National Center for Toxicological Research (NCTR)

Science Advisory Board (SAB) Meeting

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P R O C E E D I N G S (9:00 a.m.)

Agenda Item: Welcome and Overview

DR. ASCHNER: Welcome, everyone, to the Scientific Advisory Board meeting of the NCTR. We have about 15 minutes and it won't take us long, but what I would like to do first is go around the table and have everybody who is on the board introduce themselves, a couple sentences. I'll start.

My name is Michael Aschner. I am at Albert Einstein College of Medicine in the Department of Molecular Pharmacology. My interest is in the neurotoxicity of heavy metals. Maybe I'll call your names the way I see them on the screen. Mary Ellen, go ahead, please.

DR. COSENZA: I'm Mary Ellen Cosenza. I'm a regulatory toxicologist. I've worked in the industry for over 35 years and now I am an independent consultant. I've been on the board for a couple of years now, and I'm happy to be here today.

DR. SAUER: Good morning, everyone. My name is John-Michael Sauer. I currently work for a small biotech company called Peptilogics. I'm also an adjunct professor for the University of Arizona. My interest has always lied in renal and hepatic toxicity. Right now I'm trying to figure out what deleterious effects peptides can have. So

I'm really excited to be here for this session and look forward to the presentations.

DR. WALKER: Good morning. I am Cheryl Walker. I am director of the Center for Precision Environmental Health at Baylor College of Medicine. I'm a molecular biologist and I do work in the area of environmental epigenomics.

DR. TROPSHA: Good morning. I am Alex Tropsha. I am at UNC Eshelman School of Pharmacy in Chapel Hill. My interests are in computational toxicology.

DR. GANEY: Good morning. My name is Patti Ganey. I am a recently retired professor from Michigan State University. My research interests have been in inflammation and drug-induced liver injury. Happy to be here, I look forward to the presentations.

DR. ASCHNER: Okay. What we are tasked to do over the next day and a half is very important. I just want to remind everybody what the purpose of this meeting is. So we are here to advise the NCTR director on establishing and implementing and evaluating the research programs at the NCTR, which assists the commissioner of the FDA in fulfilling the regulatory mission of this agency. Our job is to provide an extra-agency review and ensuring that the research programs at NCTR are scientifically sound and pertinent. There should be nine of us, not everybody, I

believe, is on at this moment, but throughout today and tomorrow morning, everybody will participate.

We are considered to be employees of the FDA for the next day and a half, as you all know, given the amount of paperwork that you probably had to do, but I take it as a very serious mission and as an honor and clearly a pleasure to be able to assist the NCTR in the research mission and the FDA as a whole.

What we're going to do today is basically we'll have brief talks by folks at the different FDA centers. This started quite some time ago, but we've changed the order. The idea is to provide you with some information on how the other centers interact with the NCTR and how the NCTR assists those centers in their mission.

Then starting tomorrow, we will hear the different divisions of the NCTR. The directors will provide with us with a brief review of what has happened within those divisions over the last year, and in the afternoon, one of the divisions will be closely reviewed by a subcommittee of this board.

So without further ado, what I want to do first is actually thank Donna and Kim and all the other NCTR employees who made this possible. I wish we were all together. I believe this is the third year at least that we are not doing so, since 2020. I believe this is my last

year on the board, but I hope that all of you convene together next week in the NCTR.

I'm going to pass the baton now to Tucker Patterson. Tucker took over not too long ago from Dr. Bill Slikker who retired. I want to congratulate you on your new capacity, and please go ahead and let us know what's new and what exciting things are taking place at the NCTR.

DR. PATTERSON: Greg is on now, so Greg, go ahead and introduce yourself as one of the board members.

DR. LANZA: My name is Greg Lanza, professor of cardiology at Washington University Medicine School, primary research interests are twofold. One, advanced biomedical imaging, particularly advanced cardiac MRI, and a wide variety of nanomedicine for imaging and targeted drug development.

Agenda Item: Conflict of Interest Statement and "Housekeeping Items"

DR. MENDRICK: I have to give the DFO language first. Good morning. I'm Donna Mendrick, the designated federal official, DFO, and would like to welcome everyone to the NCTR Science Advisory Board meeting. We appreciate the time and diligent work of our board members in preparing for this meeting and for the forthcoming deliberations. I and the board wish to thank the FDA regulatory centers for their participation in this meeting

and my FDA colleagues for all their efforts preparing for this meeting.

Let me say a word about my role. As the DFO for this meeting, I serve as liaison between the board and the agency. I'm responsible for ensuring all provisions of the Federal Advisory Committee Act, FACA, are met regarding the operations of this SAB. Also in my role for the board, a critical responsibility is to work with appropriate agency officials to ensure that all appropriate ethics regulations are satisfied, thus the paperwork. In that capacity, board members are briefed on the provisions of the federal conflict of interest laws. In addition, each SAB participant has filed a standard government financial disclosure report.

Regarding the meeting operations, with a full agenda we have strived to provide adequate time for the presentations, public comments, and board's deliberations. This is a special note for all presenters, board members, and other participants. Please keep your video off and mute your phone until you speak. Announce your name when you do so, for this meeting is being recorded and a transcript will be posted on our website. Be sure to turn off your video and mute your phone after you're finished.

During presentations and discussions, if board members require greater clarification on issues regarding participation of attendees in the audience, they may request such information during the meeting through the chair or myself.

You will notice that pursuance to FACA, we have scheduled a one-hour offering the public the opportunity to provide comments about the topics being considered before the board. No one expressed an interest in speaking, but we did receive a comment from the Montelukast Side Effects Discussion Group as well as individual letters from some members, and these have been posted on our website.

Now I need to mention the meeting minutes. In accordance with FACA, minutes of this meeting will be prepared as well as transcript. This meeting is being recorded. All will be posted to the website.

So in closing, I wish to thank the board for their participation in today's meeting.

Tucker, it's all yours.

Agenda Item: State of the Center

DR. PATTERSON: Thank you, Donna. I'll go ahead and get my slides pulled up here. Thank you, everyone. As Miki mentioned, I am Tucker Patterson. I am the current acting director at NCTR. Bill Slikker retired at the end

of March and so this is my eighth week on the job as the acting, but who's counting?

I'm going to give you a high-level overview today of the things going on at the National Center for Toxicological Research. I'd like to feature some of our studies that we have going on right now, and I'm not going to lie to you and say that you won't see some of these slides again later in some of the division presentations, because of course they're the ones that do the work and generate this data. So I appreciate them and their efforts in what we do here at NCTR.

Our mission of course is to address FDA's needs with high-quality research and serve as a global resource for collaboration, training, and innovative scientific solutions.

Our vision statement is we want to conduct scientific research to provide reliable data for FDA's decision-making, and you're going to see that today. It's very much a collaborative effort in what we do here at NCTR, and also to develop innovative tools and approaches that support FDA's public health mission.

So NCTR was established in January of 1971, so we celebrated our 50th anniversary last fall. We're an integrated toxicological research facility. We want to foster those collaborative efforts with other government

entities as well as academia. We also have some industry collaboration through all of our cooperative research development agreements.

We have five offices, six research divisions, and approximately 500 employees, and I'll show you the breakdown of that a little bit later, about a million square feet of space here on campus with 30 buildings, about 125 experimental laboratories now, and we have also AAALAC-accredited animal facilities.

For those of you that have been here, you know that we're located on 500 acres of fairly wooded property here in south of Little Rock. We are owned and operated by the FDA, so that makes us a unique facility in that we are actually owned by the agency.

We recently renovated office and laboratory spaces in buildings 14 and 53. Three of our research divisions, biochem tox, genetic and molecular tox, and systems biology, were able to move into some greatly needed updated facilities in the last couple of years.

We've recently renovated animal housing in buildings 5 and 53. If you come out to the campus now, you have to be very careful because you may have to walk through hot asphalt that was just laid down, because we are doing a complete infrastructure renovation and so that includes sidewalks, roads, culverts, lighting, and there

are quite a bit of improvements going on right now on campus. We've put down a new water well. We're self-sustaining when it comes to that. We have a new roof partly on building 5, and we'll have some new laboratory space going into that area as well.

For those of you that have been here, you know that cell phone reception is a problem. We've installed a distributed antenna system and now we have Verizon online and our other two primary carriers that we have on campus are coming online this year. So that's greatly improved our cell phone reception, which improves our safety and security here at the facility.

And we have funds that are coming down the pipe for an agency data recovery center that will hopefully start construction next year, and also some new pathology laboratories.

So our organizational structure, you can see the office of director there and the offices that are underneath that, but of course today we are focusing on the six research divisions there. Later in the day you'll hear from the first three divisions listed in the bottom there, biochem tox, bioinformatics and biostatistics, and genetic and molecular toxicology. Then tomorrow morning you'll hear from the division directors of microbiology, neurotoxicology, and systems biology.

So this is a little bit more granularity on our staff. So you can see we're approaching 300 government employees now and the bulk of that of course are research scientists and support scientists.

We have a little over 200 contractors now. That would be our animal care staff, our maintenance contract, our pathology contract, our instrument maintenance contract as well. We typically run between 40 and 50 of our ORISE postdoctoral fellows.

So with our science at NCTR, we have approximately 250 ongoing research projects at any one time, and over 85 percent of that research is collaborative in nature, and I'll show you a breakdown of that on the next slide. We also, in addition to our FDA product centers and ORA, we have research partners in 22 academic institutions and 9 other federal agencies.

We have a unique memorandum of understanding with the state of Arkansas that's been signed by the commissioner of the FDA as well as the governor of Arkansas that really encourages collaborative efforts within the state here.

You can see the goals there, just kind of a snapshot of some of the goals here recently. I'll point out of course the COVID work which has been going on for the last two years. We were very early on in getting those

projects initiated after the pandemic began. We'll go into a little bit more detail later on that.

So a breakdown of our collaborative work, and of course this is not funding dollars per se, this is just that we have collaborative projects with the different product centers. You can see that we collaborate with all the product centers as well as ORA and then university, academia, and then we have these broad agency agreements and tech transfer agreements with other institutions.

In terms of our productivity, we certainly continued this activity during the pandemic. This is our internal research metrics that I'm listing here. It certainly does not capture all the engagement that NCTR staff had in providing direct expertise to the product centers and also participation in internal and external working groups, but we average typically 150 peer-reviewed manuscripts a year that are published. We kind of want this technical report and the new protocols to sync because our protocols are on a three-year timeline. So we want to be closing out our protocols with technical reports and moving onto new protocols, and so those numbers are running around 50 per year.

This is a partial laundry list of our research portfolio. You can see that our foundational expertise there of course is both an in vivo and in vitro metabolism

PBPK modeling that we've done a lot of work in, animal models and chronic bioassays, but as we move down the list there, you can see that almost every division is represented here and that we have this expertise across the center.

Looking at some of the more emerging technologies that we have here at the center, nanotechnology, advanced imaging capabilities, of course we have MRI, MRS, CT, Micro-PET, and also MS imaging, artificial intelligence that we'll get into a few more slides on that later, innovative computation modeling, and also our microphysiological systems and stem cell systems.

Then our outreach and support that we have, of course we have our global activities with our regulatory sciences group, and I'll talk about our meeting that's coming up this fall in Singapore, and then also we develop international standards methodologies.

I know everyone's heard about the predictive tox roadmap, and this really articulates the need for a comprehensive strategy to evaluate new methodologies and technologies. Of course, we have that capability here at the Center. We try to not only collaborate across the agency, but we collaborate across research divisions in house. So we can pull that expertise in and make more of a

comprehensive-type project instead of operating in our silos.

But we know that a toolset is needed to enable sound comparisons of the value and also the limitations of these currently accepted testing paradigms. Also, these new methodologies and approaches that are under consideration have to be validated and compared to existing data or new data that's generated.

Moving more into bridging these toxicology paradigms that we of course want to extrapolate across the human animal models and also these in vitro testing platforms. We all know that's challenging if you've done this type of work.

So understanding dosimetry and metabolism is crucial to enable this exercise. NCTR assists the agency and other public health agencies in the conduct of these rodent bioassays, pharmacokinetics, modeling, and we've also increased our involvement tremendously in the testing of these new in vitro platforms.

Out of those 250 projects that I mentioned, we're probably looking at about one-fifth of those projects actually being in vitro projects with the rest of them using in vitro assays and other testing paradigms.

So of course, what we've heard over the last two years, COVID-19, and I want to just give you a snapshot of

what we've done in this space, and we were early in the game on getting projects up and running. But we were first looking at what are the needs in this space with our COVID projects? What are the significant scientific gaps that pertain to COVID-19 and related to the infection and disease progression? What clinical evaluations would be useful? Do we want to look at biomarkers, tissue characterization? What questions and gaps can we address using nonclinical studies?

So this included risk assessments on disease backgrounds, risk assessments related to perinatal health, that's during pregnancy, pre- and post-natal exposure, and also juvenile exposure. So we designed a battery of projects to really address these different areas.

One of the first projects that we generated was wastewater surveillance. This was Dr. Camila Silva in our Division of Biochemical Toxicology. This project was in collaboration with the Division of Microbiology and also the Arkansas Department of Health and the University of Arkansas for Medical Sciences, which is our med school located here in Little Rock. This was funding that was supported by some of the COVID supplemental funds that came down through the agency, and of course what we're doing here, the goal is to monitor and help estimate increased viral spreading without individual testing.

So we're surveilling wastewater and we continue to have this project going, and it's a complementary tool for estimating viral spread in the central Arkansas area. So we're looking at different wastewater sources here in the central Arkansas area. Right now, we know that this early detection and continuous monitoring is going to help federal and local agencies respond more quickly to help the spread of COVID.

A lot of people have jumped on board with this type of monitoring and now there is, for lack of better word, a consortium across the country that posts this information about their wastewater monitoring, and also has the ability to look at the variants in that. So we have a manuscript now that's in our queue for clearance that was the first one out of this study.

Of course, artificial intelligence, we wanted to use that technology that we have here at NCTR, a very robust group that works on that. So with this approach, we wanted to prioritize drugs by their potential to interact with the human proteins that are bound by the SARS-CoV-2. This was using network pharmacology, computational drug repositioning, and also artificial intelligence. This project was funded by the medical countermeasures group at headquarters, and it was in collaboration with CDER, CDRH, and also NCATS. Approximately 20 scientists were involved

on this. There's a manuscript that's in preparation on this work.

Looking at our perinatal health risk assessment, nonclinical perinatal SARS-CoV-2 infection hazard risk assessment is what we're trying to evaluate here. So what we're looking at on this project is looking at transmission of the infection, how infection affects pregnancy and overall maternal health, looking at vertical transmission of the virus from the mother to the pups in utero. Of course this is in a rat study.

Evaluation of the pups prior to birth also allows us to assess for any possible malformations that could affect fetal/embryo development. These changes may be anything from a birth defect to reduced litter sizes to reductions in fetal weight. They're going to be also assessed for developmental delays and undergo limited neurobehavioral assessments to determine if COVID-19 has any long-term developmental effects, because we know that long-COVID is real. We see that in articles almost on a daily basis that people are suffering from long-COVID.

So what we hope to learn from this study will then serve as a starting point to help guide risk determinations, clinical evaluations for children infected with SARS-CoV-2, treatment, or possible treatment options.

So this project is characterizing the effects of viral load and immune cell infiltration in autopsy tissues taken from COVID-19. This is a MALDI IMS, our imaging mass spectrometry study. That technology is label-free and it's rather robust, which allows for spatial distribution for a variety of analytes across tissue cells. That includes looking for small molecule drug metabolites, lipids, and N-linked glycans.

With these samples, multiple analytes can be assessed with a single imaging run and down really to resolutions up to 10 micrometers. So in this particular slide, this example, the MALDI IMS is being utilized to assess COVID-19 lung autopsy tissues. So multiple lung tissues of COVID-19 are normal or currently being assessed, but in this example, you can see how imaging is being utilized to assess the different glycan structures across the tissue. This can be paired with immunohistochemistry to get a better understanding of the microenvironment.

This study is ongoing, but interesting trends are being seen, including this example of a high mannose glycan which tracks with the CD8 down here at the bottom.

Our Division of Systems Biology is evaluating COVID-19 patients to differentiate immune system response. We know, we see in the literature, that some people are resistant to COVID; some people may test

positive and be completely asymptomatic. So the goal here is to determine biomarkers of asymptomatic and mild responses to COVID-19 infection. So we're going to be looking at clinical plasma samples that are generated at our med school here in Little Rock and using an omics approach in our Division of Systems Biology.

Then using our human in vitro airway tissue model to look for coronavirus antiviral drug screening and drug repositioning. So the objective of this project is developing in vitro airway models, which we've done, and screening assays for assessing candidate drugs for coronavirus treatment. Hopefully findings from this work will provide a better understanding of the effects of the ACE2-modulating drugs on SARS-CoV-2 entry and infectivity.

I'm going to move on and talk a little bit about our Bioinformatics and Biostatistics Group, and you know that our subcommittee will be evaluating this particular division beginning tomorrow. But, of course, bioinformatics develops software tools and methods for storing, managing, and analyzing large quantities of data. We develop and provide training for and make bioinformatics tools available to the agency and also the global research community. We have to have software databases to manage this large amount of scientific data

that's generated to improve product development and safety assessments and risk analysis.

One very successful collaboration that we've had with CDER is FDALabel. That's a tool that is used by the CDER reviewers, but it's managed here onsite at NCTR. So it's currently undergoing a CDER survey.

You're going to hear a lot more about AI and what its capability is when Dr. Tong talks later on in the SAB, but we're using AI for drug safety, food safety, genomics, for text mining and FDA documents, we have several projects with CDER, and we also have projects with the Office of Regulatory Affairs that are in play.

Then you'll also hear more about the AI4Tox program which consists of these four initiatives, AnimalGAN, SafetAI, BERTox, and PathologAI. This program is designed to advance FDA's toxicological research, and it applies to the most advanced AI methods and hopefully will develop new AI methods to support FDA regulatory science and improve the safety review of FDA-regulated products, and hopefully the products from these endeavors will undergo a qualification process through the FDA Innovative Science and Technology for New Drugs, or the ISTAND program, for their potential application in the FDA drug review process.

Then another collaborative work we have with CDER is doing consults that are requested by CDER reviewers for assessing drug-induced liver injury. The reports generated for consults for the assessment of DILI risk potential and suggestions for further studies of potential dealing mechanisms of action for liver injury.

Just an example of this type of work, one of the reports that was generated by our Division of Biostatistics and Bioinformatics put a clinical hold on the sponsor's response and eventually led to the sponsor to discontinue the development of the drug candidate because of this potential for DILI.

Moving on into the new alternative methods, which there has been a lot of focus on this in the last couple of years moving into these new alternative methods, we have a variety of models that are being used here at NCTR including neural stem cells, cardiomyocytes, bronchial epithelial cells.

I do want to talk about a few of the platforms that we're using. One of the platforms we're currently evaluating is the Alzheimer's disease Brain-on-a-Chip. This is a cooperative research and development agreement that we have with the company Emulate. We're trying to standardize a battery of endpoints here related to these clinical findings and we know the A-beta and tau

accumulation and transport, blood-brain barrier integrity, neuronal, astrocytic, and endothelial cell health, and functionality.

We also want to evaluate the development of chip pathology and compare this to human pathology. So the overall goal here is to see how these compare to existing, eventually, human data and see if we can rely on this type of data as we're moving forward with our regulatory decision-making.

Another platform we're using is looking at 3D-bioprinted human skin models. We know that the in vitro, excised human skin is considered the gold standard to quantify skin permeation of chemicals of interest. However, limited supplies are associated with high cost and variability of human skin explants. This is a major challenge.

So Dr. Luisa Camacho in the Division of Biochemical Toxicology is the PI on this particular project that's in collaboration with CFSAN, CDER, CVM, NCATS, and NICEATM. So hopefully the increased availability and reduced cost of bioprinted skin is going to enable us larger and continued studies and also ultimately offer an opportunity to be used as a tool to support regulatory decision-making at the agency.

Another model in our division of genetic molecular toxicology is being headed up by Dr. Dayton Petibone and that's in collaboration with our medical countermeasures and our CBER colleagues. This is a testicular model, and we know that these conventional tests are very labor intensive and use large numbers of animals. So we're trying to develop a 3D testicular model. We're going to link that to 3D liver models and hopefully evaluate the product safety and may complement animal study studies about providing information for study design, agent prioritization, and dose selection before committing to animal studies.

One example that we're using is the Zika virus, and of course it's primarily spread by mosquitos but can spread sexually after infecting the testes and can also cross the placenta and result in negative pregnancy outcomes for both the mother and developing fetus. So hopefully this model will help us answer some of those questions before we would move into an in vivo model.

Another chip system that we have is our liver chip system. This is Dr. Qiang Shi in our Division of Systems Biology. This is in collaboration with CDER and also with a CRADA with Emulate. So as you see here, again, we are trying to identify biomarkers that would distinguish

between benign and serious outcomes that can be used in the clinic.

I mentioned earlier our air-liquid human airway tissue model. Dr. Xuefei Cao in our Division of Genetic and Molecular Toxicology has established this model, and so we're looking at this model in collaboration with CTP and CDER and CBER and also our NIEHS/DNTP colleagues to evaluate aerosols, cigarette smoke, and anything that would infect our human airway.

With our imaging approaches, I mentioned earlier we have MRI, MRS, and PET capabilities, but also one newer technique that is being utilized in our Division of System Biology is MALDI tissue imaging. Really unique instrumentation here that we can track compounds of interest or metabolites or lipids right in the tissue by using this technology.

I do want to mention our National Toxicology Program which was established in 1978. You've all heard about that who have been on the board and know that we've had longstanding collaborative efforts with our counterparts at NIEHS and NIOSH to form this NTP triad. Our agreement goes back to 1992 when NCTR was actually deemed the research arm of the agency when it comes to the NTP. So I will point out recent examples here and also ongoing studies, brominated vegetable oil, which was

recently submitted for publication, and then some of the historic compounds that we've analyzed like melamine and the cyanuric acid issue.

I want to finish up by talking about our Perinatal Health Center of Excellence. This center was created a few years ago with Dr. Slikker who was a driving force behind this. This center is managed out of NCTR, but it is open to all of the agency researchers. So we're looking at maternal/fetal, it's a unique regulatory responsibility. We all know that it used to be we looked at the pediatric population as just being little adults, and so we know that they present as a vulnerable population, and they're really understudied when it comes to the clinical issues just for obvious ethical reasons. But this particular center provides about $2 to $2.5 million a year for various projects. I think we have 14 projects that were funded last year with multiple centers represented.

I already mentioned that we celebrated a half century of research last year, so we hope to continue for at least another half century.

Our global coalition food regulatory science research activities, this has been an effort that's driven by Dr. Anil Patri, Dr. Weida Tong, and of course Dr. Slikker before he retired, so it really fosters these just

discussions with groups around the world that have the same type of issues that we're dealing with as an agency, and these are these regulatory bodies from across the globe.

So I do want to point out that our meeting is coming up in October of this year in Singapore. We have some information; they have a website available there if you want to register or see what that meeting is about.

I'll finish up by saying that NCTR conducts toxicology research that supports and anticipates future FDA needs. We develop novel translational research approaches for safety assessment, continue to use guideline approaches on emerging technologies, provide research technology and resources for all the FDA product centers which you'll see as we move through today, multidisciplinary training and regulatory science with our ORISE postdoctoral fellows, also an ORISE summer student program, and we foster national and international collaborations with scientists from government, academia, and industry. And I believe that's it, Donna.

DR. MENDRICK: Thank you. Terrific. Time for questions from the SAB.

DR. ASCHNER: Thank you, Tucker. There are a lot of exciting new things happening at the NCTR, obviously driven by COVID-19 primarily. My question to you is whether all this emphasis on the COVID-19 has been at the

expense of some other projects or -- recognizing that you have been on this job only for eight weeks, but do you feel that you can maintain the other projects at full steam with all these new added responsibilities that's been levied on the NCTR?

DR. PATTERSON: I have only been on the job for about eight weeks, Miki, but I'm still serving as the deputy director for research which is a role that I took over about two years ago, so I see all the research projects that come through. I think initially there was a big focus of course in the COVID space, but we had a major pivot there. We had supplemental funds that were earmarked for COVID projects, and so we took advantage of that and of course that was needed at the time, but I've seen us slowly move back.

I think the pendulum has moved back and I think we are heading in the right direction with a very good balance. We have a few projects that are going to continue, but I've seen lots of projects coming through the queue. We had recent applications for our intramural-funded projects, and there were a few COVID projects there, but the majority of the projects now are different and outside that COVID space. So I think there is a good balance there.

We have enough work going on in that area. I don't think we really need to expand much more but we have a lot of other projects. As I said, we have 250 ongoing projects, and I would say less than 25 are in the COVID space. So 10 percent.

DR. ASCHNER: Thank you, Tucker. Does anybody else from the board have any questions?

DR. LANZA: I have one question. I am very impressed with the improvements you've made in AI in the advancements, especially since the NCTR is kind of a central organization to the full FDA. I wondered how much the other agencies are interacting with NCTR to concentrate and share the capability versus having their own AI programs separate from NCTR.

DR. PATTERSON: I can tell you that Dr. Weida Tong, of course he's on our panel here, but they've done an excellent job of reaching out to the other centers and expressing our capabilities here, and we have a lot of interaction within that group. I think DBB has a very robust collaborative program, and they primarily work with the other product centers is where most of their collaborative work is in. So I think they are aware. I think with the different working groups that I sit on; I think they are keenly aware of the capabilities that we have here.

DR. LANZA: I personally think that NCTR can be like a pivot point or center that can steer advances in one center to the other centers with more ease than it would be from one center to the next directly. So I look forward to seeing how you are able to really not only make advances yourselves but integrate the total FDA effort, because it's probably one of the most important technologies that you'll have in the upcoming 10 to 20 years.

DR. PATTERSON: Yes. I can state, Greg, that over the last three or four years I've seen our collaborative efforts increase tremendously with the product centers, and I think there is a lot more interaction. Maybe it's that everybody is used to the new virtual technology now and it's easy to set up meetings and reach out and not have to be in-person, but I know that we have interactions with all the product centers, and they've been a really fruitful force.

DR. ASCHNER: Did you have a question, Donna?

DR. MENDRICK: I just wanted to remind people to please state their name when they are asking questions for the transcription. Thank you.

DR. TROPSHA: This is Alex Tropsha. Thank you for the presentation. Very, very impressive progress. My question relates to what you had mentioned about the use of new approach methods, and I know it has been quite a

priority for the agency, but of course ultimately the challenge is to actually rely solely on new approach methods in making regulatory decisions. I think one of the approaches was actually in skin sensitization. That drug approval was actually made as a sole tool to be relied on. Last summer I remember seeing a publication, I was trying to just to find it. I'm wondering in this whole transition from the development of the emphasis versus actually relying on them, how far are we from research to implementation?

DR. PATTERSON: That is a good question, Alex. I think everybody is trying to look in the crystal ball and see where we are on here. I think there will be some areas that progress a lot quicker than others, as you said.

Maybe the skin is a great example of maybe sooner rather than later, whereas when you're looking at total body physiology, you may be looking at 80 different platforms to try to recapitulate one mouse or one rat right now just because of the complexity there and I think that's the challenge that we have, is can we take small bites and use those and move forward and make advancements as we learn more about these different platforms?

But I've seen great progress over the last year or so, even in the midst of the pandemic, and I really predict now that probably within two to five years we're

going to see really almost exponential growth in this area, but I would say two to five years before we're really comfortable after we have those comparisons that are made.

DR. ASCHNER: Thank you, Tucker. Are there any other questions? If not, just another comment to follow up on what Alex and Greg said. The EPA, which obviously is another federal agency, has really focused on the NAMs as well, and you didn't touch upon any interactions between the NCTR and the EPA. It may be fruitful to approach them and see what they're doing, because I know that they're doing a lot of chip work.

You mentioned specifically Alzheimer's, but they're probably doing it with respect to other tissues as well and not just the brain. They might be able to learn from you, you might be able to learn from them, just ensuring that there is no duplication of effort, basically.

DR. PATTERSON: Definitely. Thank you for the comment.

DR. ASCHNER: Okay, any other questions from advisory board members? We are a few minutes ahead of time, which is okay, I'm sure we will fall back in a few hours.

Okay, if not, thank you, Tucker, for a very interesting presentation and the exciting work at the

NCTR. I have to switch to my agenda, so bear with me just a minute.

We are going to move on now to the subcommittee review of the Division of Biochemical Toxicology. This was reviewed last year. We will hear from Patti Ganey on the recommendations from the subcommittee and then following a break, we will hear from Fred Beland, his response. Patti, please go ahead, you have 35 minutes.

Agenda item: Subcommittee Review of Division of Biochemical Toxicology

DR. GANEY: Thank you, Miki. Good morning again, everyone. As part of the annual Scientific Advisory Board review of NCTR for 2021, the Division of Biochemical Toxicology, or the DBT, received an in-depth review of the subcommittee comprising myself, another SAB member, John-Michael Sauer was co-chair, and subject matter experts Drs. Bodour Salhia, Cecilia Tan, and Neera Tewari-Singh. This review was held virtually on May 12 and 13.

The mission of the DBT is to conduct fundamental and applied research design to define the biological mechanisms of action underlying the toxicity of FDA-regulated products. Their goal is to characterize the toxicities and carcinogenic hazards associated with chemicals, specifically those of interest to FDA. They use a variety of techniques to address this goal.

So with respect to the DBT in general, this is a productive unit that engages in basic research in important areas and provides support to FDA product centers. Their strength is recognized internally as well as externally, as evidenced by collaborations established within NCTR, with other centers, and other federal institutes and agencies, as well as with universities. Their funding, both intramural and extramural, was relatively constant over the period that we reviewed, which was five years, and their publication record over the last five years has been quite good, averaging about 50 publications per year. So the big picture is that this is a strong division doing a good job.

They had had some recent changes that posed challenges. One was a change in the interagency agreement with the NIEHS and NTP that supports infrastructure like animal facilities and personnel that are necessary to conduct studies that support the FDA product centers.

The subcommittee recommended that a strategy be devised to prevent a disruption in service or a need to rebuild these capabilities as once they are eroded, they are difficult and expensive to rebuild.

Other challenges related to retirement of three key scientists, Drs. Jeffrey Fisher, Mary Boudreau, and Daniel Doerge.

Before I begin talking about our recommendations in the specific focus areas that were discussed, I wanted to make one more comment about the division in general. With the explosion of alternative approaches being used, we just heard a little bit about that, the subcommittee thought that the DBT was missing an opportunity to take a leadership role in providing results from animal studies that could be used to evaluate the usefulness of these alternative approaches. This point was made in the general SAB meeting last year as well.

Five focus areas were reviewed: COVID-19, dermal toxicology, toxicological assessments, epigenetics, and computational models. I'll share some of our major findings and recommendations in each of those areas. I want to mention on the outset that many of the projects that were presented were in initial planning stages as the SAB has requested the center to present ongoing and future projects for our input as opposed to those that are well underway or completed.

The first focus area that was presented was COVID-19. At the time, we commended the DBT for moving so swiftly to address pandemic issues. On the other hand, in the lens of May 2021, we also thought the relevance of the focus area would be short-lived, and we were really wrong. So apologies for that.

One project was one that you've heard about from Dr. Patterson just recently, surveillance of SARS-CoV-2 in wastewater as a complementary tool to estimate viral spread in Arkansas. This approach for the detection of virus in wastewater is now being used in other areas of the country. The studies were fairly general, but the approach was thought to have merit.

The one recommendation was that there was a need to assess the sensitivity of the assay. For example, how many people need to be excreting the virus for it to be detectable and for results to be meaningful, and also to garner some epidemiological data for support of the approach.

The second COVID-19 project presented was a flow cytometric analysis of anti-SARS-CoV-2 antibodies in human plasma. The goal was to be able to assess simultaneously SARS-CoV-2 IgM, IgG, and IgA in human plasma to determine if there is a difference in viral protein recognition among the antibodies. The subcommittee struggled with this project, because the value in knowing the immunological profile as opposed to the currently used antibody methods was not articulated. Furthermore, the longer-term goal was not explained. For example, could this be used to understand pathogenesis or to direct therapy?

The second focus area was dermal studies. These studies were considered to be important. Some of them were being performed to support other centers like the Center for Tobacco Products. One project that was presented is the effort to evaluate biodistribution and transplacental transfer of tattoo pigments using pregnant and nonpregnant mice. Results from these studies might be useful in addressing some knowledge gaps but there was no evidence of epidemiological data to support the hypothesis that tattoo pigments have effects on pregnancy. That was considered a weakness of the project. Furthermore, the subcommittee thought that pigs or minipigs would be better models than the mouse, although we try to be sensitive to the fact that cost is a consideration.

Two projects related to percutaneous absorption were presented, and these were thought to be highly relevant. Both projects were in early stages.

One related to characterizing the pharmacogenetics of cannabidiol, or CBD, and its metabolites in rats after dermal exposure using both oil and cream vehicles. Some of the technical challenges were discussed. For example, formulating the CBD and the cream for dermal exposure, analytical methods for quantification, et cetera. The DBT was encouraged to consider interspecies

differences in pharmacogenetics for extrapolation of results to humans.

The second project was related to evaluating 3D bioprinted human skin equivalents for in vitro absorption studies. You just heard a little bit about this from Tucker. There was enthusiasm for these studies, a lot of enthusiasm for these studies, and it was recommended that absorption data obtained at least initially from the bioprinted skin be compared with available in-human absorption data just to see how well that platform will work.

The third focus area was toxicological assessments. These studies are highly integrated into the mission of the DBT and the NCTR and efforts in this area have produced highly impactful publications since 2014.

Three projects were presented. The first was an evaluation of whether cannabidiol or its metabolites were responsible for male reproductive toxicity. The question is relevant for choosing an appropriate animal species for further studies as there are species differences in CBD metabolism. One endpoint that was presented was cytoskeletal reorganization, but the rationale for the use of this as an endpoint and its relevance to toxicity were not articulated.

Part of the project involves the planned use of primary human Leydig and Sertoli cells to compare the primary murine cells and cell lines. At the time, human Leydig cells were not available, but it was not clear whether the group planned to develop them from iPSCs in collaboration with others in the center as has been done for other cell types. This was identified as an opportunity not addressed by the DBT. Another opportunity that perhaps was overlooked is to look with the modeling group to develop PB/PK models to define the relationship between testicular cell injury and exposure to both CBD and its metabolites.

The second project was metformin/glyburide in male reproductive toxicity, and this was in the initial planning stages. A pilot study was presented. It was well-outlined and well-designed.

The third related to developing metabolically competent liver cells for use in high throughput systems. As liver cells are known to lose their differentiated function rapidly in culture and drug-induced liver injury represents an important problem for human health and drug development discovery, this effort was seen as having potential for high impact.

The aims were ambitious, perhaps not unreasonable. It was recommended to give thought to how to

engage the pharmaceutical industry to use these platforms early in the drug discovery and drug development process and also to give consideration to issues related to FDA acceptance of data generated with the platform.

The fourth focus area was epigenetics which was viewed as essential to support a mission to develop translational research approaches that provide the FDA with science and database methods to improve public health. A recurring comment within this area was the projects, although important, were broad and somewhat unfocused, that the novelty was not clear, and in some instances the methods proposed were outdated.

Three projects were presented. The first was a genomic and genetic determinants of the susceptibility of non-alcoholic steatohepatitis, or NASH, in mice and development and evaluation of a novel in vitro noncoding RNA-based screening model system for hazard identification. Non-alcoholic fatty liver disease is the most common chronic liver disease in the United States and can progress to NASH. Molecular mechanisms are not fully understood and there are no approved therapies or biomarkers for NASH. So this is an important area to be exploring.

Data were presented identifying phenotypic and epigenetic changes in non-alcoholic fatty liver disease resistant and prone mice. As there are several

publications on NASH and DNA methylation, the subcommittee thought it would be important to highlight the novel findings within the study.

Some questions were raised regarding data interpretation. For example, there are studies planned using collaborative cross mice on high-fat, high-sucrose diet to investigate strain- and sex-related differences in susceptibility to non-alcoholic fatty liver disease and its progression to NASH. These studies were reviewed positively, but it was unclear how the investigators planned to differentiate between the occurrence of differentially methylated regions, or DMRs, that arise due to sex- or strain-related differences versus those induced by the specific diets that are being used.

They planned to use pathway analysis, but the subcommittee thought a more in-depth analysis of DMRs to cross sex and different diets would be important. Finally, the subcommittee thought the use of human samples would strengthen these studies a lot.

The second project was assessing epigenetic effects of nanomaterials in human cells. Most research has been conducted with nanoparticle models such as a commercially available P25. The difference in the plan for these studies is to assess DNA methylation, global histone modification, and microRNA expression after exposure in

vitro to the human food additive food grade titanium dioxide.

This study aligns well with the mission. Two concerns were raised. One is that it was not clear that there was a plan to evaluate the potential impact of epigenetic alterations. So there are changes but are they different or are they deleterious or not? It was recommended to evaluate gene expression as well.

The second was that a BeadArray or sequencing-based approach should be used for DNA methylation rather than PCR array, and it was also noted that again, effects on human tissues would be valuable.

The third project was entitled the role of epigenetic mechanisms in re-expression of estrogen receptor or ER, progesterone receptor or PR, and human epidermal growth receptor or HER, in triple negative breast cancer and specifically the effects of vorinostat and indole-3-carbinol. So the purpose of this study is to enhance the understanding of epigenetic regulation of triple negative breast cancer and expand the paradigm of treatment of current FDA-approved target therapies.

Studying the potential for epigenetic therapies in the treatment of triple negative breast cancer is valuable. These studies will be performed in cell lines, and this model was questioned. So would the results have

much of an impact since they were being generated in cell lines?

Vorinostat and other histone deacetylase inhibitors have been widely studied in triple negative breast cancer and clinical trials have been conducted as well. Re-expression of ER and HER have been reported previously after vorinostat treatment in triple negative breast cancer. So those previous findings were not acknowledged in the study design that was presented, and furthermore the subcommittee thought that the study really needed to define its novelty over these previous findings.

A second aspect of this project was the epigenome-wide association study of peripheral blood mononuclear cells in systemic lupus erythematosus, or SLE, identifying DNA methylation signatures associated with interferon-related genes based on ethnicity and SLE disease activity index. The etiology of SLE remains unknown. So this is a potentially important study looking at methylation signature in SLE and comparing between African Americans and European Americans.

The choice of biomarkers was not explained. The use of filtering data values in BeadStudio was criticized as inadequate and maybe even incorrect. What methods would

be used to conduct the proposed studies outlined in Aims 1 and 2 was not made clear.

The last research focus area presented was computational modeling, a capability deemed to be essential to support the mission of NCTR. A major organizational change to the computational modeling program is the retirement of senior scientist Dr. Jeffrey Fisher in 2020. The pharmacokinetic modeling program established by Dr. Fisher has grown and the program has demonstrated its ability to establish effective collaborations with internal and external partners, as well as to secure extramural funding.

White the computational modelers are well-trained and skilled, the program would benefit from recruiting a senior scientist who has safety assessment experience or if that's not possible, allowing the current scientists to train with advisors in the regulatory program to gain experience in safety assessment.

A major concern about some of the research efforts was not about the quality of the model, but about the purpose or design of the research. For some of the presentations, it was not clear how the computational models addressed the science questions being raised as motivation for the research.

The first project that was presented was multipath physiologically-based pharmacokinetic, or PBPK, model for nicotine in humans. The stated motivation for developing a multipath PBPK model for nicotine in humans is to support the FDA's plan to consider lowering nicotine levels in cigarettes to, quote, minimally or nonaddictive levels, end quote.

Since it is important to predict delivered dose available for interaction in tissues, a respiratory tract component was included in the PBPK model to simulate a route that is important and relevant to human exposures to nicotine. But there is no discussion on how to use the PBPK model to predict an internal dose metric that is relevant to addiction endpoints, nor was it clear how the model outputs would be linked to toxic effects or addictive properties of nicotine. So here is where we thought some of the investigators could use some senior input.

The second project was a description or development of a first-generation in-house FBA PBPK model-based tool to enhance the safety and efficacy of therapeutic agents in the perinatal-relevant life stages. It's proposed to have potentially a graphical user interface and to address current challenges related to model transparency and accessibility.

This tool has the potential to be a core product of the computational modeling program at DBT and can be used to support various centers in the FDA when internal dosimetry predictions are needed for pregnant and pediatric populations.

The third project presented was a development of an artificially intelligent virtual pregnant woman modeling suite. The subcommittee had questions about whether the artificial intelligence and machine learning were proposed to inform selection of model parameters to be included in the PBPK model, or were they to be used to predict some PK data, for example, area under the curve, or some PK changes? It was also unclear if the purpose was to replace PBPK models with an AI/machine learning informed platform or to support the development of PBPK models.

Finally, the subcommittee thought that there was a valuable opportunity for a joint effort for this project with the in-house PBPK model-based tool that I just mentioned.

Other specific recommendations for the modeling group are to develop a core competency. One that was suggested was to consider developing a modeling suite with modular design and capability to customize for predicting pharmacokinetics of substances in pregnant women, fetus, and infants, so perinatal life stages. This

product/modeling competency could facilitate FDA approval and review of safety and efficacy of drugs and chemicals.

A second recommendation is to develop a strategy regarding time allocation to developing modeling capabilities and to offer modeling services to other units. So the latter, offering modeling services, is part of the mission, part of the mandate of the Division of Biochemical Toxicology, but in order to be able to continue to do that effectively, they have to continue to develop modeling capabilities so there is kind of a bit of tension between those two activities. So a strategy regarding time allocation will be useful.

So overall, the division seems to be doing quite well. We found some areas for improvement, and the subcommittee also identified some opportunities that might have been overlooked, and we hope those were helpful to the division.

John-Michael, would you like to add anything, correct anything I've said, or just have any additions?

DR. SAUER: Patti, first of all, really great overview of the report and the meeting last year. I thought that was really thorough. I'd just like to highlight a couple pieces.

Number one, I think all of the projects were really well done that we reviewed. Likewise, I think they

are relevant to FDA's mission, and it's really my hope that the division will take these recommendations as they are, recommendations to be able to use then to basically better the science that's going on.

So again, thanks a lot, Patti, for leading this. You did a great job of putting that report together and all of those pieces, so thank you.

DR. ASCHNER: Thank you, Patti and John-Michael. I think this exemplifies actually why we are doing these meetings. It's a wonderful report and hopefully Fred and the division found it helpful. So again, I commend the subcommittee for doing a wonderful job.

Before we move on, Donna reminded me that we have to vote on this report. So I don't think we can vote electronically. I'd just like to ask somebody from the advisory board to motion that we accept the report by the subcommittee.

DR. WALKER: This is Cheryl Walker. So moved.

DR. COSENZA: This is Mary Ellen Cosenza. I'll second.

DR. ASCHNER: With that, all in favor of accepting the report, please say aye.

(Ayes.)

DR. ASCHNER: Any objections? Any abstentions? Okay, with that, the report has been accepted.

DR. GANEY: Thank you.

DR. ASCHNER: We are running a few minutes ahead of schedule, about 15 minutes ahead of schedule. I suggest that we do take a break for 15 minutes. So we'll get an extra 5-minute break as we are pretty efficient today.

Why don't we reconvene at 10:45 and we'll hear the response from the Division of Biochemical Toxicology? Donna, anything else you want to mention before we break?

DR. MENDRICK: No, that is great. Thank you.

DR. ASCHNER: Okay, we'll see you all at 10:45 Eastern time.

(Break)

DR. ASCHNER: I think we are at 10:45 Eastern time. And I would like to ask Fred to respond to the comments.

Agenda Item: Response to Review

DR. BELAND: My name is Fred Beland. I am the Division Director for Biochemical Toxicology, and it's my task now to respond to the review that Dr. Ganey presented right before the break.

What I've done is, well, all the division members, have gone through the written comments that were provided to us in the latter part of last year. So what I'm going to do is I'm going to go through the report, some of which Dr. Ganey covered, others that she did not, and what I've done is I've paraphrased where there were questions or recommendations, and then I will respond to those comments.

The first one, which was nice, is that the subcommittee considered us to be a valuable resource. But then, and this is a reoccurring thing, how do we establish priorities? And I think Tucker Patterson mentioned that the Division of Bioinformatics and Biometry have a lot of collaborative studies with the product centers, and our division, DBT, is the same, and perhaps to a greater extent than any other division in NCTR. We interact with the regulatory centers, and we currently have studies on cannabidiol, tattoo pigments, in vitro dermal penetration, brominated vegetable oils, PFAS, nattokinase, lumbrokinase, and nicotine.

We have regular meetings with the product centers, with CFSAN, with CTP, the Center for Tobacco Products. We meet every two weeks to discuss our projects and to plan new studies. So we do have DBT-initiated

studies, but I think the subcommittee needs to recognize that really we exist to support the product centers.

Then Dr. Ganey mentioned about the loss of Jeff Fisher, Daniel Doerge, and Mary Boudreau, and the committee had specific recommendations concerning the replacement of these individuals. Dr. Ganey talked about Jeff Fisher, and Jeff Fisher was in charge of the modeling group, and there were two recommendations, either that we go out and hire a senior modeler, or alternatively we provide additional training to existing personnel to gain experience in this entity.

The NCTR, traditionally we've had a very long tradition, ever since I've been here, which has been since 1976, recruiting young scientists and let them develop their research programs. Examples include Bill Slikker, who just retired, Tucker Patterson, who is the acting director, Fred Kadlubar, Carl Cerniglia, and so forth.

With Jeff Fisher retired, Annie Lumen stepped in to lead the group, and Dr. Lumen was a very dynamic individual, there's no question about that. Unfortunately in November of last year, she abruptly left NCTR to take a job in industry. I personally think that it was a mistake, but that's the way things happen.

We currently have three staff fellows in the computational modeling group, three staff fellows and three

postdoctoral fellows. So there are six individuals. These individuals work together as a cohesive unit and have established productive collaborations with scientists at the FDA regulatory centers and also at academic institutions. As such, it is my personal feeling that the second of the two pathways recommended by the committee, that we provide opportunities for existing personnel to gain more experience by training with senior advisors is the pathway that we will follow.

With regard to Dan Doerge and Mary Boudreau, let's start with Mary Boudreau. She was in charge of a study that was discussed about the tattoo pigments. What we have done there is Dr. Svitlana Shpyleva, who's an MD/PhD, stepped in to complete this study and is now in the process of expanding the scope of the study, at least we have a protocol that is being developed to look at minipigs, which was one of the recommendations by the subcommittee. We will have to have, of course, discussions with CFSAN, because the tattoo ink project, this falls under the purview of CFSAN, and again, we need to -- the studies have to be designed such that they meet the regulatory needs.

With regard to Dan Doerge leaving, he had a study where he was looking at polyfluorinated alkyl substances, or PFAS. This is in collaboration with investigators at

CFSAN. We have completed that study; we have furnished them with a draft report that is undergoing review at CFSAN. So I think we're under control with the two studies that these individuals were responsible for.

With regard to, we have additional pharmacokinetic studies concerning cannabidiol, this was briefly mentioned by Dr. Ganey. These were being conducted by Dr. Luisa Camacho and Dr. Qiangen Wu. These are ongoing, I will show data this afternoon, and I think they're going satisfactorily. Other studies are being conducted by Dr. Goncalo Gamboa da Costa, so I think as far as, I think we have the adequate personnel to meet the needs of the product centers in this era.

They brought up the funding of the NTP. Funding, as we discussed when we had the site visit from the NTP, has decreased rather dramatically. But through the interactions of, through the actions of Bill Slikker, Tucker Patterson, Goncalo Gamboa da Costa, we have received FDA funding to make up for this loss. So the infrastructure will stay in place. We will keep the animal care; we will keep the pathology. And we are in the process now of developing protocols that will utilize these funds in a manner that will support the FDA product centers.

One example is a project being put together by Luisa Camacho, which will look at in vitro and in vivo skin irritation at the request of the Center for Devices and Radiological Health, CDRH.

Before Dan Doerge retired, we had done an extensive amount of pharmacokinetic studies with arsenic, in vivo pharmacokinetic studies with arsenic in mouse model and also in nonhuman primates. Now, this has been expanded, what we're looking at zebrafish, in collaboration with investigators in the Division of Neurotoxicology, and we're also looking at C. elegans in collaboration with individuals at CFSAN.

Dr. Ganey brought this up, about taking the lead for in vitro and in vivo extrapolation and I believe we're doing this. The protocols that I just mentioned, the one that Luisa Camacho is putting together, the ones with C. elegans and zebrafish. We will continue in that manner, and hopefully use the funds very effectively.

Dermal studies, the subcommittee was quite enthusiastic about the dermal studies with what we're doing with cannabidiol and also the 3D-bioprinted skin. These are being led by Luisa Camacho. I will present some data later this afternoon, but I think we are going in the right direction.

The subcommittee was also enthusiastic about the in vitro, the metabolically competent human liver cells, and this is being led by Dr. Lei Guo. She is good at interacting with people, and has made substantial progress.

Dr. Ganey didn't bring up the last comment on this slide, and this was a recommendation that -- I think I used the term perplexed -- that we should hire an expert in epigenetics. I really didn't understand this. The three individuals presented, Dr. Igor Pogribny, Dr. Beverly Lyn-Cook, and Dr. George Hammons. Dr. Pogribny has 260 publications. He has a paper, just received a paper of the year award from Toxicological Science. He has 13,000 citations. Even more important, he's a member of the Senior Biological Research Service within the FDA. The FDA has and 15,000 employees. Only 50 people within the FDA are in the SBRS. So he's a very senior person.

Dr. Beverly Lyn-Cook trained under Dr. Lionel Poirier, and Dr. Poirier was here at the center. Dr. Poirier was one of the pioneers in epigenetics. She has extensive publication record that focuses on breast and pancreatic cancer and lupus. So again, I consider her -- she's currently being reviewed for certification in the SBRS. So I consider her to be a very senior person.

Dr. George Hammons for many, many years has held a joint appointment between NCTR and Philander Smith

College in Little Rock, which is a historically black institution. He was the chairman of the chemistry department. So he's a very senior person. All three of these individuals have extensive external funding. Dr. Pogribny gets funding from the National Cancer Institute. Dr. Lyn-Cook gets funding from the Office of Women's Health. Dr. George Hammons has external funding from what's called the CORES program. So this is a recommendation I and the rest of the division totally disagree with.

With regard to the computational modeling, I will admit, at the moment we're sort of struggling. We've lost two people. Jeff Fisher we knew was going to retire. Dr. Annie Lumen was going to step in, and I considered her to be a tremendous leader, but then she, as I mentioned, left. The problem is that Dr. Lumen had put together, had received external funding for two projects, so we need, we have an obligation to finish these projects, and Dr. Miao Li and Dr. Kiara Fairman are currently doing that.

The third person, Dr. Darshan Mehta, Dr. Fisher had CTP-funded projects and again, we need to finish these projects, and Dr. Mehta is doing so.

These three scientists are engaged in conversations with individuals at the regulatory centers about collaborative studies, and I think that is going very

effectively. For instance, Dr. Mehta will be involved in two projects, one that deals with PFAS and another deals with C. elegans and heavy metals. These were both out of CFSAN. So I think we're just going to have to see how things progress over the next year or so, but I have hopes that we will continue to be very productive.

As Dr. Ganey mentioned, we presented four focus areas. The first one was COVID-19. You have to remember, we have to go back two years, right? I don't think anybody -- I certainly didn't, I think most people didn't realize what the pandemic was going to be like. I naively thought that -- I was headed for Portugal in March, I had to cancel the trip, and I thought that I would be going in the summer, this was 2020, which of course didn't happen. So anyway, we did, as Dr. Patterson mentioned this morning, we have one project that deals with SARS-CoV-2 in wastewater. This study was being led by Dr. Camila Silva. She's done a really a superb job. I will present some data from her study. Dr. Patterson presented some data this morning, I'll show another type of slide this afternoon. And she's -- one of the recommendations was that she engage an epidemiologist, which she has done, through the Arkansas Department of Health. They have just completed a paper that has been submitted for publication.

The second project, the flow cytometry, this was being led by Dr. Jia-Long Fang, and again, you have to put this in the context of something that was started two years ago when we didn't understand the magnitude of the problem. This was an outgrowth of a study that he had conducted using flow cytometrical analysis with high-molecular PEGs, and we thought we could apply it to this. He's made excellent progress, but given the worldwide response, the worldwide scientific response to COVID-2, we think our plan is just to finish what we had outlined to do, and then he's going to move onto other things. Again, I think we received money; we have an obligation to complete the project.

The tattoo, the subcommittee, this as I mentioned, Dr. Mary Boudreau retired, Dr. Svitlana Shpyleva has taken over. She's done an excellent job, in the process of completing the report that will be submitted to CFSAN later this week. We've also put together; we have a draft protocol on minipigs that we're still working on. This was recommended by the subcommittee. I think in early discussions with CFSAN, they wanted us to use minipigs. We convinced them to start with mice. It was the wrong recommendation. And I will show data as to what happens in mice later this afternoon.

The subcommittee thought very highly of the percutaneous absorption studies. There's two components to this. One is, we're doing, there is an in vivo component where we're looking at the pharmacokinetics of cannabidiol. This is being led by Dr. Luisa Camacho. We're currently making measurements by tandem mass spectrometry. These data will be furnished to CFSAN.

The other component is the 3D-bioprinted human bioequivalence, I guess we'll call it. This also is being led by Dr. Luisa Camacho, and she has recruited a very talented postdoctoral fellow. They've done an excellent job, and they're working quite closely with NCATS to improve this 3D bioprinted human skin equivalent. The recommendation was that we make comparisons to human skin, and this is being done. As to other in vivo platforms that are commercially available.

Last week, we had a visitor from NCATS. It's our first visitor we've had in two years, through the pandemic, so it was very nice, and it was a very close interaction. I think it's progressing in an excellent manner.

Then we move to the second area, which was toxicological assessments. This project, the first project was mentioned by Dr. Ganey. This was a project being led by Dr. Si Chen, and I think this was mentioned, the cytoskeletal reorganization and in vitro to in vivo

linkage, and in our written response, I believe we addressed these.

They wanted us to look at potential mechanisms. Dr. Si Chen is doing this. There was a concern raised this morning by Dr. Ganey as to whether or not we have access to Leydig cells, and indeed we do. Dr. Chen has already published one paper in Food and Chemical Toxicology on the Sertoli cells, and now she and her postdoctoral fellow are conducting similar cell studies with the Leydig cells. So it's going very, very well.

The second project on the metformin and male reproductive tox, this is being led by Dr. Barry Delclos. This project was well received by the subcommittee. It was recommended the inclusion of a pilot study. The pilot study was conducted. It was very informative. And now the protocol has been modified based upon the results of the pilot study, and the developmental and reproductive study should begin later this year.

The committee, as Dr. Ganey mentioned, really liked the metabolically competent liver cell project. This is being led by Dr. Lei Guo. Dr. Guo has been quite good at let's call it advertising her in vitro models. They're being used by investigators throughout the world. I expect this will continue, and the recommendation was to make

recommendations primary human hepatocytes as to other in vitro systems, and she is doing this.

There was a lot of comments regarding the focus area four, which was the epigenetics. The first comment was it was important to highlight what would be new about this study. This is a study being led by Dr. Igor Pogribny with funding from the National Cancer Institute. This is a longstanding looking at epigenetics with regard to liver toxicities. It's been a longstanding effort in his laboratory.

So the main goals, and perhaps this was not articulated very well in the meeting, perhaps we tried to present too much information in this focal area, and it didn't come across. But again we had provided written material that hopefully would have provided adequate background. The main goals of this study are to investigate the role of epigenetic disturbances and the pathogenesis of nonalcoholic fatty liver disease and its progression to NASH. So this is what Dr. Ganey mentioned earlier today.

And then to identify epigenetic alterations that can improve the diagnosis of nonalcoholic fatty liver disease and be a target for drug development. To do this, Dr. Pogribny and his group use multiple animal types, multiple endpoints, from the DNA methylation, histone

modifications, molecular cellular and metabolic alterations. He has multiple publications, and he's continued to receive funding from the National Cancer Institute.

The subcommittee liked the use of the collaborative cross mice, but it was unclear how they were going to distinguish between how they would differentiate between susceptibility of differentiated methylated regions as opposed to those induced by the specific diets. The way Dr. Pogribny proposes to address this is the analysis of the DMRs in disease-resistant and disease-sensitive control mice, the emphasis here is on control mice, will indicate DMRs associated with increased susceptibility to nonalcoholic fatty liver disease. And then if he looks at the DMRs and resistant in these disease-sensitive mice that are fed the high-fat, high-sucrose diet, this will allow the identification of DMRs associated with the development of -- so the control mice will talk about susceptibility, the high-fat, high-sucrose diets will be associated with development.

As far as the, he's already identified several DMRs containing genes linked to the development of nonalcoholic fatty liver disease. and these have just been published in the paper in Epigenetics that just came out.

The subcommittee thought that a pathway analysis is useful, but a more in-depth analysis of DMRs is important. We agree with this recommendation, and as stated in the written materials and perhaps not in the oral presentation, but that Dr. Pogribny's team is using sophisticated techniques and data analysis tools. Examples of these techniques can be found in this paper that I just mentioned that appeared in Epigenetics.

The subcommittee, and Dr. Ganey mentioned this earlier today, about human samples. We agree with this recommendation, and Dr. Pogribny has established a collaboration with Dr. Arun Sanyal at Virginia Commonwealth University to obtain human samples and conduct molecular analysis.

The subcommittee recommended additional types of data measurements be made, and he is doing this. A recent paper that just appeared in the Journal of Nutritional Biochemistry describes alterations in the fatty acid composition of collaborative cross mice fed a high-fat, high-sucrose diet. And then this can be applied to other tissues, other organs, such as kidney, muscles.

The nanomaterial project was presented by Dr. Hammons. The committee felt it was well aligned with the FDA, but thought that some of the techniques were outdated. The committee thought that it was important to address the

potential impact of epigenetic alterations. We agree with that recommendation. But I think the committee, we recognize that this is not going to be easy to establish a causal link between an epigenetic effect and an adverse outcome.

With regard to the outdated techniques, remember, this project was funded by a protocol that was submitted to us called the CORES program. Dr. Hammons has an obligation to follow what is in the protocol, and you can quibble about techniques, but I think if the technique addresses the question that you're trying to address, then it is not necessarily outdated. He has successfully completed what was outlined in the project. He has a paper that has just been submitted to Nanotoxicology.

We are moving to the project presented by Dr. Beverly Lyn-Cook. The subcommittee thought the goals of the research needed to be better defined as they pertain to the mission of the FDA. Again, this may have been a problem of trying to present too much in a very restricted time period. With regard to, if we could start with lupus, this is a priority area for FDA's Office of Minority Health Equity and FDA's Office of Women's Health because of the lack of drugs for this disease and the high incidence of lupus in women, especially African-American women. I think Dr. Ganey mentioned this.

The importance of epigenetic regulation of critical genes in the interferon pathway has been shown by Dr. Lyn-Cook's team, as well as others. And this pathway is currently being investigated as a target for a number of clinical trials using several epigenetic drugs. So I think it is, it perhaps was explained better in the written material than perhaps in the presentation, and we apologize for that.

The second comment, again, the concern about the lack of strong epigenetics expertise in the division, we do not agree with this recommendation at all.

So then Dr. Lyn-Cook discussed two projects, one on triple-negative breast cancer and one on lupus. Much of the data on the triple-negative breast cancer Dr. Lyn-Cook has already published, and this was presented in the references in the written documentation. With regard to lupus, there was criticism over the data analysis, and this, the data analysis was conducted by a biostatistician who Dr. Lyn-Cook has collaborated with for a number of years. We believe, or she believes, that the analysis is done correctly, that study was published in the Journal of Autoimmunity, so apparently the reviewers also considered it to be done correctly.

The last focal area was the modeling. The first one was presented by Dr. Darshan Mehta, and this was well

received by the subcommittee, but they questioned, and this, Dr. Ganey mentioned this, that there was no linkage to toxic effects or addictive properties in nicotine. The subcommittee needs to recognize that these projects are defined by the Center for Tobacco Products. You could say we're on a very short leash. We meet with them every two weeks to discuss the progress, and they are the ones who define the endpoints that are important to them. Remember, we are providing data to product centers that they can use for regulatory purposes.

The endpoints, we were requested to develop a PBPK model for nicotine to assist reviewers at CTP to predict internal tissue dosimetry of nicotine and its major metabolites, cotinine and trans-3-hydroxycotinine, after exposure to various types of tobacco products, cigarettes, cigars, and so forth. This model will be used by CTP reviewers to determine internal dosimetrics relevant to addiction endpoints.

They questioned how we were just going to go from this human model; what about animals? And we simply didn't have the time to discuss it, but a similar model is being developed using in-house data from rats that have been exposed orally, IV, and by inhalation. So this model will be combined with the human model. I think we've addressed that.

As I mentioned, this first-generation model, this was a project that Annie Lumen put together, and then recruited Dr. Miao Li to work on. When Dr. Lumen left, Dr. Li stepped in to take the lead on this. As far as the subcommittee recommended that we use more in silico and informatic tools be incorporated, and we agree with that recommendation, and in the written response we have provided to indicate what specific open source software we will be using.

The second comment on this slide, the subcommittee felt that all the research projects have the potential to contribute significantly to the FDA's public health mission. We agree with that recommendation.

Perhaps the committee, the last comment here, the subcommittee commented these modeling efforts will have a much higher impact if investigators have a clear and specific scope and purpose and testable hypothesis prior to deciding -- the models that Dr. Li is working on, these were case studies that were related to perinatal in-house PBPK modeling to look based drugs that are currently used off-label at different life stages. The drugs for the case studies were carefully selected based upon several criteria, such as current concern about use in the perinatal life stages, the lack of dosage guidance for off-label use in these life stages, and previously reported

perinatal clinical PK data. The main goal of the project will be to support dose administrations for perinatal life stages to ensure drug safety and efficacy.

The last comment on this first what's called project 2 was how do we divide investigators' time for projects that are developed within the division as opposed to projects that are in collaboration with the product centers or helping to support the product centers. And again, this gets back to the focus of the division is to provide support to the product centers, and so I would say this will be our major effort. We have to, again, these two projects, this first-generation model and also the project 3, the artificial intelligence, these were projects that were initiated by Dr. Lumen. Dr. Miao Li was recruited to work on project 2; Dr. Kiara Fairman was recruited to work on project 3. We have an obligation to finish these studies, and then at the same time we can decide are there other areas to which we should expand.

With regard to this project 3 which is being led by Dr. Fairman, I think the question is how is this project put together, what is the focus, what is the pathway, and I think Dr. Fairman has outlined a pathway to meet our obligation to the funding agency, which in this instance is the Office of Women's Health.

What we intend to continue to complete this project by December of this year, and at that time a decision will be made, will we continue with regard to artificial intelligence and machine learning, or will she follow a different pathway? At the moment, we just want to make sure that we complete our obligations for this project.

The idea behind the question was, it was not clear to the subcommittee if the purpose was to replace PBPK models with an artificial intelligence/machine learning informed virtual pregnant woman platform, or to support the development of PBPK models. It was not to replace PBPK models, but rather to support and inform the development of PBPK models.

With regard to this last thing, the subcommittee felt that this should be integrated with the inhouse PBPK model, and we agree that a joint effort between the pregnancy PBPK project and the artificial intelligence project would be of value. But currently, it's working to achieve, Dr. Fairman is currently working to achieve the artificial intelligence/machine learning portion of the project and has enlisted experts in the division of bioinformatics and biometry to help with this research area.

There's a fourth project. This was presented by Dr. Lumen, just to show the extent of collaborations across the various product centers, and also other agencies, other federal agencies. Again, when Dr. Lumen left, we had to decide how we wanted to continue these engagements, and Dr. Miao Li is going to be involved heavily with the Tox21 project that Dr. Lumen had initiated. Dr. Kiara Fairman is going to be involved in the in vitro in vitro extrapolations as part of the botanical safety consortium.

As I mentioned earlier, Dr. Darshan Mehta is going to be involved with CFSAN projects regarding PFAS and the use of C. elegans in toxicological assessments. So these collaborations will continue. Just because Dr. Lumen left us doesn't mean that we're just going to drop them altogether.

The last was project 5. This was presented by Dr. Goncalo Gamboa da Costa. Goncalo has recently accepted a position as a senior scientific advisor to the center director, so this project is now being directed by Dr. Suresh Nagumalli. We agree that it doesn't really fit with PBPK modeling, computational modeling, but we thought it was a rather interesting approach, which involves multivariate statistics, including principal component analysis and hierarchical cluster analysis, so it is -- there is a very significant computational effort to this

project. The project, when it was presented a year ago, we were just formulating it. The protocols has been prepared, has been extensively reviewed by CFSAN, has been modified at the recommendations, is in the process of going through the final approval process at NCTR, and it's our hope that this approach will guide a selection of complex mixtures as test articles for toxicological studies.

That is the end of my response. I'll be happy to address and questions that the committee or others may have. Thank you.

Agenda Item: Discussion

DR. ASCHNER: Thank you, Fred, for the extensive response. And I'd like to open the floor now for some discussion. I know there are some people that want to comment. I will first ask Dr. Walker to comment, and then if there are others, please let me know or raise your hand. Thank you.

DR. WALKER: Thank you very much, Miki. And Fred, I just want to say that was a very comprehensive and full disclosure. I'm looking at the original committee report and your response as an outsider, as you know, I'm new on the committee. But I was actually reminded when you were talking about the excellent folks that you have there, that I actually beat Dr. Pogribny by two years in the

epigenetics space. I was reminded recently that word epigenetics was in the title of my thesis in 1984.

So the comments that I want to make is, first of all, I want to congratulate you for appreciating and moving into this area of epigenomics, and particularly for doing it in the space of NAFLD and the effects on the liver. I think this is an incredibly ripe area for research and I think you putting together epigenomics and NAFLD -- you know, a lot of times these epigenomic studies come under the purview of a systems biology group, so the fact that you all have embraced this I think is really important.

What I want to say from my perspective, though, is I can tell you, even having all of the time in the epigenetics field that I've had, I am still pedaling as fast as I can to keep up with the new next-generation approaches that are being used now and are really become state-of-the-art for epigenomic analysis, and the reason I want to mention that is because where you are in the liver I think is just the right place to be to apply these approaches, because basically we're doing things at the single-cell level now, and as you're well aware in the liver, the contribution of the hepatocyte, the stellate cells, the NK, Kupffer cells, they all are being targeted by different things, they're having different contributions, and so the ability to be able to tease out

at the level of their epigenetic responses I think is going to be transformative, and I love that you're doing it in that space.

But what the new technologies are, where you have things like the spatial transcriptomics, the spatial mass spectrometry on tissue, can be now paired with the new single-cell epigenomics, and not just single-cell RNA-seq, but now things like cut and tag and cut and run, to really bring this all together in a way that maybe couldn't happen in very many places, but your shop is one of those.

That's the comment. My question to you, and perhaps even a recommendation for consideration, is making that happen, almost no one investigator can do it. It does take epigenetics expertise, but then it takes this add-on of the technical expertise, which requires equipment that may or may not be present in any one lab. A lot of places have core facilities. It does require, I think, specialized bioinformatics expertise for doing some of these multiomic integration events. And so my recommendation or my question might be whether or not you all have the ability to move into these areas with the existing in-house technical expertise and equipment, or whether this is something that would need to be done in partnership with somewhere where this is all together, or

whether this is even the direction that you think you want to go?

DR. BELAND: Igor is probably a better person to address this. As far as equipment goes, yes, he's in the same building that the, we were talking about the imaging, the MALDI capability -- they're just down the hall from one another. Hopefully they'll talk to one another.

As far as bioinformatics capability, yes, we have a large division directed by Dr. Weida Tong, and is this the direction where Igor will go? I really can't address that today. It's clearly worth considering. And again, as I will bring up this afternoon, this has been a difficult time for us, it's been hard to conduct experiments, and we've actually done a remarkably good job I think. But again, the problem we have is getting enough personnel, and for being able to expand doing things. We lost people to retirement, but we've also have had difficulty recruiting new individuals because of restrictions on who we can have within the Food and Drug Administration.

DR. WALKER: Thank you.

DR. ASCHNER: Thank you, Cheryl. Thank you, Fred. I don't see any raised hand. Does anybody else have any comments? I don't know if the original members from the subcommittee want to say anything. Okay. Hearing none, then thank you again to the subcommittee and thank you for

the response. Again, I'll say at the expense of being redundant, I think this was a thorough review and an excellent response. I hope you'll take all of these issues that were mentioned into consideration, and I guess we look forward for another review in five years down the road.

DR. BELAND: I do not.

DR. ASCHNER: You don't, but perhaps Igor will do it. Okay. With that, we're ahead of time, but that's okay. I think we'll move onto the statement from the acting FDA chief scientist, Dr. O'Shaughnessy. I believe she's online, so please go ahead.

Agenda Item: Statement from the Acting FDA Chief Scientist

DR. O'SHAUGHNESSY: Thank you very much, and good morning, everyone. I'm truly delighted, of course, to be with you today for the NCTR Science Advisory Board meeting. NCTR's cutting-edge research and contributions can be found just about everywhere in our centers, and they are often central to some of FDA's highest scientific priorities. As we've often said, NCTR holds a unique and foundational position at FDA, as the only center that supports all FDA offices and product centers with essential toxicological research.

With that important responsibility, NCTR continues its focus and efforts to make remarkable

contributions both within the agency and with our domestic and international stakeholders. I'd like to make note of some of those efforts.

It goes without saying that over the last few years, FDA's response to the COVID-19 pandemic has been at the forefront of center priorities. NCTR was among the first centers within the agency to initiate COVID-19 research, and NCTR has taken many additional actions since the start of the pandemic to support FDA's regulatory role.

To give just a few examples, NCTR leadership organized routine conference calls with research and medical leaders across Arkansas and with the University of Tennessee Health Center to conduct and complete research to support efforts to address the COVID-19 pandemic. As you've heard, NCTR's Division of Biochemical Toxicology developed a method to detect SARS-CoV-2 RNA in wastewater, applying the type of One Health approach that we use to detect and respond and effectively to future epidemics, national disasters, and pandemics. NCTR researchers are applying this method to selected metropolitan areas in Arkansas to monitor the presence and the extent of the COVID-19 virus.

NCTR's Division of Bioinformatics and Biostatistics developed two AI-related projects focused on drug repurposing to fight COVID-19. One, funded by OCS's

medical countermeasures initiative, is a collaboration with CDER, CDRH, and NCATS, together with over 20 external collaborators. The project aims to prioritize drugs, approved or investigational, to study according to their potential to interact with human proteins that are bound by SARS-CoV-2 using network pharmacology, computational drug repositioning, and artificial intelligence.

NCTR has also made considerable progress in advancing approaches needed for a strong foundation for precision medicine. Genomic technology has evolved rapidly, and demand growing for genome-wide association studies and next-generation sequencing methods, all promising tools for identifying biomarkers for discovery and precision medicine.

Under NCTR leadership, the challenges have been met by large consensus-building teams of scientists from academia, industry, and government working together to provide standards, approaches, and bioinformatic tools to determine the best scientific practices for the use of omics data. These are important achievements, and I will say that the Office of the Chief Scientist has been and will continue to be fully committed to supporting NCTR in its work, to protect public health and advance the innovative tools and approaches that are critical to FDA's predictive capability and our ability to predict risk and

efficacy, as well as raising awareness around NCTR's scientific research and its impact on our regulatory decision-making.

NCTR senior leadership plays a key role in supporting the Office of the Chief Scientist in the planning and development of FDA's biennial Science Forum. At the 2021 Science Forum, NCTR researchers presented on a range of topics, from the use AI in predictive toxicity assessment to an FDA product labeling tool enabling patient and consumer safety in combating COVID-19.

NCTR's research also continues to be a regular feature of our public-facing monthly FDA grand rounds, webcasts that OCS launched in 2016. The grand rounds have played an important part in raising the visibility of FDA's research in the scientific community and showcasing how FDA is applying that research to its regulatory activities.

Most recently, NCTR provided an overview of tattoo ink research at NCTR, and as another example, featured the Nanotechnology Task Force report on nanotechnology over a decade of progress and innovation, published in 2020, that highlights FDA advancements in the field of nanotechnology since it released its last report in 2007. The Nanotechnology Task Force, spearheaded by NCTR, consists of representative from across FDA product centers and offices. The Office of the Chief Scientist

supported the development and publication of the report as well as Collaborative Opportunities for Research Excellence in Science, or CORES, grant funding in nanotechnology to conduct regulatory science research on emerging topics of interest to the agency.

Of course there are tremendous opportunities presented by modern techniques for NCTR. Science has progressed from primarily observational science to include many modern techniques that NCTR researchers have been advancing, from genomic technology and bioinformatics to artificial intelligence. At FDA White Oak headquarters, NCTR chairs the cross-agency artificial intelligence working group, which is dedicated to the field's study and application in FDA scientific activities. The working group hosted educational seminars featuring internal and external speakers, supported scientific progress at FDA by identifying agency science and regulatory gaps, needs, and challenges, and provided FDA AI representation on government committees.

Also, a newly proposed AI initiative is under way at NCTR to enable generating data for improving analysis of real-world data that can be used to address unmet regulatory needs, advance product development, and facilitate access to treatment for patients and rare diseases.

NCTR scientists are also developing new and alternative approaches to toxicity assessments, such as microphysiological systems and in silico approaches. Investment in developing and evaluating such models that may have higher predictive value and clinical performance and a shift from animal to in vitro or computational models would potentially reduce the time and cost of evaluating and developing products, while potentially also allowing for replacing, reducing, and refining animal testing.

NCTR co-chairs the alternative methods working group, which has cross-agency representation, and in January 2021, published its report, Advancing New Alternative Methodologies at FDA. The report details FDA's activities and collaboration with stakeholders to advance alternative methods that could reduce the time it takes for new treatments to move to human testing and approval.

NCTR is also the point of contact for FDA's cooperative research and development agreement with the companies supplying equipment and organ chips for several FDA research projects. As you heard, NCTR is studying brain-on-a-chip using brain cells differentiated from human-induced pluripotent stem cells from patients with Alzheimer's disease, and is also using a liver chip system with human hepatocytes to predict individual susceptibility and adaptation to drug-induced liver injury.

In addition to supporting this alternative research through the CRADA, the working group has held many seminars from internal and external experts, as well as hosted webinars as part of its FDA webinar series on alternative methods. The webinars provide an opportunity for platform providers to present their new methods and methodologies exclusively to FDA scientists.

So in closing, I've highlighted a number of areas in which NCTR has ongoing and active engagement to advance FDA's regulatory science mission and priorities. I want to thank all of you for your commitment to protect and promote public health and for the work you do in support of FDA. Thank you, also, of course, for the effort in pulling together an outstanding program and having us all convene virtually.

Now I look forward to hear the centers' perspectives and more about other toxicology projects under way. Thank you.

DR. ASCHNER: Thank you, Dr. O'Shaughnessy. Are there any questions for Dr. O'Shaughnessy? I'm sure she'd be happy to answer one or two questions if there are any. Okay, hearing none, as mentioned, let's move on to the FDA center perspectives. As I mentioned before, we've changed the order of these presentations. In the past, this was the last set of presentations, after the individual

divisions. And we've come to realize that this is more helpful, because we can get the perspectives of the different centers on the interactions between the NCTR.

The first one is going to be -- they're all about 20 minutes. The first one is going to be from the Centers for Biologics Evaluation and Research. And I apologize, I don't have the names on the agenda for the folks who are going to present.

Agenda Item: FDA Center Perspectives

Center for Biologics Evaluation and Research

DR. ELKINS: Good morning. I'm Karen Elkins. I'm the associate director for science at CBER, and I'm also a principal investigator at CBER. I'm a microbiologist, an immunologist, by trade, and my lab studies immunity to intracellular bacteria.

It's really a delight to be with you all today for this meeting, and I'd like to tell you a little bit about what we do at CBER in our day jobs, as well as the intersections between our research programs specifically and the NCTR research programs.

Our regulatory mandate, as you might guess, is to regulate biological products, which have a particular definition in law, but in essence it comes down to ensuring the safety, purity, potency, and effectiveness of vaccines,

allergenics, blood and blood products, and the ever-expanding universe of cell tissue and gene therapies.

We also have a heavy interest and investment in protecting the public against emerging infectious diseases and bioterrorism. There was a phase when bioterrorism was preeminent. Now, as you might guess, emerging infectious diseases are pretty much at the top of our list.

To break down a little more specifics about the products that we are responsible for, our office, we have three product-related offices: our Office of Vaccines regulates not only vaccines, preventative and therapeutic, but also allergenics and live biotherapeutic products, such as fecal microbiota transplants and phage-related therapies.

The Office of Blood regulates blood and blood components, derivatives, devices related to HIV diagnostics especially, and blood substitutes, and has a growing interest in blood pathogen reduction.

And then we have the Office of Tissues and Advanced Therapies, which regulates plasma-derived proteins, and their recombinant derivatives, intravenous immunoglobulins, polyclonal immunoglobulins -- monoclonal antibody treatments are regulated by the Center for Drugs, OTAT then focuses on gene therapies, human tissues, and

xenotransplantation products, which has been a small niche but has been much in the news lately.

All of our product-related offices are supported in their work and with research that comes from the Office of Biostatistics and Pharmacovigilance, which cuts across all three product areas.

Our research interests are described in our current strategic plan, which was released in 2021. One goal is to develop and evaluate technology and tools that support the nonclinical evaluation of our products. Nonclinical, meaning not only safety-related aspects, but also effectiveness in mechanisms of action. Another goal is to enhance the validity and efficiency of clinical evaluations, improving clinical trial designs, statistical and analytical and modeling approaches to analyzing clinical data.

Proactively addressing public health challenges and emergency infectious diseases. We're living that one at the moment. And in general advancing our capability to assess technologies and products in ways that inform our regulatory oversight of those products.

Our research programs cut across the product-related areas, as outlined here. We have research in viral, bacterial, and parasitic vaccines. This includes not only specific vaccine-related topics but interests in

pathogenesis, immune response, and correlates of protection for the respective diseases.

We have programs in allergenics, phage products and FMT, which is also a growth industry, CAR-T cells, viral gene therapy vectors, and CRISPR systems. We expect CRISPR systems to be coming down the line fairly soon.

We have programs that evaluate polyclonal immunoglobulin treatments, blood substitutes, several aspects of vascular biology and pathogen reduction, and blood-related storage issues which is a giant practical concern in the blood industry. Overarching many of those areas are our studies on the epidemiology of diseases and adverse event analyses to the products that are out there in use.

Consistent with that portfolio of research interests, our expertise ranges across those areas to include all facets of microbiology, immunology, biochemistry, and molecular biology. We have a good cadre of cell and developmental biologists and folks with interests and training in tissue engineering and microphysiological systems. We have expertise in epidemiology, particularly meta-analysis of large healthcare databases, which is helpful in our postmarketing product oversight, and underpinning all of that, expertise in biostatistics and bioinformatics, as well as all of the

technologies that are used to produce the data, not only in our research programs, but the data that we see in regulatory submissions.

Right now we have about 15 active collaborations with NCTR. I have perhaps unfairly but in the interest of time tried to group these as outlined here. They tend to fall into areas of interest that include lipidomics and metabolomic analyses, microphysiological systems and airway tissue systems, which has already been mentioned, of course, today, genomic analyses, and alternatives to animal testing.

I should pause to admit that the pace of our research activities has, like everybody's, been impacted by the pandemic. We have had for the last two years some rather large restrictions in building occupancy alone, which has been challenging to navigate. Some of our researchers with the applicable expertise and interest having started COVID-related programs, that has, as asked earlier, in some cases, come at the expense of the previous programs or in starting up other new activities.

Also, our researchers are all reviewers. Our researchers serve primarily as product reviewers, CMC reviewers, on our regulatory applications, and I think it's fair to say that the regulatory workload, particularly in the areas of vaccines and immunoglobulin treatments has

been quite heavy over the last couple of years, such that that too has impacted the pace of the research activities.

But that said, I'd like to tell you a little bit about three examples of our ongoing collaborations that in spite of everything have been productive and enjoyable.

The first example comes from the lipidomics arena. Mustafa Akkoyunlu has had a long-running collaboration with colleagues at NCTR based on Mustafa's longstanding interest in a phenomenon that's been known for a very long time, but is still poorly understood. And that is that infants respond rather poorly to polysaccharide-based vaccines. For encapsulated bacteria, the polysaccharide capsule is often a major immunogen and a major source of protective responses, but infants, unlike adults, respond very poorly when inoculated with polysaccharide alone.

In a practical sense, this problem has been addressed in part by polysaccharide protein conjugation. Nonetheless, this is not always applicable and useful, and so the mechanisms underpinning the poor response of infants remain of wide interest to us and many others. One of the observations that Mustafa has worked on for some time now is the fact that in vitro, neonatal macrophages that are cultured in neonatal sera develop a very obvious anti-inflammatory phenotype. They also form these rather

dramatic-looking large lipid bodies. So Mustafa sought out colleagues at NCTR to understand the nature of these lipid formations, because NCTR has expertise in lipidomics, and that is not a strength of ours.

So the results to date suggest that in fact neonatal serum itself does dictate early innate immune responses, and it also dictates the uptake of lipids; how those lipids are metabolized once they enter the cell, and how they are broken down. And that in turn impacts the ability of macrophages to present antigen and to perform a number of other innate immune responses, including cytokine production, that then impacts the character of subsequent in vivo immune responses. So this observation is developing mechanistic information that we hope will be useful in both understanding the poor responsiveness of infants to polysaccharide-based vaccines, but then doing something about it.

In the microphysiological systems, Kyung Sung has had a longstanding collaboration with Noriko Nakamura. Kyung is interested in MPS systems, and particularly in a human placental barrier model, but one of our areas of gap is development toxicology per se, which is what NCTR knows much better than we do. So this joint project is using the microphysiological placental model to evaluate the toxicity

of drugs on immune functions. This is in comparison to in vivo work.

This is both an example of exploiting the MPS systems under study, but potentially leading to an alternative to animal testing down the road.

In the genomics arena, Zhaohui Ye is our go-to guy for CRISPR-related technologies and systems, and he has been studying off-target effects of CRISPR-based genome editing. This can obviously be a safety issue for CBER-regulated products when CRISPR systems are worked into the product world, which we certainly expect.

NCTR has expertise in applying NGS to toxicology-related issues, so this was a natural collaboration as well. So far, this collaboration has uncovered a very interesting off-target base editor effect that appears to be independent of the better known CRISPR-Cas activities of the constructs. So this is being actively pursued as a particular problem for future products, but as an example of what to watch for and how to apply these products and how to monitor their safety profiles in people potentially.

These examples were chosen just to highlight existing areas, but I think we have a number of topics on which we could do each other some good. We are not toxicologists, and you are, so everything related to toxicology I think is fertile ground for our interactions,

and especially reproductive toxicology. Many of our biological products either are already used during pregnancy or would like to be used during pregnancy, but as you well know, this is a challenging area and so I think this is fertile ground for our interactions.

As already mentioned, we do not have in-house expertise in lipidomic or metabolomics, so this is a good area overall, and then you are all working on a number of in vitro cell culture alternatives that are of interest to us. The airway model was mentioned, that's also an area in which we have a mutual interest, not only for safety but also for the efficacy evaluations and mechanisms of action.

Not surprisingly, I think we can mutually leverage our complementary expertise to support the evaluation of our products, particularly aspects related to safety but perhaps not undersell the mutual interest in efficacy in our mechanistic studies. Thank you very much, and I'm happy to answer any questions, if I can.

DR. ASCHNER: Thank you, Dr. Elkins. Are there any questions from the Scientific Advisory Board?

DR. ELKINS: I see a hand. Dr. Lanza?

DR. LANZA: In this lipid area, are you looking or are you involved in the inhibition of acute neutrophil activation with specialized pro-resolving mediators like protectants and resolvents? Is that part of your scope?

DR. ELKINS: That product line is not part of CBER's mandate. I believe it's in the Center for Drugs. Our research programs are for the most part closely aligned to the products for which we are responsible, so that is not one of our areas of interest currently.

DR. LANZA: Thank you.

DR. ELKINS: Dr. Tropsha.

DR. TROPSHA: Thank you for a very comprehensive presentation. I think you mentioned you have about 15 ongoing projects with NCTR. I'm wondering if you care to review the historical connection between two units -- what's the dynamics of the joint project development, and what's the mechanism of generating new joint projects?

DR. ELKINS: I am probably not the best person to speak to the history, since I'm relatively new in this particular role, for about a year, but it's my understanding and from what I have observed in the last year or so, I and the NCTR leadership have ongoing conversations about the work going on in our respective centers. NCTR will send us specific projects for review and outreach, and we in turn bring specific projects to their attention, and we look actively for areas of overlap where we can help each other.

There are also several programs within FDA, as a larger agency, such as the shared resources program, where

we are aware of each other's instrumentation capability, especially, that comes into play and can also uncover a particular project-related area. Does that help?

DR. PATTERSON: I would like to chime in really quick there for Alex's question. Alex, what we do is we solicit comments from the various product centers. When we receive either a concept paper or a protocol within the Office of Research, we look at which center it best aligns with, and then also if there's already research collaborators; a lot of times our researchers will have already reached out to the researchers at the product centers. So if we have collaborators, it just makes sense that it goes to that product center for review and for comment, and we apply that input back. So it's kind of like a peer review manuscript process where there's back and forth with tweaking, especially with the methodology, and trying to finetune the research project with the particular product center that it best aligns.

DR. ELKINS: That was a much better explanation than mine. Dr. Cosenza.

DR. COSENZA: Hi, thanks. Great presentation. Just a quick question, you mentioned interest in reproductive toxicology. I was just curious whether there was a particular class of therapeutics or compounds that you were --

DR. ELKINS: Vaccines. So vaccines are always I guess of a different standard than some of our products because they are unique in going into healthy people, and going into healthy pregnant women just ups the ante that much more. So that's probably the leading example.

DR. COSENZA: Thanks. I would have guessed that, but I just wanted to get confirmation.

DR. ELKINS: Right on the money.

DR. ASCHNER: Any other questions? If not, thank you very much, Dr. Elkins. And we are going to move to the next presentation from the Center for Drug Evaluation and Research, and the presenter will be Dr. Peter Stein

DR. MENDRICK: I don't see him signed in yet. I did alert him we're running early.

DR. MARGERRISON: I am here, I can go next if that's alright.

DR. ASCHNER: So I'll move to Dr. Margerrison. And he will present the Center for Devices and Radiological Health. And then we'll come back.

Agenda Item: Center for Devices and Radiological Health

DR. MARGERRISON: Good morning, everyone. I'm going to take a slightly different tack from the one that Karen did. It's really a little bit of an extension from last May, when I last spoke with the board, about some of

the progress that we've made in that. And I'm going to just use one example, which is one that we've been discussing with Tucker for the last couple of months or so. So I think it represents a really important step forward for CDRH and for the way that we work with NCTR.

But before that, I'm going to give you a little bit of an update on where we're at, broadly, with our overall tools program. I think I've met you all, most of you, last time we were in Arkansas, but I'm the director of Office of Science and Engineering Labs at CDRH. CDRH, as I've probably explained before, is responsible for the devices, radiological health, and in vitro diagnostic side of the FDA, so as you can imagine it's also been pretty busy at CDRH in the last couple of years.

We've had somewhere around 5,000 pre-EUA applications, and well over 5,000 EUA applications relating to the pandemic. Those have involved things like PPE, masks, and suchlike, of course all the in vitro diagnostics, and ventilators comes under our wing as well. So we again have been hit pretty hard by a lot of these things.

We've actually managed to maintain capacity pretty well during this, mainly because -- and this is exactly the same as Karen's colleagues in CBER as well -- that we've discovered that our reviewers are all

superhumans in not very good disguise. They've been amazing, they really have been working around the clock to try and get things up and running again.

As a center, we regulate just over 230,000 types of medical device. I think we're close to a million SKUs right now, and the breadth is very large, as I've said to you all before. My part of the organization is involved with about a third to a quarter of the premarket reviews that we do as a center, which is in excess of 3,000 a year now. We have about 160 federal employees, and when we spoke last year in May, we'd recently realigned our research into specific program areas.

We're very, I think, a little different from some of the other centers. We no longer have a PI-driven model for our research. We now have 20 program areas that have very large objectives, and we unleash the program teams then to try and solve those problems. So it's a little different from the historical way that things have been done in FDA.

Like with all the other centers, we make lots of presentations and publications and things like that. We have a very large facility that is partly empty right now. We've had about probably 50 people a day tops in our facilities over the last couple of years. It's remained actually a very safe working environment, and I'm very

proud of my staff for doing that. Typically, we find that people will go in to do their lab work and then go to their home office to do the analysis and reporting. So it feels a little empty, even though there's up to 50 people a day coming in and out. I was in last Friday, and the whole campus still seems a very strange place, but the geese are happy, but everyone else seems to be a little bit socially distanced, to say the least.

Most of our folk, again, like with CBER, are half research and half regulatory review. And a lot of what we've been doing over the last two years is to make sure that the balance is correct for people. It's good for our researchers to get involved in product reviews, because they really understand what products we're dealing with, and I really enjoy trying to get as many reviewers as possible involved in the regulatory science, because I think there's a great long-term investment there that I want to take you through a little bit. And it also is very good for our reviewers to understand what are the new technologies coming forward, because that's one of the fundamental functions of my part of the organization is to do technology planning and predict what technologies are coming through, and to make sure the center is ready for those.

With devices, I think it's a little easier, because devices tend to evolve. You don't get the big things that are more like a genetic shift rather than a genetic drift. Ours tend to be much more gradual. Things get better and better. And that actually means that it's a slightly different benefit-risk ratio that we have to look at sometimes as a center.

Regulatory science programs, I think I shared this list with you before. The main couple of takeaway points from here is that some of our programs are product-based, so for example ophthalmology, orthopedic devices, cardiovascular. Others are more technology-based. So that would include artificial intelligence, machine learning. A lot of the work that we do in that area, for example, relates to diagnostics, particularly in medical imaging and digital pathology sorts of areas, because that technology is a fundamental one for all of those products.

I was very surprised a year or two ago to learn that the first diagnostic device that we cleared as a center which had something like artificial intelligence/machine learning in it was actually in the 1990s. I know it's something that Greg referred to earlier on, and I think it's a fascinating area. Very interesting for CDRH, because we not only use AI/machine learning and adaptive algorithms and the rest of that great science for

helping us from an infrastructure perspective, it's actually built into a lot of our devices, and we recognize different types of those. For example, software as a medical device, where the software actually is the thing that we regulate, and we also have software in medical devices, where it's an intrinsic component of the final device.

One change over the last year is that we've actually retired our regulatory science program in nanotechnology. That may come as a bit of an eyebrow-lifter for many of you, because clearly there's a very active and successful nanotechnology program within NCTR. But from the CDRH perspective, we actually don't have any reg science questions that we want to prioritize at this point in time. One of the reasons for that is that in devices, if a sponsor does not say in their premarket application that their device has some component of nanotechnology and they're making a claim based on that, then we don't even ask. So it's not something that comes up day by day for us at all at this point in time. Different for other centers, of course, but I think it’s important for me to address why we've retired that particular research program.

Obviously, I'm happy to answer questions on the details of any of these programs.

When I last spoke to you, we'd been really pushing forward with our regulatory science tools, as we call them. I want to put these in perspective, because it leads me up to one of the collaborations that we're putting in place with NCTR right now, that as I've said is going to be enormously important.

We recognize that there's a variety of different ways of evaluating and assessing the effectiveness and safety of a medical device. What we're trying to do, broadly, is to standardize that approach, with a small s, so that a novel, innovative company who are really much more of a startup don't need to waste their time in developing both a technology and a product as well as the science of how to evaluate it. That's massively important for them, because that effectively will halve their workload.

We have had a lot of discussions with the NIH over the last couple of years, and they're at a stage now where they really get this. They understand it extremely well. To the extent that we are now working with a current count of four of the component institutes of NIH who are directly funding the development of medical device development tools and regulatory science tools. I'm going to explain the difference between those in a minute. But what we're aiming at is to try and develop testing

methodologies or things that will help early innovators, to get them going at a slightly faster pace, to keep them alive for a little bit longer so we can play the probability game, and get more of these products out. And also to standardize the testing to some extent.

Not only does it reduce the burden on early-stage companies, but it allows them to benchmark their technology or their product early, which allows them to de-risk things very early, and also means they can go to potential investors with something that is tangible. Because if they can say to a potential investor that they've evaluated their product or their technology on an FDA-endorsed or blessed methodology, that actually will give the potential investor a significant degree of confidence that they might well invest in them.

Again, we keep more of these people going. That's one of the things that's very important in the device world. Most of the innovation comes from small companies, not from the large ones, and so part of our mission at CDRH is to promote that. The way that my part of the organization promotes that is by trying to keep as many of them alive as possible for longer, so that we play the probability game.

We're trying to give them some tools they can use, and these are voluntary, of course, with things like

virtual and physical phantoms, which are used an enormous amount in the medical imaging and diagnostic space. A great example of one that we developed there is a phantom that's used in photoacoustic imaging, which is a relatively new technique used for imaging, fairly deep imaging, of blood vessels, so it's very good for early detection of cancers. And we developed a phantom in that area, and about I think it was last January or so, we received our first premarket application which used our phantom, and it came from a small startup company. So that was very pleasing to see.

We do an enormous amount in computer modeling and simulation and related datasets. We were the first organization, I'm sure I've boasted about this one before, first organization to publish a fully in silico clinical trial, and we publish a lot of lab methodologies, as you'd expect. Extractables, leachables, testing is something I'm going to come back to before I finish talking to you today. That is one of the absolute heartbeats of how we do biocompatibility assessment for novel devices. Incredibly important. And we really need to standardize that testing methodology so that we can properly undertake the review of the safety of those devices.

We're also pushing ahead much more with best practices now. It's kind of a policy-lite sort of approach

to things. Where we might not want to actually issue guidance on a technical methodology, we can actually still sort of say that, yeah, you don't have to do this, but this is actually the way that we would like to see it done. And we're doing that with a lot more methods right now.

Different evaluation tools that we use at CDRH. The regulatory science tools really are things that we can push out really early. There is no regulatory guarantee of any sort of guarantee of acceptance, whether they're used appropriately or not. But very useful for early-stage companies and early technology. Usually developed before things like international consensus standards can be developed, and as they grow up and get used more, I like to think that they will mature into what we call medical device development tools. These are enormously important for us, and will be a centerpiece of allowing people to get efficient regulatory clearances and approvals as well.

Difference really there is that the various sorts of methods and tools, CDRH has qualified them for use within a very specific context of use. In other words, if you use them in the right way that we recognized,, we are not going to ask questions on the way that a device was evaluated, because we've already qualified that for appropriate use. And based on that, we can just review a summary dataset rather than having to look at full study

reports. So the difference in burden for both our reviewers and also for product developers is absolutely enormous in that way.

Of course, we still have an enormous use for recognized international consensus standards. Really, what we're doing is, from the CDRH perspective, is saying that it's, as we would say in England, it's horses for courses. And as a technology is very new, there simply won't be medical device development tools or consensus standards that are appropriate. So we're trying to, in our own way, provide those in a certain way.

Why are we doing all this? As I've said, we think it's really important for early de-risking, for technology and product development. It allows us to start up, and I've run startups, and it's not a fun activity sometimes, but it allows developers to focus on how good their innovation is, not how well it's tested, and that is enormously important for the whole community that we deal with.

Much more efficient use of very scarce resources. That's very important, for example, not just for the startups, but also for organizations such as NIH. They have enormous small business innovation research grant programs that they undertake as part of the federal government, and what they want to do is really promote as

efficient an investment model as they possibly can. We think the MDDT method is a good way of doing that, because once you have developed methodologies, it really is a multigenerational gift and can be used by multiple companies when they're in their innovative early-startup sort of phase. And that's why we've actually found at NIH now is putting significant resources with dollar signs and quite a lot of zeroes on the end, towards our programs. And we're thrilled to see that, of course.

It's also important for us because CDRH is -- I know every center claims to be unique, and it's my turn now -- but the breadth of technology that we're seeing really is coming a lot from other industries. We're seeing new players in our space a great deal, so what was traditionally considered a medical device company, they all exist still, but now we've got the digital health people coming in. We've got the augmented virtual extended reality companies coming in. And they are companies that are not used to a regulatory market in the same way that we regulate. So we might have, for example, and do have, Apple and Google and people like that coming into our space, who traditionally haven't had to develop things in a medical device regulatory framework. So the more that we can help them, the better it is for patients at the end of the day, and that's what it's all about.

We need to know, for example, if something's coming, a great deal, increased use of augmented and virtual reality in this space. We need to know that that technology is sufficiently robust for a medical application, of for example, it's being used as an extended reality application in an OR during a surgical procedure. If your screen suddenly goes fuzzy, that's much more of an important event if it's in an OR than it would be on a gaming console.

Overall, these common methodologies, overall drive predictability through our whole process. So as you can see from the CDRH perspective, we've really defined startups in my part of the organization as our key customer, and we're trying to make a lot of these things as public as we possibly can. We've now pushed out well over a hundred of our reg science tools on the public-facing website, and we see that as expanding over time in a big way.

That's sort of background kind of where we're at right now. I want to give a shoutout to Tucker Patterson on this one. We've been working with Tucker and his crew to really define some of the big areas that we can address, and this is an example of one. It's a relatively straightforward project. I think we can do it -- it's hard work, like they all are -- but this potentially, one

project, I think has the potential to be more important than all the other collaborations we've ever done with NCTR. I think this is a really exciting time for the relationship between NCTR and CDRH.

Biocompatibility of medical devices remains one of the most difficult and irritating parts of premarket clearance and approval, for both industry and for CDRH. As I've said, we've got 233,000 types of medical device, and a lot of the tests that are typically needed, we start off very often with three basic tests: cytotoxicity, sensitization, and irritation. Those are three that are pretty much done on every device.

But biocompatibility remains the area where a very large number of deficiencies are having to be raised by our review staff. That clogs up the entire system. So we're trying to simplify a lot of that testing. We're trying to, as we've heard many times this morning, trying to get substitute methods that are just as good using in vitro methodologies rather than in vivo ones. That reduces the burden. Obviously it's good because it reduces the use of animals in premarket applications. We want to see all that. And it also helps, again, standardize the approach.

So we're looking very much to with NCTR, and I think we're getting toward the stage of knowing how we're going to do it and have a much better plan for specifically

addressing it, but we're looking to develop a skin irritation test collaboratively so that we can then qualify that and that means that we can put it into day-to-day use. And that's where it really takes on its regulatory significance. From my personal perspective, publishing papers is great, but that doesn't give it any sort of regulatory utility in any practical sense.

So what we're trying to do is to make them officially recognized and endorsed by CDRH in my case, as a center, which means pushing it into the medical device development tool arena. Once we've done that, it becomes an official product and officially blessed by CDRH, and at that point people are going to use it. And if they don't, they've only got themselves to blame, because we're almost giving them, we are giving them, a much more efficient route to getting their product onto the market.

The study protocol is relatively straightforward. Using a model that has had quite a lot of work done on it before, and then compare it with the in vivo site, to check it gives the right answers. And it's important to note that by the right answer I don't necessarily mean that it gives exactly the same answer as the in vivo. From our perspective, what it means is that we make the same regulatory decision; even if the specific answer is slightly different, are we going to reach the same

conclusions from a benefit-risk perspective and either grant a premarket clearance or not grant a premarket clearance and ask more questions?

So that's a very exciting area, and I did want to share that with the board. So where do we go now? We're building a regulatory science library, both for MDDTs and our own internally developed tools. Eventually, we want this to be the go-to place when any startup company wants an evaluation methodology for their technology or their product. We're expanding a lot of what we call the owner's manuals. We've learned that to use our tools and products properly, a publication isn't enough. We know about reproducibility issues in science. We need people to use our tools in exactly the correct way, so we're starting to produce videos on how to use them and things like that, which is beginning to be very useful for us.

The other thing I'm trying to do, it's a little outside what we're talking about this morning, but I think it's worth mentioning anyway, from a CDRH perspective, we need to increase our capacity to develop tools by at least two orders of magnitude. A few hundred here and there is very nice but just not enough. So I'm now starting discussions with external companies as well as NIH to start generating really significantly increased capacity, and for me one of the interesting things is that these tools

actually do have a tangible value. If they didn't then the whole CRO business wouldn't exist.

So starting those sort of discussions now and trying to catalyze the development of a market in these reg science tools and MDDTs, because we recognize that one day there will be a set of companies who develop them, and then a set of companies that write checks to use those tools or use the services of the CROs who are offering them.

With that, thank you all very much. I will shut up and be very happy to answer any questions that anyone may have.

DR. ASCHNER: Thank you for a very nice presentation, exciting times. Obviously a lot of work, given the pandemic and everything else. Does anybody have a question for Ed?

DR. LANZA: Greg Lanza. Very nice presentation, Ed. I wanted to ask you again about this situation with AI, because what's apparent to me is that your organization has the potential to have a tremendous medical impact, because AI is being used now and can be used to remove barriers that have prohibited studies like we might do here in a tertiary center, from being done in rural America, or other centers. I give you MRI as one of the ones I spend a lot of time on. The situation is that I was wondering, I'll just say one more thing about that. Clearly, some of

the barriers are the medical technologists' dependency, another barrier is the time it takes accelerating it -- so you can get more studies scheduled, so your talent is not as jammed up, but also so people can tolerate the whole thing better.

And then the amount of what we know now is important information for making medical decisions requires often post-processing that just simply doesn't happen. And many of these things are not in the equipment, but artificial intelligence and machine learning, some regular programming, could actually have a tremendous impact on moving this forward, and I just wondered how the center is looking to help facilitate this. Because people are being treated today for cancer, for instance, and they really are just using an echo, which I do, too, every week, and I can tell you, they're missing out on better medical decision-making because of these barriers.

DR. MARGERRISON: I couldn't agree more. There's two areas that leap to mind, Greg, and I'll also just mention the working group that Donna actually runs across the agency that is looking cross-center at how we actually use AI and machine learning and all the other aspects of it together. That's an important part of it. From the CDRH perspective, at least, we have a strategic priority now recently published of being able to -- so what about health

equity and health equality, fundamentally? How do you drive it into the rural communities? Because not everyone lives 10-minutes' drive from a big teaching hospital. And that clearly is an inequality that's not acceptable today.

I think one of the other key driving points that goes hand in hand with this is 5G. Not because you can download your video faster, but because you can do things in real time remotely. And Huawei showed this about three or four years ago -- they used a, I can't remember which surgical robot, but one of the well-known ones, but they actually did a liver procedure in real time remotely. And the latency was only 300 milliseconds from a surgeon who was 100 kilometers distant from the patient.

So that's another thing. It's AI-machine learning, absolutely, in all its flavors, is going to be massive for things like computer-aided triage, if people are in rural environments, all those good things. And I think the information flow and the credibility of the information and validation of the information over long distances is going to be really important, too.

So I couldn't agree more. We could probably talk the rest of the day about it. I think when we have these meetings back face to face, we will do exactly that. I don't think we've got any great solutions right now, but it is becoming increasingly important that we know we've got

to find the right solutions for it. There's no doubt about it.

That's kind of the best answer I've got for this morning, Greg, when literally we could talk about this for hours, you know that, and we had that discussion before. The best answer I've got for the board this morning is that it's one of the three key pillars of the new CDRH strategic priorities, so at the very least we know we've got to get this right, and we've recently formed a lot of specific plans within CDRH for addressing it. It has to go hand in hand and be built into all the other medical technologies that are being developed, as well. Does that help a little bit?

DR. LANZA: It does, and I just have one other brief follow-up question, and that is that if you allow AI to do a multistep process, you have to assume that it always does it right. So one of the things that we do is that we actually have breaks at every key point that a medical technology -- say, segmentation, that it's actually segmented right before it goes and analyzes it or something -- so that you get into a situation from approval that if you had these checkpoints, then you have a way of getting without it all having to be perfect, particularly if there are multiple steps. How does the center look at segmenting the process, versus --

DR. MARGERRISON: One of the ways we are addressing that is that one of our research programs, I don't know if you noticed it, was in closed loop monitoring systems, which is effectively what we're talking about. And that really grew out of the debacle that is the alarms situation in every hospital in the country, that there's alarms going off every two seconds, and a lot of our closed loop monitoring system came from that, of understanding when you can let something go on automatic, and when you need either a checkpoint or a manual intervention, and these things are all very tied together, actually, in terms of the decision-making processes that you're having to undertake. So we're looking at it, all of these things are kind of multiaccess problems, they're fascinating, makes them very difficult to solve, of course.

It also leads into things like adaptive algorithms. Right now, we know -- at least no one's told us -- that a lot of algorithms are being adapted in real time. We don't think there are. They tend to be more like bulk updates from the manufacturer. They tend to be bulk updates, so we know about that. But it introduces similar parts of the decision-making process, such as when you need to revalidate a whole software. Is it a slight tweak? Does that count as a slight tweak, or is that a sufficient change to need a full validation?

We're going through that, and that's one of the reasons that at CDRH we established the Digital Health Center of Excellence, because this is a classic example of a team sport, if ever I've seen one. We can't look to just FDA to have these answers. We need to get involved with academia, with of course the physician community, and most importantly, the patients, because they're the people we're really all serving. But we're taking it massively seriously, that's the short answer.

DR LANZA: Thank you. We could talk, as you say, all day.

DR. ASCHNER: Thank you, Greg, and Ed. What I'm going to do next, actually, I'm going to -- Peter, if that's okay with you, I'd like to have Jonathan Kwan present first. There's an emergency that he needs to attend to, so if it's okay with you, I'd like to move onto Jonathan Kwan. He will inform us on the Division of Tobacco Products. There is no public -- we'll have lunch after that. There's no public session. So we'll make up an hour after that. And the folks that are presenting in the afternoon have been informed.

DR. MENDRICK: You'll still have Peter present before lunch, correct?

DR. STEIN: Actually, I blocked out this time so if it would be possible, my presentation isn't terribly

long. If we could do that before lunch, I hate to stand between me and lunch. But I may not be able to come back afterwards.

DR. ASCHNER: No problem. Let's do Jonathan first, because he has to rush out, and we'll do Peter after that, we'll be 10 minutes late for lunch. Hopefully everybody's agreeable to that.

Agenda Item: Center for Tobacco Products

DR. KWAN: Thank you, Peter, for being very flexible, and everybody else, I appreciate the accommodation here.

Hello, I'm Jonathan Kwan, with FDA's Center for Tobacco Products, or CTP. I serve as the team lead in the Research Operations and Advisory Resources Branch in the Office of Science. Thank you for the opportunity to talk with you all about our center and our research programs, as well as how we are collaborating with NCTR to address some of our research gaps.

Unlike the other FDA centers, who typically look at regulated products against a standard of safety and efficacy, CTP evaluates tobacco products using a public health standard. That is, we consider the population-level impact to both users and nonusers of tobacco products. CTP assesses the health risk of tobacco products and how the tobacco product is being used.

We also consider the likelihood that existing users of tobacco products might stop using tobacco products and look to understand the likelihood that nonusers might initiate tobacco use. And this is important when we think about youth and young adults who are particularly susceptible to tobacco product use.

We also want to look at former tobacco users and their potential to relapse. All these considerations come into play when we think about tobacco regulatory activity.

To make sure we're all on the same page of what tobacco products CTP regulates, in 2009 the Family Smoking Prevention and Tobacco Control Act gave CTP authority to initially regulate cigarettes, cigarette tobacco, roll-your-own tobacco, and smokeless tobacco. In 2016, Congress passed what we call the deeming rule, which allows CTP to regulate all products that meet the statutory definition of a tobacco product, which include the components or parts of those products. This rule essentially allows CTP to regulate electronic nicotine delivery systems, or pens or vaping products, cigars, pipes, hookah, and other tobacco products, to include future tobacco products that may come from the ever-evolving tobacco landscape.

Speaking of changing landscapes, there have been an increasing number of ENDS products that use or claim to use synthetic nicotine, instead of nicotine derived from

tobacco. The manufacturers' deliberate attempt to evade FDA regulations in this manner are concerning, particularly with regard to potential ENDS use in youth. So on March 15, 2022, President Biden signed a bill that amended the tobacco chapter of the Food, Drug, and Cosmetic Act to bring synthetic nicotine, and for that matter, nicotine derived under any source, under FDA's tobacco statutory authorities.

CTP has five strategic priority areas that we focus on. Those include product standards, which is about advancing a strategy that yields strong standards to improve public health and that can withstand legal challenge. Along these lines, CTP issued earlier this month two proposed product standards that would, one, prohibit menthol as a characterizing flavor in cigarettes, and two, prohibit all characterizing flavors except tobacco and cigars.

Our second strategic priority is around a comprehensive FDA nicotine regulatory policy. What this really means is that we are focused on establishing an integrated FDA-wide policy on nicotine-containing products that is public health based.

A third strategic priority is focused on pre- and post-market controls that are looking at regulations and product review. To date, the work here has been around

exploring developing rules and guidances for product review pathways, tobacco product manufacturing practices, and analytic test validations.

Our fourth strategic priority is compliance and enforcement. This includes inspections, investigations, monitoring and other review activities, and also includes taking enforcement actions when appropriate and when the evidence supports a violation to the guidelines, rules set forth in the Tobacco Control Act.

And finally, our fifth strategic priority area is public education. We have an entire office, the Office of Health Communication and Education, that focuses on educating the public, particularly at-risk audiences, on the dangers of using tobacco products. I've provided a link at the bottom of this slide that provides a bit more information on our strategic priorities, and I encourage you all to visit the page to learn more about our work, especially if you have young children. CTP has developed award-winning materials aimed at educating youth about the dangers of tobacco use.

The research priority areas that CTP focuses on has been narrowed down to eight priority areas. I won't go into depth on each of these, but there is a link at the bottom of the slide that you may visit to find out what falls under each of these areas. That said, I will note

that the areas of addiction, toxicity, and health effects have all been areas of research where we have collaborated with NCTR in the past.

In order to address these research priorities, as you can imagine, it takes a number of disciplines and scientific expertise. At CTP's Office of Science, we have scientists that have backgrounds in product science, nonclinical science, health science, and population science, and this shouldn't be too different from how the other FDA centers function, using a broad and scientific approach when conducting product review and addressing outstanding research questions.

Similarly, how other FDA centers function, CTP uses scientific data to inform its priority areas and regulatory activities, whether that is through compliance and enforcement, rulemaking, application reviews, or creating our communication and education campaign.

And because the science is so important for the CTP mission, we have been investing dollars into a number of tobacco research projects since 2010. Unlike some of the other FDA centers, CTP does not have its own laboratory, so most all of our work is done in collaboration with various partners, whether it be another federal agency or through a contract, or with non-HHS

organizations that have a particular expertise that can help to address a longstanding research question.

I'd like to take a quick moment to highlight a particular area of collaboration and that is with NIH. With NIH, we have established a partnership known as the Tobacco Regulatory Science Program. And in this partnership, CTP provides research dollars to NIH, and NIH then puts out funding opportunity announcements specific to tobacco regulatory science. These are NIH grants and managed by NIH, and they do have the rigorous NIH peer review process, but the dollars that are used to support this research come from CTP.

The second collaboration we have with NIH is the Population Assessment of Tobacco and Health study, or PATH study. This is a large longitudinal cohort study in partnership with NIH's National Institute on Drug Abuse, or NIDA. In this, CTP dollars are funding a NIDA contract that then oversee and run this large study.

This graph here is meant to give you a sense of where the CTP research dollars go. In fiscal year 2021, the majority of the research funds did go to the NIH tobacco regulatory science program to support the research portfolio I just mentioned. We have also conducted research through the FDA CERSI mechanism, interagency

agreements, and performance agreements, which includes our collaborations with NCTR.

The research projects funded and active in FY 2021 covered all our research priority areas of interest. Toxicity and carcinogenicity was our number two most coded research domain. It is important to note that a project could be coded for more than one research domain, so a project could be coded as both toxicity and health consequences, and then having this level of information though does provide us with a sense of where our research dollars are going and highlights areas where we are perhaps most active.

Now you have a sense of CTP's research program and our tobacco regulatory science interests and collaborations, I'd just like to take the next few minutes to briefly discuss some of our ongoing collaborations, specifically with NCTR, so you can get a sense of where we are currently working with the center.

The CTP has research interests that certainly align with NCTR's mission and focus and expertise. As I mentioned earlier, we have ongoing collaborations around toxicity and addiction. In addition, we have done work in the past with NCTR, and again now, around informatics. Although this work hasn't really fallen into a specific research priority area that we've noted, it is certainly an

area that we are interested in. Informatics is incredibly important in supporting our regulatory mission and activities around product reviews.

When we look at the work that we're doing with NCTR now, we have been looking in three different buckets. We have an inhalation group, a group that is looking at ALI modeling, and a group looking at the informatics piece. I'll briefly touch on the active collaborations we have in these three areas.

CTP and NCTR have a number of research projects currently active in inhalation group. This one is on pharmacokinetic analysis and nicotine in a rodent model. The purpose is to establish PK data on the distribution of nicotine exposure across three modes of delivery, including intravenous, oral, and inhalation. The data from this is meant to help inform the dose response of nicotine in blood and other organs, to help us understand toxicity, and build out a model that hopefully will help perform product application reviews. The PK analysis has been completed but there's still some telemetry analysis and some other work that is ongoing.

We have also a pilot study in a rodent model looking at not only nicotine inhalation across five days. The pilot was set up to help identify the dose tolerability and assess the possible adverse events from the increased

nicotine exposure. The in-life work has been completed, and data analysis is ongoing.

Finally, as relates to the inhalation work, we have worked to develop a simulation of a tiered inhalation exposure system to deliver equivalent aerosol flow rates to all exposure ports of an exposure chamber. The hope is that these simulations will inform the optimization of the inhalation exposure chamber, so we can decrease the methods development phase of new studies looking at inhalation models, to reduce the time and costs associated with this type of work.

We hope to be able to use this information for current and future tobacco products to include both combustible and noncombustible tobacco products. The work is ongoing as the team continues to refine the model to try and identify the best parameters for the aerosol flow rates in a nose-only inhalation system.

Next I'd like to talk to you very briefly about our ongoing collaborations with NCTR on the ALI modeling work. The purpose of this study is to validate the in vitro exposure system in establishing what we hope to be reproducible and repeatable generation of aerosol from the machine itself that produces the smoke and aerosol, and then having a set model to look at genotoxicity and cytotoxicity. The system, while it is up now and on track,

I will say that this project has had a significant delay due to the COVID pandemic, and much like the inhalation work, this ALI work has the potential to be very helpful to CTP to evaluate current and future tobacco products.

And finally we have the informatics collaboration. This project is focused on using artificial intelligence and a natural language processing model to develop deeper search capabilities within a body of text. This work is meant to have a new search tool that will enhanced review capacity by increasing the speed and accuracy of analyzing tobacco product applications and supplemental data sent to CTP to help inform regulatory decisions. You can imagine the thousands pages of documents we receive, so having a tool to help us more effectively and efficiently search through them is quite beneficial. This model is being done with assistance from the various scientific disciplines at CTP.

I'd like to note that, to date, CTP-funded projects have resulted in more than 1,500 peer-reviewed publications and have been cited in almost 16,000 unique publications, many of which are collaboration with NCTR researchers serving as the lead author.

Here are a few of the more recent CTP-funded NCTR publications, just to show that these collaborations are

really having an impact on tobacco regulatory science and CTP's regulatory activities.

As I come to the end of this presentation, I do want to briefly touch upon three potential areas for future collaboration between CTP and NCTR. These areas do focus on the inhalation toxicity work and the ALI modeling about which we spoke about earlier. Being able to look at flavors in tobacco products, whether it is in the e-liquid themselves or perhaps after they are heated and aerosolized, depending on the heating system, different chemicals, the constituents, some of which are harmful or potentially harmful, may be released in the aerosol.

Synthetic nicotine, as mentioned as well, is something that's on our radar. So these are just a few areas where CTP hopes to continue conversations or research collaborations with NCTR that will meet both CTP's and NCTR's mission statements.

I do want to take thank all my colleagues at both CTP and NCTR. I would be remiss if I didn't mention the liaison team for the two centers listed here. these projects would not be possible without their support and guidance.

Here are a few of our key outreach points for CTP should you have follow-up questions or comments after today's meeting. And with that I thank you for your time

and the opportunity to share a bit about CTP's research program and our collaborations with NCTR.

Again, thank you for accommodating my emergency here as well. I appreciate pushing me ahead of the schedule. I'll take any questions if there are any.

DR. ASCHNER: Thank you, Dr. Kwan, for your presentation. Are there any questions for Jonathan?

Let me ask a question. I've actually heard it through some of the presentations. I fully understand the move to in vitro and NAMs, but how do you go about making sure that the in vitro data that you are collecting on interface between e-cigarettes and different cell lines are actually extrapolatable to human data or animals?

DR. KWAN: I would have to ask some of our subject matter experts in that area that specific question, but I will say a lot of this is a starting point for some future research as well. I'll just leave it at that.

DR. ASCHNER: Okay, thank you. Any other questions for Jonathan?

Okay, we'll let you go, Jonathan, we wish your daughter the best.

DR. KWAN: Thank you very much.

DR. ASCHNER: We are going to move now to Dr. Peter Stein. Peter comes to us from CDER, Research Needs and Activities is the title. Go ahead.

Agenda Item: Center for Drug Evaluation and Research

DR. STEIN: Thanks, and sorry to be standing between you all and lunch. CDER is an organization that obviously is responsible for evaluation of drugs. Our mission is to provide safe and effective drugs to the American people, but we're a large organization, and what I'll present is information from a number of our different component parts of CDER, all of which work with NCTR, as well as other research organizations, which I'll say a few words about.

The work we do with NCTR, with others, as you'll see, overlaps across the offices, so we collaborate across our super-offices within our center, and then externally as well. Of course, and I'm sure this is familiar to you, the work we do, we've sort of refer to it as regulatory science research. It really just means it's research that directly is applicable to how we can regulate and provide direction to sponsors developing drugs. So it really addresses specific knowledge gaps and drug development tools, models, and ways that we can interpret safety and/or efficacy data. Obviously, it's focused on our current needs, and it has outcomes that we hope have a direct regulatory applicability to specific programs or classes of drugs being developed, or specific safety issues that we see

across classes, or sometimes within a class. But the intent is to develop tools and approaches that help us to understand and evaluate drug safety and efficacy.

Let me go through some of the overarching CDER research. In terms of the CDER research goals, it really emerges from what I just said in terms what our research overarching goals are. First of all, of course, it's to develop and improve scientific approaches that aid in developing new drugs and evaluating their safety before we approve those drugs. So this looks at in vitro and in vivo methodologies, ways to apply and evaluate safety aspects, whether cardiac safety or hepatic safety, or safety -- dermal safety, a whole range of different things -- tools that can make those assessments more efficient.

There's been, for example, huge inroads in how we can evaluate cardiac safety with increasing utilization potentially of in vitro methodologies, that therefore can spare clinical trials and make programs more efficient and potentially give us even better information.

Of course, we want to improve the scientific approaches to enhance safety of marketed drugs as well. We want to be able to figure out how the huge information that occurs in the use of these drugs after they're marketed can be harnessed and understood to be able to help us make regulatory decisions. Manufacturing quality, our ability

to improve the way we both manufacture, test, and surveil, to make sure that the drugs continue to be of the highest quality.

We also want to facilitate patient access by developing more and efficient and rapid methods that can assess this ability to compare and enable the approval of generic drugs and biosimilars, generic drugs, we've had experience for a number of years, biosimilars is a newer program over the past years, and we're continuing to learn how to enhance our analytic capabilities and understand these biological and more complex molecules better and better to be able to make good regulatory decisions about them and obviously hopefully to enhance patient access by addressing costs.

Of course, COVID has taught us certainly that we need to enhance our readiness and our ability to create the right infrastructure, collaborations, and develop the right tools to be able to address a rapidly emerging public health threat such as COVID, but many others that can occur. We need to be prepared as an organization to be able to develop drugs that can address those threats.

What I'm going to do is in the next few slides talk a little bit about some of the work within each of the super offices, those are the big units within CDER. My office is the Office of New Drugs, and I'll just say a few

words about that, and then I'll talk about the other super offices, the other super offices kindly shared their information with me, and as you can see, our focus has been on the use of ORISE, those that do specific projects in collaboration with our divisional clinical staff and other staff within our organization, as well as our outreach into our extramural research projects, including work with NCTR.

The Office of Generic Drugs has a number of areas of focus, and I think you'll see some overlap between our areas of focus, and I just wanted to say that even though I'll show you overlap, what I can say is we work very closely on these things with our colleagues in the Office of Generic Drugs, the Office of Product Quality, and in my office, so that we're working integrally, and then with our external partners such as NCTR.

Nitrosamines is an area of course of terrific interest, I'll be talking about that theme throughout, and so we want to gather increasing information; we have already a lot information about these impurities, but we need to know more and more about how to screen them and how to evaluate them. NCTR has been working on a number of very important avenues in this way, already has made progress, but there's a lot more that we need to know, the ability to be able to evaluate specific nitrosamines for their risk, be able to set the appropriate specifications,

and to also communicate to industry how those specifications should best be set and what kinds of screening tools and evaluative tools they need to put in place.

NCTR has also worked with OGD on the FDALabel, which has been a very important way of providing access to information and thereby making our reviews more efficient, as well as the use of the Smart Template, which has been enabled through work with NCTR that's really helped our Division of Pharmacology and Toxicology Review. This allows us to put information into a standardized format which can be then extracted into databases, searched.

One of the things that we are constantly challenged with across our entire organization, and I expect this is true of other centers, is we have a tremendous amount of experience in information, but managing that information and being able to search and extract it when necessary is an absolute critical piece, and knowing similar information from, for example, an impurity that we might have seen years ago when an evaluation was done, or what safety profile has been seen with a particular class in a particular target. Those kinds of information that we can readily pull is critical, and endeavors such as Smart Template really enable that effort by putting this information in our reviews into an

extractable type of database that can be searched and then pulled to enable our future regulatory decision-making.

Turning to our Office of Translational Science collaborations, there's been a number of efforts working closely with NCTR including statistical tools for sequencing. Obviously this is really critical for enabling precision medicine, has been so impactful in oncology, but it's been impactful in rare diseases and many other areas, as well. We are always looking for improved ways that we can evaluate safety, and drug-induced liver disease is a significant concern, and we've done better and better over the years at identifying early compounds that have that risk, but more and more information and more efficient ways, including in -- I'll talk about microphysiological systems in a bit, moving right to harnessing information that might occur from real-world evidence on comparable structures or on the same drug can be very helpful.

AI models to support review activities, trying to use machine learning to be able to find patterns in what we've seen, particularly as we have big data, for example post-marketing and in other areas, can be exceedingly helpful.

I've talked about the tools such as FDALabel and IND Smart Template, which is also used in our Office of Translational Sciences, and then of course other

collaborations have also occurred. One, in terms of patterns through using AI, looking at repurposing of drugs for diseases, working with NCATS along with NCTR and OTS, as well as looking at diversity differences, racial differences, that relate to delivery of critical care to patients with heart failure.

Turning to our Office of Product Quality, I've just provided a range of different programs and projects that have been ongoing, looking at PEG toxicity. This is something that's used to solubilize as a linker to a wide range of products and understanding the toxicity of PEG in different PEG moieties is quite important. Evaluation of neurotoxicity, of course that's something that a key issue for many drugs that we have to be concerned about and have to be able to have efficient ways that we can evaluate.

Nitrosamine, I'll come back to that. Nanotechnology. Cardiotoxicity, I'm going to come back to that as well, as well as reproductive toxicity, are all collaborative areas between OPQ and NCTR.

In terms of future areas, again a wide range of things that really can be on the launchpad for further collaboration, toxicology of biological product constituents, the cardiotoxicity evaluation including drug impurities, looking at how bioinformatic studies to look for potential risks related to manufacturing changes. We

know that with biological products, unlike small molecules, there are small changes that occur over time and looking at understanding how those changes might impact safety or efficacy is a critical area, how much change in a particular aspect of a biologic molecule matters or will lead to anything that's clinically relevant, that's really an important area to understand.

Novel impurities, again, the theme of nitrosamines keeps coming back, and the ability to look at, be able to predict adverse events, looking at the large database that we have.

In terms of, again, within my office, just pulled two areas of collaboration that I think worth mentioning, but again it comes back to a theme that I've mentioned several times, which is nitrosamines. The first was looking at a collaboration that looked at doxorubicin, a chemotherapeutic agent, and the toxicity on stem-cell-derived cardiomyocytes, and this is really part of a larger effort to be able to develop in vitro methods that might reduce the need for in vivo clinical studies, but also find early signals of cardiotoxicity that could be exceedingly important in understanding for the safety of drugs that we might approve. And again, this is one approach to reducing the need for animal models, and at the same time the

potential to have an even more sensitive system to detect this risk.

I mentioned that this is around mutagenicity, the project looking at mutagenicity of nitrosamines. It's obviously rather a clinical issue, and the idea is to find better in vitro methodologies, as well as potentially in vivo shorter term methodologies that can detect the carcinogenicity risk -- predict, I should say -- the carcinogenicity risk of specific nitrosamines. And this is going to be essential in being able to inform what appropriate specifications there are as well as determine when we need to ensure that a product has essentially no nitrosamines for a particular structure.

Just again, I know I've sort of harped on nitrosamines, but there's clearly a lot still that we need to know, even though we have a lot of information. We need to figure out what the alternatives to animal model testing might be able to predict human risk. That's going to be a key value if we can find better in vitro methodologies to place in vivo requirements. We need to be able to do this -- part of the value will be conducting studies that help to correlate in vitro and in vivo animal models. Doing things like carcinogenicity studies are very time-consuming and resource intensive, but even that may be necessary so that we can begin to understand what structures within

nitrosamines predict risks, predict longer-term carcinogenicity risks. So the ability to develop these in vitro, validate these in vitro methodologies, will be essential.

One of the kind of interesting areas, I think is the potential for further development of these MPS systems, and one of the things that OND has been interested in is trying to be able to bridge between the animal data that we see, so toxicology in animals, for example, liver toxicology in animals, with whether the MPS systems are correlative with that. There's been a lot of work, and I think useful work, looking directly at human cell to look for risks such as hepatotoxicity, but of course we then don't necessarily have the correlative human data, often those drugs are stopped before they go into humans, so we do have information from animal toxicology and to correlative microphysiologic system data in correlating with the animal studies that we typically do could be very helpful, but it also could help direct us to what animals studies might be the most useful for seeing a screening in vitro test suggesting that this toxicity in a mouse model but not a rodent model, or different species model. That may be a model that we then would look to to get more in vivo information in a more focused way that we might otherwise be doing. So this kind of information to bridge

between the animal toxicology we have, and in vitro information could be exceedingly helpful.

Again, sorry to keep coming back to nitrosamine-related research, but again, there's a lot we still need to know on understanding the risks both to reproductive and developmental toxicity. Again, there's more and more information that's needed there.

We've generally been considering a weight-of-evidence approach, which means that we have to increase the amount of data we have both from in silico models, structural activity relationships and in vitro data, so that we can support our understanding of development of reproductive toxicity, as well as our risks of carcinogenicity. So those are areas, continued areas of research that need to occur.

I mentioned before already as I was talking about interactions with OPQ and NCTR, within OND we also see the need for development of an optimized in vitro mutagenicity assay for nitrosamines. This is something that's already I know projects that NCTR is working on and sort of made progress on, but this is really essential. One of the things that we really see as important is the ability to develop our database of QSAR modeling so we can basically have enough information based upon in vitro results and hopefully some in vivo results that are either completed or

may need to be done in the future, to be able to really understand and evaluate the range of impurities, nitrosamine impurities, that we see.

What would really be optimal is if in the future we have a very well-informed QSAR database where we could say okay, here's a nitrosamine, here are surrogates for that nitrosamine, and we understand that mutagenicity and carcinogenicity risk for that particular structure, and here's appropriate specifications for that. That's sort of the future status that we need to get to. A lot of information needs to be developed before we can be there, and although we do already have some models, some information from SAR that we can use, that really needs to continue to be improved and enhanced. Again, the development of in vitro and in vivo correlations to be able to really identify what are the best assays that could help to characterize the risks of nitrosamines, are really going to be essential.

And I'd add, also, need to know more about the human risk of nitrosamines, in terms of what the repair mechanisms are and any biomarkers that we can develop that might help us assess whether exposure to a particular nitrosamine is leading to changes in a biomarker that might predict human risk. Those are going to be important factors as well.

I'll stop there, and as I said, we collaborate with NCTR widely on a wide range of projects. I think at last count I think I saw over 70 different projects across CDER, with collaboration with NCTR, but as I mentioned, areas that I think are particularly important, nitrosamines that I've emphasized in a number of the slides, but also the work that I mentioned with regard to the Smart Template and FDALabel. So a wide range of different and diverse projects that we work closely with NCTR on. I'll stop there, happy to answer any questions.

DR. ASCHNER: Thank you, Peter, for the exciting presentation. Does anybody on the board have a question?

DR. LANZA: I seem to be dominating, but I wanted to follow up on your question. In particular with regard to looking at cardiotoxicity in cell culture, without recognizing a bigger organism response, because we know these are poisons, they're life-saving poisons. But the impact on the heart itself could be just mild edema, could be some changes in the stiffening of the heart, could be that it leads to some toxicity. But some of the endpoints that you would see in a cell, like even leaking out components, or other things, can't be seen and translated to the clinical implication. Plus, it doesn't take into consideration the cardioprotective approaches we're using to minimize that on the net outcomes.

So I applaud the idea that it's critical to look at these things, but I wonder if you're going to get enough information screening these sort of drugs or any new drugs simply through cells, without taking the bigger picture into consideration, the organismal, organ-level picture.

DR. STEIN: Greg, that is a great question, and what I would say is that I've probably said the same thing about a million times, because I couldn't agree with you more. But I think that's, I think we should understand is there a different role for these in vitro assays. I don't think the intent is that they're the end-all and be-all for looking at broad organ safety. It's really put into the context of a much broader range of information.

So for example, if we screen something and we see cardiotoxicity that raises in an in vitro system, and maybe we don't really see that in the animal toxicology studies, but we still have that concern, it can allow us to be more directive in our early clinical study, in our guidance to sponsors with regard to early clinical studies. We may say maybe there's additional preclinical studies we would want, more focused animal studies, with perhaps more intensive cardio evaluation of cardiotoxicity. In the clinic we might say, okay, we want to look at cardiotoxicity more carefully, we may want to do more ECGs, we may want to do cardiac imaging. So it's not that it would replace by any

means our thorough evaluation of any kind of toxicity. It's really an issue of the ability to help us direct our safety evaluations, both preclinical in vivo, and clinical, in the right way to enhance.

The other piece of it is, of course, if you see, and we believe there's good in vitro-in vivo correlation, it's also important at the discovery level for companies to be able to say this particular chemotype I'm seeing this kind of cardiotoxicity in vitro, I'm not going to take a chance of moving that drug forward, so I'm going to go with this other that doesn't share that same toxicity.

So it can be used in a number of ways, but to your point, I think it's really important to emphasize one isn't going to simply say we're seeing this, we're done, that's the only information we need. It's really the starting point, and it helps us focus the information. I'd say that's true of all of these kinds of assays.

They really help us focus, they can make very early, they can help enable early decisions. But they really help you decide what kind of monitoring and what kind of safety information you need to subsequently collect. They may also suggest that a certain exposure is a concern, and so that may also tell us we need to be very careful about how we increase dose exposures as we go forward into the clinic.

So yeah, thoroughly agree with you, we don't just look at the in vitro and say we're done.

DR. LANZA: Thank you, Peter. I just wanted to say one last point about this, though, is that what you're going to find in general, whether it's cardiac imaging or not, our ability to interrogate the problems you're finding in an animal or in a person, are limited. And unfortunately severely limited. So you may be providing insight into what needs to be achieved, whether it's with medical imaging or blood test, or so far, to get at -- what you're talking about is getting at the earliest biomarkers of toxicity, that's the point I think you're trying to make, but I don't think the other part of the equation, the clinical part of the equation, is sensitive enough to it. Or it might be, but not without more processing or more information.

DR. STEIN: And that goes into the decision-making as to whether a drug can even be taken forward in the first place. So if there's a toxicity that looks very worrisome, that may mean that drug isn't going to go further, because if we can't properly evaluate in the clinical or if we can't get more preclinical evaluation of it -- on the other hand, for example, a lot of the cardiotoxicity has been very carefully focused on channel effects of drugs that can lead to arrhythmias, because as you know that can be very

carefully evaluated, going from in vitro to thorough QTC studies, things of that sort.

There are certain findings in the in vitro that can be very thoroughly investigated in preclinical and then in clinical studies. And there are other findings that might be worrisome enough that we say this drug shouldn't go forward, or really, a sponsor is likely to say that before it even comes into the regulatory hands, because if a toxicity can't be properly monitored in the clinic, that's a real watch-out. Again, there's always a benefit-risk there, so if it's a drug for a severe cancer that could lead to rapid mortality, we may be more open to allowing some progression of that drug, but ordinarily a drug that has a toxicity that we don't have the tools to thoroughly monitor, that drug isn't going to be able to go very far.

DR. LANZA: Thanks.

DR. ASCHNER: Thank you, Greg, and Peter. Any additional questions? If not, we'll take a 40-minute break. We'll reconvene at 2 o'clock, and we have a couple more presentations from the centers. I think the first one is going to be Suzy. And as I said before, we don't have the public session, so we'll make up some time and hopefully finish a bit earlier today.

With that, have a good lunch, and I'll see you in about 39 minutes. Thank you.

(Luncheon recess at 1:20 p.m.)

AFTERNOON SESSION (2:00 p.m.)

DR. ASCHNER: Okay, we're scheduled to have a break after Suzy, but I think we're going skip it and keep going and we'll see how far we can go before we take the next break. Hopefully, a couple hours. So it's 2 o'clock Eastern time, and I welcome Dr. Suzy Fitzpatrick, and she will give us an update on the Center for Food Safety and Applied Nutrition.

Agenda Item: Center for Food Safety and Applied Nutrition

DR. FITZPATRICK: Thank you very much for inviting me today to talk about CFSAN Partnerships with NCTR to Advance Regulatory Science. I'm going to concentrate just on what we've done with NCTR, which is quite a bit.

So what's the regulatory mandate of the center? First of all, we oversee about 90 percent of the food supply. We look at direct food and color additives, food contact material, we oversee cosmetics, dietary supplements, botanicals, contaminants in food such as toxic metals. Our products touch every American every day, more than once, and Americans want a safe food supply and when we don't, you see articles in the newspaper like you've seen recently.

So it's a pressure for us to do it and part of the difficulty for overseeing much of it is that we don't

have any preapproval authority. So if we have preapproval authority like most of our medical centers, we can ask for data before a product gets on the market, but for cosmetics, for dietary supplements, botanicals, contaminants, or constituents in food, we don't have any preapproval authority, and we have to do research in order to show if there's harm.

NCTR is a valuable, valuable partner in doing that. One of our biggest programs is Closer to Zero, looking at toxic elements in infant and children's food, food labeled and commonly eaten in children or infants, and it started with looking at arsenic, but we know arsenic, cadmium, lead are there in mixtures, and we had some epi data that indicated that all of these are developmental neurotoxicants, but not enough to set safe levels or reasonably tolerable levels in foods for children. So we needed to do some research.

We were fortunate that Dr. Beland and Dr. Doerge gave us a very good platform in which to start our arsenic research. They have done a lot of research in the last few years on arsenic metabolism and toxicokinetics, and they were -- you can see the number of papers they published in the last few years. They have looked at exposure to carcinogens during prenatal life. They've looked at a lot of the metabolism, the toxicokinetics and age-related

exposures and arsenic species. So they gave us a good starting point, a very good starting point, to look at additional research.

So one of the things we wanted to look at, we said that we saw in epi studies, but in other countries that didn't match the U.S. population demographics, and which were exposed to very heavy levels of arsenic in water, we saw the indication that they were developmental neurotoxicants, but the data wasn't good on how much exposure there were. So we wanted to do a study of developmental neurotoxicity in Sprague-Dawley rats, and we did this in Dr. Talpos' group, the study designed to look at arsenic in the Sprague-Dawley rat. We used pregnant rats, gavaged them twice a day, treated the offspring up to day 21 post-delivery, and looked at doses that were not that far off from what we'd seen in our total diet study.

This is what we found. They actually did a very good job on this study. We did a lot of different -- in our preliminary study, we saw interestingly, we saw reduced water consumption because the rats could taste arsenic in the water. So we had to gavage, and the big study we saw not too many changes according to weight. Some weight changes, and then we did a bigger study where we looked at -- you could see we looked at male, female weight. We

looked at cognition, memory, and motor function, and we really didn't see a lot of differences in these studies.

And this study was really great, but this study also took us many years to complete. We started talking to the NCTR in 2017, and we just published the paper a couple of months ago. So while it's a good study, we realized quickly that we can't use it, we can't use animal studies anymore, when we find something in the food supply, because the public does not -- would not accept the fact that we have to get back to them in four or five years to tell them whether things in their food are safe, especially in food for children.

So we wanted to look and see if we could find an alternative that would give us some of the data that we wanted to see in this very excellent animal study. We got a perinatal health grant to look at the developmental and neurotoxicity of inorganic arsenic in zebrafish to see if that could be a model instead. We did some work also in our lab in C. elegans. We wanted to compare it with zebrafish, again Dr. Talpos's lab did this. We exposed the zebrafish for 24 hours with arsenic. We looked at various endpoints and, in the next slide, you can see this is sort of the protocol, and in the next slide you'll see what we found. We found initial exposure for 24 hours. We showed no obvious morphological defects. We looked at exposure

for longer periods and higher doses and found some changes; you can see them listed here.

So we did see changes in the zebrafish at concentrations that were not that far off from what we see in food, but how do we interpret this? How do we take this data and interpret it to develop a tolerable dose level for, say, infant rice cereal with arsenic or cadmium or lead, and that's the quandary that we're in right now in that we're looking at this, we're looking at what we saw in C. elegans and what we saw, what we might see in other models, and decide how can -- we need a fast, quick model, but we also need to know what the relevance of this data is to humans and children especially, and that's what we're working on now.

Another study that we're working on looking at developmental neuro and mixtures of metals is a study that is being done by the CERSI, which is in the Office of the Chief Scientist, and they're partnered with Hopkins to look at their brain organoid, and we're looking at what we can see in the brain, the brain, the neural cells, the support cells, when they are exposed to different concentrations of metals to try to understand what's going on here.

And we're trying to look at the impact of metals, mixtures, gene environment interactions, on individual susceptibility and the developmental neuro in these brain

organoids. Here again, we call on NCTR to help us. This is -- I'm a regulator, not a researcher. I can't get in all the details. It's been a long time since I had a lab coat on. So we asked Dr. Talpos to not only help us conduct research, but help us interpret research from other laboratories, and I think that's a second very valuable function that NCTR does for our center and for other centers is really supply SMEs or science matter experts to some of the studies we have in partnerships with outside stakeholders.

So this study is going on, and it's an exciting study, and we appreciate NCTR helping us with interpreting some of the things that we see.

So metals are not the only issue that we have at CFSAN. We have asked NCTR to look at tattoo inks. Now tattoo inks are considered cosmetics, and therefore, there's no preapproval authority, no real regulation of tattoo inks, and the inks that they use for people aren't really designed to be used for humans. They're the same inks you would put on your car or some other metal object or toys or something like that, but now they're used in humans, and we want to see really what's going on with these so we can have some sort of regulatory oversight of them.

NCTR has done some really great studies to look at the microbiological contamination of these tattoo inks and found that a significant portion of them have harmful constituents, that they have metals, they have microbiological things, and we also want to look at how much are the distribution of these and where they go in the body.

So those are ongoing studies that we couldn't do ourselves and which really will give us a chance to see what's going on with these tattoo inks. Remember, you can see a lot of women of childbearing age have tattoos, and quite a number of tattoos, and are they -- how are they affecting the function of their body and possibly their child's body. So I think this is an interesting and exciting work that we're doing at NCTR, too.

Remember, any product that we don't have preapproval authority over, we have to find harm in order to be able to do something about it.

The next thing that we're doing with Dr. Camacho is to look at 3D-bioprinted skin. We want to try to find a better nonanimal human-relevant skin model. So if you're looking at cosmetics and any other dermal product, the first regulatory question is it dermally absorbed. If it's dermally absorbed, then we have to do testing. If it's not, then we can stop right there. So we want to try to

find a better model than excised skin, which has been very limited to get, especially (inaudible) and costly, and also may not really be relevant to most of the population.

So Luisa is working on this study for us with NCATS looking at bioprinted skin and see if it will be a good model. We are expanding that project to look at other models besides bioprinted skin, some of the other in vitro models, and we've also started working with other centers, with NIOSH and other centers, to work on this project. So this is another one that will give us some information on what type of regulatory actions would we need to deal with dermal products.

Cannabinoids have been in the news quite a bit lately, and she's also working on looking at dermal exposure to cannabinoids in cosmetics. So this is another area where she's looking at how much -- we're doing an animal study to look at the pharmacokinetics of cannabinoids and their major metabolites in male and female Sprague-Dawley rats exposed to CBD in cosmetic-relevant formulations, oil, and cream vehicles. So that's again something we want to see how much is crossing, what's the absorption, and then depending on the level of absorption, we would look at what other toxicology tests we need, but this is a first step. What's dermally absorbed? So we're doing this one in Sprague-Dawley rats.

We also are looking at developmental neurotoxicity of cannabinoids in Sprague-Dawley rats. We're gavaging them daily with a certain concentrations and looking at the neuroimmune effects of CBD. So you can see the protocol here. This is the study that's ongoing and it's part of a bigger project that we're working on with NCTR on cannabinoids, because these are compounds that have been really of interest lately to a large segment of the population. You can see them in a lot of products in pet foods, in botanicals, in other products. So we have heard about some of the toxicities, and we're trying to decide what's going on here.

And we have heard that cannabinoids can have male reproductive toxicities, so NCTR is looking at an in vitro evaluation of male reproductive toxicities induced by cannabinoids and their major metabolites, and this is done by Dr. Chen. He is investigating the effects of CBD and their main metabolites on human Leydig and Sertoli cells. So this is an ongoing study. You can see some of this preliminary things here, data here, and this is one that we're really interested in it because of people wanting to put cannabinoids in different foods and food additives.

We also wanted to look at the fetal and neonatal toxicokinetics of the 6:2 fluorotelomer alcohol. So this was a study done in Fred Beland's lab to establish methods

and criteria for fully characterizing biopersistence of potential substances. This was paid for by a grant from the Perinatal Health Center of Excellence that NCTR and that's a really great program that gives out grant money to the different centers to look at different perinatal health concerns and this one was looking at studies, in vitro studies, PK studies in pregnant rats in parallel in vivo studies in rat and human kidney cell models to look at the toxicokinetics of these important compounds. Next they're analyzing all the data and looking at in vitro and persistence of these compounds. So that's another one that NCTR is doing for a problem that is going on in our food additive arena.

One of the other ones that's going on is brominated vegetable oil. That's something that's been permitted for use as a food additive in fruit-flavored beverages since 1970. It keeps the citrus and stuff from going up to the top of a beverage. And it's been a problem, and recently Dr. Goncalo did for us -- (audio issue) toxicity of dietary exposure to the accumulation of these brominated species in food additives, and it's really going to give us a chance to do something about this problem of what should we set a level for BVO in food, in beverages. He's planning on publishing two papers, we're about to put out some questions and answers on this. So

this was a really great study to help us again work on a problem that CFSAN has with an area in foods.

Not wanting to give Goncalo a chance to relax after doing this great study, he also leads the NCTR as the NCTR, the FDA interface to the National Toxicology Program, and this alone is a fulltime job, because a lot of the NTP monographs deal with CFSAN products and without all of the work that Goncalo does on this, we really wouldn't be able to comment on documents that then would have implications for products that we either regulate or are found in our foods. So we are very grateful, CFSAN is very grateful, to all the hard work that Goncalo does making sure that CFSAN scientists as well as other scientists interact with NIEHS, with NTP, and with NIOSH, and also help us get some valuable research done in partnership with these two other agencies.

So I don't know how we would be able to do this without all of his hard work, and we at CFSAN are especially appreciative to what he's done for us.

And because we're so appreciative, we gave him another job. We actually asked if he would be on -- we are starting a globalization of food safety, it's called ILMERAC. It was started by EFSA, and it brings together all the EFSA countries and then we also have Canada, Korea, the Germans, and Netherlands are particularly active, and

CFSAN FDA, and we formed a working group under this that CFSAN, EFSA, OECD, and JRC chair, co-chair, on new approach methods for food safety, and NCTR, Goncalo, again is an active member and really gives us a lot of support on what we can do and what we can't do.

Currently we are focusing on mixtures, which is a very big area for food. Food is a mixture, but right now we look at one commodity and one contaminant and one commodity at a time. So for example, there's the mixtures of metals and baby food, but they're looked at one chemical and one commodity at a time instead of looking at the aggregate of cumulative exposure. That would require new methodology mostly in new approach methods, and we're working globally to look at that issue, as well as looking at the relevance of NAMs for risk assessment. I think one of the biggest challenges to use NAMs at least in the food area that I think is how relevant is the data to humans? That was not a question that we thought about too hard, at least until recently, on the relevance of animal data. We looked at animal data and added safety factors, and used it in risk assessment.

Now we're being asked, and in the food area we really need new approach methods, because, like I said, if we find something in the food supply we don't know anything about, it's not acceptable to say, well, we're going to

have to do an animal study and we'll be back to you in a couple of years to tell you whether this product that you and your children are eating now is safe or not.

So I think NCTR has been a really big partner not only talking and doing research for us and some of these areas that I pointed out, and I'm sure I'm not the best person to tell you how it goes, because again, I'm not a researcher, but a regulator, but I know the importance of it, of what NCTR is doing in order to assure that we have a safe food supply. And I don't think there's any way that we could do it without their help.

And the last thing is, next slide, and then doing this globally will allow us to do it, also NCTR is really kind enough to let Donna work with this with me on the alternative methods workgroup, which is a cross-agency workgroup, and Donna is really the engine that makes this go. She keeps everyone straight. She helps with the meetings. She runs a seminar series which she's actually giving a seminar after this meeting at 4 o'clock.

So we are grateful in CFSAN to have NCTR allow Donna to work on this, as well as everything that she has to do for CFSAN, but this is an alternative methods workgroup. We're committed to development and use of new approach methods. We work with our global partners here

and abroad, and we do a seminar series where they can bring in new approach methods.

We have a website for alternatives at -- if you type in Google at FDA alternatives, you'll get the website. This is the report that we did last year on advancing new approach methods in FDA, and again, we are really thankful that NCTR allows Donna with all her other responsibilities, including taking care of all of you guys here today, to work on this area, because I think it's really making a difference at FDA.

With that, I thank you. Sorry I bumbled some of the research. But I put a lot -- they put a lot of data, NCTR made my slides for me, or many of them, so that's why they're so nice, and I think it will give you a good idea of what they do for the FDA foods program and their valuable role really in keeping the food supply safe for all of you, everyone here at that meeting and everyone out in our country. We're lucky that we can work with them so closely.

With that, if you have any questions. If it's about the studies, you might have to ask the researchers themselves that are all here.

DR. ASCHNER: Thank you, Suzy. Are there any questions?

Okay, we'll move on. We've heard before from Jonathan, so we'll move next to the Center for Veterinary Medicine, and I believe this will be shared by two individuals, Dr. Regina Tan and Dr. Daniel Tedesse.

Agenda Item: Center for Veterinary Medicine

DR. TAN: Thank you. Hello again, everyone. I am Dr. Regina Tan. I'm the director for the Center for Veterinary Medicine, Office of Research, at the FDA.

I've talked with you in the past, and so what I'm going to do is recap our last discussion, give you progress since our last discussion, and talk about some of our next steps. From my perspective, coming from one of the regulatory partners for NCTR, I have shared a lot of the same struggles that I've heard discussed here by the SAB in the past, really ensuring that we have an understanding of how our research is supporting our regulatory mission.

So what we have been working on is really with our own research in-house making sure that works and what I'm going to talk with you about today is how we have rolled NCTR into the process with us and welcomed NCTR as a partner at the table.

Okay, so just as a reminder, our strategic goals are to support the availability of safe and effective animal drugs, advance food safety and safe animal food products, support One Health monitoring, investigation, and

response, advance emerging technologies, and innovation, improve business processes and operations to enable excellence in science and research, and to foster a One CVM culture across organizational boundaries. I will talk with you today about an example.

Now, Suzy just talked with you about a working group that she and Donna run at the agency level about alternative methods. The example I'm going to give to you is the preparation work that we at CVM, the Center for Veterinary Medicine, do so that we can come to the table and support Suzy and Donna when they need the center perspective.

What are we doing? We are working to align the center's research with the center's regulatory functions, and that is safe and effective animal drugs for companion and food-producing animals preapproval, monitoring safety and effectiveness of animal drugs on the market, making sure animal food is safe, made under sanitary conditions, and properly labeled, making sure that food additives used in animal food is safe and effective preapproval, and helping make sure more animal drugs are legally available for minor species and infrequent and limited use in major species.

This is our working style. It looks really simple on this slide, but it's actually a tremendous amount

of work to do it. The work is worth it. We are very hardworking to make sure that our portfolio of research is aligned with the rest of our sister offices and the center level at the Center for Veterinary Medicine. This means that we're coordinating discussions all the time at the center level so that our developed science is aligned with our regulatory mission. We enable the One CVM perspective, and that is offices working collaboratively together. This process does take a lot of time, and it takes a lot of time to get all the players at the table.

We work with our other offices for visibility and coordination, and coordination with the center's senior research council. I will talk with you more about that in a moment. And the end goal is mission alignment with the center's priorities.

Okay, again, we're going to use the example of CVM senior research council, which brings the offices from across the center -- for us, that's new animal drug evaluation, surveillance and compliance, and research -- to discuss scientific issues that touch all of our missions. Otherwise, what we find is those conversations have all been -- what happens then is there is the chance that if we don't have the intentional conversation, that there, because we're all so mission-driven, those intentional conversations can fall through the cracks, and we don't

want that. Those intentional conversations, we find, within the center are those things that make it extremely important and extremely available for us to tailor our research to the mission, and it allows us to weave together those issues that are specific to different offices together for a center-specific focus.

Okay, where are we with this? If you go to our insideFDA website, you go to the CVM, and then you go to the Office of Research, so this is only available within CVM -- sorry, this is only available within FDA. That's our splash page. There we go. And if you scroll down, what you'll see beneath our mission statement is you'll see our research and right there is the CVM/NCTR collaborations. Can you click on that, please?

So this is my backup slide. If you're within FDA and you can get to this slide, what this does is it brings you to the live grid of all of the different projects that NCTR and CVM have together. It tells you who the PI is. It tells you who the CVM collaborator is. And what this enables us to do, then, is that anyone from the Center of Veterinary Medicine can have access to that information. This is what helps us stay together. It also helps us support NCTR.

The impact statement from my perspective is the most important statement. It's not that the research isn't

good. We trust. We know the research is good. We've been working with NCTR for a long time. We understand the quality of the research. What's important is that impact statement helps us understand the utility of the research to the regulatory mission, and we insist that for every one of the projects that we have ongoing together there is an impact statement that very much makes sure that the work is supporting the regulatory mission.

So how do we support a conversation that helps everyone across the Center for Veterinary Medicine understand the NCTR collaborations? Making the projects and information available to everyone is the first step. It's not the only step that we take.

I'll talk to you first about how we actually do it within the Office of Research. Within the Office of Research, we do division portfolio reviews. Every three months, the divisions discuss their research portfolio with the Office of Research management team. This would be myself, my business manager, my business process recruitment manager, and my deputy, and what this helps us do is it helps us keep in touch with the science, but also we're ensuring the best support for budget, milestones, and contracts, because research is a beautiful thing, but it doesn't happen without support.

What we have found is we can, if we are working together and tied tightly with the division and the researchers' needs, do the best we can with the amount of budget that we have to support that research to be successful. Once a quarter, the Office of Research brings a division portfolio to the senior research council at the center level to discuss the research portfolio's applicability to the center's regulatory mission from all offices' perspectives.

Now, what we've started to do this year is welcome NCTR to the senior research council for exactly the same discussion so that our center's senior research council looks at NCTR's portfolio, which it just looked at portfolio of research exactly the same way they look at our internal research, and on March 16, we welcomed Tucker and Donna for that discussion.

The SRC feedback from that discussion was that it's helpful to know that my perspective of the research matches theirs. Now, it's -- you can take that very lightly, but the truth is, at the center level we're dealing with a lot of science, we're dealing with a lot of research projects, and that extra surety that we are all marching in the same direction is what helps make it possible for us to ensure that our science is supporting the regulatory work.

Helpful to know that I really did know about the research ongoing, and I will say, when we have a large research portfolio and there is a lot of research that goes on between NCTR and CVM, it's a lot, and for us as managers and as center leadership team to know that we really are keeping track of everything, and we are endorsing everything, and everything really does support the mission is very important.

And the research council welcomed NCTR to come back in the next year, and I will be honest with you, I'm hoping that this is a practice that we can just make sure is something that happens every year.

What these NCTR portfolio reviews allow us to do. It helps CVM understand the totality of scientific development at the center level. That means also that CVM interacts with our partners together as one CVM, rather than separate office voices, rather than new animal drug evaluation, research, and surveillance and compliance, you get us all at the same time, understanding the different perspectives. It helps facilitate a direct interaction with offices that have a more regulatory forward mission. Our new animal drug evaluation and research, we're dealing with evolving science all the time. Our Office of Surveillance and Compliance may not otherwise be present for that discussion.

That said, from my perspective, scientists need to benefit from understanding direct knowledge from the Office of Surveillance and Compliance how their innovative techniques are going to be used in the regulatory space. If they're going to develop an assay, they need to know how that assay is going to be used. If they have that understanding up front, they can tailor their science to fit the need and save us research time.

This collaborative discussion supports direct application of scientific development. Within the Center for Veterinary Medicine, we're starting to have those conversations earlier and earlier, and have them more often, so that we can ensure that our science stays on track with supporting the regulatory mission, and welcoming NCTR as a partner on that just makes sense to me.

Our next steps are actually I'm going to talk with you about an alternative methods effort that we have. Again, this is at the center level, and what alternative methods has looked like at the Center of Veterinary Medicine is spots everywhere, right? You have the Office of Research, which is really focused on replacement, reduction, and refinement research. We're the Center for Veterinary Medicine. We are going to seek replacement, refinement, reduction methodologies, but we are not ever

going to be able to work animals out of research, because of our mission.

We also have the Office of New Animal Drug Applications, and they're looking to support efficient and effective reviews. We have the Office of Minor Use and Minor Species, which sometimes because they're a small office may not be heard at the table, and the Office of Surveillance and Compliance, which often has a regulatory need for research but again, because their main focus is not research, can be excluded from the conversation, not intentionally, but they are a huge partner, and they are missing when they're not available.

So what we're looking to do is the CVM alternative methods community of stakeholders, and what this does is it provides a platform, not that much different than the portfolio review process that I talked to you about a little bit earlier. It's a two-way street, the conversation. It's important that everybody knows who everyone is. It's important that we all understand our slightly different missions in support of the center.

Here's the goal. That all of us come to the table with an equal voice. We're all different offices. We're all needed for the center's mission. That each perspective is needed to support that holistic voice. It allows CVM to identify work and researchers with impact of

alternative methods that are otherwise not known to management, and it prepares CVM to support FDA's higher level mission. So when Donna or Suzy have a need for alternative methods, that alternative methods working group at the agency level, they can come to CVM and they know the person who supports them is not just representing one bubble, but representing one center.

It all comes down to our strategic goals and how CVM prepares itself to be a better support to the agency.

Okay, we're going to give you an example. Dr. Daniel Tadesse is joining me today. He does some alternative methods research. Dr. Tadessecomes to us from the Division of Animal and Food Microbiology. They generally have three different types of research, method development, molecular techniques, and bridging studies.

And if you want to know where this division sits within the organization, the Division of Animal and Food Microbiology is one of three divisions. Its sister divisions are the Division of Residue Chemistry and the Division of Applied Veterinary Research.

Daniel, please.

DR. TADESSE: Thank you so much, Dr. Tan. One of the alternative methods efforts in our center is the use of organ-on-a-chip model. We are exploring the potential of

intestine on a chip model as an alternative to animal testing, specifically to predict the effects of drug residues on the human intestinal microbiome.

As Dr. Tan said, our research focuses on the mission, and the effect of drug residues on the human intestinal microbiome is an important human food safety endpoint of concern that must be addressed during the new animal drug approval process. There are two endpoints of concern. The first one is disruption of microbiome colonization barrier, and the second one is antimicrobial resistance development among microbiome residents.

There are in vivo and in vitro approaches to measure the effect of drug on microbiome. For example, the animal model is used to investigate the human microbiome, because it's much easier to manipulate animal models. However, there are important caveats, including difference in anatomy, physiology, diet, microbiome itself between humans and animals are different.

On the other hand, the widely used in vitro system like the cell culture bioreactors ignores or lacks the host contribution to the drug-microbiome interaction. So what we are trying to do here is to leverage the recent advance in tissue engineering, microfabrication, and stem cell biology to enable the development of the organ-on-a-chip model to recapitulate the key microenvironment that

characteristics of the human and animal organs and mimic their primary functions. In our case, we are focusing on the intestinal microenvironment.

So the goal of this effort is to optimize the intestine chip model for co-culture human intestinal cells with gut microbiota to create a more human-relevant model. In addition, we are planning to use a metagenomics and meta-transcriptomics approach to measure the change in microbiome and resistome as a result of this exposure. We are hoping this model enables us the creation of more in vivo-like in vitro models, and as a potential to reduce and refine the use of animal in research.

So we are interested the host microbiome drug interaction, which does require a longer contact time between the microbiome and the drug to see the impact. So as part of optimization of this model, we tested whether we could sustain the epithelium and endothelial cell for 10 days on the chip without losing the structure and function, and we compared the performance of day 10 chips with that of day 8 chips, which is normally done in Emulate system using three critical parameters.

The first one is for the creation of tight barrier function. As you can see on the top left figure, we had a monolayer on day 4 and the tight endothelial epithelial barrier function was maintained until day 8. So

we were able to give the same results both on day 8 and day 10 chips.

The second parameter that we used is we evaluated whether we were able to recreate and sustain the epithelial and endothelial tight junction until day 10. If you see the top right figure shows a nice tight junction creation for both day 8 and day 10. In addition, we were able to demonstrate the production of mucus even on day 10.

The third key parameter that we used to compare day 8 and day 10 chips is we used a gene expression assay to assess the differentiation of epithelial cells to absorptive goblet cells, enteroendocrine cell, consistent with what's happening in normal epithelial cell growth cycle. As you can see on the bottom figure, the expression of indicators for the mature colonize gene, for example, Muc2, alkaline phosphatase, sucrose isomaltose, and chromogranin A, increased over the course of the culture, whereas the expression of cyclic stem cell driving gene, Lgr5, decreases as expected. The differentiation of the epithelial cells were consistent for both day 8 and day 10 and mimics what's happening in normal epithelial cells.

This is an ongoing project, and as I indicated, we have completed the first phase. The next phase of the experiment is introducing the microbiome and then the drug

and see the impact of the drug on the microbiome-host interaction.

There are several efforts across different centers within the agency. In addition to the intestinal model, CVM is also working on developing a canine organ chip for toxicity study for new canine drug evaluation. As Suzy said, Donna is leading an Emulate system user group, and we constantly meet to discuss and exchange ideas among the different PIs who work in the different centers.

So what I shared is about the work that our division, which is the Division of Animal and Food Microbiology working on in terms of creating the intestine chip to measure the drug residue effects on the human intestinal microbiome. As you can see on this figure, CBER, CDER, and NCTR are working on different organs, and we are close to working together to learn from each other and share experience.

I think, Regina, it's you.

DR. TAN: Thank you, Daniel. So points of contact if you're looking to talk with us, collaborate with us, have further questions about this. You can reach myself and my deputy director, Dr. Chris Whitehouse, at CVM-OR-Office of the Director. Actually, the Emulate working group, one of the things that I wanted to show you is there is so much benefit from us working together, and I think

the Emulate working group is a very good example of that. I want to thank Donna for her leadership on that, and this is something that it's very important across all of our missions. We can see one technology that touches multiple areas in the agency, but benefits from coordination. So, thank you, Donna.

If you have questions about Emulate technology use at the Office of Research, please reach out to Dr. Daniel Tadesse, whom you've heard from, or Dr. Haile Yancy, who is not with us today but also is working with Emulate.

Thank you very much.

DR. ASCHNER: Thank you, Dr. Tan and Dr. Tadesse, and I'll open the floor for questions at this point. I'll ask a question. I may have missed it, but can you give us some specific examples on the interactions between your division or center and NCTR?

DR. TAN: Sure, I can. What we have been working with to this point is there's a lot of PI-to-PI interaction, researcher-to-reviewer interaction, and that's historically how we've been talking together. That's historically how ideas come about. And there is a beauty to that, right? Because there is an innovation that happens when you have that level of research.

We want to be able to honor that. We want to be able to encourage that. What's important is for us at the

management level to also stay in touch, and that's something that I think at least from the CVM perspective we haven't been working with. For us to support our regulatory needs, we have to have those conversations I think in a smaller, more intimate environment and be able to do it so that, as when Tucker and Donna visited with us, essentially you have senior research and senior scientists from all over the center, we're sitting around, of course, a virtual conference table.

Tucker is going through I think there's maybe 16, 16 to 20 projects that are ongoing between NCTR and CVM. He goes through each one of them. He talks about each one of them, and the senior scientists who have an understanding and -- this is important -- who have an enterprise perspective of what the mission is at their office level and who also have an understanding of what the centers' needs are. They can ask questions, make sure they understand the research, make sure that research is in tune.

Where I would like to go next is, frankly, bringing the Office of Surveillance and Compliance more to the table, because the Office of New Animal Drug Evaluations, they have a very healthy, already they have a very healthy research component working with NCTR. I think of these 16 research projects, I would say probably 12 of

them are from our Office of New Animal Drug Evaluations, but I think what we really need to do is engender discussions with the Office of Surveillance of Compliance.

Again, because I have an understanding now with my own research portfolio, that talking to the Office of Surveillance and Compliance up front and often, how is this assay going to be used? Is it going to be -- does it need to be handheld? Is it something that is going to be used in screening? Where is it going to be used? Who is going to be using it? Those are all different nuances to the science that help the PI understand how better to construct the work, and those are the conversations that I would like to nurture, again, between NCTR and the Office of Surveillance and Compliance.

Did that answer your question?

DR. ASCHNER: Yes, it did. Thank you. Thank you, Regina. Are there any other questions? If not, we'll move on to the last presentation from the centers, the FDA centers, and the next one is from the Office of Regulatory Affairs by Dr. Selen Stromgren.

Agenda Item: Office of Regulatory Affairs

DR. STROMGREN: Thank you, Dr. Aschner, and good afternoon, everyone. Thank you for your attention. I know it's late afternoon on Eastern time, and last presentation.

For today's presentation, I know that I have been presenting to this group for a few years now. I've been a guest at this forum and have obviously heard very interesting information. For today's presentation, I wanted to do a little something different and show a slightly different part of ORA today, to all of you on the phone, and sort of homing in on a topic that's sort of in the greater context of information technology collaboration we have with NCTR.

Again, as a reminder, ORA, which is sort of a -- its name starts with Office of Regulatory Affairs. It's not really a center per se. But it is a large component of FDA, more than close to 5,000 employees. It is -- the regulatory mandate of the ORA is really supporting the regulatory mission of the agency by producing science-based evidence. We are not a guidance setting rulemaking nor a preapproval component of FDA. And the science-based evidence that we're talking about that ORA is responsible for generating comes in two flavors, our inspectional findings, and our analytical findings in our laboratories.

We have an 80 percent inspectional workforce who is out there inspecting firms, collecting samples, either from domestic points or import entry points, and 20 percent of our laboratory workforce is distributed across 12 labs nationwide.

Our strategic science plan is really focused on defining some long-term tactical goals to uphold our mission. So it's a lot of practical goals are in our strategic science plan. Some of those highlights are there's a big focus in ORA laboratories on the quality and integrity of science, because we need to be able to obtain defensible results when we test products in our laboratories and assess their compliance with the FD&C regulations.

We try to maintain laboratory, a healthy laboratory capacity and achieve maximum efficiency. We do it of course through how we structure the workflows in our laboratories, methods we use looking as multiple analytes in one trying to extract as much information as possible from one sample. So look at a whole variety of analytes, chemical analytes, microbiological analytes.

We do this by partnering with other laboratories, extending our lab network. We do this by focusing our limited lab resources on higher risk items. So we have been working on implementing effective point-of-entry testing for better targeting of import products, for instance.

We try to develop methods that are also investigative in nature, because sometimes we get products in our laboratories that are associated with some adverse

event, but it's not known at first what exactly is wrong with the product. So we need to be able to approach from a more investigative angle.

We spend a lot of time trying to do horizon scanning, which is actually a very hard thing to do in terms of what new capabilities we need to develop to be ready for the next public health event. New outbreaks, new contaminants, new products, are always out there for us to prepare for. There could be new risk perception on old products, new legislation, that could require us to come up with new methods. So our horizon scanning efforts, we're always trying to improve it as much as possible by sort of brainstorming, looking at past events, looking at open source intelligence, but again, working, getting the risk priorities from the product centers.

We focus on data that can cross borders. We have a lot of international partners. Timeliness, speed, streamlining decision-making of course is important for our regulatory testing. We modernize our technology pace annually, and we have performance standards for our laboratories because their work is used for FDA regulatory decision-making.

We also have some efforts that go beyond our laboratories. We work with some state labs and foreign labs for purposes of lab capacity-building.

So, this is a map of the location of our laboratories, and we actually have one new laboratory that's going to be operational in August. We're very excited about it. This is a $62 million investment, and I just visited this laboratory. It looks quite amazing, and I just, for all those enthusiasts in the audience who recognize city landmarks, so I put one landmark of this city this lab is close to so you can guess what it is, maybe you can drop it in the chat, and I'll reveal the destination at the close of my talk.

So I talked about our laboratories doing regulatory testing to support enforcement actions by the agency. The main work product of our laboratories is what we call laboratory analytical packages. So when a sample, an official sample collected by ORA, FDA ORA investigators, are sent to our laboratories, the sample is analyzed for a variety of agents, and all the findings need to be documented, what we call evidence development, in a very thorough fashion.

What I wanted to do today is sort of hone in on that aspect of what we do. The reason I chose that is slightly different from what I've presented before, but it also is sort of very related to a project, a collaboration we have with NCTR. We've been working with them for a couple of years now, trying to build an automated

laboratory information system with their bioinformatics team.

This will centralize -- this will network all our laboratories and it will allow -- it will replace a lot of sort of disjointed systems we have right now where different documents are posted in different databases, and it's just, it's really hard for them or for a reviewer to look at a package and all the associated documents all in one smooth swoop. So hopefully this ALIS, this Automated Laboratory Information System, is going to make it much easier to organize our laboratory analytical packages and all associated information, and it is an effort that we are very happy to be partnered with NCTR. We've been at this, chasing this laboratory information management system, for a while now. We've had negative experiences with outside contractors. But now that we've partnered with NCTR on this, a lot of progress has been made in getting this system up and running. So we're very excited about this.

But I do want to hone in on this laboratory packages. We've recently initiated what we dubbed as Operation Checkers in our office. These ORA laboratory work packages, they are -- because so much rides on them, they represent our official work product -- they are available to the public through the FOI, Freedom of Information process -- we want to make sure that they

reflect the best science and they're free from defects, especially egregious defects. So to that end, our laboratories that are operating on their ISO-accredited quality management system framework are required to shepherd these packages through an internal peer review process before they go -- they're finalized and become available for FOI requests.

So we wanted to check how robust the current peer review process is at catching any defects, errors, in these packages. We initiated this Operation Checkers project. It also addresses a number of our strategic goals, executive performance goals, and we formulated the project. We wanted to take a look at analytical work packages produced in the past couple years or so, and just like anybody else, our output statistics looked quite different over the pandemic. There's been a drop in sample collections. But there is still quite a healthy amount of data for us to harvest from.

So we focus our efforts in the last couple of years, we wanted to look at a more recent history, rather than go way back, because we've made some improvements to how we put our packages together. So we wanted to sort of focus on the most recent way of doing things.

I mentioned a peer review process that's mandated by our quality system framework, and that's conducted by

the laboratory management, as well as some headquarter personnel. So there's a multi-tier, multiple number of people who look at these analytical packages that are constructed by the analyst. We have SMEs looking at them, supervisor of the analytic group, branch director, all the way to lab director. In some instances, headquarters scientific liaison or coordinator can be involved as well. So we just wanted to evaluate how this multiple review is working out, how effective is it at catching any errors?

Just to give you a visual idea of what exactly is an analytical package, that's produced by our laboratories. These packages can vary quite widely. They can be as short as three pages, or they can get up to more than 300 pages, depending on if it's a chemical analysis, multiple analytes, versus this may be a more observational analysis such as field analysis, perhaps, just looking at the sample under a microscope describing some features. So there is also quite a number of pages are also dedicated to describing the sample, how it was received, the firm it was collected from, any special conditions. So there is some administrative aspect to these analytical packages, but the bulk of these packages is analytical information.

So again, the Operation Checkers, we wanted to get people from laboratories who are used to reviewing these packages to sort of come detail with us. It's a

headquarter-led project. We wanted to look at multiple program areas where we're testing samples for microbiological contamination, or maybe toxic metals, or maybe some persistent organic pollutant. So these are all different analytical program areas in our laboratories.

We wanted to again sample from these different areas. We wanted to make sure the reviewers working for Operation Checkers came from the lab, so they do this, they're familiar with this. We actually had a lot of interest in this project. So we opened it up to all our laboratories, and we got over 70 people who were interested in coming to work with us for multiple weeks to look at these packages.

Again, this is just some more details about how we constructed our team, the detailees. The project had many sort of facets to it. So we had to plan to make sure it ran smoothly, working remotely with the teams, and they were rotating teams that each served in a couple of weeks, then we rotated. That way we could also look at different program areas.

We also had a tiger team, a core team, that served to give the detailees some marching instructions on how to get started. Also, the tiger team was there when detailees had questions about how to conduct the reviews.

Now, as far as the actual reviews, so we have as I said before, our analytical packages are currently housed in a database. So we constructed a master spreadsheet where we put live links to the various packages, where the detailees, the reviewers, could go pick these, claim them, and then work on reviewing them.

The reviewers were asked to fill out a checklist basically, and that's where their observations were captured. There are a lot of specific questions about that trying to get to the completeness of the package, completeness of the information, integrity of the data, traceability of equipment, reagents used, any quality controls, calibrators. So a lot of those are sort of in this rather long checklist there inquired about item by item. But then there's plenty of room for any comments to be entered as well.

The teams, the review teams, were encouraged to work together and harmonize their review criteria as much as possible. The checklists are supposed to be checklists that the reviewers use -- are also supposed to be used by the local peer review process. There is -- it's not requirements. So there are differences in how the different labs perform their peer review.

We've actually completed wave one. We looked at three program areas. A lot of data was generated. There

are 426 laboratory packages were reviewed by our detailees. So we are sort of right now at a pause and going methodically through the information to try to capture any trends so that it can inform. First, we would like to get an idea how effective our current review process is at catching any errors, whether there are some errors our detailees uncovered, and how we can improve the review process going forward.

These are the three program areas we looked at: pesticide analysis, toxic metal analysis, and microbiological analysis, and we tried to sample worksheets from across the different laboratories in as balanced a way as possible. And these worksheets we looked at, they were mostly worksheets that reported violations, violative findings, but of course we also sampled worksheets that were just about samples that were found to be compliant. Because those worksheets also need plenty of data to show nothing was missed and the sample was indeed compliant.

Just a preview of we're just sort of diving into the data, but some interesting observations came out. We actually found a discrepancy in the rounding, of all things, which was interesting, because most of us know rounding to be -- if the decimal, this relates to significant figures, as well. We were also looking to just make sure consistency of significant figures, and that it

was sort of reflected realistic significant figures, but most of us know rounding as if the decimal place is the one you want to drop is 5 or bigger, the previous one increases by 1, and you drop the trailing one.

But apparently there's a very austere -- I guess a purist group of mathematicians out there that a more official, a more accurate way of doing it is looking at the decimal place in front of the trailing, the decimal place you want to drop, and depending on it's odd or even, you do different things. So I actually didn't even know about this myself, but there was a discrepancy in how the different labs were rounding. So we got an opportunity to streamline it to the all general well-known practice.

Another observation we had was there was varying levels of quality control information that was being included in the packages. So we will need to get together and come up with some specific guidance to give to our laboratories, how much of this quality control information they need to include. It's a fine balance, because we don't want to be too onerous and tie up the labs forever getting stuff together to add to their packages. But on the other hand, we want these analytical packages to stand on their own. If they're ever in a court of law, they need to have all the key quality controls included in the package.

We came across some important variations in the amount of raw data, such as chromatograms included in the packages. Again, we need to come up with some specific guidance. And some issues we've observed regarding portability of validation status of different matrices for the different -- for various methods. So if a matrix A or a method X is validated in a lab, can another lab using that method assume that matrix is validated and what sort of verification they need to do? So we're sorting through those.

Our next steps of course is just to go through this data as carefully as possible and come up with ways to strengthen our peer review process. I think there's room for it to be made more robust, and it's also clear that we need to come up with some more specific guidance to the laboratories so we can minimize these variations in certain aspects of these worksheets, and I think this will be timely, because we are making progress with NCTR on the construction of the automated laboratory information system, the more streamlined our packages are, the easier it will be for them to be turned into these harmonized digitized worksheets that the labs can fill out, and we can just achieve that next level of standardization across our laboratories in terms of the evidence that the work product may produce.

So with that, I think that was my last slide. I will conclude my talk there, and I'd be happy to take any questions if you have any.

And I'll just, I don't know if there were any guesses, but the city of the new laboratory was Boston, and that was the Longfellow Bridge that goes across the Charles River there, connecting Boston to Cambridge. Thank you.

DR. ASCHNER: Thank you, Selen. Are there any comments or questions from board members?

Okay, hearing none, I suggest that we go to the first presentation by Fred Beland, the Division of Biochemical Toxicology. We have three scheduled for today. I erred actually earlier in the morning when I said they're going to be all tomorrow. So three of them are today, and the other three will be tomorrow. They are 30-minute presentations, with a 15-minute discussion for each of the divisions, and Fred, kindly go ahead, and then we'll take a break and finish with the last two.

Agenda Item: NCTR Division Directors: Overview of Research Activities

Agenda Item: Division of Biochemical Toxicology

DR. BELAND: Good afternoon, everybody. I'm going to provide you with an update on the Division of Biochemical Toxicology. The first few slides will be used for each of the presentations, I believe. We've been asked

to provide this information. This is a slide that I've used in the past. The numbers have changed slightly.

The major point is we have 49 staff members; the majority are research scientists, staff fellows, and visiting scientists. We have currently I have two administrative, we have two administrative staff, 10 support staff. We have currently five ORISE postdoctoral fellows that are supported entirely by extramural funds. We don't have any graduate students. We have a total of 46 people.

By the way, I think this is important here is 27 of these individuals -- so this is 60 percent -- are people who are not originally from the United States who received their training outside of -- almost all of their training or the great majority of their training -- outside of the United States, and I think this will be important right at the end of my presentation.

We already talked about collaborations. This has been sort of discussed throughout the presentations today. Each of the divisions at NCTR really work with other divisions. We also, in particular, this division, but also the other divisions, work quite closely with the product centers, because remember, NCTR does not have a regulatory mandate. We're supposed to do research, and this research is supposed to support the mission of the FDA.

We also have interactions with NIEHS through the National Toxicology Program, the National Cancer Institute, NCATS -- the next one I've never quite known how to pronounce, NICEATM, it's part of the NTP -- EPA, CDC, and so forth. We're involved with IARC, we do monograph reviews for IARC, we're involved in various working groups for the World Health Organization, EFSA, and OECD.

Our mission of the division is to conduct fundamental and applied research designed to define biological mechanisms of actions underlying the toxicity of FDA-regulated products. So we characterize toxicity and carcinogenic hazards. We don't do risk. We provide data that the product centers can then do risk assessment. I think that's important.

The way we approach this is we have a long history of conducting bioassays. We also at the same time do mechanistic studies so that we can determine whether or not what we're observing in the bioassay is pertinent to humans, and we also do computational modeling where we try to put all the pieces together to come up with a complete package and extrapolate to humans.

As far as metrics, 20 years ago, we would emphasize manuscripts, and this has evolved in that we've really come to the realization that while it's nice to have manuscripts -- and I think Ed Margerrison pointed this out

in his presentation, really what we need to do is provide the product centers and regulatory centers with data they can use in a regulatory setting.

So if we can provide data, that is important. If we can publish manuscripts, that is also very nice. But I don't believe, at least within this division, that is the primary objective.

When we met a year ago, I outlined three different project areas: tattoo pigments, cannabidiol, and then COVID. At that time, I said, okay, this is what we're going to be doing in the next year. So I'd sort of like to bring you up to date on where we stand on these projects and also then where we envision going with it.

So the tattoo pigments, and I talked about this earlier today to some extent. Remember that a year ago, Mary Boudreau was the principal investigator. She subsequently retired and has moved back to Louisiana.

I was fortunate that I work with an investigator whose name is Svitlana Shpyleva, and we were talking, and I asked if she could take over these projects, and she's done so with great enthusiasm. She's trained as an MD and a PhD. Having her MD perspective has really been a tremendous advantage to me.

So as was pointed out, I think maybe Tucker Patterson in his presentation mentioned it, or I guess it

was more Suzy Fitzpatrick, said tattoo is considered mainstream now. And it's very prevalent in young adults, aged 25 to 39, and interesting that women now have higher rates of tattooing than men.

A lot of pigment is put in. The average tattoo, and this is in the United States, is 100 centimeters squared. So you have a quarter of a gram of pigment in this, and 30 percent of U.S. adults have more than four tattoos. So they have a gram. There's a report out of Germany that 16 percent of Germans have tattoos that are greater than 900 square centimeters. So there's a lot of pigment.

These are organic compounds, represent the majority of the tattoo pigments, these are the things that give them great color, and they're, the majority of this are azo pigments, so these can undergo degradation to give aromatic amines. They're intended to be permanent. When you get a tattoo, you want it to be permanent. The problem is that there's been reported decreases let's say around 90 percent reported in skin.

So the idea behind this project was to assess the placental transfer and biodistribution of three commonly used azo tattoo pigments: pigment orange 13, pigment yellow 83, pigment red 22. The first two compounds have two azo linkages, you see here and here. They're drawn in a

different tautomer, but trust me, they're azo. And this is the pigment red 22 is a mono-azo compound.

We have to -- this project was supported or funded by the Perinatal Health Center of Excellence, and it was done in collaboration with investigators at CFSAN, and when we were putting this together, we had discussions of what should be the animal model, and we convinced CFSAN that based upon studies that had been done previously here by Paul Howard and Neera Gopee that we could use an SKH-1 mouse. This mouse is hairless, which is a distinct advantage, and has been used a lot in phototoxicity studies. So we thought it would be a proper model to use.

We gave radiolabeled material, well, we tattooed -- and I'll show you pictures of this in a moment. And then we evaluated the distribution of radioactivity using scintillation counting in organs, tissues, and the fetuses.

All right. There was two studies. There was a long-term study where we monitored the tattoos for up to 20 weeks after administration. That is shown on the left-hand side. On the right-hand side, there was a perinatal study where we tattooed the animals with pigment red 22 on gestation day 1 or pigment orange 13 or pigment yellow 83 on gestation day 5. These animals were then euthanized on gestation day 17.

This is how we tattooed -- by we, this was Mary Boudreau and Michelle Vanlandingham -- this is how the tattoo was applied. It was a rectangular area. It was 2 centimeters across and 3 centimeters down the back of the animal. So this is pigment red 22. This is pigment orange 13. This is pigment yellow 83.

The interesting, what was interesting to me and totally unexpected, was that if you look at this rectangular area, if we look on the left-hand side, this rectangular area dramatically changed in shape, and on the top of this what we're showing is the area, we started with an area that was 6 centimeters squared, and you can see by 8 weeks, it is down -- it's reduced down to 30 percent of what we started with.

It then remains relatively constant. In fact, this happens very rapidly, because if you look at an animal that's tattooed on gestation day 1, by gestation day 17, it's down nearly the same amount. If you tattoo the animal on gestation day 5 and look at it on gestation day 17, it's not down as much, but it's about 50 percent of the material has been -- the tattoo has decreased to about 50 percent of what we initially started with. This seems to involve the healing process of mice, and from that perspective this is not a particularly good model, because obviously this does not occur in humans.

The other interesting thing was, okay, what was the distribution of the pigment? Did it remain in the tattoo? And this is what I'm showing here is from the long-term study. So please note, okay, this is pigment red 22 -- please note, this is a log scale. Ninety percent of the radioactivity, and we assume it's still the parent pigment, but we have not investigated that as yet, is located in the tattoo. However, this is only about 10 percent of the material that was initially applied. The majority, 90 percent of the pigment that was initially applied has disappeared. And apparently this occurs very, very quickly.

The second most prevalent location for the pigment is in lymph nodes, and other people have detected tattoo pigments in lymph nodes. This stays relatively constant. This is across from 4 weeks to 20 weeks as relatively constant.

Then much lower levels were found in nearly every other tissue examined, and some of these tissues, it decreases with time.

So these observations have led us to conclude that -- I think it provides valuable information, but it also indicates that we need a better model. So what we're in the process of doing and we're having -- okay, so this study, we've completed, we've prepared, we have a draft

report that we will send to investigators at CFSAN, hopefully later this week. We're going through one final check. It will be a draft report, and then we'll go through iterations with them to make the report final.

But at the same time, we're preparing a draft protocol, again which we will have discussions with investigators at CFSAN to make sure that it meets their needs, but this will focus on the use of minipigs. For those of you who aren't familiar with minipigs, they're actually rather substantial animals. They weigh about 30 kilos. So it's about half a human.

But the healing process, the skin of a pig, a minipig, and the healing process is similar to that in humans, and we believe that this would be a better model, and this was suggested by the Science Advisory Board subcommittee a year ago. We agree with that, and so what we have to do is we are drafting a protocol. We hope to submit it for discussion with CFSAN in the next month or so.

And then we would hope to be able, with their approval and with funding, we would hope to start later this fall with this study.

The second area I discussed last June, also Suzy Fitzpatrick discussed today, was the interest in cannabidiol. Cannabidiol is an approved drug. The FDA,

through the Center for Drugs, has approved Epidiolex for the treatment of epileptic disorders. During the review of this drug, there was concern that it was used in a rodent model, okay, this is cannabidiol. It's oxidized at this methyl -- carbon to a 7-hydroxy CBD, which goes under further oxidation to 7-carboxy CBD. There was concern during the review of the drug by CDER that in rodents this tends to be the major metabolite. In humans, this is exclusively found. So the question was, was the data being determined, found in rodents, applicable to humans? So that's one of the driving things.

The other thing as far as, you know, with the passage of the 2018 Farm Bill, CBD and other hemp-derived products have been added to food products, and again, but the data are limited. This is what Suzy Fitzpatrick was saying earlier today.

It's being used in cosmetics, and again, we don't know if it goes in -- it's absorbed through the skin, but what is it being metabolized and there are large data gaps. There's also, as Suzy Fitzpatrick mentioned earlier today, there was indication that it may be a male reproductive, it may impair the male reproductive, toxic. So I showed the metabolism.

So there's two projects or two areas that we're investigating. The first, and then Suzy mentioned this

project, and this is being led by Dr. Si Chen, and this is with funding from CFSAN, and in collaboration with investigators at CFSAN. This is the -- CBD is metabolized as 7-hydroxy CBD and 7-carboxy CBD, but from the data that CDER had worked with, there was a question of what is the toxic metabolite? Is 7-hydroxy just as toxic as CBD? And what about 7-carboxy, and the trouble is that in the rodent model, there was very little carboxy.

So what Dr. Chen is doing is looking -- since it's supposedly impairs the male reproductive system, she's conducting in vitro incubations with testicular Leydig cells and Sertoli cells, from both humans and mice to compare the species. The other endpoint is do these metabolites; do they have equal toxicity? Or is CBD more toxic than carboxy?

She and a very talented postdoctoral fellow, she was able -- they have actually been quite productive, have worked throughout the pandemic. We've been very fortunate at NCTR to be doing, to be able to do this. She's completed cytotoxicity evaluations of CBD, 7-hydroxy and 7-carboxy. This is in mouse and human Sertoli cells. These data were just published in Food and Chemical Toxicology.

When we were reviewed last year, at that time she did not have access to Leydig, human Leydig cells. She now has these cells, and so she's doing, she's conducting the

same type of incubations that she did with the Sertoli cells with the Leydig cells.

What I'm showing on the bottom of this slide is the human Sertoli cells exposed to CBD. There's both a time and dose dependence.

And there was questions of whether or not the dose is, the concentration she was using, was relevant to humans, and she goes as high as 10 micromolar and serum levels in humans can be around 3 micromolar. I think the concentrations she's using really are relevant. So she will -- in the process, there was a suggestion that we should be doing mechanistic studies, which she's doing. She has just completed a second paper, which is undergoing review here at NCTR.

Then she's in discussion with investigators at CFSAN as to what should be the future directions for this project.

Then the second area, so Dr. Chen was doing with human Leydig cells and Sertoli cells, and the second area was the dermal CBD pharmacokinetics. This is being led by Dr. Luisa Camacho. Again, Suzy Fitzpatrick mentioned this this morning. The idea was we need -- I think it's important to have good pharmacokinetic data. Then that helps you interpret potential toxicities and so forth.

In this study, the CBD applied to the -- this is in Sprague-Dawley rats. They'll be applied dermally, serial blood samples will be collected for up to six days, and then tissues will be collected at terminal sacrifice, and then quantifying CBD, 7-hydroxy, 7-carboxy, by tandem mass spectrometry.

The pilot study was conducted just to see was it possible -- first of all, we needed to develop a tandem mass spectrometry method, which was done. This was done by Qiangen Wu. And then we then needed to see what do the levels look like, can we detect the metabolites. So a pilot study was conducted, I'm showing you the data from the pilot study. The gray area is how long the CBD was applied, stayed on the back of a Sprague-Dawley rat, and then after 24 hours, the area was cleaned.

This left-hand side is showing -- this is the parent drug as a function of the concentration in the cream that was applied, and you can see we see a very nice dose response with the peak occurring around 8 hours. The right-hand side is showing from the 10 percent CBD, we're still able to detect the metabolites, and again, in CBD max is at around 8 hours. But we were able to detect a 7-carboxy, which seemed to reach maximum around 24 hours.

We're in the process of improving the sensitivity, and then likewise, we're in the process of

taking delivery on, ordered a new mass spectrometer, which will also increase our sensitivity.

In addition to doing dermal CBD, we're doing oral CBD. Again, Suzy Fitzpatrick mentioned this in her presentation. As far as the background goes, she talked about this, there was a rat toxicity study was being conducted to look at neurobiological, neurobehavioral, hepatic, and testicular effects of oral CBD. The original proposal -- this was being conducted by investigators in our neurotoxicity division -- did not include dosimetry, and we think it's important to have dosimetric data so that it helps to explain the findings and also extrapolate to humans.

So again, the idea was to assess the pharmacokinetics of CBD and 7-carboxy -- I'm sorry, 7-hydroxy, 7-carboxy, CBD in pregnant Sprague-Dawley rats and their pups following oral exposure. The design was that pregnant Sprague-Dawley rats were dosed daily by gavage with CBD on gestation day 6 or from gestation day 6 through 17. That was a gestational exposure.

Then there was a gestational and postnatal exposure, and the pregnant animals were dosed from GD 6 through delivery, and then their pups were dosed from postnatal day 1 to postnatal day 4 or 21, then blood samples were collected from both dams and the pups, tissues

were collected. Again, we're going to assess CBD, 7-hydroxy, 7-carboxy, by isotope dilution tandem mass spectrometry.

Where we stand on this study is we're in the process of a lot of samples to analyze. So we have one mass spectrometer, and an individual is conducting all of the dermal studies, and then we have another mass spectrometry individual who is doing all the oral studies, and we're just in the process of going through the samples as quickly as we can.

Okay, the last area that I'd like to talk about, and Tucker Patterson did mention this, and this is the wastewater surveillance. This was, as he pointed out, at the start of the pandemic two years ago, we were asked what could we do, and funds were provided. Dr. Camila Silva is trained as a microbiologist, and so she -- we talked about the possibility of her doing this surveillance. She put together a very nice protocol. She has spent the last two years collecting samples every two weeks in four different sewage treatment plants in the central Arkansas area.

These are the data. This is just from Adams Field. Adams Field is the sewage treatment by the Little Rock Airport. What's interesting here is, okay, so these colored lines, she's assessing three different genes. The gray area is the COVID incidence in this region.

Notice this is a log scale. What's important is that you can see that this S-protein drops out right here. She wasn't able to detect it and the reason for this is this was when the delta variant occurred. Apparently, the way they were screening for ORF, S-protein, and N-protein, they were simply there were mutations in the S-protein, and they were no longer detected.

If you look here on the gray line, you can see it's dropping. Remember, this is a log scale. This is the N-protein, and what's happened here is with the rise in the omicron variant, they are no longer able to detect the N-protein. So the important thing here is that you really need to be detecting multiple genes in order to get a true idea of what's going on.

Dr. Silva and I, we were talking about -- does she continue doing this? We had a nice two years sampling, and she has just submitted a very nice paper for publication, and we're trying to come to some conclusion as should we continue this, should we turn her over to someone else, and we really, at the moment, we don't have an answer.

Just in my last three minutes, these are the last challenges. In vitro, you know, we've talked about the NTP funding and how that's decreased. I think, as I mentioned earlier today, that through the interactions, the actions

of Bill Slikker, Tucker Patterson, Goncalo Gamboa da Costa, I think we now have adequate funds. Now we have to just put protocols in place.

Changes in personnel. We lost three people. We're in the process of recruiting an immunotoxicologist, which is something the SAB has recommended for years. The other big change is I finally, I've been asked to have a deputy director for a number of years, and I finally came to the realization that this was a good suggestion. So we put out a job announcement. Of the people who applied, Luisa Camacho is clearly the most qualified. So she now is my deputy director. We chat a lot about what should be the direction of this division and how -- who we need to recruit and so on.

Then the last thing, and this is a plea I've made before, as I told you at the beginning of this talk, 27 of the 46 people in this division are scientists who have trained, born outside the United States, and trained outside the United States. We are currently not allowed to hire anyone who has not been in the United States for three years. This seems to be an FDA policy. We've talked to people at the NTP, we've talked to people at NCATS, and they're not -- they are not subjected to this restriction. This restriction I think is damaging our ability to conduct studies. It's clearly damaging our ability to hire people.

We used to have a very vibrant visiting scientist program. That's completely disappeared, and the plea I continue to make, and I will keep making it until this policy changes; we need to go back to a program where we're allowed to bring visiting scientists in because I think it's good for us, it's good for the FDA, and it's good for the people we are trying to train.

With that, I will be happy to answer any questions.

DR. ASCHNER: Thank you, Fred. I was just going to reiterate that I don't know where the restrictions on hiring originate, but I would have thought actually that all the federal agencies would have the same policy. But we've also learned through COVID-19 that that's not the case with the travel restrictions. So maybe we shouldn't be surprised. But I guess that's where we are.

Okay, are there any questions about the science specifically? I see Ken joined us. Ken, why don't you introduce yourself and then ask your question?

DR. RAMOS: Thanks. I have actually been listening for the afternoon session. So, I'm Ken Ramos from Texas A&M University based in Houston and a member of the board.

Fred, thank you for the presentation. I actually have a question that's not really science-based, but it's

more a process-based, based on the comment that you made before. You made reference to provision of data for the centers in response to the needs of the centers, you know, provided, presented to you as perhaps a top priority for any one of the divisions. Obviously, I think right on point.

My question is how is it that you provide the data to the centers? Do you prepare a formal report? Do you just hand in the data and let them manage it? Could you tell us a little bit more about that?

DR. BELAND: Okay, Suzy Fitzpatrick mentioned this PFAS work we're doing for CFSAN. In that instance, we have provided them with a formal report, which took -- Dan Doerge had started that study, and I felt the obligation to complete it.

In the case of the tattoo project, that was funded by the Perinatal Health Center of Excellence. We owe that center a report, and so Svitlana and I have been preparing the report. This will be submitted to our coinvestigators at CFSAN for their input, and then once this collaborative group is happy with the report, that will be submitted to the Perinatal Health Center of Excellence. We'll also hopefully prepare a publication.

Other things, for instance, Goncalo Gamboa da Costa just completed a paper dealing with brominated

vegetable oils in collaboration with CFSAN. That is being published, and that will be the report, the full report that will be used by CFSAN for regulatory purposes. So it just depends on the specific center. We have a long history of preparing reports for the NTP, for instance. That's sort of the model we've followed.

DR. RAMOS: But it is not consistent that a report always gets generated.

DR. BELAND: No. Sometimes it's a manuscript and that will suffice. Sometimes -- but in these last two, the PFAS and the tattoos, we actually have prepared reports, very extensive reports.

DR. ASCHNER: Thank you, Ken. I think Alex has a question.

DR. TROPSHA: Very interesting presentation and very interesting endpoints. I guess I'm curious with the tattoo study, looks like you looked at the distributions. Are there any plans to go beyond pigment distribution into physiological effects? That's one question.

And second, for instance, or other biochemical events, and related question, this seems to be a topic -- the number of people that have it are staggering across the United States. So that certainly is very valuable study, but there have been studies elsewhere. I found a report from GRC on possible toxicity of some of the ingredients

and claims that some alternatives are less toxic than what's been used historically. So do you plan to expand the study and kind of evaluate it in the context of what else has been done in the world?

DR. BELAND: Well, to a large extent what we do will depend upon what CFSAN needs. Remember, we're providing data that they will use. As far as this study goes, we did not look to see do the pigments get degraded, because the way you assess the tissue is you digest in a strong base solution for up to 36 hours. So when we do the pig study, we'll have sufficient tissue that we can attempt to look for metabolites.

These compounds are not easy to work with. I've never dealt with anything that's quite so insoluble. This is people -- why it's a tattoo pigment is because it's insoluble.

As far as measuring toxicity, that again is going to depend upon what CFSAN, their primary interest and the draft protocol, we were going to look at various mechanisms of why the tattoo disappears so quickly, assuming that the same thing occurs in the pig. But in humans, it's the same -- you know, the data that I showed on the first slide about the 90 percent decrease. That's human data. So apparently it must be part of the healing process.

What we think on the mouse is when we tattooed the mouse, we put oil on the skin and maybe some of that, they were oiled every few hours for the -- and maybe that caused some of the loss.

Right now, we were just drafting the protocol with minipigs, and we will have to have extensive discussion with CFSAN to make sure to see what their interests are and the direction we will go.

DR. ASCHNER: Thank you, Fred. Are there any additional questions for Fred?

Okay, hearing none, I suggest it's 3:54, 3:55, I suggest that we take a 10-minute break and then we come back at 4:05 and we finish with the two last presentations for the day. So 4:05 we'll be back. Thank you.

(Brief recess.)

DR. ASCHNER: Our next presentation is from the Division of Bioinformatics and Biostatistics. Dr. Weida Tong, the floor is yours. Go ahead, please.

Agenda Item: Division of Bioinformatics and Statistics

DR. TONG: Thank you very much. Let me just get straight to what I need to talk about today. This division was established in 2012. So this year marks the tenth anniversary of the establishment of this division, and on top of that, the division is going to be reviewed by the

SAB subcommittee tomorrow and the day after tomorrow. So we already prepared the wine and champagne for the celebration over the weekend.

The division has four branches. The Scientific Computing Branch is an IT unit that supports the entire NCTR. Both Bioinformatics Branch and Biostatistics Branch focus on regulatory science research by developing hypotheses and methodologies, and Research-to-Review Branch, or the R2R Branch, takes these research outcomes and they translate to the regulatory application.

The last year, and actually it's the beginning of last year, we also established a special team under the immediate office called the AIRForce team, and the AIRForce stands for AI Research Force to develop an AI for the FDA.

So our vision is to make the division an indispensable resource to FDA. So for that, we try very hard to ensure that everything we do in this division has some way related to the FDA review process, such that our linkage with the FDA product center continue to be strengthened and our capabilities evolve to meet the current and future needs of FDA.

And consequently, our division is extremely collaborative with the centers, and we are literally collaborating with all the FDA centers, and particularly in

the last year and we added three new projects with CDER, CDRH, and CFSAN.

Currently we have ten collaborative projects and five projects that have been around for several years now, and we also have five new initiatives I'm going to talk a little bit about these projects in the next few slides. Most of our collaboration still is with CDER, particularly we are working with the Office of Translation Science and Office of Computation Science since the beginning of establishment of our division, and nowadays we also work with the Office of New Drugs and Office of Generic Drugs and so on and so forth.

We are also very successfully to obtain the funding from our centers. Right now we received a little bit over $1.5 million, and we are on the path to receive over $2 million as just as well we did in the past several years by the end of this year.

This slide summarizes the five existing projects. I'm not going to spend too much time on it, because most of them have already been mentioned by our center presenters, such as like Smart Template Systems, FDALabel, and Dr. Selen also mentioned about ALIS, the project that we are working with the ORA. For the CTP, we have ASSIST4Tobacco project was mentioned by Dr. Kwan.

As I said, starting tomorrow, our division is going to be reviewed by SAB committee members, and we have a session specifically talk about the background information on this project, as well as the progress we have made so far. So if you are interested to hear more about this project, I really highly encourage you to attend our subcommittee review starting tomorrow.

So this slide summarizes the four new collaborations we started last year. The first one with the Office of New Drugs of CDER to develop a DASH-like system to support the FDA Safety Policy Research Team. The second one with the Office of Computational Science of CDER, we have a new initiative called the SafetAI. This initiative was funded by CDER SRIG program. The SRIG program addresses the drug safety-related regulatory science needs and priorities. I'm going to talk a little bit more about this project later on in my presentation.

We are very excited to have a new collaboration with CFSAN on the CFSAN initiated a very large program called the New Era Blueprint initiatives, and within it there is an AI component, and our group has worked with CFSAN to develop and evaluate an AI component of a new era of food safety.

The next two projects is more on the PI-PI interaction related to FDA intramural grant applications,

and those are the two grants funded by intramural grants in FDA, and one is by Chief Scientist's Challenge Grants, another one is by Office of Women's Health, and our team is providing the technical arm to support this project.

Now, speaking of the FDA intramural grant applications, I don't remember we really talked about this grant, but actually it's a very important mechanism for the regulatory science development in FDA. There are a number of intramural grants available. I just list here a couple of them, which are important to our division. These are the Chief Scientist's Challenge Grants, the Medical Countermeasures Initiative, or the abbreviation is MCMi. Nowadays the MCMi mainly focuses on the COVID-19 related projects, and another two grants was one offered by the Office of Minority Health and Health Equity, and another one is offered by Office of Women's Health, and as the name suggested, one is focused on the minority health, another one is focused on the women's health.

Now, those are the four grant opportunities that are most relevant to our division, and those are -- these grant opportunities are very competitive, and NCTR only selects three proposals from the NCTR to compete at the FDA level, and I believe the last year we selected more than three for the MCMi and Office of Women's Health, but only

three from the Office of Minority Health and Health Equity and the Chief Scientist's Challenge Grant.

But anyway, on the last year, we have six proposals from the DBB, from our division, was selected to compete at the FDA level, and four were funded and two by the Office of Women's Health and one by Office of Minority Health and Health Equity, and the last one is by MCMi.

So now I'm going to switch gears a little bit and just talk about the science, and more specifically I'm going to talk about a specific AI program we started last year to use AI to advance the toxicology study at NCTR, and this program we called AI4TOX.

As you know very well, AI has been around for many years now. However, the most significant advancement in this area of AI actually only occurred in the past five years. So our AI4TOX program is really focused on the new AI methods, and I just emphasize the new, because the machine learning, particularly machine learning has been around for a long time, but this is not what we focus on this project. We focus on the new AI method to advance the regulatory application in the FDA.

This program consists of four initiatives, and initiative number one is to ask AI to learn from the existing animal study data in such a way that the derived animal, derived AI model, will be able to generate the

animal study results without really conducting animal study.

The second initiative is called SafetAI, I already mentioned in the previous slides. This initiative was started by CDER, and we are trying to develop a list of the AI models for the toxicological endpoints that are important in the FDA review process. Now, since FDA review mainly involves the text document, so the initiative number 3 is an AI-powered natural language processing to support the toxicity assessment using the FDA documents.

The last initiative is about how we will be able to effectively and accurately to process histopathology data from the animal experiment. I just wanted to emphasize that those are the research program and it's not for the regulatory use yet, but certainly this is a direction we are heading to.

So let's just talk a little bit about the initiative number 1, which is focused on generating animal study data using AI that we called the AnimalGAN initiative. As you know very well, animal studies are an important part of regulatory framework to assess the safety of the consumer product in FDA. But we also are aware that animal studies are expensive, time-consuming, and labor intensive, and besides, many people just don't like to kill the animal. So there is a paradigm shift in the toxicology

to find a way of replacing, reducing, and refining the animal studies.

We have been doing animal studies for many, many years now, right? That means we have a lot of data from the past animal studies stored in some sort of database. So, here we are asking the question of whether AI can learn from the past animal studies in such a way that we can generate animal study results for the new and untested chemicals without really conducting real animal studies.

So for that, we developed the AnimalGAN, using a methodology called generative adversarial networks, also called GAN. This methodology was introduced I think about five years ago, and if you are not familiar with this name, you have definitely heard about the Deepfake. Deepfake is exactly using this methodology, and Deepfake is capable of making you say something or doing something and actually you did not do it.

In other words, Deepfake can generate new data. So we took this concept to develop the AI models we called AnimalGAN, which is capable to generate animal study data for the untested chemicals.

On the right side, you can see when you conduct animals studies, you first have a study design, for example, which particular compounds have been used at which dose and which treatment durations. Then in the end of the

studies, you will have study results. In this case, like a clinical chemistry readout or hematology data.

So what we did is nothing really different from the real animal study. In this case, there's an in silico model, called AnimalGAN, we feed the in silico models by the chemical structure information, treatment duration, and dose. We are asking AnimalGAN to make a guess what is the study results look like.

We compare the predicted results versus the real results. In this case, we have 38 experimental measurements from the clinical chemistry and hematology, and of course, in the first few tries, AnimalGAN does not really get results correctly, and when we compare it, we see the difference. We feed this difference into the AnimalGAN, ask AnimalGAN to include themselves. So after we trained AnimalGAN on the 6,500 rats, which was treated by 110 compounds in three different doses and five timepoints, and in the end of day, we found AnimalGAN can generate very accurate results for the 1,500 rats that were treated with 28 entirely different compounds.

Now here is the results slide. As I said, we have 28 different compounds associated with 1,500 rats, and we looked at 38 measurements from the clinical chemistry and hematology, and we compared our results, the AnimalGAN

generated results, versus the real experiment results. We achieved a 98 percent concordance.

Now, keep in mind, both the 28 compounds and the 110 compounds used for training are from the same study design. So the next question we asked, can these models predict the results from a different study design. In this case, we used the DrugMatrix as the external validation set and the training we used in the TG-GATES.

So luckily, there is a common chemicals were tested by both TG-GATES and DrugMatrix. So we will be able to compare these 38 measurements between these two experiment designs, and you can see the concordance for the common chemicals tested by both study designs only around 68 percent. And then we asked AnimalGAN to generate the results, and for the chemicals tested in the DrugMatrix but is not tested in the TG-GATES, and we found that the concordance is 71 percent. So it's really not bad.

So the next question we asked, okay, the AnimalGAN can generate the data. Fantastic. Can we use it for the real world applications? So we look at the FDA guidance on how to assess the hepatotoxicity, and the guidance says if the ALT elevations is more than 3 of the compared to the normal condition, indicating the intermediate hepatotoxicity, between 1 and 2 is considered minor hepatotoxicity. We looked at these two situations,

and we found for the situation 1, we have 100 percent in agreement. For the minor hepatotoxicity, we have 83 percent in agreement between the experiment data and AnimalGAN generated experiment data.

And the next one we looked at the liver injury patterns, because this is quite important that this information dictates what kind of treatment we're supposed to receive for the patients with the hepatotoxicity. In this case, we involved two different parameters. One called ALT, another called ALP. If ALT divided by ALP larger than 5, indicating this is hepatocellular injury, and between 2 and 5 is a cholestatic injury or less than 2 is mixed injury. So you can see agreement also very high.

Now, the AnimalGAN is not the only GAN models we develop. Actually this is the second model we developed, but the first one we called ToxGAN for the toxicogenomics data, and the paper already being published in Tox Science earlier this year. So how we are going to use this AnimalGAN or ToxGAN? I want you to put your mind aside, don't think about the whole in silico science. You just consider this is the real experiment, and if you do the real experiment, what are you going to do? You use this experimental data for the toxicity assessment for the mechanistic understanding of the underlying toxicity, or develop a safety biomarker. So we can do all of that.

However, AnimalGAN and ToxGAN in my mind could be much more significant to contribute to the read-across, which is part of the NAMs. When we do the read-across, we mainly base on the chemical structure information, okay? That means the guilty by association. We assume if two chemicals are similar, that means these two chemicals share the similar biological or toxicological profile.

Well, the concept is good, and actually I can point out so many different examples this is not true. So the read-across, based on the chemical structure, is really by the convenience, not by the science. Of course, ideally we do the read-across based on the experiment data, but we don't have it for all the chemicals. But now using AnimalGAN, we will be able to generate experimental data to support read-across, and then we can use the ToxGAN to generate the genomics data to support the read-across.

Actually, we have several projects that were going on, and one of them, called HistoGAN, which would generate the histopathology data, and in one presentation I think I give to the NIEHS, they asked, okay, do the animal studies will only be able to measure several organs? But when we talk about toxicity, we talk about the entire organization, not just several organs. Can you predict based on the several organs, predict that the toxicity in other organs? Actually, we can, and we are not completed

this project yet, but a manuscript indeed is in preparation. We called it TransorGAN, and of course this is still far way to go, but the preliminary results is really, really exciting. We also developed BERTox, which I will talk a little bit more later on.

Okay, now I'm going to shift gears a little bit, talk about the second initiative, which is a common way to use the AI. That means we apply the AI on the experimental data to extract the patterns as a biomarker to predict that the toxicological endpoints. In this case, we develop a list of the AI models for the toxicity endpoints, which are important for the drug review, and these are the liver toxicity, carcinogenicity, mutagenicity, cardiotoxicity, as well as kidney toxicity.

Now, I need to point out that those endpoints have been widely studied. It's not we are the first one to study these endpoints. Particularly we are not the first one to use the in silico approach to study these endpoints.

The reason these endpoints have been widely studied is because they are important. However, traditionally when people use AI methodology to study these endpoints, using the approach I called the single model of prediction. That means you develop -- you select the one specific AI algorithm to develop a model to predict the risk.

So, quickly, we realize that for the different endpoints, you need to use different AI algorithm. We do not have one AI algorithm fit all the different toxicological endpoints. So for the best practice and people just develop all those models based on the different algorithms and pick the one they feel the best models, and then get published.

Since you already developed all the models, the next step is a very natural which is combine these model results, and this gives rise to a new approach called consensus modeling, basically combine all kinds of different model results, using some sort of demographic way to summarize these results. You can use average results from these models, or using the winner-take-all, but we here take a step further. We implemented AI to look at all the different combination of these models, which tailored back to the chemicals you're going to predict.

We found our results performed very, very well. But this is the slides I showed before about the drug-induced liver injury. This is the first model we developed, and we construct a model based on the drugs approved by FDA before 1997, and then we predict drugs approved by FDA after 1997. We reach 70 percent accuracy.

And then we compare other methods and using the same dataset, and our models really showed great improvement.

Then we take this methodology, apply to the carcinogenicity, and we develop the model based on around 700 compounds, and the challenge by 171 different compounds, we yield 75 percent accuracy, and we compared different methods and used it in the literatures, and our results have improvement around 37 percent.

So the paper already been published, and most recently, we finished another project focused on the mutagenicity, but this is in collaboration with the National Institute of Health Science of Japan. They have a very really large mutagenicity dataset, a little over 10,000 compounds. We developed models, and then we received a blind test, and we don't know whether the results of a little over 1,500 drugs, and then we did a prediction, we have 84 percent.

All right. So let me just talk a little bit about the initiative number 3. I'm going to skip initiative number 4, just stick to the conclusions, so we have time for discussion.

Initiative number 3 is to deal with the text documents. As you know very well, FDA regulatory decision-making largely involves reviewing and summarizing the

documents submitted by the sponsors. So this is certainly a time-consuming and labor-intensive process. Meanwhile, one of the most rapidly advanced technologies in AI actually is how we will be able to effectively extract the information from the text document? For example, Google developed a model called BERT, B-E-R-T. This has generated tremendous sensation in the research community, and we advanced BERT by applying a toxicology we called BERTox, and we found that the BERTox can perform very, very well for the document we are working with.

Okay, so this is how we do it. You look at the document, you extract the sentence from this document. You feed it in the BERTox, and then the BERTox puts all the sentences into the mathematical representation, and if these three sentences are similar, but that means these three sentences are talking about the same things. If one sentence says the king like apple, the queen like banana, and the third sentence says the royal family like fruit, and if you put the inquiry, you say does the king like banana, and then you will get results that says royal family like fruit. This is really allows us to walk away from the standard terminology and data standards to link the sentence in the semantic way. So we have a number of projects going on. If the document has a positive and a

negative -- I'm just going to skip that, and there are many ways to use it.

Last initiative was dealing with the pathology data, and we are trying to ask the question by compared to the pathologists, how AI will be better to determine whether it is injured or not, where the injury occurs, what type of injury it is, how severe is the injury.

And this shows the pipeline, the manuscript in preparation is another thing to go to the details to explain how the whole things works, but just trust me on it, that this is really cool. At the end of the day, we will not be able to understand the severity of the injury but also we will be able to pinpoint the location of the injury.

So here's the summary. I just wanted to reiterate AI is a research program. We are trying to leverage new AI approaches for the regulatory science, and right now, we have four initiatives that they are pilot studies, really promising, and the next step we're going to continue to develop it, evaluate these tools, and by focusing on potential suitability to support the regulatory decision-making. So that means in the next few years, you are going to hear more about this program.

Before I leave, I just don't want to leave an impression that AI and machine learning is the only thing

we do, and we actually do a lot of things. Those are the four projects that were funded by FDA intramural grant, and you can see they are everywhere.

I skip this summary, and I give you the beautiful NCTR campus, and I'll be happy to address any questions you may have.

DR. ASCHNER: Thank you, Weida. This was really exciting work. I open the floor now for some questions, and I don't know why I predicted that Greg would ask the question.

DR. LANZA: Thank you. I just have one question. When you say 84 percent or 83 percent accuracy, is the inaccuracies that you're getting more false positive, false negative, or balanced?

DR. TONG: Great question. Actually, I should not say accuracy. I should say concordance. That means that using the experimental data, and we say this is the intermediate hepatotoxicity. Then we use an AnimalGAN data also says this is an intermediate hepatotoxicity. If both point the same direction, we consider it is in agreement. If it is not, we consider it is not in agreement. In this case, 100 percent agreement, that means both experimental data and predictive data pointed the same conclusion.

DR. LANZA: Is there an actual truth data set that you're comparing to that one is the actual truth?

DR. TONG: Yes. We do the experiment data and predict data side by side for these 28 compounds, which is not included in the training set. Thanks.

DR. COSENZA: Just one question on the digital pathology. I know you didn't have a lot of time to talk about it, but it's become quite a hot topic amongst the CROs, the toxicology CROs, and there's a number of companies that are sort of advocating use of more digital pathology. So I'm just curious the relationship between what you're doing and what they're doing in terms of just straight slide reading for toxicology studies.

DR. TONG: Excellent question. Actually it gives me the opportunity really to add a little more detail and what is the thinking behind our project and what the difference between what we do compared to others. You look at the preclinical histopathology image, which is very different from the clinical pathology, and because clinical pathology, you took the tumor, the majority of the tissue is the tumor, and when you compare the tumor versus normal tissues, and this is a massive difference between the clinical samples, you know, the tumor samples versus normal samples. But in the toxicology study, we treated the rat, and all the injury was occurred very small portion of the entire image, and so that means inherently this is much more difficult to do.

And then we label it, when we do the label, we call the weak label, which is the entire image observed that injury. But actually, it's only a very small part of the injury.

So the entire community really struggling on how to deal with these so-called weak label issues. Now what's the difference we did compared to others is what we did is we are in between. We took the entire image, and we are using the GAN approach to digest this image in such a way we will be reproduced the digital image, and then we can condense the information in such a way we are not only be able to look at the severity, but we also can look at the location.

DR. ASCHNER: We have a question from Alex as well.

DR. TROPSHA: Weida, thank you, great presentation, great diversity of tools that you develop, and I know we have the entire day to look deeper into your division, so I will save most questions for that day. I loved your abbreviations, by the way, especially interorgan. That's extremely clever. I congratulate you on this.

Quickly, when you call methods and use methods and call this AI, is the key difference in the use versus traditional machine learning is the use of deep learning

algorithms, or there is more to this? With the exception of NLP. And if so, have you compared the performance of new, if you will, versus old?

DR. TONG: Absolutely. If you draw a line between new and old, separated them in a dichotomy way and this is pretty difficult. And we also know a lot of deep learning and new methodology does derive from the conventional machine learning methods. But in my mind, when we developed this program and with the four different initiatives, we do use the deep learning. We consider deep learning is the new AI method, but we consider like supporting the machine, you know, KNN, all of these methodologies are conventional machine learning methods, and we did compare with the conventional machine learning method with the deep learning, as well as much more advanced deep learning method.

DR. TROPSHA: So do you get better statistics? So we'll look more into this. My second question is somewhat on top of Greg's, and another sub-question of that. I guess the more global question is what accuracy is quote/unquote good enough for the models to be useful, and sort of on top of what I think Greg had in mind, I do think that total accuracy is insufficient when you report model, especially when you talk about toxicity, right? So I think

it would be useful to dissect sensitivity and specificity for this type of datasets, and this type of endpoints.

DR. TONG: Excellent questions, and I wish I had time to talk a little bit more about it. In the FDA's mind, we always talk about context of use, and it's not about overall accuracy. It's how we're going to use it, and besides the context of use, we also need to understand that the applicability domains, prediction confidence, and on top of that, we also get investigation of so-called adaptivity or adaptive behavior, and that means if you have new compounds coming in, whether the model is going to be evolving, become better, the context of the use remain the same or going to evolve, and we did all of that.

This was actually essential to be qualified in the ISTAND program in FDA. So I cannot really get a chance to talk about it, and I totally understand that the overall accuracy is just oversimplified to characterize the utility of the models, and I wish I had more time to talk about it, but just want to let you know that we do thought about this, we did this sort of assessment.

DR. TROPSHA: Thank you, I'm sure, and we'll have more time the next two days. Thank you.

DR. ASCHNER: Thank you, Alex and Weida. I have one more quick question, a general question, and I have asked the same question before. So sorry for being

redundant. But these are very novel methods, and my question is whether there's an effort to share this methodology with other federal agencies. You mentioned that you presented some of this at the NIEHS. So I'm wondering if NTP or other agencies such as the EPA are talking to you and taking advantage of these kinds of things.

DR. TONG: I am very glad you asked this question, because this is my fault I did not mention about NTP and NIEHS, and this program, actually we are collaborating with NTP and NIEHS particularly for the AnimalGAN and initiative number 2, the SafetAI, and as a matter of fact, we're jointly writing proposals to have a student to translate our methodology to the NIEHS. They have a program called ICE, and ICE is the platforms they want to incorporate and our methodologies and datasets into their systems.

We also work with the Swissmedic from Switzerland to translate the BERTox for their application.

DR. ASCHNER: Thank you. This is obviously very laudable. I'm glad to hear that. Okay, are there any other questions from the board members?

DR. TROPSHA: I may have a quick suggestion and we could address it. So NLP is an extremely rapidly growing area, and I don't think we could be confident that BERT would remain on top, right, and you probably use BERT as a

starting point anyway. So perhaps NLP tox will be more general, just to allow you to not depend on the particular software or method or technique in the future.

DR. ASCHNER: Thank you, Alex. Thank you again, and we're going to move to our last presentation for the day. This will be the Division of Genetic and Molecular Toxicology, and Dr. Bob Heflich will present.

Agenda Item: Division of Genetic and Molecular Toxicology

DR. HEFLICH: Hello. I'm going to try to get through this as quickly as I can, because when I tried it yesterday, it took me 40 minutes, and I sense that Donna has a pretty quick hook at 30 minutes.

My name is Bob Heflich. I'm director of the Division of Genetic and Molecular Toxicology, and Dr. Mugimane Manjanatha is our deputy director.

This is just an overview of our division staff. We have about 35 individuals all told, and that has held steady for a number of years. Very little net change this past year. But that sort of hides a lot of comings and goings. We lost three government FTEs last year, which is pretty unusual for us, and we've made a number of other activities including acquiring three support contract support scientists for FY2023, 2022 and 2023, through the funding from CDER.

So here are some of our collaborations with other divisions at NCTR, and other regulatory centers, and government agencies, both here and abroad, and universities and other institutions. I'll just let you read that if you're interested, but that's there for your information.

Here are some of our outreach activities where we've actually taken a lead in some of these collaborations. HESI, we've been workgroup chairs and co-chairs on the steering committee. IWGT, which is a big international organization in the genetic toxicology world, and OECD which has a lot of activities in tox. We've been leads for activities and committee chairs, and of course on expert groups.

Let's go down to the bottom, journal editors. We have two journal editors in the last couple of years, one current lead editor on Mutation Research and I've been editor-in-chief in the past of Environmental and Molecular Mutagenesis, which are the two specialty journals in our area, plus many people on editorial boards and publication policy committees.

So here is our mission and goals. Our mission is to improve public health by providing FDA with the expertise, tools, and approaches necessary for the comprehensive assessment of genetic risk, and how we try to do that is enunciated in these goals. Respond to agency

needs for expertise and chemical specific data. Maintain our tradition, the division's tradition of leadership in regulatory assay development and validation. After 15 years of effort, I'm happy to tell you that we're on the cusp of getting an OECD test guideline approved. It was approved by the WNR a couple of weeks ago for the Pig-a gene mutation assay. So that's a big event here at DGMT.

Develop better methods for carcinogenicity testing and translation of rodent studies to human risk, and we do that through the development of an error-corrected next-generation sequencing method called CarcSeq, and we like to incorporate our gene-tox methods into other toxicological models, especially in vitro toxicological models. We put a lot of effort into that in recent years.

And number one, we try to engage FDA product centers, the NTP, and other national and international organizations to set research priorities. We try to develop better biological models for assessing human risk and human in vitro models mainly in liver and airway have been our two major activities in that area, and develop more comprehensive approaches for monitoring genetic variation, and that's been in the realm of sequencing methods, and I'm going to say something about that hopefully if I get to it at the end of this talk.

Here are those metrics that you've been given for different divisions, and as Fred said, we like to think our major contribution is contributing to FDA's regulatory decisions and addressing their regulatory needs. So that sort of comes at the top of the list, but there are all kinds of numbers we can give you.

Here are some of those numbers, and I'd like to point out a few of these things here. Two of us were on -- we represented FDA in explaining the genetic toxicology safety assessment at the Molnupiravir Advisory Committee meeting last November. You may recognize that drug as being a COVID-19 orally administered drug, the first of its kind that was given emergency use authorization. It turns out to be a genetic risk, but I'll get to that a little later.

A lot of projects which are listed in the fifth bullet have external support, and that's I think sort of indicates that these are important enough to the product centers to put some money up for us to actually do them.

I'd like to go into the couple of projects we're doing, and Dr. Stein mentioned this first one is important to CDER, looking at nitrosamine impurities. We started this project I'd say about a year and a half ago, and it's sort of developed in a large effort, those three contract support scientists were hired to help us with this project.

So here's the title of one of the experimental protocols that's involved with this mutagenicity of N-nitroso drug-substance-related impurities in the Ames-Salmonella gene mutation test optimized for evaluating immunogenicity of N-nitrosamines, and there's a lot of little elements to that title that I'll try to explain to you.

But I'm working as the PI on this, and there's here listed from NCTR. Point out the CDER collaborators, that mostly members of what's called the CDER nitrosamine task force. Aisar Atrakchi is our main contact person, but there are a lot of people involved in this effort, and also I have to mention Rosalie Elespuru from CDRH. She's got I would say 40 or 50 years of experience in immunogenicity of nitrosamines, and she's an expert on microbial mutagenicity assays. So she's been really useful for conducting this project.

Some background to this. CDER uses the Ames bacterial mutagenicity assay to classify drug impurities and degradation products for the risk of causing cancer. Mutagens are suspect carcinogens and controlled at low levels.

Nitrosamines have been found increasingly in drug products as impurities formed during the synthesis or storage, including as derivatives of the drug substance itself, and these are referred to as nitrosamine drug-

substance-related impurities or NDSRIs. These are totally novel in the world of nitrosamines. So this problem is estimated to potentially affect 15 to 20 percent of all marketed drugs. So it's a huge potential problem for CDER.

Nitrosamines in general are cohort of concern compounds. Most of them that have been tested -- and I'm talking about 90 percent, upwards of 90 percent -- are carcinogens and appear to act through a mutagenic mode of action.

So when you get a negative one, you sort of wonder whether or not that's accurate or not, and the literature indicates that the mutagenicity of nitrosamines in the Ames test is affected by the protocol used for testing, making Ames-negative compounds challenging to classify as to their potential risk, and several of these NDSRIs, these new compounds, for which there are little data, certainly no carcinogenicity data, are claimed to be negative using standard Ames methods. So CDER came to the conclusion that there's a need for a version of the Ames test optimized for detecting nitrosamines that will increase FDA's confidence in the negative findings that the drug companies are sending to us in applications.

So the objectives of this was to develop recommendations on the best way to evaluate nitrosamine mutagenicity in the Ames test, to improve the in-silico

prediction of nitrosamine mutagenicity by filling in gaps and generating optimized data sets for standard nitrosamines and NDSRIs. As Weida sort of alluded to, a lot of the decisions are made using in silico tools, and there's a lack of good data in the datasets for this particular class of compounds.

And when called upon, we contribute to regulatory evaluation of NDSRIs and other nitrosamines.

Here's a little schematic of the Ames test. Up in the left-hand corner is an NDSRI that if you're a chemist you might recognize, but what we're doing is evaluating what variations to this standard kind of method that's been around for 40 or 50 years will optimize the response and sensitivity of the test of these particular problem test agents, and on the right, here are some of the variables we're looking at.

Okay, so, as I tell people, this is a work in progress. So far we've confirmed literature reports that different Ames test protocols affect the mutagenicity of standard nitrosamines, sometimes by orders of magnitude.

Developed a strategy to evaluate the most promising of these variants with a series of nitrosamines and NDSRIs that are of interest to FDA, and we've conducted so far over 10,000 assays, that's just in 2021, on approximately a dozen of these compounds. Each assay

includes 1,080 plates, and those are big numbers, and really each plate requires hands-on activity by the person doing the assay. So it's a lot of labor involved in this.

So our additional support scientists have increased throughput to approximately 600 assays per week. So our immediate plans are to test 15 to 20 nitrosamines that have been prioritized by CDER and develop a strategy for efficiently conducted the Ames test and perhaps we'll have to test more if we can develop a strategy. And then the immediate plan, at least what we're planning on right now, is to use this golden strategy to test 27 reported Ames negative nitrosamines that have positive, negative, or unknown cancer findings. So these are particularly worrisome to the CDER regulators.

Here are our Ames testers, and I like to show some smiling faces in contrast to my face, and this reminds me to thank the Office of Pharmaceutical Quality, OPQ, who are actually paying for these people to be at NCTR and Dr. David Keire, who sort of led the charge to get the money to fund these people. So we can actually work our way through these mountains of testing to get the data that CDER needs.

Here's some of what we're doing, just as an example. On the table on the left, shows some dose response data for a nitrosamine called N-cyclopentyl-4-nitrosopiperazine, which is an impurity found in certain

drugs. It's a little complex nitrosamine, and you can see -- well, maybe you can't see, but the data that's italicized and dark, bolded, in the table indicates a positive response in a particular test strain, and you can see at the top there are five test strains that we used, and you can see dark, bolded responses in some columns, not in others. So this is very specific type of mutations being induced by this particular nitrosamine.

So we'd like to know which, what works best. So we looked at these dose responses quantitatively using some software called PROAST, which is available through the Dutch regulatory agency online, which converts using a hill and exponential models, coverts this data into dose response relationships. We can see that on the right, the two models that are used, the two sets of graphs.

And then we use these data to do what's called benchmark dose potency ranking. We look for a dose that produces an increase in mutant frequency, a predefined increase in mutant frequency, which in this case is 50 percent, and we calculate from the curves, the two curves, what kind of response the benchmark dose and the confidence interval for each tester strain, and you can see on this graph on the right that there are sets, pairs of confidence intervals, and that's because there's two models that are used.

And you can see on the left is the responses that are more potent. So TA1535 is obviously the most potent detector of this particular nitrosamine, followed by TA100. Both of these detect mutations at GC base pairs, and then WP2, which is an E. coli strain, again a base pair detected, but this time at AT base pairs, and the two dotted lines the least effective at detecting mutagenicity, and there 1536 and TA98, which are both frameshift detectors. So this is a clue as to what kind of assay you might want to run to detect nitrosamine. Of course we have to do a lot more testing to see how well this clue holds up and looking at different classes of these compounds.

So, beyond Ames testing. There was a public workshop held on nitrosamine impurities last year by CDER and then of the things that the experts who were queried came up with is there's a lack of data confirming that screening in these rodent-based systems -- and the Ames test uses rodent activation, mixes liver homogenates from rats usually, and hamsters -- has any relevance to human risk.

Also there was a need to express for developing in vitro and in vivo follow-up assays that can be used to further investigate Ames test findings for nitrosamines, especially the negative findings. So we're conducting a number of additional projects using human-based

metabolically competent systems to evaluate nitrosamine genotoxicity and mutagenicity in vitro, and we're conducting some in vivo studies to determine relative sensitivity of the different in vivo type genotoxicity endpoints, and they're listed here as transgenic gene mutation, Pig-a endpoint, micronucleus, Comet, next generation sequencing endpoints, and which are the best endpoints that we can use for detecting the mutagenicity should it exist for unknown nitrosamines in rodents. So all this is going on in various projects.

The in vitro human cell project was given RSR funding from the CDER Office of New Drugs last year. So we're very happy about that and pushing that along. And actually, this is a worldwide issue and we're collaborating with people different pharma groups and contract research organizations, including the Fraunhofer Institute and the European Medicines Agency, have projects going in Europe and we're comparing our list of compounds we're interrogating for mutagenicity to see whether or not we can sort of collaborate and coordinate what we're doing and sort of because there are literally hundreds of potential nitrosamine genotoxic impurities in drugs. So big problem. It's not going to be solved overnight.

So I'm up to my second topic for research and that's error-corrected or error-avoidance NGS. So as you

probably know, mutagenicity generally in genetic toxicology assays is determined indirectly by evaluating a phenotype associated with mutations in a particular gene, and that's why these events are called gene mutation. But these assays often employ very specific cell types or a particular kind of animal model like a transgenic animal model; I also list Ames tester strains here, which are kind of engineered type bacterial cells.

All this has been successful and obviously useful for identifying hazard and potential carcinogens, but if I could have the next slide, if you could look at mutation directly in the DNA, I'm talking about rare somatic cell mutation, you could potentially measure mutation in anything, in any tissue, in any animal, in any sequence, including the entire genome. So you could use generic animals, for instance, used in general tox assessments, and you could do it in humans and perhaps even archived samples. You could measure mutation in cells difficult to assay by traditional means, like these in vitro NPS-type organotypic cultures that are being increasingly used.

You could measure mutation in any sequence, like cancer driver mutations, which may have specific value in evaluating carcinogenic potential, and also various kinds of somatic mutations. Like somatic mosaics, which little attention is given to, and then there's germ cell

mutations, as well as in neutral genes that get a good idea of mutational load in an organism.

And one interesting thing about these methods, they generate spectra or fingerprints as part of the assay, because you're measuring mutations directly. So you can understand mutation mechanisms as well as associating cause with effect, and all this may be useful for conducting molecular epidemiology applications when you're looking to associate a disease with a particular exposure.

Here is something I probably showed this group before, last year, but the problem with doing this is standard NGS has a very high error rate, and that's sort of shown on the top left-hand graph, where about 1 percent of the bases have incorrect read as far as the nature of the template base. So it looks like the mutation rate at any one position is about 1 percent, which is ridiculous. It's tremendously above the level of actual somatic cell mutations. So the method itself is not useful for measuring rare events.

But if you apply some filters and do some fancy computational kinds of things and use special approaches, you can clean the system up so you can measure one real sequence change in approximately 10^8 normal bases. So that's the kind of sensitivity you need to actually do mutagenesis using sequence analysis.

So we've used this, we started adopting these kind of methods several years ago, but we have used this for a number of projects which I'm not going to go into, but this is right now considered to be an emerging technique, and FDA does not look at these data for regulatory purposes as yet, but in the coming years, they will. The world is changing here.

So I am just going to feature and talk to you about two projects on the bottom that are in bold. One is called PacBio sequencing. It uses the platform produced by the PacBio company, the PacBio Sequel II smart sequencer, or sometimes called HiFi. It sort of has a different chemistry than Illumina sequencing and I'll go into that in a little bit.

And I want to talk a little bit about analysis of mutation in highly differentiated in vitro organotypic tissue models also if Donna doesn't stop me from going on.

Okay, here's a proof of principle study we've run evaluating the mutagenic potential by PacBio error-corrected NGS, and this is being conducted by Javier Revollo, Vasily Dobrovolsky, and Jaime Miranda, and on the bottom here it sort of explains how the system works. You generate fragments. You actually fragment the whole genome into about 5 kilobase chunks, and then you put these linkers on the ends of these chunks. You can see they form

hairpins on double-stranded duplex DNA. So it looks like a barbell, and that's exactly what PacBio calls these smartbells, and they're sequenced in these linkers that enable you to identify this one particular model, and then you can go ahead and sequence it, and because this is a circle, you can just go around and around and generate multiple copies. So computationally, you can subtract out all the errors that are induced randomly by the sequencing process.

So if you go on to the next slide, this is a proof of principle study we conducted with molnupiravir-treated cells in culture, and you can see on the bottom left here that the method was very sensitive to detecting molnupiravir-induced mutations. There's little dose response. NHC is the active metabolite of molnupiravir, MOV is a prodrug, molnupiravir is a prodrug, and you can see they produce pretty comparable responses at microgram quantities, and over on the right, you can see that most of these mutations were A to G mutations. NHC is a base analogue which produces mispairings in the DNA which produces specifically A to G mutations. So here you go. That's an example of how this assay can be used.

And you get spectra out of this. These are called fingerprints, and potentially these could be used to

fish out a molnupiravir signal for instance in a treated animal or a treated human even.

And we've applied these kinds of error-corrected NGS systems to organotypic tissue models that are highly differentiated and don't lend themselves to normal genetic toxicology endpoints. Work done by Yiying Wang and Xuefei Cao on the ALI airway model.

And this enables us, this is a little picture of the airway model in the upper left-hand corner, to integrate mutation analysis into other kinds of physiological endpoints in this model. So it's kind of nifty the way you can look at multiple physiological and molecular endpoints in this one little tissue model that sort of replicates the lining of the human airway.

In the lower right hand, we can do histochemical evaluation of cells and all kinds of things. The bottom line is in the lower right-hand corner is a mutational dose response we got after 28 days of dosing. So we did a subacute study essentially in this in vivo-like model and got a mutational response. You know, sort of parallel to what you might do in a transgenic rodent assay.

So, we're using these methods in a bunch of projects, both starting up and proposed, one is a CRADA with an industry partner; one is hopefully a project with CTP looking at mutations induced in ALI airway cells.

So I have a lot of challenges here, most of which were enunciated by Fred Beland as far as dealing with the restrictions placed on foreign nationals, which is a major problem for us. Also, the ORISE contract that we use for postdocs, even if someone has been in the United States for three years and got their PhD at a U.S. university, they come to NCTR as a postdoc on what's called an OPT or a optional professional training, but ORISE only accepts one year OPT. So after one year, they're sort of on their own, and they have to apply for a visa to continue their studies, and often there's a big delay in this. We've had people sit at home for months at a time waiting for their visa to come through. It's really pretty bad.

We deal with a lot of international suppliers, including suppliers that have ties to Asian countries that are on the FDA disapproval list, and they tend to fall on and fall out. And this is particularly true of genetic analysis contractors for next-generation sequencing where you can do that kind of analysis a lot more economically than we can at NCTR or in FDA.

And the last one is I've actually I resigned three years ago and have been waiting to be replaced, and it looks like that's going to happen fairly soon. People are being interviewed for the next DGMT division director, and I just hope we get someone who's a leader in genetic

toxicology and not someone who's just a manager, and that's my only suggestion to the people making the choice of the next DGMT leader. This is a big opportunity to get someone who can really help the division and advance the science in the division.

That's the end of my talk. Thank you.

DR. ASCHNER: Thank you, Bob, for an interesting presentation, a lot going on in your division as well, and good luck with retirement. Hopefully, it will come soon.

DR. HEFLICH: Well, I'm not retiring yet. Just stepping down. I really would rather work at the bench than push papers around. That's the bottom line.

DR. ASCHNER: Thank you. The floor is open now for questions. We have 15 minutes. No questions for Bob? That's why I like face-to-face meetings much better than Zoom.

DR. HEFLICH: I don't know how to take that, guys. Either we're totally disinterested, or I just blew you over and you can't think of anything.

DR. COSENZA: Hi, it is Mary Ellen. I am interested in the nitrosamine project, tweaking the Ames assay. I have to say I'm very impressed by the number of assays that your group is doing a week. I hope they are able to maintain those smiling faces after all that work. But when do you think that project will be done?

DR. HEFLICH: It is sort of open-ended at this point. I mean, we're just discovering this problem, and I think this taskforce was formed by CDER to just find out what we can do about it, how CDER can address it. The pharmaceutical companies are discovering the NDSRIs in particular. They haven't looked for them before in drug products, and really they don't -- they are applying standard techniques to evaluate them, and they just might not be the best way of doing it, and I think everybody recognizes that there's a need to sort of rethink the whole paradigm of looking at drug impurities and try to come up with something that's better and tailored for these things that are apparently everywhere, 20 percent of marketed drugs. So I really can't tell you that. But it probably will be going on after I retire, even if I don't retire this year.

And yes, I did an Ames test today right before we had this talk, and we try to push out 200 assays three times a week. So if we keep that up, we'll work our way through a pile eventually and maybe we'll get to a space, a spot where we can make some decisions about how much more we need to know.

DR. ASCHNER: Thank you, Bob. Thank you, Mary Ellen. Are there any additional questions? Hearing none, Donna, do you have any comments?

DR. MENDRICK: No.

DR. ASCHNER: Okay, so this will conclude the first day. I want to thank all the presenters, the board, everybody else that was online. I was monitoring the number of participants. Some points we had over 150. So great interest in the work, very exciting work. We will resume tomorrow morning. We still have three different divisions that will present for the general public. I guess the full board will be a shorter day. So please come back tomorrow at 9 o'clock Eastern time. Thanks, and have a nice evening.

(Whereupon, the meeting adjourned at 5:20 p.m.)