin had developed as a number of proofs of concept models related to RBCs transfusion and this proof of concept model could be quite useful from a clinical point of view and as a preclinical animal model. And also, they have developed in the process a number of established mechanisms, in some cases, several endogenous healing, binding, and clearing mechanisms. And with that, I

think, that summarized the presentations that we had at the site visit. Thank you.

**DR. KAUFMAN:** Thank you. Are there any questions from the committee for any of the previous speakers? Dr. Lewis.

**DR. LEWIS:** I was going back to the very beginning sort of the overview of the purpose of the Internal Research Program, and I heard two things: one is to develop science, a regulatory science, that informs regulatory decision making. And another was to attract, retain, and train scientists who then participate in the regulatory process.

Is there any information on the degree with which the scientists who perform in this work perform regulatory activities? Or if I misunderstood one of the purposes of the program, it would be good to clarify that as well.

**DR. ALAYASH:** Do you want me to answer this? I mean, from our point of view, research point of view, and regulatory point of view, the whole idea is obviously to produce so-called researcher reviewer

model whereby your research effort is really directly related to the product or the product you regulate. With time, you will eventually produce a well-informed reviewer. And both tracks are evaluated. Both tracks are appreciated by the upper management, and we feel the impact of these pathways.

**DR. LEWIS:** So, what I was looking for -- and I don't think the question was stated very well -- what I was looking for was some sort of quantification of the activity. So, for example, if I run a lab and I spend, you know, four days a week running the lab and one day a week in review activities, that would be sort of 80/20.

I'm looking for some sort of understanding of the effort of the senior scientists who are working in these labs.

**DR. ILLOH:** If I think I understand your question, you're asking how much time they spend on both activities. It's half, 50/50. Yeah, generally.

**DR. ALAYASH:** Ideally, we look for the model 50/50, but this is really almost impossible to

accomplish. Sometimes the people who work in my lab, which I know, could be a week could be entirely preoccupied with regulatory issues. And you have to wait as a supervisor until that task is accomplished and done. Then you go back and nag and push as far as the research.

So, it's always a delicate balance really. But obviously, the primary function here is to take care of the review and address all the issues, be it attending a meeting, be it writing follow-up memos, and so on and so forth. But also at the same time at the same track, we are also evaluated by the degree of publication, on productivity.

**DR. VERDUN:** There's some fluidity with the model. What Orieji gave you was the average, which we try to stay around 50/50; but as was pointed out, there are regulatory issues that pop up that need attention. And we also have to balance that with research work and overall workload. And so, it ends up being about that. But on any given week, it could be a little bit different, if that answers your question.

**DR. KAUFMAN:** Okay. So, any other questions? Okay, so we'll take a 15-minute break and why don't we reconvene at five minutes to three.

**BREAK**

**DR. KAUFMAN:** Okay. So, for the remainder of the time for this afternoon, we will have a closed committee discussion of the program that we've heard about. And I'd like to invite Dr. Tom Ortell, who is the site visit chair, and ask him to present his comments on the site visit report and to make specific recommendations on each individual that had undergone the site visit review.

And after that, the full advisory committee, that is us, will have the opportunity to ask questions on the overall scientific directions of the program, new research directions that could be considered, productivity of the program, administration of the program, allocation of resources, and personnel action, recommendations to be addressed. And well, let me stop there.

We'll be asked at the very end to vote to

accept the site report or not; and potentially, to vote to accept the report with the attachment of any addendum that reflects discussions by the advisory committee members. So, Tom, why don't I turn it over to you.

**DR. P. ATREYA:** Can you check if Dr. Meera Chitlur is online?

**DR. KAUFMAN:** Yes. Meera, are you are online?

**DR. CHITLUR:** I am here. I'm online. Thank you.

**DR. KAUFMAN:** Thank you.

**DR. P. ATREYA:** Okay. Thank you.

**OPEN SESSISON TOPIC II ADJOURNED**