UNITED STATES OF AMERICA

DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

+ + +

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

+ + +

115TH MEETING OF THE BLOOD PRODUCTS ADVISORY COMMITTEE

+ + +

April 5, 2017
8:30 a.m.

Tommy Douglas Conference Center
10000 New Hampshire Avenue
Silver Spring, MD 20993

COMMITTEE MEMBERS:

CHRISTOPHER P. STOWELL, M.D., Ph.D.	Chair
SUSAN F. LEITMAN, M.D.	Voting Member
THOMAS ORTEL, M.D., Ph.D.	Voting Member
SRIDHAR V. BASAVARAJU, M.D., FACEP	Voting Member
MICHAEL DeVAN, M.D., FCAP, CDR MC USN	Voting Member
NORMA B. LERNER, M.D., M.P.H.	Voting Member
ROBERT J. REES, JR., M.H.A., MT(ASCP)	Voting Member
KATHLEEN SULLIVAN, M.D., Ph.D.	Voting Member
MIGUEL ESCOBAR, M.D.	Voting Member
JAMES MAGUIRE, M.D.	Temporary Voting Member
JUDITH BAKER, D.P.H., M.H.S.A.	Consumer Representative
LCDR BRYAN EMERY, M.A., RN, USPHS	Designated Federal Officer
JOANNE LIPKIND, M.S.	Committee Management Specialist
DENISE ROYSTER	Committee Management Specialist
ROSANNA HARVEY	Committee Management Specialist

This transcript has not been edited or corrected, but appears as received from the commercial transcribing service. Accordingly, the Food and Drug Administration makes no representation as to its accuracy.

FDA PARTICIPANTS:

CAROLYN A. WILSON, Ph.D.
Associate Director for Research
Center for Biologics Evaluation and Research

C.D. ATREYA, Ph.D.
Associate Director for Research
Office of Blood Research and Review
Center for Biologics Evaluation and Research

HIRA NAKHASI, Ph.D.
Director
Division of Emerging and Transfusion Transmitted Diseases
Office of Blood Research and Review
Center for Biologics Evaluation and Research

SANJAI KUMAR, Ph.D.
Chief, Laboratory of Emerging Pathogens
Office of Blood Research and Review
Center for Biologics Evaluation and Research

LYNDSAY MEYER
Press Contact

INDEX

MEETING

(8:30 a.m.)

DR. STOWELL: Good morning, everybody. Welcome to Topic II of the 115th meeting of the Blood Products Advisory Committee. Thank you all for coming this morning. It looks like we have a fairly intimate group. So I believe Bryan has some comments to make to begin with.

LCDR EMERY: Good morning. I am Bryan Emery, the Designated Federal Official for today's meeting of the Blood Products Advisory Committee. Mrs. Joanne Lipkind, Ms. Denise Royster, and Mrs. Rosanna Harvey are the Committee Management Specialists, and they can assist you with any needs at the tables located in the back hall.

I'd like to welcome you all to the second day of the 115th meeting of the Advisory Committee held in the Thomas Douglas Conference Center.

Dr. Christopher Stowell is the Blood Products Advisory Committee Chair. The CBER press meeting contact is Lyndsay Meyer, who is in the audience. Tom Bowman is the transcriptionist.

I'd like to request that everyone please check your cell phones and pagers to make sure they are turned off or in silent mode. Please also remember to speak directly into the microphone at all times, and please identify yourself. It is helpful for the public, people attending by webcast and the

transcriber, for the members around the table and the audience.

For the members of the Panel, there are refreshments in the back room, 9225. All committee topic and update discussion needs to be done at the public forum and not in groups at break. The FDA and the public needs your advice and expertise.

The public and industry must stay behind the stanchions and in the audience area. Please do not enter into the FDA or BPAC committee table area. Please wait until the Open Public Hearing designated time to make any remarks using the center aisle microphone.

Now I'd like to read into public record the Conflict of Interest Statement for this meeting. The Food and Drug Administration (FDA) is convening today's meeting of the Blood Products Advisory Committee under the authority of the Federal Advisory Committee Act of 1972. With the exception of the Industry Representative, all participants of the Committee are special Government employees (SGEs) or regular Federal employees from other agencies that are subject to the federal conflict of interest laws and regulations.

The following information on the status of this Advisory Committee's compliance with federal conflict of interest laws including, but not limited to, 18 U.S. Code Section 208 of the Federal Food, Drug and Cosmetic Act is being provided to participants at this meeting and to the public. FDA has determined that members of this Advisory Committee are in

compliance with federal ethics and conflict of interest laws.

Today's agenda includes an overview of the research programs in the Laboratory of Emerging Pathogens, Division of Emerging and Transfusion Transmitted Diseases, Office of Blood Research and Review, Center for Biologics Evaluation and Research. This overview is a non-particular matter. Based on the agenda, it has been determined that this overview presents no actual or appearance of conflict of interest in closed session. In closed session, the Committee will review and discuss the report from the FDA site visit team.

Roger Dodd is serving as the Acting Industry Representative acting on behalf of all related industry. He's employed by American Red Cross. Industry representatives are not special government employees and do not attend closed sessions and do not vote.

The conflict of interest statement will be available for review at the registration table.

We would like to remind members, consultants, and participants that if discussions involve any products or firms not on the agenda for which an FDA participant has a personal or imputed financial interest, the participant needs to exclude themselves from such involvement and the exclusion will be noted for the record.

FDA encourages all other participants to advise the Committee of any financial relationships that you may have with

firms that could be affected by the Committee discussions.

Thank you. I'll turn it back over to Dr. Stowell.

DR. STOWELL: Maybe we can start by introductions and just to go around the group, maybe start with you, Mr. Rees.

MR. REES: Good morning. I'm Robert Rees. I am the manager of the regulatory program for the New Jersey Department of Health.

DR. ORTEL: Good morning. Tom Ortel from Duke University. I'm the Chief of Hematology there.

DR. LERNER: Norma Lerner, pediatric hematologist at NHLBI/NIH.

DR. BAKER: Judith Baker, Public Health Director with the Center for Inherited Blood Disorders in Orange County, California, and UCLA. Thank you.

LCDR EMERY: This is Bryan Emery, DFO for the BPAC Committee.

DR. STOWELL: Chris Stowell, Chair of this Committee and Director of the Blood Transfusion Service at Mass General.

DR. DeVAN: Mike DeVan. I'm a pathologist at Walter Reed and Director of the Transfusion Services.

DR. MAGUIRE: James Maguire, infectious disease specialist at Brigham and Women's Hospital.

DR. BASAVARAJU: Sridhar Basavaraju, CDC Office of Blood, Organ and Other Tissue Safety.

DR. LEITMAN: Susan Leitman, Director of the Medical

Research Scholars Program at NIH, and before that, the Deputy Director of Transfusion Medicine at NIH.

DR. STOWELL: And also I believe we're going to have two people join us by phone. Dr. Escobar and Dr. Sullivan, are either of you there?

DR. SULLIVAN: Yes, this is Kate Sullivan. I'm the Chief of Allergy and Immunology -- Philadelphia.

DR. STOWELL: Okay, thank you.

Dr. Escobar.

DR. ESCOBAR: This is Miguel Escobar. I'm a hematologist and Director of the Hemophilia Center at the University of Texas, McGovern Medical School in Houston.

DR. STOWELL: Very good. Thank you for joining us.

This morning's topic is a review of the report of an external review of the research program in the Laboratory of Emerging Pathogens, and the team leader for that was Dr. Maguire, who will be presenting their findings in a bit. To sort of prepare for that, we have a series of presentations from people from that laboratory, as well as those divisions, so starting with Dr. Carolyn Wilson.

DR. WILSON: Good morning, and thank you. As was noted, we'll be doing a series of presentations starting with an overview of the Center and then drilling down to Dr. Kumar's presentation that will give you an overview of the laboratory that was reviewed in December.

I want to start by thanking Dr. Maguire and Basavaraju, who were the co-chairs of this site visit.

So with that, I'll just give you a quick review of the kinds of products that we regulate. Obviously, you're most familiar with blood and blood components and blood derivatives and certain related devices, but we also regulate things like cell and gene therapies, certain human tissues, xenotransplantation products, vaccines both preventive and therapeutic, allergenic products and therapeutic probiotics, as well as live therapeutics, biotherapeutics such as fecal transplantation

The complexity of the products themselves and the challenges of the raw materials that are used to manufacture them, the fact that they are not terminally sterilized, so there's risk of infectious disease, as this Committee knows very well, means that we really need to have very strong scientific understanding of the products we regulate, and for that reason, we use science and regulation to advance product development. And this is our -- a graphic that I'll be going through that helps us understand or communicate how we use science.

And so we think of things as starting with a public health issue that drives the development of a new product. But oftentimes at the start of development, when it comes to the Agency, we don't always have all of the information that we

might like to as regulators to have to make benefit-risk decisions about first-in-human clinical trials or later-stage development as well.

And those regulatory challenges are where regulatory science or research can, through a combination of discovery research as well as targeted development of new tools, help us generate the information we need, perhaps as development of reference materials as, you know -- especially in this arena of the Office of Blood, there's a lot of need for reference materials, panels of sera and so on, but also it may be that there is a need for a better mechanistic understanding of products, better animal models. But what we try to do is focus on areas where the work we do is addressing underlying problems that can advance a whole class of products as opposed to an individual product.

And so as we generate this information, puts us in a better place to provide advice to sponsors, provide policy, and do our decision making. And as we get better guidance to sponsors, they're providing us better data that allow us to make benefit-risk decisions that hopefully, in the end, license a product or allow products to go forward that have a positive impact on that public health need.

And then, of course, postmarketing, we need to continue to be vigilant with regard to surveillance, and we have robust methods development trying to improve and identify better ways

to do that as well.

And so really, we consider this a collaborative approach to the regulation of biologics, where it's not just review of the data coming in from sponsors, but our active research, the surveillance program, obviously internal CBER discussion, and equally important, advice from external experts like you helps us to make sure that we're really bringing the best science to the table as we regulate these complex products.

And I wanted to mention that in the Center, our research scientists are what we call researcher-reviewers, and what this means is that they not only conduct research, but they also do the same activities as full-time reviewers. This means that they are reviewing data that's coming in through regulatory submissions. They're going out on inspections, they're organizing advisory committees and presenting here, they're organizing scientific workshops and so on.

And because they firmly have one foot in their world of science and going out to professional and scientific meetings, they can see things that aren't yet here at the Agency and help us to be proactively prepared for what's coming, but while also having the other foot firmly planted within the Agency, seeing what's going on behind closed doors, if you will, to be able to identify those cross-cutting issues that can help move product development forward.

So to just sort of summarize this, the purpose of our

research is to contribute to regulation of biologic products by addressing scientific aspects of critical regulatory issues. As I mentioned earlier, we establish methods and standards to ensure safety, purity, potency, and effectiveness of our products, recruit and maintain highly trained scientists with the appropriate expertise, and provide that scientific expertise and leadership to the community to facilitate development and introduction of new biologic products.

So we do this through a variety of scientific expertise within the Center. We've invested heavily in the last decade, I would say, in a number of applied technologies to improve our analytical capabilities: NMR, mass spectrometry, flow cytometry, microarray, high-throughput sequencing or next-gen sequencing, and most recently, in building the bioinformatics and IT platforms we need to evaluate and analyze these large and complex datasets.

As you can imagine, with the products we regulate, we obviously have to have good expertise in microbiology, immunology, biochemistry, and molecular biology, as well as cell, developmental biology, and tissue engineering. Epidemiology is obviously critical, as is biostatistics and bioinformatics.

So this creates a nice environment for interdisciplinary collaborations, but we also collaborate heavily with the outside, with academia and other, and this is just data

captured from the last research reporting database showing across the country. We have collaborations across the world, and these collaborations are not just government laboratories but also represent a very robust collaboration with academia and, in some cases, nonprofit and industry.

So I want to just spend the last few minutes talking about the research management process and how the site visit that you'll be reviewing later in closed session fits into that. I'll briefly touch on some new governance bodies that we started a little over a year ago, our science and research goals and impact framework that we stood up about a year ago, and how we evaluate the role of the site visit in that.

So the two new governance bodies are shown in these red boxes, the Resource Committee and the Regulatory Science Council, and these committees are advisory to the Center Director, and what they do is -- and in particular, I'll focus on the Regulatory Science Council, which is composed of leadership from all the Offices that have a research component. And this council is involved in overseeing the development of Office-level goals and objectives, as well as Center-level goals, and providing portfolio review of the research program. And they interface with the Resource Committee to ensure that resources are aligned with our needs and goals.

And so this year we developed four goals which are advancing 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 nonclinical models and methods predictive of clinical performance with respect to toxicity and effectiveness; improving clinical evaluations pre- and post-licensure through use of big data, innovative designs/statistical, analytical and modeling approaches; and finally, but very important as well, preparing for future regulatory and public health challenges.

As I mentioned, we have a research impact framework which we apply at both the portfolio and individual project level, and at the portfolio level, we are really looking for alignment with those goals that I mentioned and making sure that we have the expertise to inform our review needs, as well having an agile set of internal capabilities. And as this body knows better than anyone, that's really been tested in the last few years with the Ebola outbreak, the Zika outbreak and, you know, we never know what's coming next. So having a diverse scientific expertise in-house has allowed us to really be responsive.

We also want to make sure that we are using our research resources to address those issues that are most critical for our regulatory mission, and of course, at the individual project level, we need to always keep in mind that has scientific merit and that the PI is productive.

So we have three levels of internal review that we started in FY17. One-fourth of all programs and any new project proposals are reviewed by an internal peer review committee as well as through their management supervisory chain.

And then the Regulatory Science Council will also do a portfolio-level review of one-fourth of those programs so that the idea is, is that everything gets a fairly good look at sort of an individual scientific review, as well as alignment with goals and objectives once every 4 years because they're all using the same impact framework.

I'm not going to read through this, but just to show that we have a research reporting database, which is a web-based interface where PIs, on an annual basis, provide program-level information that provides us an ability to track how it's relevant to CBER goals, FDA priorities, staffing, space, equipment, and we also capture their relevant output, like publications, presentations, things like development of reference materials and standards, guidance, and so on.

At the project level, we get information about the Office goals and objectives, and then how it's supporting our review capability, outcome, and impacts that are expected, and then more detailed information about the experimental design; progress and plans are provided on an annual basis and various administrative information.

So in addition to these processes, we also have this

external review which we refer to as site visits, which is a peer review by scientific experts, and these are subcommittees of existing advisory committees, and you'll be hearing the output of that process today.

The report that's generated by the site visit goes to an internal peer review committee called the Promotion, Conversion, Evaluation Committee, and these occur every 4 years for each PI.

The career pathways for research scientists include starting in a temporary track where they can stay for up to 7 years, and we have sort of two groups of senior staff fellow or visiting scientists, which can eventually become a permanent principal investigator or support scientists, which are called staff fellow or visiting associates, which can eventually become permanent staff scientists.

During that 7-year period, they have to undergo at least one external site visit, and that report, along with a much larger package about their regulatory work and other accomplishments, goes to review for the PCE, and then they make a recommendation that this person is eligible to compete in an open competition for those permanent positions.

So what you'll be reviewing today is a draft report that the site visit team developed. The outcome of your work today can either be to accept the report as written -- you may find that you want to make some minor changes to language, in which

case we have an option to amend the report, and if there's a major revision, then you have the option to reject the report and send that back to the site visit team for revision. Once it is approved by the full Advisory Committee, the final report is really useful. It is, as I mentioned, used for the PCE review. The PIs take the input very seriously for improving their own research program. And then, of course, management relies on these for informing them with regard to resource allocation decisions pending resource availability.

So, finally, I'll just start where -- finish where I started, which is a big thank you again to the co-chairs and the site visit team and to you today for your evaluation of this report. This input is really critical for making sure our research programs are fulfilling our regulatory mission.

So I'll stop there and answer any questions.

(No response.)

DR. WILSON: Okay, thank you.

DR. STOWELL: Thank you very much.

Our next presentation will be from Dr. Atreya, representing the Office of Blood Research and Review.

DR. ATREYA: Good morning, everybody, and thank you for, you know, being here, and it's valuable input that we are receiving from you, from the Committee, for the site visit of the Laboratory of Emerging Pathogens. I'll give you a brief overview of the Office of Blood Research and Review.

Our Office mission is to ensure the safety, efficacy, and availability of blood and blood products, and we achieve this through regulation of blood and blood components for transfusion and plasma for fractionation and then devices used in the manufacture of blood and blood components, for example, like BECS and automated cell separators, blood grouping and cross-matching reagents and devices, HLA tests, etc.

And we also do the blood collection containers, review them, and also the additive solutions, for example, like anticoagulants; and then plasma volume expanders like albumin, dextrans, and hetastarches; and then oxygen-carrying solutions like HBOCs and perfluorocarbons; donor screening tests and confirmatory tests for transfusion-transmissible infections and also pathogen reduction devices; and lastly but not least, diagnostic tests for human retroviruses.

To do our mission and to fulfill it, we establish policies and standards to assure donor safety and the safety of blood and blood products. We review applications for investigational and commercial use of blood products and related devices and retroviral diagnostics. We perform establishment inspections and assist the Agency in regulatory compliance actions. Perform health hazard evaluations and risk assessments of blood and blood products.

We engage in the emergency preparedness, as Dr. Carolyn Wilson just mentioned a minute ago, like for example, with

Ebola or with the Zika virus outbreaks. And where feasible, we also participate in global outreach and cooperation, and we do organize scientific workshops on timely topics important to CBER. For example, there's one tomorrow on the tick-borne infectious disease workshop. Most of you are, I think, attending it. And we also conduct research to facilitate the development, manufacture, and evaluation of blood products and retroviral diagnostics.

Our vision for research is to support FDA's initiatives in regulatory science including medical countermeasures to facilitate product development, to focus on scientific questions critical to effective regulation, concentration in areas where our unique role as regulators is most contributory, and we have a provision of an infrastructure for investigation of product limitations and failures, advancing innovation in research areas that enrich FDA's regulatory science base. So these are the visions for our research.

And then resources to do such research in our Office, we have subject experts in virology, retrovirology, bacteriology, parasitology, prions, cell biology, immunology, biochemistry, and physiology.

We have 17 investigator-initiated programs located in two divisions under five laboratories, and one of the laboratories is the one that you are doing the site visit and having this report here today.

And the programs are mostly funded by both internal, that is FDA intramural research funding, and also with external sources, something like NIH-NIAID, NHLBI, NCI, Clinical Center of NIH, and CRADAs. And sometimes we do get funding from BARDA.

Our research goals for the next 4 to 5 years are assess and promote safety and effectiveness of transfusion products and related devices and technologies. And under this Goal 1, we do have a few objectives, for example, evaluation of ex vivo stored platelets and red cells for safety and efficacy, toxicokinetics and development of biomarkers of product quality including omics-based approaches, and microparticles-associated toxicities.

We have projects on evaluation of safety and effectiveness of oxygen-carrying solutions, platelet-like products, and related biologics; development and evaluation of reference panels for molecular typing methods for blood groups and HLA antigens. The last objective under Goal 1 is facilitate development of pathogen reduction technologies applicable to whole blood and blood components.

Goal 2 is to assess and promote safety and effectiveness of transfusion-transmitted infectious disease agents, donor screening and supplemental tests, and retroviral diagnostics. The objectives under this Research Goal 2 are 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; facilitate preparedness for blood safety from emerging infectious agents and other pathogens of global significance through investigations of mechanisms of transmission and pathogenesis.

Our global outreach, where it is feasible, our staff participates either as a member or an observer in several WHO initiatives. There are a bunch of them listed here: Collaborating Center for Biological Standardization; Expert Committee on Biological Standardization; Blood Regulators Network; Prequalification Program for diagnostics; European Directorate for the Quality of Medicines & HealthCare, Blood Transfusion Sector; and so on and so forth.

And so, in conclusion, what I would like to say is that research is integral to the mission of our Office, our Center, and the FDA. And our office research facilitates product evaluation and development and is aligned with and fulfils the regulatory science mission of CBER and FDA.

Thank you. If you have any questions, let me know.

DR. STOWELL: Thank you, Dr. Atreya.

Our next presentation is from Dr. Hira Nakhasi, who will give us an overview of the Division of Emerging Transfusion Transmitted Diseases.

DR. NAKHASI: Good morning. So my job is to really give you an overview of the Division, which the lab which you will, you know, hear the lab which was reviewed with that Division for the site visit, and you'll be hearing the report from the chairs of the site visit committee.

So the Division of Emerging and Transfusion Transmitted Diseases is part of the Office of Blood, and this presentation that was done at the site visit and, you know, we presented the thing. So the Division of Emerging and Transfusion Transmitted Diseases is organized in the Office of the Director, and then there are three research laboratories and one Product Review Branch.

What do I mean by that? The three research review laboratories are organized in the discipline of where we basically talk about PIs, the principal investigators, do research and review of activities in bacterial and transmissible spongiform encephalopathy agents, and Dr. David Asher is the Chief of that. Laboratory of Molecular Virology, Dr. Indira Hewlett is the Chief of that. And the Laboratory of Emerging Pathogens, Dr. Sanjai Kumar is the Chief of that.

The site visit, the scientific program evaluation, which you'll hear today, is from the Laboratory of Emerging Pathogens. Again, it is not for all the PIs there. The PIs which will be -- you will hear the report part today will be Dr. Alain Debrabant, Dr. Duncan, and myself, Hira Nakhasi. I'm

also part of that. Even though I'm the director, I'm also part of -- as a PI in part of that in the lab.

And then we have full-time reviewers in the Branch of -- we call it Product Review Branch, and Dr. Leiby is the Chief of that.

The mission of the division is to plan and conduct research, as Dr. Atreya mentioned, and one of the goals in the OBRR is to really work on the development, pathogenesis, and proof of concept for detection assays for bloodborne pathogens. It may be viral, which is parasitic, it is bacterial, biodefense agents, transmissible spongiform encephalopathy agents.

And also in addition to that, we have a program which is basically development of biomarkers of vaccine safety of bloodborne parasitic pathogens such as malaria and *Leishmania* -- and you will hear some of it today -- to fulfill the FDA's commitment to ensure global public health safety; it's basically a CBER priority.

And then we basically look at proactively ensuring the safety of the blood supply through the regulation of the test kits; evaluation of new technologies, you will hear some progress on that; development of policies, the guideline and guidances for blood screen and donor screening.

In addition, we develop reference materials for the test kit manufacturers. We also collaborate with WHO as a part of a

collaborating center to develop international standards. Then we also have a mission to investigate testing failures, if there are, at the inspections or other consultations. Then we also have advisory role. We provide advice to other agencies, such as government components, CDC, Department of Defense, and DHHS.

And we have quite a bit of outreach to our stakeholders, such as blood product -- and to blood establishments and other -- you know, we have a liaison to both blood establishments and device manufacturers and also as a part of the collaborating center, WHO collaborating center

So the blood safety and availability impact on the U.S. public health is, as we all know, that there are approximately 14 million units are transfused annually, and then the risk of transmission has been significantly reduced by the introduction of several tests, both NAT, nucleic acid test, or serological tests, and the list is there.

In addition, blood is being screened now under investigational testing, and it means those tests have not been approved yet, you know, licensed yet. These are Zika, as many of you know that part last year. So we have been testing Zika using NAT assay nationwide, it started in December of last year, and *Babesia* using NAT and serological assays in high-risk areas.

Now, what are the challenges for blood safety and

availability? Obviously, you know, there are always reemerging pathogens, emerging pathogens. You know, every year we hear something of a new agent coming up, like the tropical diseases. People travel all over the exotic places and come down with infections from there, such as parasitic infections, arboviruses, you know, dengue, chikungunya, and yellow fever.

Then what are the other challenges? We don't have assays for all the pathogens to detect them. We want to have assays which can be multiplex so that we cannot have each assay for each pathogen; lack of confirmatory assays, and then potential impact of travel-based deferrals on adequacy of the blood supply, and lack of knowledge on pathogenesis of emerging pathogens also. And that's why, as Dr. Carolyn Wilson mentioned, CBER's goal is really to have in-house expertise to know about the pathogenesis of many of these agents.

This is a list of mission-relevant regulatory research. We do research on pathogenesis and standards development of HIV and other retroviruses; studies on pathogenesis of arboviruses like West Nile, dengue, Zika, and hepatitis viruses. We have a program on advancing detection technologies, we have a program on detecting TSE agents, and we have a program on parasitic infections as well, which is malaria, *Babesia*, and *Leishmania* and then also *Trypanosoma cruzi*.

I select examples of significant mission-relevant research publications. I don't want to go into details of each

publication, but you can see from the list that it covers a gamut of things from parasites to viruses to detection assays to, you know, also TSE agents.

In addition, our major workload is the development and evaluation of reference reagents and standards for assay development. And you can see from the list that we have a large number of lists for the -- which we have developed in-house these panels which are used now, given to the industry for validating these tests, starting from *Babesia* antibody and RNA, *Babesia* RNA, and dengue virus RNA, chikungunya RNA, Zika, vCJD, *Plasmodium*, HIV genotypes, and *T. cruzi* antibody. Actually, it's *Babesia* DNA on that, not RNA.

So all the things -- you know, the PIs, which are 11 of them in the division under these three laboratories, and we support a total staff of 52 FTEs and contract fellows, mostly these are funded through either through FDA intramural program or extramural program, as Dr. Atreya mentioned.

And last year we had approximately 44 publications in peer-reviewed journals, and we had a workload of reviewing applications, more than 400 applications, which included all sorts of types, all types of applications such as BLAs, PMAs, BLA supplements, PMA supplements, INDs and IDEs. The PIs in the division spend quite a bit of time in developing guidance documents for the industry, how to use those tests, how to implement those tests, and what type of different policies they

have to have.

As you can see from the workload, both the people who do research as well -- mission-relevant research as well as the regulatory functions.

Thank you for your attention. I can take any questions.

DR. STOWELL: Thank you, Dr. Nakhasi. Any questions for Dr. Nakhasi?

(No response.)

DR. NAKHASI: Thank you.

DR. STOWELL: Our next presenter will be Dr. Sanjai Kumar, who is the Director for the Laboratory of Emerging Pathogens.

DR. KUMAR: Thank you, and good morning. So I guess I'll be here for a while. I am going to summarize the research programs of four PIs in the lab, in the Laboratory of Emerging Pathogens, including myself, as presented to the site visit committee last December.

So you heard quite a bit about our public health mission, so I'm not going to dwell on it really. What I'm going to talk about here a little bit, we do our best to align our research programs with the public health mission of the laboratory, so the programs in the blood safety, and also you will hear quite a bit about our programs on the biomarkers of immunity and parasite vaccines. That falls in the arena of the overall public health goal, public health mission of FDA's safety, health safety. And I would like to also say that these four

programs you will hear about, they're unique programs within FDA that, you know, the PIs were working on these pathogens within FDA and within the Center, so we tried to attempt to address the issues also related to vaccines.

Okay, so I'm going to talk first about my own research program with *Babesia* and malaria. So malaria, notwithstanding the global public health safety, we have our own challenges with malaria here. Around 1,700 clinical cases reported to CDC every year and exclusively in travelers, immigrants, and prior residents of endemic areas that is known of transmission in this country.

But a vast majority, I think this is the tip of the iceberg here because the vast majority of these individuals who come from endemic areas, malaria symptomatic, present a challenge to blood safety.

Over the last 35 years or so since the data was collected, there have been over 100 cases of transfusion-transmitted malaria. And just to emphasize the risk of transmitted malaria is not gone, we had two cases of transmitted malaria in the last year or so really. Other than this direct risk of transmitted malaria, we have significant blood donor loss, around 100,000. There are a lot of donor loss from self-deferrals also.

So what we need is a donor screening program, which is not in place, and mostly just because the challenges related to

detection of malaria parasites in blood because of the infectious nature; the assays work for other viruses, just not that simple to work for malaria. And also, we need highly sensitive antibody assays. Then also, my own program on novel vaccine candidates and assays to evaluate vaccine efficacy are needed, and I'm going to talk about that.

So the next pathogen that I work on is *Babesia.* That's closer to home, and I would like to begin by emphasizing here the highest number of clinical cases of transfusion-transmitted babesiosis cases are reported in the United States. So it's not -- I mean, it's a global problem but more problem in America. You try to test the pathogen, you cannot -- unless you're trained in microscopy, you can't tell the difference between malaria and *Babesia* under the microscope.

Around 1,800 clinical cases each year reported, and again, the vast majority are asymptomatic. Those are blood safety challenge. There is what you call the so-called *Babesia* belt, the hardest parts. Most of the cases, both transfusion-transmitted and clinical cases in these seven states here, but it's not to say it's not a nationwide risk. Transfusion cases have been reported from 22 states and clinical cases in 27 states, mostly from the troublesome area.

So what we need here is we still don't have -- we have, as you heard, investigational testing, but we don't have a licensed test, and always there's a chance, time, and place for

improvements, so we need highly sensitive nucleic acid and antibody tests, and that's what my program is trying to address, okay.

So these are my own research programs. Blood safety, we're trying to develop superior nucleic acid tests for malaria and *Babesia,* and then we have a program for antigen discovery for *Babesia* so we can develop antibody tests, because we're looking around the literature, we found that there was a major scarcity of antigens, immunodominant antigens that we can use for antibody assays.

I briefly mentioned about our program on discovery of novel malaria antigens, and we are targeting the transmission-blocking stages, these stages that develop in mosquitoes, and also we are trying to develop superior assays so we can detect if these are viruses of infection.

And that's a really important point here. These are viruses of infection. That means those are the results were gametocytes, I mean, they are carrying the gametocytes, and it's really important to know because that's where the target intervention assays need to be addressed, whether it's the vaccine testing there or other work to control elimination efforts.

We are also working to identify the correlates of malaria immunity and pathogenesis so we can find the targets of anti-disease vaccine. So far there is not a single malaria vaccine,

investigational even, which targets the disease pathogenesis. So we are attempting to address that, and also we are trying to develop the assays of vaccine efficacy.

So first looking at the nucleic acid test for *Plasmodium falciparum,* what you see here, there are two things here. We're evaluating technologies here; one is a bead-based assay which is a non-amplification assay, and the second with the PCR amplification, comparing these two methods. And also, we're trying to improve the sensitivity of existing nucleic acid assays.

Because the idea is the overall -- we want to improve what is out there in the field really in terms of detection of a nucleic acid-based assay because how does one detect flu-infected lymphocytes in a unit of blood? That's the challenge here. So as sensitive as we can get, that's the goal here.

So by this bead-based assay, what is based on two probes here, which target the same target sequence of *Plasmodium*, one is linked to the bead and the second to fluorophore here. To our surprise, this non-amplification method worked very well.

So here the typical 18S ribosomal RNA gene, which is five copies in *falciparum,* and the other one we target, this EMP1 gene is 60 copies in genome. And sure enough, we have significant gain in sensitivity using this high target gene more than -- superior most times.

And then also clinical sensitivity. What I call clinical

sensitivity here is these are the asymptomatic adults which are clinically immune to malaria and live in Ghana. So EMP1 target improves sensitivity here, which is not surprising, but this is the population which is more akin to the asymptomatic blood donors also. By PCR, again, a high target -- high target, high copy number target improves sensitivity and also in terms of analytical sensitivity and clinical sensitivity of the assay.

So I think we -- using the existing technologies, we have gotten as far as we can. The idea is to validate the assays further and using the similar sort of things for the nucleic acid-based test for *Babesia microti*, which is also our urgent need.

With 18S ribosomal RNA by bead-based non-amplification assay, we can detect over 100 parasites per mL. But when we use this high copy number target, sensitivity becomes four parasites per mL. I think also this is one of the best we have seen in the literature.

For *Babesia microti* by amplification, there is not that much difference because I think amplification compensates, but it is again in terms of sensitivity, clinical sensitivity. I think that has to do where you can use more sample volume here.

Okay. In terms of discovery of novel antigens, we did spend significant time looking at the genome-based, bioinformatics-based immunodominant antigens, and nothing worked. Then we went back to the old brute force method of

genome-wide immmuno-screening of phage display library, and we came up with 60 immunodominant antigens. Then for the immunological ranking, we selected 30 highest-ranking antigens in *E. coli,* and what you see here is the results of 15 of these antigens here, and they are ranked based on the activity in ELISA. So it's hard to see for me very well here.

What's happening here is at least three or four antigens give us high sensitivity, which we see according to what is published in the literature, and one antigen is reaching almost 100% sensitivity. What makes it better, when we mix these antigens, we get 100% of babesiosis presence here, and the results have been exposed to *Babesia* also. So this is the assay we are really excited about; we are working to validate it further.

So just to sum up and tell you the future direction, I'll just talk about the future direction only. For the NAT assays for *Plasmodium* and *Babesia*, we are determining the clinical sensitivity in larger cohort samples from endemic area, and also we determined the specificity in a large number of U.S. blood donors.

Similarly, for *Babesia*, we are optimizing the single antigen-based ELISA and multiple antigen-based ELISA because if single antigen works, that's the best in terms of actual making donor screening assay with this. And also, we are looking at these antigens in terms of vaccine efficacy. I'm not going to

show the results, but the results are very encouraging.

So that's where we stand with this. I'm going to move on to the next part of my talk, which is related to discovery of novel transmission-blocking vaccine candidates.

So the transmission blocking, what I mean is disrupting the parasite development in the mosquito here, mosquito recta. So these are the malaria gametocytes here in the human host, male and females. They are picked up during their blood meal. Then they -- male, female in the form of zygote and gamete, so forth, okay. So we are working on these gametocyte stages here.

So there are three major antigens I have listed here; those are the vaccine candidates, hundreds of millions of dollars in investments so far over the last 2 decades or so, but none of those produced significant transmission-blocking antibodies that will become a sustainable vaccine. So we thought we need to go back and look for -- discover novel antigens, and we did that. And the same antigens that we discovered while working to look at the reason why, so detection, because that's a major requirement -- of that.

Simultaneously, we are trying to develop an assay as part of our efforts to improve vaccine efficacy methods to develop malaria vaccines. At this part of FDA mission, we are developing immunological methods that will replace the microscopy as a method to measure parasite burden in

mosquitoes.

And I must say all this work has been funded for the last 8 years by PATH malaria vaccine CRADA.

Okay, so biomarker detection by microarrays and antigens of vaccine efficacy. Through microarrays, we identified 56 gametocytes in these antigens, which is more than 50-fold up-regulation in expression, and also exclusively we wanted to make sure they're not expressed in blood stages.

And after very extensive screening, we came up with this one molecule which has the sensitivity of 10 gametocytes per mL, where the gold standard is this molecule Pfs25, which has 25 gametocytes per mL. Using this detection marker, we detected around, in 20 persons, more blood smear negative Ghanaian children as gametocyte positive. These are -- negative, so these are the added detection in terms of who are the children who are gametocyte and potential reservoir for transmission.

And also, it really is a lot more -- we detect twice as many asymptomatic adults with this new molecule. So we are pursuing this further.

In terms of novel vaccine antigen discovery, we have selected 16 antigens which have dual expression both in the asexual stages and in gametocyte stages, because the idea here is not to exclude the blood stages; we have a multistage vaccine here. And also there's the natural boosting if we have

blood-stage vaccine.

And at least one molecule -- so this is the candidate antigen which has been developed for many years, 48/45, and we have several molecules which are equal or superior reactivity than this antigen.

So I would just like to move along from here now quickly. I just want to talk about the last part of my project here, correlates of malaria immunity and pathogenesis.

Over the last several years we have worked and defined the role of Th1 CD4 T cells in malaria immunity and pathogenesis and published this and moved on pretty much. We found a really significant role of CD47 molecule in recognition of self on macrophages in malaria virulence and regulation of parasite density.

What I am going to present today is our work on characterizing a novel population of T cell receptor-expressing macrophages during malaria infection. Usually, macrophages are part of innate system and are not known to express TCR.

So here's the model here. We used *Plasmodium berghei* ANKA parasites in C57. We have six mice. They have the symptoms of cerebral malaria. This is the closest it gets really to human cerebral malaria.

So we worked with doing this strain, what we call the ECM, experimental cerebral malaria phase during Days 6 to 10 post-infection, and we tried to find the new markers that induce

this pathogenesis of malaria.

So these are the brain-sequestered cells isolated by perfusion. So in brain, on Day 6, we find around 20% of these cells are CD11 high; these are the marker of monocytes. So probably these are macrophages when we defined them further for CD14 and F4/80. So there's triple positive cells for CD11 high b, CD 14 and F4/80. Eighty percent of these express all these three molecules.

So knowing the current immunological markers, these are macrophages, but what is curious there, more than 80% of these express also TCR better expression, which is unconventional and unknown, and also these are negative for CD3, so the CD3 negative TCR-expressing molecules.

And also, when we look in the spleen at the same time point, the numbers don't show here, is 2, only 2% of these are here, and 37% of these macrophages express these triple markers here.

But similar to the brain-sequestered macrophages, they also expressed -- more than 90% express TCR and the CD3 negative. So probably what's happening, they have left the spleen by that time without sequestered in the brain.

We look for TCR rearrangement here, whether these are classical TCR and macrophages, and even though there are a predominance of certain TCR types here, there is certainly -- there's heterogenicity in T cell receptors here

that's actual TCR rearrangement going on there. The TCR expression correlates in parasite burden, so it seems like this is antigen driven. But then finally, we wanted to know what is the clinical significance of all this? What is the biological significance?

So what we did was we took the -- there are three post-infection, mixed them in equal number of APC-labeled infected lymphocytes, and then we stained them for these all sorts of things: CD14, CD11b, after incubation with CD4/80. And then also, we excluded them for Ly6G marker, which is a neutrophil marker. So these are macrophages and not neutrophils.

And on Day 3, we did the assay on Day 3 because it's almost equal distribution of TCR positive and negative cells at this time point. And then we looked for the APC positive infected lymphocytes, and 30% of TCR are expressing macrophages had phagocyte-immune infected red cells. In comparison, less than 20% of TCR negative. So it seems like those macrophages which express TCR are more effective or more capable of phagocyte-immune infected red cells. So that's where that stands.

And just to summarize the future direction, we are looking at the macrophage TCR rearrangement program during malaria infection at molecular level. We are trying to find out what are the malaria antigens that induce the proliferation of TCR-expressing macrophages. And also, we are looking at the role

of TCR-expressing macrophages in malaria immunity and pathogenesis.

So I will move on to the next program, Dr. Nakhasi's program here, his approaches to reduce the risk of leishmaniasis through vaccination and in the process will reduce transfusion-transmitted *Leishmania* infections. His program has been devoted for the last more than a decade to oversee generation and evaluation of efficacy of genetically modified *Leishmania* vaccines, and also, he's doing studies that will fulfill the target product profile, and we'll talk about that in a minute, okay.

Similar to malaria, *Leishmania* has really significance, global public health significance. At least one-fifth of the population lives in areas of *Leishmania* transmission risk, worldwide infections and deaths every year. But what is important here is if one talks about the control or eradication of *Leishmania*, mass administration is probably not going to do it, unlike malaria, where it can be effective in the local area because the drugs are not that effective. A radical cure is not possible, and also then, excessive use leads to increased drug resistance.

So vaccination is not only most cost effective, probably the only effective method for controlling visceral leishmaniasis and also in the process reduce the burden of asymptomatic leishmaniasis in a unique area and that eventually

will lead to blood safety.

Here I would just like to focus on a few things similar to malaria. Significant millions of Americans travel to areas where leishmaniasis -- *Leishmania* is transmitted, and in the process they acquire infections. The high proportion here, similar to malaria, it is asymptomatic and also does not reveal itself until it is too late in terms of blood safety.

And in response to all this, and also what was really during the Gulf War and a large number of soldiers came back with leishmaniasis here, the Infectious Disease Society of America has produced new guidelines on how to solve this and do this to protect them from leishmaniasis, and there's no donor screening assay available, okay.

So what has worked for vaccination against leishmaniasis? Recombinant proteins have been tried with drugs with no success. DNA vaccines have not been a success. Inactivated whole parasite with or without adjuvants were not very effective.

So what probably will work, what is known through literature and through recent history, is that leishmanization is a process where a scab is taken from a patient, from between the leishmaniasis, and use that to inoculate into contacts and other healthy volunteers, they do not get malaria -- *Leishmania*.

But the downside here also, they develop the pathogenesis

in some cases. And also those were healed leishmaniasis cases; those are not prone to reinfection of leishmaniasis. So there is sort of reinfection immunity.

So what it does really -- all these things did not work here with those approaches, but the whole parasite is a good vaccine if it can be delivered in a proper way.

So here is the genetically modified live attenuated vaccines program. Dr. Nakhasi, *Leishmania donovani* centrin knockout, that's the molecule, and he has generated a whole slew of these genetic mutants, but this is the one work I'm going to present here today. I'll start from the bottom here.

So the advantage here is through homologous combination, they produced this vaccine. So these are genetically defined mutations, they are well characterized, they know where the mutation is, and they can go back and check whether the mutation still exists.

Controlled persistence without causing disease: This is the data provided from an experimental model, so I'm going to show you today a complete array of antigen vaccine. You can have one -- vaccine, one antigen or two or three antigens here, all the thousand *Leishmania* antigens are expressed. And also, it mimics the natural course of infection the best possible way, okay.

So about centrin molecule, just very briefly, it's part of cell cycle. It's involved in the duplication and segregation

of centrosomes.

So this is the earlier work from his lab. This is the centrin expression in wild-type parasite. You knock out the centrin through homologous combination, and the expression is gone. You add it back by epigenetic expression, and this is the same thing, the expression. And here we add it back in wild-type parasite.

When you look at the growth profile in these modified parasites, where all these other forms have grown normally in culture, in macrophages, the knockout parasites undergo one cell cycle, one cell division, and then they have arrested growth here. So they failed to grow these amastigotes in culture.

Looking in the microscope, here's the normal cell division in wild-type parasite, but the knockouts, they fail to undergo mitosis; they have mitotic arrest. They don't undergo cytokinesis, and they in turn just program cell death.

Okay, so what is the virulence profile, safety profile of these knockouts, centrin knockout parasites? So this is in hamsters here, wild-type infection. You will see all hamsters die around post-22 after infection, but the centrin knockouts, they live on. There's no mortality here.

Looking at the histology, you see this pathology in granular formation with wild-type parasites, but not so in the knockout parasite.

What is more important here is also they do not grow in the vector sand fly. What that means is, after blood meal, you can see parasite here, both wild-type and -- but post-Day 4 or Day 8, you don't see any knockout parasite. So the significance of this is that, in field, they're not going to mix, the vaccination is not going to mix with the wild-type parasite and create some super new -- and also they looked at the effect of this vaccine.

The immunosuppression, they will turn to virulence; it did not happen. These are mice maybe immunosuppressed during infection following injection with wild-type and centrin knockout parasites. The knockout parasites still retain their growth activation. So if the vaccine is given in immunocompromised people or people become immunocompromised post-vaccination, the vaccine sort of still -- in those.

These are some of the results of this new mechanism and protection in three different experimental models, in mice, hamsters, and dogs, so looking at the immune mechanism profile here. So as the immunity was defined for *Leishmania* more than 2 decades ago, this is exclusively a cellular response, Th1 mediator, multifunctional T cells. The similar thing we see in mice, hamster, and dogs, and all of these are published in a series of papers, this finding.

But also what you see here is the protection, so immunization with *Leishmania* centrin knockout parasite. They

protect against homologous challenge, but also in mice, they're protected against this heterologous *braziliensis* and *mexicana* parasites. In hamster, the protection is against homologous, and in dogs, the immunization is *donovani*, but following challenge *infantum*, which is the dog counterpart, but they're still protected.

But this is cross-species protections. So the many species transmitted in endemic area, vaccination with one species might lead to eradication of other ones, too.

Okay, so this is what is the target profile I had mentioned earlier.

So this is the recommendation of an NIAID panel in 2015. So they came up with five concepts here or recommendations. Somebody who is starting to make malaria vaccine, what is the target product profile they must meet in order to become ready for clinical studies? And Dr. Nakhasi has addressed them all one at the time here, so -- but I'm going to present the first three here only.

So the first part of the work from Dr. Nakhasi, and also the presentations by Dr. Ranadhir Dey and Dr. Sreenivas Gannavaram, and I'm going to indicate when it comes to that, okay.

So this is, what are the biomarkers of centrin vaccine-induced immunity? So they find strong co-stimulatory signals, CD40, CD80, CD83, and CD86. The classical activation of M1

markers on infected macrophages, so these are the macrophages which are the pathway of a cellular Th1-mediated immunity, and which makes sense here because this is all Th1-mediated immunity.

There's a significant expansion of antigen expressed with CD4 T cells after vaccination, the CD4 T central memory cells that can be restimulated upon antigen stimulation in vitro, and also CD8 cells are found to be involved in protective cellular response, as been defined earlier. And Th17 cells were necessary for protection against visceral leishmaniasis. So all of this fits within the classical paradigm of immunity against *Leishmania* in mouse models, experimental models.

So next, I'm going to move and tell the research which was presented by Dr. Ranadhir Dey, efficacy against natural sand fly infection.

So traditionally, so over the decades, the papers published looking at the efficacy of *Leishmania* vaccines, they use a needle challenge infection and show protection, okay. I mean, but in nature that's not going to happen; it's not even natural sand fly challenge. So their research at NIH about a decade ago, I think, he showed using the *Leishmania major* model in mice that the comparable needle challenge with a direct sand fly challenge, and he found that the protection was abrogated with the sand fly challenge.

So what was protected before the needle challenge was no

more protected when they used sand fly challenge. So that showed that the sand fly challenge is the one that should be used in evaluation of vaccines, and the needle challenge is too artificial and is not rigorous enough.

So all the work Dr. Nakhasi's group is doing was really done with *Leishmania donovani*, but there was no sand fly model. So Dr. Dey took it upon himself, and working in concert with NIAID, he developed this sand fly model.

So to feed them infected spleens, they put in these ice cream cartons here, sand flies here, and by Day 11 they find mature infection. So I guess it's the gut, the sand fly gut. And then they used these infected sand flies, they can detect parasites there with parasitological methods, and they used those to fill these mice and hamsters here, and they did compute the cycle; the infection was transmitted.

So here is the model that Ranadhir developed for *donovani* which did not exist before, and they have gone on to do other things here, identified the virulent factors, which is IL-1 beta. And also they have looked at the effect of gut microbiota of sand fly and *Leishmania* transmission. None of these we need to talk about today, but they are expanding this research further, okay.

So what happens when they used this sand fly model recently that was developed here? So here are the results. So these are the immunized mice with the centrin knockout parasite

when the challenge is -- when they challenged with the infected sand fly, these mice remain protected. They don't succumb to infection, whereas unimmunized mice with the live sand fly challenge, 60% of the hamsters -- actually, these are hamsters -- develop severe visceral leishmaniasis and die. So the challenge model is working in terms of measuring vaccine efficacy.

If you look at the parasite burden here, there's significantly higher parasite burden in the spleen of non-immunized mice compared to immunized mice. So the parasites, there's a significant reduction in parasite burden following sand fly challenge in vaccinated mice, okay.

So next and last piece of this program is evaluating the biomarkers of safety and efficacy for human trials. So the idea here, this is short of clinical studies because there's no human challenge model for *Leishmania*, unlike malaria. I mean, one has to go through the expensive human challenge. There are certain biomarkers that can be evaluated prior to human studies are undertaken to make sure the vaccine is worthy of human studies.

So this is the work from Dr. Sreenivas Gannavaram. What he did was he took human PBMCs, coats them with GM-CSF to transform into monocytes, macrophages. Then he infected them with wild-type and centrin knockout *Leishmania* parasites. Twenty-four hours later produced RNA and did microarrays in a

human chip, so these microarrays here, and I'm going to focus on the relevant results here only.

So what came out, which seemed important, was the differences between the wild-type and centrin parasites in terms of miRNA, micro-RNA, and four of the -- four which were uniquely expressed within these two here. And these are the results here. This is wild-type or centrin knockout, and so the three of the four are plotted here, and what stood out really was miR-21. Because it has immunological significance, miRNA-21 is involved in the -- it's a feedback mechanism in the negative regulation of IL-12 expression. So it degrades miRNA for IL-12, and IL-12 is the primary mediator of immunity for *Leishmania*. So it does make sense that things fit together when he expanded this further.

So these are the U.S. blood donors here, these are individual donors, looking at the miRNA expression in individual donors here. And when they use inhibitory oligonucleotide, the response comes down. The centrin knockout, there's lower response here for miRNA. So the less miRNA means enhanced expression of IL-12 by these macrophages here, and probably hence, more immunity by this vaccine, which produces less parasite burden. It still gives very significant immunity.

So just to put this together in a cartoon, what this miRNA-21 is doing is pushing the immune response to a more

robust response to Th1-type responses compared to a Th2-type response. And that's why the centrin knockout vaccines are so efficacious.

Okay, so the conclusions I'm not going to read, just to focus on the future studies here. They are working on the evaluation of centrin knockout vaccine efficacy in co-endemic infections, in malaria and Chagas disease.

The further characterization of early biomarkers of innate response for monitoring immunogenicity/efficacy of this vaccine.

The further characterization of induced immunity against sand fly mediated challenge because most of the work so far was done by needle challenge.

Product characterization of next-generation sequencing to see where else the effect might be of centrin knockout deletion in the parasite genome.

And also, those could be used as a marker of manufacturing consistency then.

Development and evaluation of GMP material, that's the next step.

And then Phase I through III clinical studies, and these studies were funded through this Japan and Gates Foundation CRADA.

I will move on to the next program here, Dr. Alain Debrabant's program, and there was a joint presentation by

Dr. Rana Nagarkatti. Dr. Debrabant is trying to improve blood safety by reducing the risk of transfusion-transmitted Chagas disease.

So Chagas disease is mostly south of the border, endemic in Mexico, Central America, and South America, in those areas a very strong presence. It's a major part of life there. In 18 countries it's transmitted. Eight million cases (2005), new infections every year and more than 12,000 deaths.

What is important about this parasite, these are, you know, mean-looking parasites here; these establish chronic, lifelong infection. Mostly they find this in heart, the skeletal muscle, and in intestine, so that's where the parasites hide lifelong. Twenty, thirty of these infections will develop cardiac or digestive complications. So it's a quite significant pathogenic burden. The transmission is via triatomine bugs.

So what is the implication here for the United States? I mean, we are aware of the amount of immigration from these countries, Mexico and Central and South America. There's supposed to be around 300,000 people in this country who were exposed to Chagas disease.

The disease is transmitted by organ and blood transfusion here. These are the cases listed here within the U.S. and Canada, but when the blood -- when the donor is screening to establish for antibody, 2,225 confirmed cases for antibody

positive blood donors. So definitely this shows the presence of asymptomatic infected donors among us here in this country.

So Dr. Debrabant's program is directed to develop animal models and imaging techniques to study the pathogenesis of chronic Chagas disease and better understand the fluctuating blood parasitemia observed in chronically infected blood donors. So that's one goal.

The second goal is to develop non-serology, non-PCR antigen detection assays using aptamers as alternative tests for the screening and confirmatory testing of blood donors. And I'll show you the reasons why, okay.

So here is the biology of parasite and how it relates to the pathogenesis. This is the acute infection; it comes and goes and disappear. So these are the amastigote forms in tissue here, and the acute infection in tissue also then disappear. This is the trypomastigotes in blood. So there's a blood form, and there's a tissue form here. So what happens is it's very difficult to detect parasite during the chronic phase in blood, although it's not impossible. But the antibody passes for lifetime. So what does it mean, presence of antibodies? It becomes difficult to distinguish whether there's an active infection from chronic infection.

So there are gaps in knowledge. So what are the gaps in knowledge? Sites of *T. cruzi* infection during chronic phase are poorly understood. I mean, they are known to be in heart

and skeletal muscle and digestive tract. What are the other sites?

Consequences: The parasite reservoirs are not known. They maintain chronic infection but cannot be assessed by drug treatment. So the measure of drug treatment is not there. How does one know the drug treatment was successful or not? And that's a way -- extremely toxic also. So blood does not appear in blood, parasite does not appear in blood, and antibodies persist, so whether drugs work or not. And also there needs to be discovery of how does one measure, short of necroscopy and measuring the entire mouse?

Okay, so what he's trying to do, identify parasite that survives during chronic disease in the mouse model. So he's using imaging, using transgenic bioluminescent parasites, and then he's developing a newer imaging method to detect biomarkers of *T. cruzi* infected red cells, because in natural infection one cannot find transgenic parasites, so one needs to know the biomarkers of infected red cells. Imaging has to work for people.

Okay, so this is imaging in mice. So he's using the nanoluciferase as the protein, and he has constructed these transgenic parasites and developed these experimental models.

So this is the imaging done Day 7 post-infection and monitored through Day 126 post-infection. So you can detect infection by imaging through Day 126, but the microscopy

becomes a negative. So the imaging is already more sensitive than microscopy.

But imaging simply does not tell what are the viruses of infection. So by necroscopy, they harvested the tissue, so the heart, lung, liver, visceral fat, the skeletal muscle, and the spleen, and what they find here, the results make sense here. They find the parasites by measuring the bioluminescence in skeletal muscle, in heart, in intestine, but also they find parasites in mesenteric fat, lungs, and liver, but not so in the spleen and the visceral fat. So it seems like the assay is doing what it's supposed to do, but the next question is how to take this for human use, and for that, they need to know the biomarkers.

So what they have done here, this is the mouse cells infected with parasites, and the cell extract from infected cells and non-infected cells. Here you see the difference. This is the one spot that they found, which was unique infected cells, and mass spectroscopy, they see a peak here and eventually, by mass spectroscopy, what they find is 39 proteins were expressed in infected cells as compared to non-infected cells.

So what are those 39 proteins? Thirty-two of those are from the host organ. Seven are unique to *T. cruzi,* and here, what they found via bioinformatics, there is information about the proteome. These are the molecules. So now they're

developing this particularly against these seven molecules and see if they can utilize to develop the assay by imaging using these molecules. So they target these expressive molecules, and hopefully they will bind to the infected cells, and they can use them for imaging, the human application, okay. But the applications, I don't know if I emphasized this clear enough for you. The implications are one could use it to monitor drug efficacy, the effect for drugs in humans by imaging.

Okay, so the next program, which is going on for many years, and Dr. Nagarkatti is spearheading this effort in Dr. Debrabant's lab, so development of non-serology, non-PCR biomarkers.

Just because the antibodies don't allow to distinguish between active and chronic infection, what one needs is one needs to find active infections and use non-serology biomarkers, which are non-PCR based because PCR has not been shown to be effective.

So the approach they have taken is to utilize the aptamers. What are these aptamers? These are small synthetic nucleic acids, single-stranded RNA with well-defined and stable 3D structures that bind with high affinity, and they're target-based on molecular recognition, and their binding affinity is extremely high, more so than monoclonal antibodies. The target does not need to be antigenic in order to be recognized. They can be easily produced, they can be

synthesized, and they can be modified and put tags on them really for detection purposes, so they are ideal for that purpose.

So this is the TESA antigens, *T. cruzi* excreted and secreted antigens. Here, these antigens are parasite secretes, and this is the infection mouse fibroblast 3T3 cells. This is the culture suspended and purified from there and then use this to screen a library for aptamers, to identify aptamers which are unique against these TESA antigens. In the process, they identified around seven aptamers or so, and I think they focused on one. And based on this, they have developed a simple ELISA, but they call it ELA, enzyme-linked aptamer assay.

So this is the demonstration to use this aptamer assay to monitor the efficacy of drug treatments, something I've been talking about many times by now.

So this is the infection in -- this is the infection is Swiss mouse using *T. cruzi* Colombiana strain. I think they consider day post-130 as chronic phase infection when this cannot be detected by parasitological methods in blood anymore, and then they sacrifice mice on Day 170 post-infection here. In between, during this window of 20 days, they give them a daily dose of benznidazole, a common Chagas disease drug, to these mice and then monitor how effective their treatment was by aptamer assay, okay.

So this is 130 days. You can see how clear the aptamer detection is here between non-infected ones, highly infected. This is a hundred -- middle time point here, detection. So you can see the aptamer detection has come down, but it's still very clear. And this is the post-drug treatment here, the aptamer detection has come down, but it still has not become negative.

So what it shows, the drug treatment did not 100% cure the resident parasites in the tissue. So one can just imagine the application of these aptamers in drug discovery programs and to monitor the drug treatment, okay.

Next is the application of this aptamer assay ELISA in real-life scenario. So these are samples they got through collaboration from Brazil. These are characterized Chagas disease cases. So this is the assay, these are control samples here. So these are the samples which are reactivated Chagas disease, for whatever reasons, and the assay detects most of these reactivated cases. So it has been effective in detection in these clinical Chagas disease samples.

These are the known cardiac patients for Chagas disease, and it detected more than half of these samples, not 100%. But where the assay was not effective was detection of the asymptomatic infections. So I mean, that's where the need is in terms of donor screening, so it was obvious to them that the assay needs to be modified further. So they're developing

another assay using two aptamer-based assays. One is the capture aptamer assay. The second is the detection aptamer. And in order to do that, and they believe this assay was more sensitive with less background, so the threshold, cutoff threshold can be lower, then, for detection.

So in order to do that, they are identifying at this time, instead of unknown targets, they're looking at the known targets of secreted proteins. So they went to the proteome here, the *T. cruzi* proteome or secretome, and there appeared six or seven molecules from there produced synthetic peptides in immunized rabbits. And using these purified polyclonal antibodies, they can detect these biomarkers, secreted biomarkers in serum. So it seems like these are secreted detectable biomarkers, and now they are trying to identify these aptamers unique to these molecules.

So that's somebody else's results so far, and I'm going to talk about the future studies. So compare the tissue distribution using chronic phase of genetically distinct transgenic parasites using bioluminescence imaging. So genetically distinct means from different geographical areas. So far the work was done with Colombiana strain.

Confirm host cell surface expression and develop aptamers against *T. cruzi* biomarkers of infected cells to detect wild-type parasite reservoirs by in vivo imaging, something I talked about with the imaging studies.

Develop next generation of target-capture ELA assays based on newly identified candidate blood biomarkers. I mentioned that also.

And then evaluate the performance of the target-capture ELA assays with endemic and U.S. blood donor populations for use as screening and/or confirmatory assays. So that will be the ultimate application of their work.

So moving along, the last one now -- just bear with me; there's more. This is Dr. Duncan's research program. So Dr. Duncan's research program is solely dedicated on technology evaluation and support the element of novel technologies that could be then brought into donor screening arena.

So if one looks at the advances on cancer, in terms of diagnostics, it's moving very fast-paced. But the same thing is not translated in donor screening arena. So what Dr. Duncan is doing, he's looking at the technologies which are available in the industry, in academia, who are developing new technologies. He's always on the lookout and collaborating with those people to evaluate them for detection of bloodborne pathogens. And eventually they will hopefully become with regarding to donor screening.

So the landscape of the donor screening is changing very fast. It's not exactly the same as it used to be, and we have seen here how many -- a handful of pathogens that FDA recommends for detection.

But again, then there's the emerging agents, there's the Zika, there's dengue, there's *Babesia*. They come and go. And then there's restricted geographic risk. There's seasonal risk; an example is dengue and chikungunya. And then cost effectiveness of screening multiple new pathogens.

So every time a new pathogen comes, how does one model that, really? And the answer probably lies in multiplex detection, and also it has to be adaptable so the new pathogens can be added and subtracted as the need is. But nonetheless, the assay has to be sensitive, at least as sensitive as the current testing. It has to be specific, robust, and adaptable. And Dr. Duncan's program is trying to address all of those.

But in the end, the advanced technologies contributes to the area of public health beyond blood safety and prepares FDA personnel to review submissions of new technology devices. And that's the ultimate goal here, to prepare all of us with the technology.

So over the period, what he presented at the last site visit, there are these five different technologies or methods that he has reviewed so far, and I'm going to go over one at a time.

So the first one is this OpenArray bloodborne pathogen panel on a chip. So the outstanding or the unique feature of this is so there are -- I think there are 16 wells on each of these panels here on this slide, so each pathogen can be

applied to this open well individually so there's no mixing there. So what that means is there's an issue of interference or cross-contamination does not exist, and also the new panels can be added and subtracted without much problem.

So these are primer and probe sets that he tested. There are 10 of these pathogens here that he had evaluated here, and the result is summarized in this table here. So on this panel here, what you see is the results of 10 licensed tests, and the last one you see the concordance with the OpenArray technology that he evaluated.

The numbers are a sample that he tested, okay. For HIV there was 96% concordance; HCV, 93; HBV was 100%. Negative control was 93, and overall 94% concordance.

So this is, you see, not ready for prime time, but the idea here is to evaluate technologies as they come, and then one can work on the improvements where the differentiation lies. And we will not know that until we go through this process arbitration, okay.

So the next technology that he evaluated is the bloodborne pathogen resequencing microarray. So this is the Affymetrix GeneChip-based microarray, which they produced a custom design based on a special need for us really, and this is based on target sequencing of the targeted region of pathogen, not the entire genome but the targeted regions, and it included the sequences for 55 bloodborne viruses, 5 bacteria, and 16

protozoan species, strains, and genotypes. So the chip itself is far beyond what you need right now, but it offers the potential of what can be done actually.

And here are the results listed here. Using the spiked pathogens for HIV, Dr. Duncan could detect all five of five times. HBV can be detected two of two times; HCV genotype 2a, three of three times. So the assay did work when we use the spiked pathogens.

When you use the donor repository, results from the licensed tests for donor HCV, it worked. HIV, it did not work at the time it was tested. And the other one worked. So they're overall negative, there is some problem, a specificity problem, and total correctness is 91%. And again, I think that work needs to be done in this, but this is how the technology exists, okay.

But the unique features of this technology, it allows one to look at the nucleotide changes in the target pathogen. So in one way, this also tells the genetic drift that may be happening or the assay is failing to detect, so that's an advantage. So it may be more suited, the way the technology exists right now, not for donor screening but more in the confirmatory tests in blood safety arena.

Okay, so the next one is resequencing microarray (RMA) for the Ebola virus genome. So this project was started in response to a request from the Medical Countermeasures Program,

and the funding was given to Dr. Duncan based on his previous work on resequencing microarrays, and the idea here is to have a method which -- to next-generation sequencing to look at the -- rapidly monitor the Ebola virus genome drift.

The technology does offer certain advantages over next-generation sequencing because this is basically bioinformatics really. Actually, you don't need sophisticated -- the results come out ready to use. You don't need sophisticated bioinformatics, algorithms, and tools here.

So here the idea is to look at the viral evolution, and eventually you can look at those sequences, genetic drift, and look at the adaptation to humans, whether in terms of reduced virulence or enhanced virulence and how the virus is adapting. And all of this came about in response to the Ebola virus epidemic in West Africa, okay.

So the way Dr. Duncan evaluated this technology, he went about by looking at the average Ebola virus viral load, which is 2.7 x 10-7 copies per mL during acute infection. So the idea is to measure the sensitivity during acute phase of infection here, and the corresponding volumes of blood samples they used was 15 µL. One detects 400,000 copies and so forth, with a reducing sample volume here. And eventually, the last one is 400 genomic copies of virus here, okay.

And using this method, when you look at this algorithm of virus quality, he scored based on how good the sequences come

out from this really by this technology. With 15 µL blood and 400,000 copy number, one gets close to 95% of quality score here. So it's correct placement on tree detection. What this means, it will allow you to identify, correctly identify the circulating viral strain here.

But what is important here, even in the four -- even with the 40 copies here, it's 22% quality score, which still allows to detect the circulating viral strain. So he's still working further to improve the quality of sequencing score, and that's where the project is. I understand he's writing a manuscript on this or the findings, okay.

By the way, I mean, all of these publications, they are generally listed in the slides.

So as a proof of concept with how good these detections are in the Ebola genome using this technology, so what he relied on is this VSV-engineered recombinant virus where the Ebola virus glycol surface protein is expressed. This is the work with Dr. Kris Konduru and Dr. Kaplan, in the Level 3.

And so what Robert did was he took the RNA from this artificially engineered VSV virus expressing GP, Ebola GP, and then monitored them for several passages and see how the sequence changes are constantly, and he was able to detect these variations after two different passages. So this kind offers the proof of concept in important viral protein that's involved in cell entry, immunity, and neutralization.

Okay, so I'm going to talk about his next technology that he's evaluating. Of all the technologies, this is more in the infancy phase. This is the earlier work so far here. So this is the laser-based, rapid multiplex detection of bloodborne pathogens, and this work he's doing with Creative LIBS Solutions. So these are the physicists, I believe, in the University of Arizona somewhere. They are using the laser technology for detection of pathogens on a filter paper. And I believe if it works, it will be a game changer, but it's a very early state.

So how does it work? The light released after a laser strikes a blood sample can be resolved by a spectrophotometer to produce a spectral fingerprint that is unique for each pathogen that may infect blood.

So the concept, I guess, is too much physics for us, but it's simple really. You spot a spot of blood on a filter paper, bake it for half an hour, I believe, and then simply strike this laser light, and based on the spectrophotometer, the tracing you get, it allows -- I believe it allows you to identify a unique fingerprint for a pathogen. So a low level of skill is required to operate the instrument, and the answer is available within minutes.

So this is the work that Dr. Duncan has so far, using *S. aureus*. This is the bacterial concentration per milliliter, 10,000/mL. He can detect it five out of five times; up to

1,000, 100, and even 10 bacteria he can detect. No blood alone. I think there's some problem here, but the detection is accurate even up to 10 bacteria per mL. And then when the coded samples are matched, 100 to 10,000, three out of five times was detected. And obviously, there's a lot of work out with this, I mean, the challenge being the cellular pathogens, he tried to take pathogens, but his work -- in the process of working on these and working out the problems, okay.

So the last piece of his project is for the standardization of spiking methods for evaluating diagnostics. So basically how the spiked blood with pathogen can be used to develop methods for validation, spiking methods, validation methods, and then those spiked samples, how they can be used for evaluation of diagnostics.

And it has really direct relevance for our work, also. This is what we do. We develop reference panels for the evaluation of donor screening programs, and this work was initiated at the request of NIAID through an interagency agreement.

So they requested Dr. Duncan to study to validate pathogen spiking methods that they could recommend to device developers that they fund. So Dr. Duncan developed standardized methods for model viruses, I believe, HIV, bacterial and protozoan pathogens, including *Babesia* and malaria and the three biothreat bacteria surrogates.

Each step in the process of characterization of the pathogenic strain was grown in cultures, so very interesting -- where the pathogen came from, how it was cultured in terms of the counting of the pathogen and so forth, harvest conditions, and catalogue them properly so the method can be reproduced, and then the quantification to long-term storage conditions were optimized. I mean, this is core to most of the things we do.

And then validate to provide a method that developers can follow to achieve reproducible results. That's part of what is published now.

And then also, then use an in-house method for validation of detection, and it's a PCR method. And then another one, target-enriched PCR-based detection methods to compare to -- again, this is published now. And this project is complete now, and the experience gained from this hopefully will help us in the future.

Yeah, almost coming to 19 seconds left for my talk, and I'm almost done now.

Just to summarize his work and his future plans, OpenArray part of work is complete. Bloodborne pathogen resequencing microarray, microarrays have been manufactured for future additional work and will complete the evaluation with spikes and blood donor specimens to take the work to completion, evaluate the technology.

Ebola genome resequencing microarray, writing a manuscript on this; test additional laboratory-cultured Ebola and clinical specimens for further validation of the microarray.

And the LIBS, the laser technology is complete. Current studies for multiplex detection of bloodborne pathogens on the bench top system so it could be applicable in actual testing settings, and collaboration on validation of assembled instrument to ensure the device is suitable for blood safety application.

So I'll stop now, and thank you.

DR. STOWELL: Thank you, Dr. Kumar, for presenting a huge amount of information in a very organized and clear fashion.

At this time we can have questions for Dr. Kumar or for any of the other speakers. Any comments or questions from the members of the Committee?

(No response.)

DR. STOWELL: Thank you, Dr. Kumar.

DR. KUMAR: Thank you.

DR. STOWELL: The next step here is for the Open Public Hearing. We don't have any names of anybody in advance who wanted to make comments, but if there's anybody now in the audience who would like to approach the microphone, please do so.

(No response.)

DR. STOWELL: And seeing no takers, I then conclude that

we have nobody who wants to make any comments, so from that we can move on.

At this point, actually, I think we are ready for the closed committee discussion of the report and also with that, of course, Dr. Maguire's presentation. So I believe the members of the FDA are dismissed with thanks.

And let's take a 5-minute break.

(Whereupon, at 10:11 a.m., the open session of the meeting was concluded.)

CERTIFICATE

This is to certify that the attached proceedings in the matter of:

115TH MEETING OF THE BLOOD PRODUCTS ADVISORY COMMITTEE

April 5, 2017

Silver Spring, Maryland

were held as herein appears, and that this is the original transcription thereof for the files of the Food and Drug Administration, Center for Biologics Evaluation and Research.

_____

Tom Bowman

Official Reporter