FOOD AND DRUG ADMINISTRATION
National Center for Toxicological Research (NCTR)

Meeting of:

National Center for Toxicological Research (NCTR)
Science Advisory Board (SAB)

August 19, 2020

(Virtual Meeting)

Proceedings by:

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TABLE OF CONTENTS

P R O C E E D I N G S (8:00 a.m.)

Agenda Item: Welcome

MICHAELKAWCZYNSKI: Good morning, everybody, and I'd like to kick this off correctly to the NCTR Science Advisory Board meeting. Welcome to day two. I'd like to introduce you to Michael Aschner, who'd like to kick it off today.

Michael, take it away.

DR. ASCHNER: Thank you, Michael. Thank you very much. And thank you to, again, all presenters and participants. I see we have a fair number, again, just like yesterday.

Without further ado, we'll have three presentations this morning from three different divisions. The first one is the Division of Microbiology, and Dr. Carl Cerniglia will be the presenter, the director of the division.

Carl, the floor is yours.

Agenda Item: NCTR Division Directors: Overview of Research Activities (Continued)

DR. CERNIGLIA: Hello and good morning, everyone. My name is Carl Cerniglia. I'm the director of the Division of Microbiology at NCTR.

Again, I just want to wish everyone a good early morning here, and I look forward to the presentations of my

colleagues later today as well, as all the nice presentations I heard yesterday from the FDA representatives, as well as the NCTR division directors. Outstanding, so I hope I can follow in that same type of path.

First, I'd like to thank each and every one of you for being here this morning at this virtual meeting, and I appreciate all the effort that the committee members make in reviewing our document, as well as the FDA representatives giving us feedback on our work. It's very much appreciated.

I also want to acknowledge our deputy director of research in the division. His name is Dr. Steve Foley.

This is the disclaimer.

The mission of the Division of Microbiology is to serve a multipurpose function with specialized expertise to perform fundamental and applied research in microbiology areas of FDA responsibility in toxicology and regulatory science.

As part of the division's efforts to identify the most important or impactful questions that we have, we have two major goals. One is we prioritize our research efforts to best serve the agency to meet the FDA mission. And there's a variety of ways that we do that. One is certainly by engaging colleagues at the other centers,

primarily in the research design phase, to assess the feasibility and the interest in the research area.

Secondly, which is very, very important to us, is the obviously enhancing communication channels. We do that in a number of mechanisms -- meetings like this, communications via the web, with our colleagues as well as participating in a wide number of working groups.

The microbiology needs of the FDA are diverse and oftentimes very complex, so for the division to be a key asset to the agency, we have different strategies that we keep in place. One is certainly in terms of the focal areas, and we have five bullet points here, but today I'll be probably spending most of my time talking on three major areas. One is evaluating the impact of antimicrobial agents, food contaminants, food additives, nanomaterials, and FDA-regulated products on the microbiome. And as Dr. Slikker mentioned yesterday, we've been doing microbiome-type related research for a number of years at the institute.

We also have a microbial contaminants program, developing methods to detect and characterize microbial contaminants in FDA-regulated products, and our food safety program, focusing on determining the antimicrobial resistance and virulence mechanisms of foodborne and other pathogens. We're also conducting research to aid FDA in

areas of women's health, tobacco products, and nanotechnology. I won't have time to probably get into the details of that, but we will be discussing these topics this afternoon at our subcommittee expert SAB meeting.

We also have studies improving risk assessments of FDA-regulated products, including by integrating systems biology approaches.

As I said, to meet these agency goals, we have three-pronged strategy. One is, and I'm starting from the bottom, is strengthening research program management. We try to spend a lot of effort actually establishing benchmarks of scientific excellence for our scientists in the division. Certainly, when asked we communicate our research in plain language. We have some of the older buildings at the institute, so there's a need to upgrade research facilities and infrastructure, and we try to do that as best we can.

The other important aspect in our strategies is enhancing FDA research interactions, and the first key is obviously to try to assess the needs and then try to conduct research critical to the FDA regulatory science mission. I think our goals and strategies, too, is to kind of continue to expand our collaborative relationships with FDA centers and ORA.

Ultimately, if we achieve those first two points, then we could contribute to FDA guidelines and regulations. This morning, I'll briefly mention a couple of examples where we actually have done that. I'm really proud of the group for their efforts.

Similar to what you heard yesterday from the other division directors, our staff is composed of research scientists, staff fellows, visiting scientists, support scientists, administrative staff, and ORISE postdocs and graduate students. At this time, our total is 39, but it can vary, it can be as high as 50 or so, depending upon the summer programs, intern programs, and other visiting scientists that we have.

I wanted to spotlight the staff because it's always very inspiring to me. I have a great staff, and it's always inspiring to me, the pride that they take in their research. I don't have time to go through each and every name, but I will during the presentation try to mention their names with the various projects. Today, later today, each of these will be giving either an oral platform presentation via the virtual meeting concept or a poster flash presentation.

It's also very admirable for me as they work as a team, or they can work independently, but as you can see from this particular slide, I kind of match the research

expertise with the various research focal areas, and how each of these individuals work together. Basically, what I wanted to say is we do the team concept on our projects based on the research expertise and the research focal areas, and it's wonderful to see how they take these projects from the concept stage all the way to completed protocols. I have a very dedicated staff.

Part of the format was to discuss outreach activities, and our staff is both very active at the international and national levels for outreach activities. This is just a kind of an explanation in each of these various boxes, some of the activities, but if you go online and look at the NCTR PI site for the Division of Microbiology, you can click on each of their biographies and sketches there, and you'll see the various specific details. Personally, I've been involved for a number of years with the World Health Organization, both with JECFA and JMPR, that's continuing, we had meetings just recently via virtual meetings.

Also at the international level on harmonization of guidelines for safety assessments with VICH and other international working groups.

We also, staff is involved with guest worker programs, a wide variety of mentors through students from a number of different international universities. Our

scientific staff also is very active in scientific societies. I've listed just some of those there. Science advisory boards, journal editorial boards, a number of U.S. government panels, and we have close relationships with our local universities here in Arkansas. The University of Arkansas at Pine Bluff, which is very close to the institute in terms of geography, so we have a number of students that come from UAPB and work in the lab. The University of Arkansas, Little Rock, we've had a number of students in a PhD program there do their research in the Division of Microbiology. Similarly, at UAMS, and also the University of Arkansas, Fayetteville.

As I mentioned, we have on our research focal areas, I'm going to talk about our top accomplishments of the year, and I kind of grouped them into the three focal areas. One area is microbial contaminants detection, and this is what's on this particular slide. We have studies evaluating the presence of microbial contaminants, including pathogenic mycobacteria in tattoo inks and permanent makeup products. Yesterday you heard from Dr. Suzy Fitzpatrick at CFSAN our involvement in these projects. This is being led in the Division of Microbiology by Dr. Seong-Jae Kim.

We have another project with CDER, and what I have tried to do in this particular slide is just a list

from representative centers. We have a number of projects, but I just kind of picked out some examples that are in various states of either ongoing or completed.

Developed and evaluated optimized methods for the detection of Burkholderia cepacia complex in pharmaceutical products. Dr. Bernard Marasa yesterday described our work there. This is led by Dr. Youngbeom Ahn in the division.

We've had a couple of projects with CBER evaluating the potential health risks associated with the contamination of fecal microbiota transplantation samples. This work is being led in the division by Bruce Erickson and Dr. Doug Wagner.

We've had projects with the Center for Tobacco Products, identifying characterization of microbial populations in smokeless tobacco products. This has been led by Dr. Chen and Dr. Foley.

We also have a project with CFSAN developing a cultural method for the detection and isolation of salmonella in spices, and this is led by Dr. Ashraf Khan. I will give examples, a little bit specific examples, on some of these projects.

Tattooing and the use of permanent makeup inks has dramatically increased over the last decade, since there's been outbreaks of nontuberculous mycobacteria associated with tattoo inks, and this occurred around 2011,

2012, the FDA has become aware of these contaminants of tattoo inks with microorganisms, and they are a source of tattoo-related infections and outbreaks. So the division has been supported by CFSAN, the Office of Cosmetics and Colors, to do surveys. We've actually completed two surveys, two very recent publications on this, and there has been significant impact on this particular work because some of the research has contributed to the recall of contaminated tattoo inks.

But basically what we found is that almost half of the inks were contaminated with microorganisms, including some species that may be opportunistic pathogens. We also did a follow-up study; the results were very similar. We're also looking at endotoxin levels in these inks, and that was also found to be in many of the samples.

In the next phase of the work, and we have very good communication with the cosmetics group there at CFSAN, and we're continuing microbial and endotoxin surveys of different tattoo ink types, the country of manufacture, and a reassessment of the previously recalled tattoo inks.

Our project on Burkholderia cepacia complex in pharmaceutical products, I won't go into a lot of detail on this because Dr. Marasa yesterday did an excellent job of actually describing the work, as well as the importance of the work in terms of how this test method could be a

compendial test method that could be used, similar to the Pharmacopeia USP method for BCC recovery detection. It turns out to be a very interesting project, and the reason, as Dr. Marasa mentioned yesterday, Burkholderia cepacia is an opportunistic pathogen and can cause serious illness to immunocompromised patients, especially with having respiratory issues. Currently what we're doing is developing a rapid PCR-based detection method for BCC from water and other pharmaceutical drug manufacturing products.

Another focal area that I wanted to highlight in terms of ongoing work accomplishments is the human microbiome work that we've been doing, and what I call the human microbiome host-interaction studies. I think you're probably all very much aware over the last decade the microbiome has emerged as one of the hottest areas of biomedical research, and it kind of represents a new frontier in environmental toxicology, human toxicology. It's very, very important for regulatory agencies to effectively integrate these new and emerging sciences into the risk assessments, and that's one of our goals, is to incorporate the microbiome data into toxicology risk assessments. The key question that we face from an environmental toxicological standpoint is how do these environmental exposures influence the microbiome composition and function and the associated health effects?

We have research teams involved in a number of projects, and I'm just listing these. Again, I did the same examples here. I just picked out the projects front he various centers. Over the years we've done an enormous amount of work in collaboration with CVM and they're evaluating the effects of acute and chronic exposure of residual levels of veterinary antimicrobials on the gastrointestinal tract. I'll talk about some studies specifically in a moment. This particular work is led by a team, Dr. Youngbeom Ahn, Dr. Khare, Dr. Kuppan Gokulan, and myself.

We also have a project with CDER led by Dr. Huizhong Chen in the division, continuing efforts to evaluate the interaction of nanoscale materials used in sunscreens on the skin microbiome. We have a very important program with the National Toxicology Program and NIEHS, research conducting studies to understand the potential impact of xenobiotic compounds. I just listed a few of those there. Arsenic, bisphenol AF, triclosan, aloin, silver nanoparticles, on gastrointestinal microbiome and immune responses research. This is led by Dr. Sangeeta Khare and team.

We also have projects which have been completed now assessing the impact of smokeless tobacco products on members of the oral microbiome, and this has been led by

Dr. Chen and also Dr. Foley has been involved in tobacco research, as well. We also have the recent project in collaboration with CDER led by Dr. Kuppan Gokulan in my program, developing an in vitro intestinal epithelial cell culture cell model for the evaluation of toxicity of nanocrystal drugs and their parent compounds. We're also looking at microbiome and other host effects as well.

What I'd like to highlight is the work that we're doing in collaboration with CVM on the impact of exposure to residual levels antimicrobials on the human intestinal microbiome. This work, and when I talked about strategies, this work fits one of our goals, and that strategy is to impact guidelines, and we have at both at the national and international level. The methodology and results from our studies have enhanced the development of VICH GL 26/FDA Guidance 159, Studies to evaluate the safety of residues of veterinary drugs in human food, general approach to establish a microbiological ADI. We recently coauthored a paper summarizing the latest developments on the guideline with Dr. Sylvia Panaro(ph.) at CVM.

One of the key things when we're doing any of these risk assessments and you're looking at human intestinal samples is that one of the challenges is that there's a lot of interpersonal variability, differences, among and within individuals which occur regarding the

microbiome composition and antimicrobial resistance effects. We have found that in all of the different antimicrobials that we studied, and we just published a whole series of papers on tetracycline effects on the gut microbiome, and microbial resistance and epithelial barrier results. And basically, results are, and this is not too surprising, that the higher levels, sometimes when you're talking about therapeutic levels of drugs, we know without a doubt they impact the intestinal microbiome composition. But the residual levels that could potentially be found in foods did not seem to impact the intestinal microbiota.

The same thing goes with resistance; the challenge with resistance is that we do have, especially with tetracycline, we have background levels of tetracycline-resistant genes in our fecal samples. If I sampled each of your fecal stool samples, you would see high levels of tetracycline, these 23 resistant tetracycline genes were assayed in our studies, and the four tetracycline-resistant genes, tetO, tetQ, tetW, and tetX, were commonly found, and the fold changes were very different depending upon the individuals.

The other important aspect when you're talking about toxicology risk assessment is bioavailability, or oral exposure to the GI tract. If these are ingested, then there could be potential exposure in the gut. What we've

been finding with most all of the antimicrobials that we've tested to date, that they bind very, very rapidly, at very high percentage. You can see with tetracycline, it ranged from 42 to 73 percent. Also the binding is very quick. It does not resorb; it remains constant over the incubation period.

The other kind of new aspect of our work on the effects of antimicrobial drugs on the microbiome and intestinal tract is actually looking at the acute and chronic exposures on permeability of the human colonic epithelial cells. We've been finding that the high concentrations could compromise intestinal barrier functions. Residual levels that you might find in food do not. The latest studies we have that are currently going on around erythromycin.

Our food safety program, antimicrobial resistance, and virulence-associated accomplishments -- you heard very nicely yesterday by Dr. Tan at CVM, she described these top two projects, multiple ongoing studies that are evaluating genetic basis of antimicrobial resistance and virulence mechanisms of Salmonella enterica, and developing and optimizing a salmonella virulence gene database and tools to utilize the database to evaluate DNA sequencing results. These studies have been led by Dr. Foley's team, which includes Jing Han, Bijay Khajanchi, and

a host of support scientists as well as students and postdocs.

Kind of a new area that we got involved in the division is what we call coinfection model, and that's the third bullet point here. This has been led by Dr. Marli Azevedo, and I'll talk about her later, but she's a virologist in the program. One of the things she was very interested in is the relationship between norovirus infections and this coinfection with salmonella, and she's been doing some excellent studies in that.

Also, from us with CDRH, we've been involved for a number of years, and we have new projects involved using multiple approaches to evaluate antimicrobial resistance associated with antibiotic-coated medical devices. We've been honing in on Pseudomonas aeruginosa infections related to antibiotic-coated catheters. And this has been led by Dr. Kidon Sung and Dr. Saeed Khan.

Dr. Slikker mentioned in his opening presentation about the emphasis of how we, as a team at NCTR, have a number of projects on COVID. And I'm very proud to say that -- and he mentioned the fact that the Division of Microbiology recruited a virologist a number of years ago based on the recommendations of the SAB, and we did a very competitive search. In fact, we had five very top virologists seeking the position, and we selected our top

choice, Dr. Marli Azevedo. And it's just maybe somewhat coincidental but it's the way science works, actually she was doing coronavirus work in her postdoctoral studies.

So when she arrived at NCTR she actually did two studies on coronavirus, so this is pre all of this everything we know about the emerging problem now. My point that I'd like to make is that we pretty much had this skillset in the facility, within the division, to take on some of the challenges of COVID-19. Basically, early on, she did study a human respiratory coronavirus to detect in patients with influenza-like illnesses in Arkansas, and so this was a publication that came out on that work.

And also, which is relevant, that study is relevant to today's issues, and else she did another study looking at the survivability of coronavirus on leads, and she did a variety of different techniques. So again, that's applicable to the work that's being done currently about the coronavirus pandemic.

So we have a number of ongoing studies that Dr. Azevedo has kind of garnered within the Division of Microbiology, and so she's also doing a team concept leading some of these projects, but also being involved in other projects within the Division of Microbiology. And I won't go through all of these now. You can read the

highlights of each particular project, but some of these were mentioned yesterday.

Another important point that I would like to make is how Dr. Azevedo actually works very closely across NCTR divisions. This has always been one of the questions that comes up from the SAB, how does each of the divisions interact with the other divisions? And I think this particular example, and I have to give Dr. Azevedo a lot of the effort as well certainly all the PIs from the different divisions that are listed here on these particular projects. And some of these were mentioned yesterday by the other division directors. As you can note here, if you just look at the division programs, every division at the NCTR, the research divisions, are involved in COVID in collaboration with the Division of Microbiology.

What are the challenges that we have, and certainly the challenge is always obviously communication, with engaging the other centers early in the research design phase, and make sure that the feasibility and interest in the research, and sometimes that can be difficult due to geography, but I think even with the COVID where most of us are teleworking now, we're getting very adept at these type of WebEx and Adobe Connect and our different formats of communicating, so I think we're

getting very good exchange of information amongst our FDA colleagues.

But also, I think another challenge is always in the recruiting aspect, we always are trying to recruit the top scientists to the agency. Certainly, central Arkansas sometimes can be challenging, but I think all in all we've done a good job.

Also, and we'll be talking about this this afternoon, Dr. Steve Foley will actually be giving a presentation in our subcommittee meeting planning the division of the future.

We always like to get feedback from our FDA colleagues as well as the SAB, are we addressing the needs of the FDA, can we do a better job of that? Are there any of our future directions, how the division could impact the FDA, any type of -- we are always welcome to hear these type of comments.

Lastly, I'd just like to thank the members of the Science Advisory Board, the representative of the FDA centers and offices, for always providing us excellent advice and guidance, so it's very much appreciated. Certainly I want to acknowledge and thank Dr. William Slikker, our director of the Institute for his scientific leadership, and also his encouragement and support, not only in microbiology but beyond. Dr. Tucker Patterson, our

associate director for science policy who's leading our Office of Research, he's been very supportive and encouraging, and without a doubt, Donna Mendrick does a fantastic job as our White Oak representative to NCTR, kind of making these connections that make some of these projects that I mentioned this morning.

Without a doubt, I'd like to compliment the staff's creativity, resourcefulness, and the high marks in conducting research. That really makes a difference for their fields and the agency.

So with that, I'd like to close and thanks again.

DR. ASCHNER: Thank you, Carl, for bringing us up to date and for a very nice presentation, and I will open the floor now for questions by SAB members.

MICHAELKAWCZYNSKI: I see we have one from Charles Kaspar. You raised your hand?

DR. KASPAR: Yes. Carl, of all the various things there are to research in microbiology, and you're looking at a number of them, which area do you feel is most significant that you're not investigating at this point? At least in the next one to five years?

DR. CERNIGLIA: That is a challenging question, because there are so many things. That is exactly, when you're doing research like this, and I think even Bob, Dr. Heflich, mentioned yesterday, how he's been around a long

time and how he gets energized and excited more so than ever, and I feel the same way. I kind of echo those comments, and there are so many. One of the things I think we should be doing, and we're leaning on it now more, is on the artificial intelligence area. I have people actually going to these workshops within the division and trying to learn more about that and how we could apply that type of technology to our research interests and goals, the agency's. So I see that as one area.

This whole area of bioinformatics I think is very, very challenging in terms of, and Dr. Weida Tong mentioned this yesterday in terms of data integration using system biology approaches. Well, as I mentioned, we're doing a lot of system biology approaches, and you got a lot of different data, but at the same time I could see us working more on the bioinformatics end to really try to make sense of this data, because this is one of the problems I've found with risk assessments in general, as I review so many studies, is it's with all the test kits that are out there, whether you're looking at cytokines or the metagenomic data, you get a lot of data, but how does that really impact the regulatory science piece of it? What is the magnitude of change of whatever you're measuring? Is it really of clinical significance? I could see really

honing in and getting kind of team approach, and honing in on these types of questions.

Anyhow, I can go on and on, in food safety. I mean, look what happened in virology. We didn't expect to see what we're seeing now with the COVID situation. So that's unearthed a whole new area of questions which we're going to be investing in. All those ongoing projects, to the credit of the Division of Microbiology scientists, they have been working on these almost immediately, so we have about anywhere from 30 to 50 percent of the staff actually coming down to NCTR during this COVID phase, so they're continuing these projects, too. So that's going to unearth a whole new area as well.

I can go on, but that's just a few examples. Thank you.

DR. KASPAR: Thank you. How has COVID impacted your research activities as a division?

DR. CERNIGLIA: I think in terms of, from a health standpoint, my staff is perfectly healthy, so that's fantastic news, so we have no issues that way. So it hasn't impacted anyone coming down to do research.

In terms of specific projects, what it's really done, I think, which is we'll have to see, only time will tell, once the data comes out, but I think it's really given us more involvement from a multidisciplinary

approach. You could see the projects we're doing, interacting with the other divisions. As well as we're linking up with medical centers. Dr. Slikker has done a great job of coordinating meetings with various other medical, university, medical schools, so kind of linking up with that has been a very good outcome, I think, in future collaborations, too. But ultimately, I think we'll just have to just get in there, and Dr. Azevedo's building a team, which is an important outcome. We did get support from the agency, significant support, which helped us hire some new people to build this team, a virology team, which moving forward I think will be very beneficial.

MICHAELKAWCZYNSKI: Thank you. We have a question from Mary Ellen Cosenza.

DR. COSENZA: Great presentation, Carl, thanks a lot. So you are fortuitously, as you said, quite prepared for the COVID crisis by having somebody in your team who already was very knowledgeable on coronaviruses. Have you thought about how, what you learned through that could prepare you for other future pandemics, that might not be of that class of virus?

DR. CERNIGLIA: Yes, I think that's an excellent point or question that you're making. Because of the fact, if these types of problems don't go away, the other pandemics, and then we would have studies on norovirus, for

example, and we had studies on other coronaviruses before that. We think we're positioning ourselves, Dr. Azevedo's team, build the infrastructure, like we're beginning to do, we can work together.

She's also, and I should make this point, too, if I didn't make it in my presentation, but in terms of some of these projects with COVID, she's linking up also with our FDA centers, specifically with CBER, with their talent that they have there and shared technologies, et cetera, and this was mentioned yesterday in one of the CBER presentations about how we link together not only working as a team on the projects, and so on, but it's kind of a shared responsibility. So I can see how in the future, if a pandemic occurs, or any type of viral outbreak occurs, we have the capabilities to kind of shift our focus, if indeed necessary.

DR. COSENZA: Just one follow-up. No one's mentioned CDC. I was just curious how the division works with CDC, if at all?

DR. CERNIGLIA: Yes, we're quite involved, we're very active over the years with CDC. I've been there a number of times and given presentations. One is the antibiotic resistance area, certainly a lot of salmonella projects and exchange of information there. Another, tattoo ink, believe it or not, obviously when that was

reportable, that those -- I mentioned those mycobacteria infections, obviously went through CDC, through the FDA. So I was involved with meetings there on that. The Burkholderia cepacia complex, the one we were talking about, the immunocompromised patients, that information, due to the infection with that bacteria, again, that information went to CDC. I kind of went over there and talked about the work that they're doing, and they're following our methodology procedures.

With COVID, there's always been connections there, as well, with Dr. Azevedo. So yes -- I didn't mention that, so I'm kind of glad that you did mention that. We also have projects with USDA and previously with EPA as well.

DR. ASCHNER: This is Michael Aschner. Has there been any directive that came up from the top, FDA or any other agency, for specific studies for the division to do?

DR. CERNIGLIA: I'd have to probably ask Dr. Slikker, because he is more privy to those particular conversations in terms of if they had specific questions that came up to COVID. In terms of the projects that we're generating, they come from both sides. They come from grassroots, and also from interactions with the other FDA centers and our colleagues within the division and the other research divisions.

DR. ASCHNER: Did I hear that you got some additional funds to do research, or --

DR. CERNIGLIA: Yes, there was supplemental funding, and I have to give credit to our administrative crew, which is Charlotte Ashcraft there was kind of our connection, in terms of managing those funds. Certainly, with the support of Dr. Slikker in terms of communication, of getting this information and our capabilities to FDA headquarters, there was supplemental funding that came in, which helped us get a jumpstart on some of these projects, as well as hiring an additional postdoc and FDA staff fellow.

DR. ASCHNER: Thank you, Carl. I don't see any additional questions. Are there any other questions for Carl?

Okay. Thank you very much, Carl, for answering the questions and for a very nice presentation. We're going to move on to the Division of Neurotoxicology, and presenting for the division is Dr. John Talpos.

John, please go ahead.

DR. TALPOS: Thank you. Before I begin today, I'd like to thank Drs. Mendrick and Slikker for organizing this year's SAB, of course the members of the SAB for their time and attention, and all the members of the Division of

Neurotoxicology for providing me with so much material to present upon today.

Now, 2020 has been a challenge. On March 13, the Office of Management and Budget encouraged us to maximize telework flexibilities. Since this time, the majority of work done by the division has been done from our homes. As a result of this, many studies were paused, while others were delayed in starting. We were also notified that we would need to purchase our own PPE for vivo work. Maintaining a constant supply of N95 facemasks has been a challenge, and will likely remain one.

Some studies did begin in June 2020, but these were considered to be higher priority because they had external funds which would be lost if the work did not begin.

The division is currently comprised of 35 individuals. We currently house 16 research scientists, staff fellows, and visiting scientists, with one open position for a neuropathologist. We have 11 support scientists, with two open positions. We have two members of administrative staff, although one will be leaving us soon. And we currently have six postdoctoral researchers with another two open positions.

The division, of course, collaborates quite a bit with the other portions of the NCTR. We currently have

several active collaborations with other regulatory centers, specifically CDER, CDRH, and CFSAN. We also work with multiple governmental agencies, such as CDC/NIOSH, EPA, NTP, and HESI. And at the moment we have studies funded by the Office of Women's Health and the Office of the Chief Scientist.

When it comes to leadership and outreach, we begin by leveraging our local scientific expertise. We have collaborations with the University of Arkansas Medical School, Arkansas Children's Hospital, University of Arkansas at Fayetteville, and the University of Texas Health Sciences Center. Of course, we also have collaborations further abroad, such as the University of Birmingham in the United Kingdom.

We're currently working with the Mayo Clinic on the Mayo Anesthesia Safety in Kids study. We have a member of the division on the steering committee of SmartTots, which is a collaborative effort of the FDA and the International Anesthesia Research Society, as well as others.

We're involved with the Coalition Against Major Diseases, and we're currently with the National Institute of Perinatology, which is part of the National Institute of Public Health in Mexico. We're also involved in European

Cooperation on Science and Technology committee for Nano4Neuro project.

You can all see our division mission on the screen, so I'm not going to read this to you, but I do want to highlight one specific goal, which is to provide the data and expertise necessary for crucial regulatory decisions made by other centers. This is really a focus of the research that we do within the division.

Despite the many challenges of 2020, we have really gotten a lot done. I need to apologize -- there's a typo on this first bullet point. We've in fact had 10 publications accepted since the last SAB, and we have another nine in review. We're currently on pace to surpass our publication totals for 2019 or 2018, and while I'm not certain, I think this might be a record-setting year for us. We also currently have three projects that have received external funding this year.

Scientifically, perhaps our largest accomplishment is the completion of a decade-long study -- the genotoxicity, cardiotoxicity, and neurotoxicity of adolescent to adult use of methylphenidate in the nonhuman primate. This was really a center-wide accomplishment, as over the years, individuals from multiple divisions have contributed to this work.

Methylphenidate, also known as Ritalin, is the drug most commonly used for the treatment of attention deficit hyperactivity disorder, and it was first approved for medical use in 1955. Although it's generally considered to be safe and well-tolerated, none of the initial safety studies were actually longer than six months, and mechanistically, methylphenidate is very similar to cocaine and amphetamines, both of which are very powerful drugs of abuse. Because of this, there's always been these lingering concerns over the use of methylphenidate in children, especially because they may be on the drug for years at a time. Areas of specific concern have included cytogenetic toxicity, cardiotoxicity, issues around sexual development, as well as neurotoxicity.

The NCTR was prompted to take action in response to a 2005 publication. This was a retrospective epidemiological study which established a potential link between cytogenetic effects in children and the use of methylphenidate.

Performing this work, the division decided to make use of rhesus monkeys, nonhuman primates, and the reason for this is that the adolescent period in the vast majority of nonclinical species only lasts for a couple of weeks, and the concern was that this period just wouldn't be long enough to model the kind of day-on-day dosing with

methylphenidate that occurs in a clinical population over a number of years, during adolescence. So the decision was made to use the rhesus monkeys to more closely model the clinical condition.

Now, we were very interested in modeling clinical use of methylphenidate. So we initially began with a series of PK studies. In the clinic, kids are given methylphenidate, typically twice a day, Monday through Friday, and it was this type of dosing that we wanted to model. What we determined was that 2.5 milligrams per kilogram of methylphenidate, given twice a day, approximated the area under the curve of methylphenidate in a clinical study. We also included a 12.5 milligrams per kilogram dose, to allow a five-times safety margin. This is a supratherapeutic dose.

The first thing we looked at, of course, was genetic toxicity of methylphenidate, and we saw no effects. We also looked at the effects of methylphenidate on cardiovascular structure and function in the rhesus monkey, and again we saw no signs of toxicity. One area where we did see an effect was on the time to the onset of puberty. The high dose of methylphenidate was associated with a longer period to the onset of puberty. However, the treated groups did eventually catch up with the control group, and then matured normally from there on in.

As for the neurotoxicological assessments, these began when we first started dosing with the monkeys with methylphenidate when they were about two years old. The used the NCTR operant test battery, also known as the OTB. This is a translational test battery that was initially developed for use in nonhuman primates and in humans, and in fact we currently use this battery of tests at our lab at Arkansas Children's Hospital. The battery is composed of five different tests of cognition, and it requires the animals to respond to respond to a series of lights or levers in order to earn food pellet rewards.

While the animals were being treated with methylphenidate, we saw no effect of drug on performance. Interestingly, when the animals stopped the dose, we did see a temporary effect on a progressive ratio task, which measures aspects of motivation. For about a month, animals previously treated with methylphenidate made fewer responses. We interpreted this to mean that they were probably undergoing some kind of withdrawal effect after having received methylphenidate for years. My colleague Serguei Liachenko also performed a T2 MRI on these animals. He looked at 22 different anatomical regions, and he saw no significant differences as a consequence of methylphenidate treatment. This was also quite an achievement for the

division and the center, as this was the first adult nonhuman primate MRI study completed on site.

My colleague Xuan Zhang has done quite a bit of work with these animals over the year as well. She had a specific interest in how long-term use of methylphenidate might change monoamine function as well as glucose utilization. She initially examined these animals when they were adults, but within the testing phase of methylphenidate. So she would scan them on Monday mornings. This allowed her to measure them during the methylphenidate phase, but also when they were drugfree, avoiding the confound of any acute pharmacology.

Of the multiple endpoints she looked at, the only one that was altered was glucose utilization within the cerebellum. However, when she rescanned animals after washout, no changes persisted. Going forward, we do plan to do some additional ex vivo analysis. This is work that will be led by Dr. Hector Rosas-Hernandez. We'll be focusing on changes to the monoamine system in the striatum, locus coeruleus, and substantia nigra. We'll be focusing on these specific areas, as this is where you would most expect an effect of a drug like methylphenidate, based on its receptor binding profile.

What do we learn from this work? To begin, we saw no sign of genetic toxicity, we saw no signs of

cardiotoxicity. And while we did see a minor delay in the onset of puberty, this was only in the high dose group and the effects did eventually dissipate. We saw no lasting changes on any of our imaging markers, and we saw very little effect on our various cognitive evaluations. So as a whole, using this long-term clinical trial-like design in the nonhuman primates, we were able to gain quite an additional amount of confidence in the safety of the use of methylphenidate in an adolescent population into adulthood.

Moving on to other work being done in the division, this is current work. Two areas of focus at the moment are expansion of our vitro methodologies, as well as providing a continual response to agency-specific needs.

At the moment we have two different vitro models that are being evaluated. The first is the blood/brain barrier on-a-chip technology as a tool for toxicological screening. This is a protocol being run by Dr. Syed Ali. And we have another one which is the Alzheimer's disease brain-on-a-chip protocol. This is work being performed by Dr. Hector Rosas-Hernandez. I'm going to talk a little bit more about this last one in detail.

This is still actually in late-stage development, and what Hector is hoping to do is work with a neurovascular brain chip that's derived from human iPSCs. Importantly, the iPSCs were from different human

populations. The first is a cognitively healthy group. He will also include an APOE4 allele group, so this is the most common genetic risk factor for Alzheimer's disease amongst the general population. Then he'll also be working with presenilin-1, presenilin-2, and in APP-derived cell lines. These are some of the most commonly studied genetic models of Alzheimer's disease that result in extremely aggressive pathologies when they do occur in the general population.

What makes Hector's protocol unique is his focus on neurons, astrocytes, as well as endothelial cells. In the healthy brain, these three cell types routinely interact with each other to keep the brain operating in an optimal fashion. However, each one of these three cell types is also capable of developing its own distinct Alzheimer's disease pathology. The majority -- in fact, probably all vitro models for Alzheimer's disease, will include only one or potentially two of these different cell types. This doesn't allow us to study the myriad of interactions that will occur between all three cell types. Moreover, there are certain endpoints which are of great relevance to the clinical situation, which can only be measured if you have all three of these cell types in place.

So he'll be comparing control chips to various Alzheimer's disease chips from these different populations. Each will contain neurons, astrocytes, and endothelial cells, which have been cultured in parallel under physiological conditions. This is work that's being done in collaboration with Emulate, the maker of the chip, as well as Suzy Fitzpatrick from CFSAN.

Hector hopes to develop a standardized battery of endpoints related to clinical findings. Specifically, he's interested in A-beta and in tau accumulation and transport, and these are endpoints that can really only be studied in this tricellular model. He's also interested in blood/brain barrier integrity, neuronal, astrocytic, and endothelial cell health and functionality. Eventually, Hector hopes to evaluate the development of chip pathology and compare this to human pathology.

I'm going to also talk a little but about our continual response to agency-specific needs. This is something that we do take very seriously within the division. At the moment we have three different projects that are underway with CDER. The first is adolescent exposure to ketamine. This was developed specifically in response to CDER's need to have safety data for the use of ketamine in adolescents. This is because they've seen an increase in clinical trial requests to use ketamine during

adolescence for the treatment of various conditions. However, there is a dearth of data about the effects of ketamine, as well as other NMDA antagonists, during adolescence.

Of course, we have the work that's being done by my colleague Serguei Liachenko on trying to get TS MRI biomarker qualification, and then we also have another project, which hopefully will be starting in 2021, on the use of acetaminophen during pregnancy. This is work that was requested by CDER in response to a series of very largescale epidemiological studies suggesting an increase in the incidence of attention deficit hyperactivity disorder as well other psychiatric issues in children who were exposed to acetaminophen in utero. Based off of the frequency of acetaminophen use in the United States and in Europe, this has potential to affect a truly huge number of people.

We also have work currently underway in collaboration with CFSAN. Sherry Ferguson is studying the developmental exposure to inorganic arsenic. This was work that was done at the request of CFSAN, and it's currently funded by CFSAN.

We also have a project that was of great interest to multiple centers. It was requested by individuals at CDER and other agencies, other centers, but eventually

funded by the Office of the Chief Scientist, and this is on the developmental exposure to cannabidiol or CBD. This is being led by Sherry Ferguson and others within the division.

Now, the cannabis plant produces more than 80 cannabinoids. THC and CBD are the most abundant of these. And CBD is not intoxicating, has no abuse potential. However, we do know that CBD has the potential to interact with the central nervous system in multiple ways. Moreover, in 2008, the FDA approved Epidiolex, which is a purified form of CBD for the treatment of Dravet as well as Lennox-Gastaut syndromes. These are disorders that result in really quite devastating seizures which can be partially managed through the use of CBD.

This, again, highlights the ability of CBD to interact with the central nervous system. The 2018 Farm Bill gave the FDA the authority to regulate CBD in foods, drugs, as well as cosmetics. What we know is that the endocannabinoid does play a critical role in development. It has an impact on implantation, placental development, as well as neuronal proliferation and differentiation, and cannabis use during pregnancy is known to have deleterious effects.

Unfortunately, many people, including pregnant women, perceive cannabis use as harmless, and this likely

extends to CBD, as well. The use of CBD has become commonplace. Fourteen percent of U.S. adults report using CBD regularly, and this is highest among adults of child-bearing age.

If you visit the FDA website, you'll see that CBD is considered to have the potential to cause liver injury, it may result in drug interactions, and it may also have issues with male reproductive toxicity. What you'll also discover is that there's almost no information in the public domain about the effects of CBD on the developing brain or the developing fetus. And it's just this data that Dr. Ferguson and her team are hoping to provide. The aim of this work is to characterize the neurodevelopmental and/or neurobehavioral deficits induced by a range of CBD doses.

They'll be working with Sprague-Dawley rats that will begin being exposed to CBD on gestational day 6, and these exposures will continue through postnatal day 21. This will allow the team to study the period between implantation, through lactation. They'll be working with a variety of CBD doses, up to 300 milligrams per kilogram, and they'll be looking at a wide range of endpoints. These include developmental landmarks like eye-opening and righting behavior, a number of adult behaviors, such as motor and sensory function, anxiety-like behaviors, and a

whole slew of learning and cognition endpoints. And they'll also include neurochemistry at postnatal day 1, 21, and 180. Moreover, tissue from these animals will be given to other divisions to allow an analysis of the effects of CBD on reproductive function.

I'd also like to note that in most instances the sample size for this work will be N=20 per sex. This really is a very large study. And I think it effectively highlights the kind of regulatory type work that we're able to do here within the division.

Moving on to the future direction of the division. I think it can be divided into three parts. The first of these are center-driven studies. These type of studies occur when people from the other centers come to us with specific needs, specific questions or data gaps, that they need filled in order to effectively regulate, and we work with them to test a critical hypothesis, to provide the necessary data, so that they can do their jobs. Typically, these studies have very large sample size, and they'll include a lot of histology, behavior, and increasingly, vivo imaging, because of the translational potential of this approach.

We also do a lot of work in what I like to think of as predicting future needs and expertise. These are studies that will not necessarily affect regulation today,

but may do so in the coming years. Really, this is what allows us to have a dynamic response to new regulatory issues. A lot of this is around methodological validation or technological trial and error. For example, this could involve new screening methods, the adaptation and interpretation of vitro assays. It may involve tissue-clearing technology as a potential substitute for traditional histopathology, as well as understanding the limitations of fluidic and other biomarkers.

Biomarkers is an area where we're currently doing quite a bit of research. Of course, we have the work being done by Serguei Liachenko on trying to get T2 MRI validation. He is currently working with a variety of compounds, such as kainic acid, cytarabine, as well as hexachlorophene. Frequently these are compounds that result in dissociable and specific patterns of damage. This allows Serguei to potentially develop fingerprints of neurotoxicity, which could then be used in a future regulatory manner.

We also have a number of researchers here in the center that are interested in lipidomic analysis, as new ways to consider neurotoxicity, but also as potential biomarkers of neurotoxicity. For example, my colleagues Dr. Cheng Wang and Fang Liu have an interest in changes in

the brain lipid composition after early-life exposures to gaseous anesthetics.

We also have several projects centered on fluidic biomarkers, for example Syed Imam is studying the effects of peripheral changes, cuprizone and rotenone. And my colleague Zhen He is looking at fluidic biomarkers of stroke, with specific emphasis on isoprostanes as well as related molecules.

An area where we're evaluating specific new technology is tissue-clearing approaches. This new work is being led by Qiang Gu. As you probably all know, the brain is composed primarily of lipids, which are not particularly transparent. Over the last decades, several methodologies have been published by which these lipids are actually replaced by a series of other materials, which result in the brain becoming transparent. You can see some examples of this here. It's really quite remarkable just how clear the brain can come.

This allows an unobstructed view of the brain, potentially allowing an intact three-dimensional analysis. It also allows what's referred to as structural expansion. A side effect of this process is that some small subcellular protein structures actually grow larger, making it easier to visualize them.

This is also an amazing tool for visualization of neural networks. Traditional histological approaches cause these to be largely destroyed, and it's really quite a bit of effort to reconstruct them after the fact. Here, these will stay intact. This potentially allows us a whole new way to consider neurotoxicity, by focusing on these networks, as opposed to looking at things just like cell death. Unfortunately, this approach is also currently very slow and data-intense, which leads us to the question of how do we best incorporate this into future projects after we complete this initial data validation phase?

Another example of us predicting future needs is Dr. Rosas-Hernandez's project looking at this vitro model of Alzheimer's disease. One, this allows us to gain needed experience with vitro alternatives. Two, there's also the possibility that this can be used to study personalized medicine approaches. For the moment, Dr. Rosas-Hernandez is working with human iPS cells from very specific models of Alzheimer's disease, if you will. There's no reason why this couldn't also be replaced with cells derived from specific human patients, which would lend this approach to personalized medicine.

We've also gained quite a bit of expertise with various comparatively high-throughput screening methods. For example, in the last year we purchased a Cytation 5

multiplate reader. This allows automated tissue analysis, and we can also use this to study luminescence and absorbance in an automated, multi-well format. Dr. Syed Imam has done some work with microelectrode array technology, which we have onsite. We can use this to measure LTP and LTD, and these are the basic units of brain function. We could potentially use this for compounds screening like is done at the EPA, or to study combinational pharmacology.

And my colleague Josna Kanungo has also done a wonderful job with our zebrafish colony. This allows a relatively fast assessment of compounds for or against a specific phenotype in a vivo setting. But again, with all of these technologies, how do we best use them? Is it to model different populations, to study combinational pharmacology, or to perform mechanistic studies?

The final direction of research within the division is work that's really driven primarily by the PIs, and this is work that isn't going to necessarily have an impact on regulation. But it's still clearly within the remit of the agency, as all this work is designed to protect the health of the general public. Moreover, I think this work is extremely important because it raises the profile of our researchers within the general scientific community. This is extremely important when it

comes to recruiting future members of the division, as well as establishing collaborations. It's this kind of work that will make us the partner of choice when it comes to leveraging the billions of dollars that are spent every year in neuroscience research.

Now, I think most of the work in this area can be grouped around three different themes, the first of which is reducing unavoidable toxicity early in life.

Some examples of these projects are the work currently under review by my colleague Xuan Zhang, looking at the effects of chemotherapy in children. We also have studies designed to look at xenon-based anesthesia. This is work being led by Cheng Wang and Xiuwen(ph.) Liu, xenon-based anesthesia as an alternative to traditional gaseous anesthetics in a pediatric population. Dr. Sarkar and myself were approached about studying the neurotoxicity associated with prophylactic HIV treatments in utero and in infants.

Another cluster is around factors modifying Alzheimer's disease. We're not necessarily looking to cure Alzheimer's disease, but understanding the factors which may exacerbate the condition. Examples of work here are some of Sarkar's looking at the gut/brain axis in Alzheimer's disease. This also provides us needed expertise with microbiome. Dr. Elvis Cuevas is looking at

sex-specific treatments and pathology in Alzheimer's disease, and both Dr. Sarkar and Dr. Rosas-Hernandez have an interest in how TBI contributes to Alzheimer's disease. That's traumatic brain injury.

Now going forward, over the last several years and going forward, neurovasculature and the blood/brain barrier will be an area of emphasis within the division, and some specific areas of research that are in the future include looking at the effects of stimulants and analgesics on TBI and the blood/brain barrier. This is very relevant when you think about all the kids that are out there that are taking stimulants for the treatment of attention deficit hyperactivity disorder and may unfortunately be concussed while playing sports. Also, then, they go into the hospital and they receive analgesics as part of a pain management strategy. What are the effects of those drugs on the blood/brain barrier and TBI?

We have also have interest in long-term pathological and behavioral changes that occur as a result of TBI, as well as understanding how the blood/brain barrier itself changes in response to traumatic brain injury.

Now, the division is facing quite a few challenges. First among these is performing work especially long-term vivo studies during the pandemic. We

have a constant worry about performing long-term studies and what will happen if COVID-19 outbreaks amongst our animal care staff.

Also, we need to come up with better ways to fund expensive maintenance contracts with high-tech instruments. Currently the maintenance contracts for our MRI and microPET account for 18 percent of our annual division budget. Also, we need to constantly find better methods for hiring. As you may have noticed, we currently have five empty positions on our org chart, which we really would like to fill as soon as possible.

And finally, we need to constantly come up with better ways to interact with the other centers. We really try to do our best to align our research with the need of the other centers. However, this does require constant and effective communication.

Some areas specifically where we would request feedback, the first of which is what pressing neurotoxicological issues should we pursue, what emerging technologies should we examine, how can we best verify those newer technologies, and as technologies mature, should we be focusing on mechanistic studies, drug by drug interactions, or vulnerable populations.

That brings me to the end of my presentation today, and I'll happy answer any questions you have. Thank you.

DR. ASCHNER: Thank you, John, for a nice presentation. Again, I should have probably said it before, but I was impressed with the breadth of the research and you've certainly pointed out to a lot of interactions between the different members of the division and outside.

I have a few questions but I'm happy to defer until -- I don't see --

MICHAELKAWCZYNSKI: We got a few here. We have Greg Lanza. You have your hand up. So I'll just unmute you real quick if you have a question.

DR. LANZA: Thank you, very nice presentation. My question refers to CBD and the potential, if you looked at the potential of CBD in regards to platinum-based chemotherapy and neuropathies. I don't know that this is maybe more peripheral in the peripheral cell bodies, but have you looked at that? Because it's a very prominent problem that we're dealing with, and is really nothing that we're doing that's ameliorating it.

DR. TALPOS: We have not done any research of that type at this time. I assume you're thinking about it as a therapeutic, as opposed to as a potential toxicant in that

format. No, we haven't, but it's certainly an interesting idea and something we could consider in the future.

DR. LANZA: I think as you're going down in your work on neurotoxicity clinically, this problem of peripheral neuropathies and sensory problems associated and actually it's a variety of symptoms associated with platinum-based drugs is one you might consider, because we're using a lot of them, and almost all of our patients are suffering from it. Thank you.

MICHAELKAWCZYNSKI: We also have Steven Stice. Do you have a question? I will unmute you as well.

DR. STICE: Steve Stice, University of Georgia. Thank you, John, nice presentation. Thanks for going over the microphysiological systems, particularly the Alzheimer's disease one. I'm a real believer in them. But I think you really on this last slide hit it on the head, nail on the head, on how do we verify these newer technologies. It's great to do multiple cells like neurons and astrocytes and endothelial cells, but getting different phenotypes of cells, sub-phenotypes, even we're missing a big void there with microglia, oligodendrocytes not being in some of these model systems, I mean, you can't get it all.

So I guess the question that I have is there is a large amount of funding that came out of DoD, NSF, NIH, to

fund these microphysiological systems. Nothing against Emulate, but there are a number of different systems out there that commercially and actually a lot of leading groups out there. How are you following what they're doing and trying to really model your activity after successes in this field, and then secondly, how are you going to differentiate yourself from the rest of the very crowded field, I feel, in the microphysiological systems?

DR. TALPOS: That is an excellent question, and it's 100 percent true. We're not necessarily leading the pack in this area. We're still trying to gain more experience. So that is a very, very good question.

I don't think we're necessarily trying to lead in this area. We are really trying to just provide the data which is going to be most of value to the agency. Now, if there's certain systems that do take off and appear to be the ones that are most commonly being used for regulation, I think that's an area where we're going to end up focusing. Otherwise, it's going to be picking systems that are most relevant for the specific hypotheses that we want to test.

Part of the reason for the Emulate system was besides availability is it also works very well with our own division's interest on the microvasculature, because it does include that key component. At the moment, we don't

have any kind of active vitro methods working group within the division, and we are relying on communications with other parts of the agency to fill us in on some of the newest developments, but I think you raise a good point, and going forward, that is something that we may need to more actively address and consider internally with what we think are the most developed -- what we think are the quickest developing interests and the areas where we can have the greatest impact.

DR. STICE: I think, just to follow up, it would be great if some of your team could be sitting at the table with the people from DoD and NSF and when they're reviewing different technologies and really getting to the forefront and understanding where are the real problems, because there are still lots of problems. I don't think anybody's saying there's a solution now for that microphysiological systems are going to replace anything for right now. But it's a good goal to go after.

Just one other follow-up question, and maybe you covered this, but great -- the group has completed the 10 years with the methylphenidate trial. Does that mean that things have been reduced or stopped? And if so, what is the next big thing? It seems like there's considerable resources to complete a study like this and should be

applauded for it, but is there something that's filling the next study?

DR. TALPOS: In parallel, mostly in parallel, we've also had a long-term study on the effects of early life exposure to anesthetics in the nonhuman primate. So we will be focusing on the remaining cognitive testing and imaging work that still needs to be done there.

Because of issues surrounding nonhuman primate testing, I think we're unlikely to start another major project until we have one that has buy-in from one of the regulatory centers, in part because of the long-term financial implications of such a study, and also, because they do last for multiple years at a time. So we want to make sure that we're fully leveraging that resource to maximize the value.

DR. MENDRICK: This is Donna Mendrick. I just want to make a comment, Steve, about your first question. As Suzy Fitzpatrick mentioned yesterday, there's an FDA-wide alternative methods working group and I run a stem cell MPS discussion group or research group under that, so there are a number of us at the agency who are following the various platforms and academics. We've had people come into the FDA and talk to us about their platforms. It is changing rapidly, but just to note, we are trying to keep abreast of the field.

DR. STICE: Thanks, Donna.

DR. ASCHNER: I have a general question, which could probably have been addressed in any of the speakers. One thing that you mentioned on the methylphenidate study was you didn't see anything, but there were some minor effects on puberty. I think you said there were some delays in puberty. You call it minor. Some people may argue what is minor, what is major. So what kind of process do you have in place when you see something -- if I recall, your first slide was about how you are to advise the FDA on regulatory issues. So what is sort of the red flag and how do you move forward when you see something in terms of, you know, getting it into the regulatory stage?

DR. TALPOS: I think there's two different prongs to that. One of course is we try to publish pretty much all of the work that we do. So we make it available that way. But of course, we regularly interact with colleagues at the other centers. Often they're collaborating in these studies, and we simply make them aware of the data as it's generated.

DR. ASCHNER: When you say that methylphenidate has some minor issues delaying puberty, that's because it's minor, you just let go?

DR. TALPOS: So I have to admit that particular finding predated me. So I can't say how it was handled.

So part of the reason why I would consider that minor is the only time when we saw an effect in the low dose group. So the vast majority of them were in the higher dose group. The change between control and the low dose was smaller in fact than the daily variations that we saw in that particular measure, as in this was on testosterone levels and how they change throughout the day. So in fact, the effect of time of day was greater than the group effect.

DR. ASCHNER: Thank you. I know it's probably an unfair question for you, but I think just in general it's something that we may want to think about and have a more general discussion when we sort of a summarizing meeting at the end. I appreciate it.

The other thing that I would mention, and I had the same thought, I mean, it seems that a lot of these methods, you know, at times it seems that you aren't making sufficient effort to go out and get some of the expertise that already exists. There's a lot of people, as Steve talked about the brain on the chip. These are very complex studies that you have to assure basically that in every prep that you're making -- and Steve mentioned all the different cell types -- that you get the same number of astrocytes and neurons and you have to prioritize, and a lot of this has been done. So again, it might not be a fair question for you, but is there any attempt to send,

for example, some of the postdocs to a lab that is already very proficient in this methodology? Or are you trying to do everything by yourself?

DR. TALPOS: No, we certainly do collaborate for that kind of thing when most appropriate. So for example, I know that when we first started doing some of our TBI work, we were working with individuals at the University of Arkansas who had more experience to leverage that.

DR. ASCHNER: Okay, thank you very much.

MICHAELKAWCZYNSKI: Ken, did you have a question now?

DR. RAMOS: I actually will be very brief, because both Mickey and Steve actually covered the question I was going to ask, and it related to the iPS model that you described. So I echo a lot of the feedback that's been provided to you.

Then the question that remains is, if in fact your priority is going to generate model systems and answer questions of regulatory relevance, why are you spending considerable amounts of effort in model development, be it in a very challenging environment, when in fact maybe a more expedited approach would be to partner with people who have developed the models or who are developing the models, and then apply those to the questions that are priorities for the agency?

DR. TALPOS: I think internally we are not necessarily trying to do model development, per se, as study the models and gain experience with the models and understand a bit more of the strengths and weaknesses of them. We are not necessarily going to be using these to truly try to drive regulation, but to better understand their limitations and as appropriate answer questions with them that come from our specific centers.

DR. RAMOS: I see. I guess, from my perspective, that does not seem to align well with other statements that you made, but I take your answer for what it is. In some ways, then, you really have answered your own question about the feedback that you wanted regarding about focus, you know, so that's perhaps I think an area where a little bit of time and thoughtful reflection might be helpful internally.

I do want to praise you for the quality of the presentation that you gave, and I think for the exciting stuff that in general is taking place within this division.

MICHAELKAWCZYNSKI: I think we have one; I want to make sure I call it up correctly. Was it Sherry Ferguson? Did you have a question? I'll unmute you.

DR. FERGUSON: Thank you. I wanted to mention something about sending the postdocs off for training. We actually had the opportunity to do that, and we considered

sending several of our postdocs off to get increased blood/brain barrier methodology training, and then of course COVID-19 happened. So we do have that opportunity to do that.

DR. TALPOS: Thank you.

MICHAELKAWCZYNSKI: Michael, that was the last one. So John, thank you so much.

DR. ASCHNER: Thank you, John. Thanks again for a very nice presentation and thank you for the questions.

I guess we're moving on to the last presentation today. This is going to be from the Division of Systems Biology. Go ahead, Bill. Thank you very much.

DR. MATTES: Good morning, everyone. Good afternoon, depending upon where you are. I think it is still morning for most of us. In any case, I again want to thank Donna and Bill Slikker and Mike for making this all possible, if not necessary, as one might say.

The other thing, I recognize I'm the last person between you and a break from this long session, but I do want to say one of the things, bad things, of being the last is I see all the ideas for slides from my colleagues and I think, gosh, I wish I'd done that. Gosh, I wish I'd done that. But I'll muddle through with what I have done. So here we go.

First off, the disclaimer.

The second thing, which is one of the templates that Donna mentioned, in terms of the staff, we have a mix of PIs, research scientists, visiting scientists, support scientists. We have three administrative staff and four ORISE postdocs.

I should note as others have noted, we have vacant positions. Yes, it is difficult to hire for a variety of reasons. Remarkably, in COVID times, we are actually in the process of I think filling two of these positions; notably we're in the process of trying to bring in a staff fellow who has extensive experience in microphysiological systems, as I will point to in our talk, in my talk, and I'm hoping to bring in a staff fellow or postdoc with experience in the data integration that we see so essential to systems biology.

So that's just kind of a where we are in terms of people. In terms of our interactions, really we have collaborations and I think you've seen that in some of the other slides. We have collaborations with all the NCTR divisions and a number of the FDA regulatory centers, we have collaborations with a number of government agencies, and a whole list of universities. Some of these will be mentioned as I go along with my talk.

I should note I did not indicate our external involvement in societies and bodies like that, but we have a number of those ongoing.

Collaborations of note, as I was alluding to, with CDER we're putting together a series of projects to look at neurological targets and neuropsychiatric effects of Montelukast, an anti-asthmatic which has been called attention to at CDER. We have been refining, have projects that seek to refine the iPSC stem cell cardiomyocyte models for cardiotoxicity prediction. Also with CDER, we have ongoing a project looking at in vitro toxicity assessments of opioids on neural precursor cell development.

We have, we're looking at developing tools, technology, to rapidly detect contaminants and adulterants in crude pharmaceutical preparations. You'll see a discussion of a project looking at metabolomics in MAIT knockout mice, and also, we have an ongoing project developing a microphysiological model of testis function. Both of these collaborations with the Center for Biologics. And finally, we have a collaboration with USDA on E. coli detection and quantitation.

This is our overall mission, to address problems of food, drug, and medical product safety using systems biology approaches and innovative technology.

Now, why systems biology? I look at it as these are tools and approaches that can bridge nonclinical models with clinical settings. That is, in the nonclinical models, the adverse events and individual responses, with the clinical settings. Again, adverse events and individual responses. We, or I, we -- refer to this as translational toxicology as well as precision safety assessment.

The systems thinking is basically used as systems -- and I use an example here pathways, but it's really a concept of systems that will link the in vitro, the animal in vivo, with the human and the population concepts. With that, what we're using then are these omics tools, transcriptomics, proteomics, metabolomics, to do this sort of linkage.

Our goals are translational, prognostic, or predictive biomarkers for improving pharmaceutical product safety, delineated mechanisms for species, tissues, sex, and subpopulation specificity, and drug toxicity that individual response mechanisms for opioid addiction, and mechanisms for the toxicity of next generation pharmaceuticals such as oligonucleotide therapies.

We're also, we talk about in vitro models for better evaluation of reproductive developmental and clinical toxicity, and in many cases, what we're doing is

examining those models with the idea of how might these be evaluated in the regulatory environment when they come across as submissions by sponsors. We also are looking at in silico models for predicting relevant toxicities, and this is consistent with other directions within the agency in different centers, such as next generation pharmaceuticals, for example, the non-natural components of peptide therapeutics. I've noted our development of technologies for drug alteration and compounding.

Our strategies is use tool compounds, classes of drugs with known toxicities. I give examples there. You characterize the systems biology effects with these tools, with state-of-the-art tools, I should say, such as the omics, integrate the data with systems biology informatics, accounting for the species, tissues, sex, and subpopulation differences. I must say, much of this is aspirational, and I noted our goal to bring on a specialist in this informatic integration, and to bring that together to incorporate in vitro, computational, and instrumental technology.

I'm going to -- when I talk about our projects, there are themes aside from those missions. The general themes are we do have projects focused on the response to COVID-19. We have projects focused on biomarker discovery,

projects on technology development as I alluded to, assay development, and precision health.

So I've not -- we were tasked actually to select accomplishments and not give a long list, and perhaps some might say we don't necessarily have that long list, but I do want to highlight two what I think are relevant and very interesting accomplishments, both publications, as you see here. One is the determination of structural factors, an in silico approach that looked at binding to the mu, kappa, and delta opioid receptors. Another one is a collaboration with CBER, looking at bile acid profiles and its responses to cefoperazone treatment in MR1-deficient mice.

So the first one is making use of an in silico modeling technology that was developed at NCTR in my division, before I came here, I might add, and uses unique aspects of a molecule, more than just three-dimensional structure. It also uses the NMR signals to identify the atomic environment of a particular atom.

In this particular modeling, it was based upon over 9,000 opioid ligands and identified features with high affinity binding to all three of these opioid receptors, and this is a first in the modeling world.

This other one that I mention is an interaction with CBER, is based upon the fact that MR1-deficient mice are kind of unique. Cefoperazone is a treatment which

allows to model the Clostridium difficile infection that is seen in fecal transplants, if you will, and what is found is that in MR1-deficient mice, they are resistant to C. diff infection. So the question is what changes are in these mice, and so the comparison was between the wildtype and the knockout mice in response to cefoperazone, and using the lipidomic technology that my division has developed and I should note, this is Jinchun Sun and Rick Beger's work and vision in expanding this type of technology within our division.

I should note that the molecular modeling was done also under Rick Beger's branch, that is with Svetoslav Slavov in his branch.

Now I'm going to talk about our current projects, because this gives you an opportunity to see where we have thought about going, where we think we're going, and some opportunity for feedback on it. So the projects I'm going to talk about are, one, the first is real-time detection and identification of viruses in body fluid. The second is to talk about predicting tyrosine kinase inhibitor induced cytotoxicity using iPSC-derived cardiomyocytes from individuals. The third is development of a mouse model for doxorubicin-induced delayed cardiotoxicity, and the fourth here is verification of novel predictive biomarkers of

doxorubicin-induced cardiotoxicity in breast cancer patients.

There are also, I want to talk about a unique technology that was brought into the division a few years ago by Ellen Jones, matrix-assisted laser desorption ionization imaging mass spectrometry, MALDI IMS, and we're going to be -- we're applying this to looking at opioids and subsequent neurotransmitters in rat brains.

We're also, I mentioned, investigating opioid-induced neural tube defects, in this case in the mouse model, and then we have a project with CBER looking at critical quality attributes to predict the safety and efficacy of CAR T cells using novel in vitro and in vivo models.

So to jump into the first one. In this case, the hypothesis is the complex mass spectrum of an unpurified sample that had virus could be transformed into a fingerprint unique for that virus and diagnostic for that virus. The methodology makes use of a compact mass spectrometer that is effectively portable, modified to deliver high spec ionization. The sample is placed on a metal holder, dried, and basically blasted with a spark, to give you these spectrums. Then that is transformed and you can see the PCA plot there showing that in fact there was discrimination between spiked samples where you had saliva

spiked with cucumber leaf spot virus and control samples. This is promising. It is a project in, let's say, in progress.

Now I want to talk about one that we have actually been working with for a couple of years now, and this is an exciting collaboration with the Medical College of Wisconsin where our collaborator there, Ulrich Broeckel, has a grant where they are having 256 -- they're generating 256 cell lines from individuals with well-defined cardiac phenotypes. That's this HyperGEN project, and you can see it's a whole variety of individuals, different ethnicities, there's extensive background on this, and the idea is then how do these different cell lines perform in vitro?

What we found is actually really important in the case of when we talk about in vitro testing, because what you can see here, and let me see if I can grab my pointer, what you can see are two different patients, okay? Two different patients here, and you can see that the responses to doxorubicin are different in those cell lines from two different patients. The same is true for this other -- ponatinib, tyrosine kinase inhibitor, and you can see the same thing for other pairs of patients for other tyrosine kinase inhibitor. So the cell lines from these two patients, the responses are drug and cell line specific.

This is, I think, a real wakeup call for as we look toward using, quote, human cell lines to screen for various safety assessments, the reality is people are different and in toxicology, there's the complaint that we use homogeneous populations of animals, and the issue then becomes if we move to in vitro, how do we address the heterogeneity of the human population.

In this particular project, it has been developed -- and I should say I'm sorry, the previous work was being done by Li Pang in my division. This work is being championed by Varsha Desai and Tao Han, and in this case, the idea is how can we model what is known clinically to be true for anthracycline treatment, which is it's a very effective cancer treatment, but the risk is that cardiotoxicity that sometimes may be delayed by years after treatment stops. So in this case, what you are seeing is that there was treatment for 24 weeks, and then after the treatment stopped, the animals were monitored, after treatment, for cardiac effects.

In this case, what we're using is ultrasounds that we can actually look at without sacrificing the animal, we can look at ejection fraction and fractional shortening, and what you can see is that there is a decrease, even at lower doses of doxorubicin. There is a decrease in cardiac function, even at lower cumulative

doses. The stars here show where you start to see an increase in cardiac troponin T. So this is really pre-symptomatic, if you want to call it that way. Our goal is with this system to look for biomarkers that can tell us, that can distinguish the onset of this cardiac dysfunction.

Consistent with that is a parallel effort to look at cardiac, biomarkers of cardiac dysfunction in a clinical population. In this case, it was 70 patients who were treated with four cycles of doxorubicin and cyclophosphamide. There are blood draws before treatment and also after cycle 2 and 3. Then the left ventricle ejection fraction was measured two to three weeks after the last cycle.

Then you group patients by did they have normal or abnormal cardiac function at that later time period, and in this case, we're looking for biomarkers before treatment that would be predictive essentially of the response of the patient to long-term treatment or delayed cardiac dysfunction with the treatment. You can see this is a preliminary experiment, there is some promising results. There's clearly more to do to confirm these biomarkers, and demonstrate their ability to distinguish those patients at risk from those who are at lesser risk.

Another and very exciting project I alluded to is this MALDI IMS, and effectively what the process is is

after drug administration, frozen tissue is collected, treated, and sectioned, and you basically look at that using a laser that blasts spots in this tissue section and then monitors the mass spectrum of those spots and that allows you to look at a number of molecules in the same spot and assess its distribution along this tissue section. So you can see what you're looking at is oxycodone distribution in this tissue, in this brain tissue.

Our goal is to look at that oxycodone distribution and assess its individual responses in individual animals, and ultimately our interest is developing a model of addiction where you have some animals showing evidence of addiction and some not, and then at that point, determining are there brain differences in the distribution of the drug and/or neurotransmitters.

Again, along the lines of asking how might we understand opioid effects is a model for neural tube defects in mice, and you can see in this case the CF mice are sensitive to morphine exposure using valproic acid here as our positive control, but you can see the effect at the higher dose with morphine.

Another project working with CBER is this developing quality control attributes of CAR T cells, and it's to look at the proinflammatory properties of these cell products in vivo. So we're going to be using beige 1

mice looking at tumor growth, asking after injection of the CAR T cells, measure toxicities at timepoints after the injection, looking at cytokine release syndrome and neuroinflammatory and neurotoxicity markers.

So I want to talk a little bit about -- those are ongoing projects -- I want to talk about future projects and this again is an area where we welcome some input.

First off is this clinical validation of the putative biomarkers, as I alluded to. Expanding the application of these technologies such as MALDI imaging and metabolomics, lipidomics. We're very interested in further exploration of in vitro technologies, human and iPSC-derived cell models, and microphysiological systems.

I'm going to stop and pause there just to say that as Donna alluded to, and as what I think is relevant is it's as much to say what does FDA need to think about when it is considering how these technologies show up in submissions and how they're used in the product development process.

We want to continue looking at the -- in mechanisms of opioid addiction, and look at -- and we're thinking, I should say planning on, investigating the toxicities with cutting edge therapies such as the oligonucleotide-associated thrombocytopenia. One of the things I noted was this microphysiological system, and I

also noted that we're trying to bring on board a staff fellow who has experience in this area.

We already have been approved for a CRADA with Emulate to establish a liver chip system, as noted here, to predict individual susceptibility and adaptation to drug-induced liver injury. It's interesting, as I think some of the folks noted, in prior talks and discussions, what do we want to do with this, and Emulate, for one thing, is a system being used in the marketplace, in pharmaceutical development, so it is relevant to say we're interested in this versus necessarily a homegrown system, in this case my real excitement about this is that we are pairing this with an in vivo experiment in rats where we showed that acetaminophen treatment, you could see transient increases in liver enzyme serum transaminases, transient increases that adapted after a period of time.

This is clinically relevant and clinically very important. So our goal is can we reproduce that particular setting in the liver chip system? I should also note that those responses, transient responses in the rats, varied among individual rats. So again, we're hoping that we can emulate, but we can replicate that individual and transient response in a liver chip system. That then poises us to ask can we replicate -- can we see this same effect in a human liver chip system?

So I'm at the point where I am interested in your feedback for the approaches we're looking at, MALDI IMS, the microphysiological systems, the iPSC cells, are there areas other than that that we should be exploring? What developments on the horizon that may impact FDA are we missing? And I kind of note about the oligonucleotide therapies, those are being used now for ultrarare diseases. So I think that that's an area that interests me and excites me in terms of having impact.

What approaches in addition to our current efforts might better inform us of FDA research needs at other centers? We have outreach efforts going on with CDER to compile and centralize FDA research needs, identified by reviewers, but there's always this question how do you drill down to the pain that the regulatory centers are finding?

With that, I'm going to back up so that I'll leave that slide up. Thank you for your attention and welcome your questions at this point.

MICHAELKAWCZYNSKI: All right, if you have any questions, please raise your hand, and I'll unmute you or call on you. Michael, I'll also make sure you're unmuted.

John-Michael Sauer has a question. I'll unmute him.

DR. SAUER: Thanks a lot, Bill, for a great talk. I have a couple of questions for you with regard to your biomarker strategy, of course. I get the idea of what you're trying to do with the predictive biomarkers, but what about the prognostic, right? So, you know, when I hear that word, it sounds like what you're trying to do, based on your presentation, is be able to understand which individuals are more susceptible to a given toxicity. Is that right?

DR. MATTES: Yes, absolutely, yes.

DR. SAUER: So what is your strategy there? So I saw a little bit of it, you know, when you were comparing some of the cell lines, but are you going to take more of a clinical approach to understand some differential responses there? Or is this going to revolve around animals, or how are you going to do that?

DR. MATTES: Let me back up from -- I showed the slide where we had, where we were looking at proteomic biomarkers, serum proteomic biomarkers, in patients treated with doxorubicin. So that's a case where you have a clinical study, if you will, and you do basically a discovery approach, one has to follow that up with -- since you're using an omics approach, you have to follow up with a targeted approach to confirm those observations and then you move from there into whether or not at some point that

can be folded into a true clinical -- and you and I both know we use the word validation carefully, but that's at the clinical level.

The iPSC cells, I think, that's a little bit early, since quite frankly none of those patients that we know of at this time have been treated for cancer or treated with any of the indicated tyrosine kinase inhibitors. So is that prognostic? Interesting question, since you don't just treat people blindly with something like a TKI.

I must admit I've asked the question of our collaborator, has there been any follow-up with those patients, in terms of, let's say, TKI treatment, and I don't believe we have done that since the study is ongoing with the full 256 cell lines, and the interesting question will be at the end of the day, then do we go back and see if there's any correlation?

And then, in terms of the animal models, that's a very relevant point. The one difficulty with the mouse model is a very tractable model and one that we have a fair amount of history with. Of course, the issue with rodents, mice, even rats, is how do you get enough blood to run assays and supersensitive assays are really what you'd love to have.

In the case of the in vivo rat experiment I mentioned, folks were able to run from tail bleeds enough sample for ALT measurements. In the case of the mice, we might have to take a different approach. So I'm aware of this issue of the in vitro in vivo clinical translation back and forth, and I think we're developing -- we're not yet in the full pipeline connection mode yet. Did I answer your question?

DR. SAUER: I think you did. I think what would probably be really helpful is to really understand what the end game looks like, right? So where are you going to be when you're successful? I think that would really help, because it seems like there's a lot of discovery going on, but I don't see exactly where you're going. I know it takes time to get there, and I see there's other questions as well. So I'll stop there.

DR. MATTES: Okay, well, I'll answer that I do think the end game is indeed the precision health concept. I really do believe that's the end game. It does indeed connect all of those.

In the case of the transient transaminases, consider that some individual animals spiked with ALT and some didn't. That's exactly what one sees in the clinic. So what we really would like there is, one, who's going to spike, and two, are they going to recover, because that's

of course when you see ALT going up, that's your big question.

The same is true in the doxorubicin, which could be applied and then you ask yourself does that apply in general to cardiotoxic treatments. Who is going to respond and then what changes in treatment might you take?

MICHAELKAWCZYNSKI: All right. We have another question from Alex Tropsha. I will unmute you. Alex, go ahead.

DR. TROPSHA: Very quick, kind of following up on the previous question. So I think it's a very interesting work on biomarker discovery, and the work with doxorubicin. My question is about the size of the cohort. For the question asked, it appears to be awfully small, and I understand how difficult it is, to enlarge it, but what are the issues there with getting more study participants?

DR. MATTES: Could you repeat that again? You're a little quiet on that.

DR. TROPSHA: I am asking the question about the size of the cohort for the study design, and it's certainly very -- I find this very interesting, this study design, and what you want to do. But I think that the number of patients is a limitation, for any significant claims, and so just wondering what are the difficulties there?

DR. MATTES: Without any question, it's small. I would say, it was very intentionally and very -- I won't say intentionally -- but very clearly a very initial discovery process, discovery study. We set up a collaboration with the University of Arkansas Medical Sciences. These were the patients they had. So what you're saying is extremely relevant. The next step is you can, in the same samples, confirm with another technology something that may be a little less omic-based. You could confirm the biomarker levels and then it would be a case of saying, okay, now do I have a study that's powered enough to give me a solid answer on any of those biomarkers. I agree with you. The cohort size is what we got.

MICHAELKAWCZYNSKI: All right, next question is from Patricia Ganey. Go ahead, Patricia.

DR. GANEY: Hey, Bill, thanks for a really nice talk. So I have a couple of questions. One is really general, but I'll ask a more specific one first. So with respect to this variability that's observed in people and also in animals, and now you tell me it's also observed in cells, with your collaborations, are you expecting that -- are you thinking, maybe expecting is too strong a word -- but are you thinking that all of that variability can be explained by genetics?

The reason I ask this is that when you take cells from different people, you're only accounting for genetics, as opposed to any environmental factors that are influencing susceptibility as well.

DR. MATTES: At best, you might have some epigenetic carryover, but I would -- I am of the camp that individual variability is -- genetics plays a role but not the only role in individual variability. That's why, quite frankly, I'm very excited by Rick Beger's metabolomics approaches, because I think that that can capture real-time individual variability.

But the fact is you do see those differences, and what is driving those is going to be one of the outcomes of the study, because while we're looking at the TKI responses, the University of Wisconsin is doing RNA-seq and whole genome sequencing, and there will be a, let's say, overwhelming amount of data that we can mine for answering the question.

DR. GANEY: Okay. Well, I'm glad to hear that you're at least open to other possibilities.

My next question is really a general one. I could ask this of everyone at NCTR, really, but it's something that's kind of occurred to me over the last couple of days, and this is coming from someone whose lab is entirely in vitro. I moved to in vitro many years ago,

and we don't do any whole animal studies any longer. But we've heard a lot of in vitro approaches and AI approaches, and a lot of things that you've discussed today, and my question is when you have something you think is a significant finding, do you -- are there any measures in place to test whether it's relevant to go to back to a human population, or an animal population and say, oh, yeah, whatever we saw on the chip really is relevant to what's happening in the whole organism?

DR. MATTES: My response is that would be exactly what I would want to do in all of our experiments, especially for instance chip experiments, but I will give you an example of that, to answer. A project I did not describe, where we were looking -- where Qiang Shi in my division was looking at tyrosine kinase inhibitors in vitro, and he was looking at one particular one, regorafenib, very toxic, and it was toxic to cell lines, cells, primary hepatocytes from liver -- I mean, from rat, dog, and human.

It turns out he also looked at the metabolites of that, which are well-documented clinically. Now, it turns out that one of the metabolites is uniquely nontoxic, and his search of the literature shows that it's pharmacologically active. So he's following up that experiment, where he shows in vitro in rats the metabolite

is less toxic than the parent, he's following that up with an in vivo experiment. The concept of following up in a clinical trial, we well understand is not within our FDA mandate. We would publish that and hope that somebody picks that up and runs with it clinically, because that becomes something that cycles back into the FDA for approval.

MICHAELKAWCZYNSKI: All right. Next question, I think we have here from Greg Lanza. Greg, go ahead and take it away.

DR. LANZA: Thank you very much, Bill, for the presentation. In regards to cardiotoxicity, cardio-oncology is burgeoning all over the country, divisions, and the mechanism that we're looking at that precedes both troponin, BNP, and certainly ejection fraction is strain, and I suggest that you start using that, and we do it both with ultrasound every day here, and in my lab also with cardiac MR. It turns out that cardiac MR is actually better, because we have less dropout.

The pulse sequence you might consider is endorsed by the Society of Cardiac MR. It's called DENSE, and we're doing this both in humans and in animals. I think that I wanted to suggest to you Frederick Epstein. Frederick Epstein is one of the innovators of DENSE. He's at the University of Virginia, and he works both with small

animals and in human situation. I think this would put you in line with what's being done clinically, and also what we see and what we're acting on, actually, is changes in deformation, which is strain, that are triggering changes in therapies, and it's part of a general trend now that's happening where imaging is not only a diagnostic tool, but becoming integrated as a therapeutic guide.

So I endorse what you're doing, but I encourage you to start using deformation imaging and since you're paying a lot for those scanners apparently for the service contract, maybe you can get more mileage out of them. But I recommend Fred Epstein to contact so you don't have a delay.

DR. MATTES: Thank you. That is a good suggestion. Needless to say, we were -- both the clinical and the mouse models were ones we were -- they were obviously driven by -- some of the design is driven by, in the clinical study, it's certainly driven by the cardiologist and the cardio-oncologist there. In the mouse model, we had a fair amount of interaction with Bob Hamlin, who is a veterinary cardiologist at Ohio State. But I'll look this up. Fred Epstein at Virginia, yes.

DR. LANZA: Yes, he's, I guess it would be the department chair for bioengineering, and he's closely associated with Dr. Chris Kramer, who's in

cardiology/radiology at the medical school, and I think they would set you in synch with the clinical, and I think the numbers -- because you can get circumferential longitudinal radial strain, but certainly circumferential longitudinal strain would be very good, and you could apply it to mice that you've knocked out or done other things to the pathway so that you can break this thing down more. Anyway, it's a suggestion, but I know you can only do so much with ultrasound.

DR. MATTES: I appreciate that. Thanks.

MICHAELKAWCZYNSKI: Thank you, and we have time for one more question. John-Michael Sauer, I will unmute you. Take it away.

DR. SAUER: Great. One broad question for you, Bill. I mean, you brought the oligonucleotide therapies that are becoming more and more popular for rare disease. That made me then think about gene and cell therapies of course, and so it really seems to me that gene therapy is still an evolving toxicological science. You know, where -- and maybe this is a broader question than just your group, Bill, but what is happening within NCTR to support those types of activities for CBER?

DR. MATTES: You saw the proposals. There have been -- actually, a lady from CDER has joined the division, who has had background in oligonucleotide therapy reviews,

and there are some ongoing proposals to address that. I think it was -- I don't know about you, but when I was in pharma a couple of decades ago, I remember Isis coming up with oligotherapies and then it kind of fell back, and I think gene therapy and those took a big hit because of some of the toxicities, and we're coming back to looking at this and how it's going to -- how can we address those toxicities in a new way? Yeah.

MICHAELKAWCZYNSKI: All right, Michael Aschner, are you there?

DR. ASCHNER: I'm here, yes. Thank you for the presentation, Bill. Thank you to all the presenters. This is the end of the presentations by the different divisions. I suggest that we take a break and reconvene at 10:45 local time. So we have lost a couple of minutes of our break, and the next thing on the agenda is discussion with the SAB. So see you in about 25 minutes, or 22 minutes, to be exact.

(Break)

Agenda Item: Discussion of NCTR Research

MICHAELKAWCZYNSKI: Welcome back to the NCTR meeting. Let's see, Michael, there you are. We are in discussion time. Let's see, everybody is unmuted because I believe we are in discussion mode. Michael, take it away.

DR. ASCHNER: Okay, so I think this is an open session where we are basically giving feedback to everybody. Being the chair of the committee, I don't mind going first. Let me give you my impressions and then we will open it up, or if you want to interrupt me as I go along, please do so, is not a problem.

From my point of view, I think we had an excellent day and a half thus far, we have heard a lot of very interesting presentations. Going from the top to all the division heads and staff, I think the leadership, the personnel, is doing a great job. Recognizing that we have at our meeting only a few months ago and we are constrained obviously by the pandemic. Nevertheless, even since the last meeting that we have had, I think we have heard some new studies and the science seems to be progressing at a fairly rapid rate.

I think the breadth of research is actually very impressive. At times, and this was mentioned before, I almost felt like saying you guys need to focus a bit more and you can't do everything. But nevertheless, the strength of the science is evident, and the breadth of the research throughout all of the divisions. I think you're clearly answering the mandate of the FDA. One of the points that I think was very important for me, and sort of reassures me that your credibility is very high at all levels, the fact

is that you have been asked to do work that is germane to the COVID-19 pandemic. I envy you in terms of instrumentation that you have, I wish I had one/tenth of this. Clearly there has been also investment in terms of the type of resources that you have. We always want more but I think you have quite a bit.

So, I can't comment really very specifically on all the research projects that we have had. We have had some specific questions for the different presenters. I'll say that one of the things that I'm missing most by not having the face-to-face meeting is that you can't visit, you know the staff, you can't go to the posters. You can't ask people questions during the break about their old research. That is missing from this Zoom type of meeting that we have.

In terms of things that I felt can be better -- I think some of it came today. I think it was part of some other presentations that we have discussed. I brought it up this morning. I feel that sometimes, I'm not sure that the resources and the time is optimized. I think, in many cases, when you are developing new methodology, you don't go out sufficiently to try to get what is available. I think it will save you time, it will save you money, and it would expedite a lot of the research if some of these techniques could be brought in at a more rapid rate. Or, I

think maybe Steve mentioned this morning, I think you should consider rather than doing everything yourself, the time might be more beneficial to contract with a university or work with them more closely on a collaborative basis to basically get the same things done.

I like the idea, and I think there's more possibility for it, I gather from being on this committee for several years, that most of the time the research between different divisions and outside with other centers, is driven by the investigator. I think Ed actually mentioned it yesterday. I think having a sort of top to bottom initiatives could be optimized, and again I think it would be for the benefit of the NCTR. Having said that, again, as a committee member for several years, I remember it seems like five, seven years ago, everybody was in their own silos. They didn't know what was going on across the hall were from them. Even on a Zoom meeting or Adobe, I could definitely sense that there is a bidirectional enthusiasm between the NCTR and all of the other centers. I encourage you to continue this.

One of the questions that seemed to be on everybody's slide at the end, is always what kind of methodologies should we incorporate into the NCTR. I'll be very honest with you, I never thought that it was the methodology that should drive the science. I think what I

would like to see everybody do is come up with a most profitable hypothesis for your mission, for the NCTR, and based on those, go out and seek a technology that is going to be the most beneficial. I don't think that science should be driven by methodology. I think it should be driven by good questions. If you have the good questions, you will find the methodology.

Finally, the other issue that I want to mention, and that is sort of general, and maybe Bill can address it later or when we finish this session. I would like to get a better sense on when we find positive things, what is the next process? How do you raise a red flag? What does the FDA do about it? Is it really just publishing a paper? There might be times where it might be more urgent than that. I'm wondering if there is any mechanism in place, or maybe there should be one if there isn't, to address those kinds of issues.

So, these are my comments. Again, I think to end up on the positive way, I mentioned few things that are not negative, but things that I want you to think about. I think the purpose of this committee is to help you see where you are and direct you to the future. So, these are not major weaknesses, but hopefully if you pay some attention to those, NCTR will become even more prominent and more important leader in the FDA.

So, thank you again to all the speakers and I'll open the floor. If you don't have a specific order for other members to comment. If there is any volunteer, please raise your hand. I don't know if Bill wants to answer my comments, I'll open up the floor. We have a hand, okay, John Michael. John Michael?

DR. SAUER: I'm sorry, I had to unmute myself. I'd like to act on many of your points, Michael. I thought this was a great set of presentations this go around. I really enjoyed the overall FDA Center presentations and how those then interrelated to what is going on within NCTR and the collaboration. The one thing that I did notice during the division discussions is that there are several themes that are actually embedded across NCTR overall. I don't know, maybe it's the format, but that really didn't come out in a clear way, those interconnections. You can kind of see them, but they are not completely fleshed out. I think what would be good is to really understand the interconnectivity between the divisions, to see how this work is progressing.

The other thing that struck me is I see that several programs are very much proactive for NCTR. In other words, you guys are really going out there and trying to figure out what the next step that needs to happen and the science for FDA, and for the toxicological community.

But I see other, and probably the majority of the activities to be truthful, where it is very much reactive. I think there has to be a balance there because when I look at NCTR, I would love to see NCTR lead in the realm of toxicology. Right? That's what this Center was created for. I'd like to see more of that. I'd like to see you guys the even more proactive to really drive the science, create the tools that other scientists need to go ahead and then be able to show off your wares and change the world. I think that is the important aspect. That's the way I look at NCTR. I think you have that opportunity with your degree of focus.

Overall, I thought it was a great SAB meeting. I learned a lot, which was fabulous. I very much liked the way that the various activities are progressing. That's all I have to say.

MICHAELKAWCZYNSKI: So, do me a favor. We have a lot of people raising their hand which is great. Turn your camera on when you're presenting. That would be helpful. Do you want me to call on the next person?

DR. ASCHNER: Let me call on the next person. I'll call the order. The next person is Patty Ganey.

DR. GANEY: Okay. I know you want me to turn on my web cam, but that mean I would have to brush my teeth and brush my hair. I'm just going to go without it on. I

want to echo everything that both Mikki and John -Michael said. I think this was an excellent series of presentations. The work is really great. I applaud the NCTR scientists for moving forward on COVID experimentation. I think whatever we can learn about that would be helpful at this point. I think it's great. I also agree with Mickey that the technology should not drive the science. That the questions should drive the science, and so that should be a secondary concern.

Also, sort of following up on something that Mickey said about wanting to know what next steps are, part of that is what I asked earlier when Bill Mattes was talking. We have seen a lot about in vitro methods and AI, and a whole lot of things. So when you get results there, how do you know that they are relevant for humans? How do you know that they are relevant in organisms? Do you have sort of a policy in place for trying to figure that out, or do you just publish it and hope that somebody else will do it? I would like to know what NCTR 's standing on that is.

Overall, really impressed and think you are all doing great, great work. It seems that you're doing timely work, and I think all of that is important. So, thank you for this day and a half.

DR. ASCHNER: Thank you, Patty. The next one is Mary Ellen.

DR. COSENZA: I also thought it was a great review. I learned a lot. I don't know if it is because it was my second time, I felt like I got more of what was going on and how things work and how the different divisions interact. So, that was really great.

I don't want to just repeat everything that Mikki and John- Michael and Patty said, so I second everything they said. The only thing I would add is I guess we learned a lot about ways to work collaboratively, but remotely, through the pandemic the last few months. So, I guess two rounds of that. One, if this continues on, how does NCTR continue to work amongst themselves and with the rest of the FDA? What good think maybe have come out of that? Have there been learnings on better ways to work, or the fact that NCTR is in Little Rock, and a lot of the FDA is in the DC, Maryland area. Are there learnings that could make the collaborations stronger? The only other thing I would add is we did hear a little bits and pieces about some work that NCTR is doing to help FDA with some of the novel therapies like CAR-T cells, gene therapies, but I think if you look at the portfolio of what is in drug development, (indiscernible) drug development. Just thinking about what else can be done to help expand the science, the basic science in those areas. That's it from me.

DR. ASCHNER: Thank you, Mary Ellen. Okay. I think the one that was next was Alex.

DR. TROPSHA: Okay, thank you. So I, somebody said, that they learned a lot. I think as a newcomer, that applies to me probably more than anyone else. I certainly have learned a lot. I think that the scientific rigor and breadth of presentations were absolutely top-notch. I think the level of science is extremely high in many aspects. That has been really very refreshing. I don't have really critical comments but just perhaps a few suggestions in three areas.

One is more of the presentation of organizational, one on strategy, and one on research. Organizationally, again, all presentations were very deep, and they were organized as Center described important. Research groups described the projects. I think it transpired that a lot of research is interdisciplinary and collaborative. I think it would be useful just to get an idea of the complexity and rigor of the network both inside NCTR, and between NCTR and other units within FDA, and elsewhere, to have a view on projects versus units. I think that would help us and the Center understand sort of the level of connectivity and collaboration. I think that would be quite relevant to have this view.

Looking at the distribution of activities and collaborations and partners, my expectation, and that is probably because I'm a newcomer, was that I thought that the portfolio of projects with academia would be larger than eight percent of the total, which was a on one of the first Bill slides. It was not clear to me how these partnerships are formed. Whether it's a strategic eight percent or whether this is sort of as it comes formation, and given the level of research within the Center and elsewhere, I would expect high connectivity within academic research and that of the Center.

Then, I think very importantly for the Center, there are a lot of really interesting projects. Research and toxicology are very important functions for the Center, which it does, but I think it's also very important to have, at any given time, a summary of best practices for any area of toxicology research that again I found summarized in one of the first slides. I think it's important for the rest of research groups in the U.S. and abroad to have the Center lead the summative publications, in any form of presentations, and what is currently considered best solid practices in any given area of toxicological research. I think it would be very helpful for the Center to leave that particular paradigm. That's it from me. Thank you.

DR. ASCHNER: Thank you, Alex. Charles, please go ahead.

DR. KASPAR: Thank you. I'm not going to repeat the positive comments that others have already mentioned. I'll try to keep it short. I would like to commend the division directors and the Center to Center communication amongst the various groups. I found that to be impressive, particularly between divisions within NCTR. It is clear that there is frequent and discussions amongst these various groups.

My primary comment that I wanted to focus on has been touched upon already, has to do with the breadth of research, and the focus of research. That is both a blessing and a curse. I think it has got to be difficult for the division heads to sort out between NCTR science driven projects versus Center projects that they get requested to work on. I would be interested to learn more how that decision process works. How they decide between a Center driven project and a PI driven project. I think that has got to be a real challenge, and I'm sure money comes into that decision process. But I think, and others have commented on, that makes it difficult to stay focused on PI driven projects.

Along with that, I think it would be of value to understand what are the one or two highest priority areas

within each of these various disciplines. Again, how do you determine those priorities? Also, the greatest needs. An example in microbiology would be in the past, pardon me, it was discussed about bringing on a virologist. They have done so and look how they are in the lead when it comes to this COVID based research. So, what disciplines, which areas do all of these groups see the greatest need moving into the future? Somebody else already used the word to be a leader in these areas. That would be one of my primary suggestions. Thank you.

DR. ASCHNER: Thank you, Charles. Okay, we have a couple more. Steve, you are next.

DR. STICE: Okay, thanks, Mikki. I'm not going to talk about the science, but many of you asked about recruiting people. Just a couple thoughts there. It is not going to get easier, it's going to get harder, as we all know. I think there is a couple things that could be done if there is the ability to do this within the FDA. We have a large NSF Center, and a big component of it is education. Particularly undergraduates as well. We have a research program for undergraduates. We are really concerned about it the summer because it couldn't bring anybody in. What we did was we did it totally remotely. So, gave them problems sets, gave them actual data to analyze. Taught them a lot about PCA plots. Different ways to statistically analyze

data. Those are the type of things that I think, if you can set your hooks into people, saying okay, these are good people to begin with, can we get them started with us at some level? Location is difficult for you guys so if you could do it remotely, by small internships, or something along those lines.

Again, I know there are lots of regulations and red tape in FDA. I'm not sure if that is possible. That's what is possible with a Center grant that you can do those types of things. Get people interested in what you're doing, maybe then longer-term they will be interested in coming and working with you. So, that's I think a really good recruiting tool to use going forward if you can do things remotely with undergraduates, graduate students, where they are going to learn something, you will benefit from it, and possibly be able to bring them in after they are excited about the work that is going on.

So, I'm not going to touch on all the other points. Those have been touched on already. Happy to brainstorm with people on more ways to do that. Thanks.

DR. ASCHNER: Thank you, Steve. Greg, you're next.

DR. LANZA: Thank you, Mikki. I won't repeat everything, but I have to say with virtually everything that has been said. The biggest thought I had, and it

occurred to me yesterday actually, is that on a granular basis, I think they are doing fine, maybe a little bit too academic. But the bigger picture is the COVID epidemic, or pandemic, has actually unmasked a vital need within the FDA. That is when they have to produce new drugs, get approvals, get things out there, they can, but they need to do it usually with a collaboration of industry, other government agencies.

I think, given the situation, and what is going on, that it may be time that we could take a little bit strategically, and a little bit bigger picture. Like for instance, an FDA Modernization Act. Within that act it would bring resources into the FDA that would tend to reduce the time to bring a drug into the market, or a test or whatever, but then, provide much more surveillance of that market use with large numbers of people involved in it to look and ensure safety. Always we want to delay to be sure it's safe, but it's a trade off on the cost for getting things out, and the timeliness of doing so.

So, I think the improved interactions between the NCTR and the different divisions, has improved tremendously. Many projects, much more interaction, then just a few years ago. The NCTR itself is working better. If the NCTR, in conjunction with these Centers, could begin to use the capabilities of AI, in this case, but in

general, to harness for instance in the case of AI, what is going on in stage four surveillance and to determine using AI, which adverse effect may be appearing in those cases, then, be prepared within the NCTR to be able to break down what the safety issue, is it real, or is it just an artifact of how it is being processed?

I just think in general scope, that right now is the time when public health, and the ability to achieve the overall function of getting products out there fast and safe, this is the time where the money could be available through the government to actually modernize the FDA procedures through academic interaction and so forth. With the idea that it is going to reduce the overall costs to produce drugs, expand the capabilities to handle so many different classes of drugs, and to ensure that the NCTR is actually on top of the problems that are evolving in the public itself with these drugs. And trying to determine this early on, not long after we know these drugs are problematic. This is a general concept but may be something that could come out of here is a much bigger strategic vision that could be applied. Not just with the NCTR alone but the NCTR in conjunction with their other FDA partners. Then, using that vision to bring in the Microsoft, or whoever, the government through the public health system, to actually implement these things. That maybe hadn't been

visualized before. That's all I got. I know, out-of-the-box. Get back in the box.

Dr. ASCHNER: Thank you, thank you, Greg. Let’s us have Ken say a few words.

DR. RAMOS: All right. Thank you. I echo a lot of the comments that have been made by the rest of the members of the panel. I agree essentially with everything that has been said. I will just focus on three or four points that I think might be helpful to the leadership of the Center.

The first comment is -- Actually let me preface any comment by saying I think the Center is functioning at a very high level. Could be stronger if there was more collaborative activity taking place. That's one point. The second point that I'd like to make is something that I think I do not understand. I think I heard several of my colleagues actually raise the same question. It is not clear to me how priorities are established by the Center, and by the different departments or divisions within the Center. In many instances, I got the sense that those priorities are established almost at the unit level.

Rather than having priorities aligned with bigger priorities and aligned with sort of some strategic vision for the enterprise as a whole, if this assessment on my part is correct, then I would encourage the leadership of the Center to think hard about how to refine and improve

upon the way that priorities are established by the Center. This is reflected in the way that projects are defined. It is reflected in terms of what the vision for the future is. It's also going to be reflected in the nature of the interactions with the other Centers. So, all of that is going to be something that could be helpful.

The third point I'll make is a recurring theme in a number of the presentations is concerns about service contracts. It is something that emerged I think multiple times. Whatever the mechanism is to fund and to consolidate contracting for service contracts, is something that I think needs to be paid attention to because in a couple of divisions, I think a very crucial part of our budget is going toward service contracts. This of course is going to be at the expense of some of the other priorities that are being sought out.

The last point I'll make is while the Center is extremely collaborative, it's clear that collaboratives locally, regionally, and across the Centers, I think that there's an opportunity for expanding that collaborative network beyond what is already there. I think there were a number of instances that projects could have been improved a lot upon if the appropriate collaboration had been in place or was set out. I think that is something that certainly beats into the first point that I made, and that

if appropriate, I think could really help the center to increase the quality of its product, and to perhaps even minimize costs attached to some of the activities they are involved in. I'll stop there.

DR. ASCHNER: Thank you, thank you very much, Ken. Steve, did you have another comment? I see your hand up.

DR. STICE: No, I did not.

DR. ASCHNER: Okay. So, I guess it is hard to summarize everything together. I think in general we have heard a lot of positive comments. We have heard from some of the SAB members, some issues that can be improved upon. I agree with all the comments that have been noted. So, I think what might be best now is perhaps to give you, Bill, a chance to respond, or gesture with your impressions. We have about 10 minutes left. So, go ahead, Bill, if you want to say anything.

DR. SLIKKER: Very good. First of all, let me thank you all for very insightful comments. It has been a packed full list of speakers and activities. I appreciate all of you paying attention and obviously you picked up on a lot of the information because you came up with some very good suggestions. I just want to thank you for your diligence in performing your role for NCTR and FDA.

Let me try to, since I have about five minutes really, to respond, let me sort of try to group some of these together. First of all, I'll make a general comment. One of those things is that I mentioned in my presentation about the need to have a way to sort of verify the utility of the emergence technology, this is a theme throughout NCTR and so essential for FDA. I made that first little comparison by looking at the in vivo exposure capability to agents such as tobacco, smoke, and other things that will affect the lungs and the various cardiovascular systems etcetera, and then moved to using the example of using the human airway model where you have human cells in culture. I was hoping that would set an example as to how NCTR is really focused on this idea of looking at the newer technology and then comparing them to the existing technology of whole animal exposures. It apparently did not necessarily hit everybody in the same way. I can see now each one of these items is in that very way. That is when you are doing experiments with brain cells in culture, you're always looking for comparisons of the whole animal response. We certainly have that capability to do both. If you're looking for issues with effects on the liver, you're not only looking at cells in culture, and stem cells in culture, but you're comparing that back to the whole animal response.

Really we think that is a driving force of our research, and one that is quite different than some of the laboratories around the world that are just developing a new technology. Our point is to be able to actually run those new technologies in our lab and then to compare them to our in vivo results that either we have already completed those studies, or we are doing them concurrently, or will do them in the future. It is always a comparison because it is not just that FDA needs new biomarkers, they need biomarkers that are validating the work in their particular fit for purpose way. That has to be done in such a way that not only are the researchers convinced, but the reviewers are convinced, that these newer technologies are ready for prime time. So, somehow, we didn't seem to get that message clearly enough across. I'm hoping that this will help to do that.

The other thing that you brought up, is about the focus of our research. We are not an academic center. We are not after the next NIH grant. We are after concerns that are within the mission of FDA. Therefore, the driving force for us, is to pay attention to where the FDA concerns are at that moment and address those issues. Many of these approaches that we are using hopefully are looking toward the future. That is, let's say the in vivo imaging, yes, we have invested in that technology. The diarrhea is to be

able to assess the nervous system for example, in such a way that it is minimally invasive and allows you to use longitudinal studies. Allows you to do developmental studies using the same animals as a control. This is the way of the future. What we are trying to do then is be able to immediately use those biomarkers developed for whole animal injuring and use those of course in a clinical setting. Actually a clinical setting is way ahead and use of imaging compared to the preclinical and off clinical use. We are reverse engineering you might say, using what has been learned in the clinical practice of imaging, and applying it to our models and getting much more out of each individual animal that we are using. And also being able to tie that to some of the other events, not only anatomical changes as with MRI, and volume changes that are equivocal there, but also the pet imaging, which gives you biochemical information and pathway information. We were hoping those examples, sort of fit what you were concerned about, which is the idea that we are really approaching FDA needs.

We are doing it in such a way that we are meeting timely requests, at the same time developing new technology that are tried and true so they can be used by the FDA decision makers. So, those are the two points I wanted to make.

The last one, since I have about three minutes, is to cover the framing aspect. This is really critical to us. You may or may not realize that for 25 plus years we have a summer undergraduate training program, which is a 10 week intensive course structure for usually somewhere between 25 and 30 undergraduates that are selected very carefully from invitations that go out and applications that come in from around the United States. They do have to be U.S. citizens. Other than that, we have individuals that have trained with us from all parts of the United States. This has been a very critical feature. This particular summer, it was not practical to run that. I appreciate Steve's comments about doing it online. You have to realize that most of our work is laboratory function, as well as online, and you really need to have both. Even training online, you would need some sort of interaction, and we were concerned about the liabilities and the safety of the students in this particular setting.

Hopefully next year we will be able to reconstitute that, and it has been a fabulous program for us. Our postdoctoral training program is alive and well as I mentioned. Some 60 to 70 post documents are at any time. Many of them do have the opportunity to look at positions within the NCTR. Many other ones also look at positions throughout FDA, throughout industry, throughout academic

centers, and this spreads the good training that they receive at NCTR, not only throughout the United States often times, but to all parts of the world. This is an important feature for us. I do appreciate any input you have on recruitment. I know that you, in academics, and other industry etcetera, are also facing the same issue as we are at the NCTR.

We would like to have more input on how to recruit, and that sort of thing. We have been successful. We have been able to hire 16 individuals just in the last six to nine months. We do have some key positions still open, and we do want to fill those, and we want to learn more about how to improve our recruitment. We appreciate those inputs that you can provide along the way. I wasn't able to really respond to the majority of questions. All of them are good. We will take a look at those. Certainly, I have to say that the collaboration between the divisions is very real. It is very robust. However, we do deliver information to you division by division. We could talk about a different way of delivering that information, perhaps providing a few examples of these many collaborative studies so that it gets across. But, I think it's more a way in which we deliver the information to you, rather than actually an issue of people not collaborating. Because there is much collaboration as you have seen, not

only between the divisions, but between NCTR staff and staff at other FDA Centers. That is really key to us. That is really an important aspect of all of this interaction and driving the research is the collaboration. It happens early, it happens often, and it happens throughout the experiment. It happens in the publication of the data. We didn't spend a lot of time talking about publications, except that those numbers are enhanced over what they have been in the last couple of years. Thanks to COVID in part, and thanks to us wanting to get all this important data out there. That really is the way that all can evaluate this. Not only do our fellow FDA members see the data as being generated, and also evaluated as this is being written up as co-authors, but of course, that information literature is critical for everybody to compare and contrast and learn from. That's an important aspect of what we deliver as part of the FDA.

I'll stop there and thank you once again for your valuable input. We certainly will respond to these, as we normally do through our normal review process. Thank you very much. I think, special thanks to Mikki for running a really great session and review session for us, Mikki. All the individuals that serve on the review board, and of course our colleagues that helped make this possible which includes Donna, as well as Michael, helping on the

technical side of this. All the staff, the division directors and the leadership. Thank you all very much.

DR. ASCHNER: Thank you, Bill.

DR. MENDRICK: This is Donna. Carolyn would like to make a few comments. Go ahead, Carolyn.

(Muted)

Michael, do you want to close it out?

DR. ASCHNER: I don't have much to say. I agree, this was a wonderful day and a half. I think the comments that we have heard over the last hour or so, 45 minutes, are great, and hopefully they will be helpful.

Here is Carolyn.

DR. WILSON: I had another phone call coming in at 12:30. I just messed up how I responded to it. I was on that phone call talking to them. Technology is not my friend. Just a quick thank you and goodbye to all of the NCTR colleagues down in Arkansas. I am sorry that I'm not able to say goodbye in person. I am retiring at the end of this year. This is the last time I will be participating in the SAB. It is bittersweet. I have been at Little Rock for over 10 years. I just wanted to thank everybody down there for the hospitality, and for the interactions and collaborations. I'm really excited that over the course of the last few years we have really gone from at the beginning, zero interactions between CDER and NCTR, and

now as you can see, we have really built up a nice collaborative portfolio. I think it has taken a while, but we have really figured out how to leverage each other's expertise, and in a great way. I'm confident that that work that we have done over the past 10 years will continue to build that momentum over the next few. Thank you and goodbye. I'm done now. Thank you.

DR. SLIKKER: Let me just say a couple words to Carolyn. She has been one of those really fantastic collaborators. She really set the benchmark on how to present information during these meetings and how to maximize the opportunity for exchange. Carolyn, we really thank you very much, for you setting the example, and bringing on others behind you that can do the same. Thank you so much, and we hope you really do enjoy your retirement. We still want to hear from you from time to time. Take care and thank you very much.

Thank you, Bill.

PARTICIPANT: Okay, I'll just wrap it up. Carolyn, enjoy your retirement. Thank you to everybody, and I think especially to Bill, Donna, and Michael for letting us have this meeting and running smoothly. Special thanks to all the SAB members and all the participants and division leaders, that presented. We are all going to quit now and the SAB members need to log into the WebEx. You

have the address. Maybe just as a last thought, as SAB members, we have signed up to help the NCTR. So, the way I look at it, it's not just the day and a half that we spent with you today, if there are any questions, if there any protocols we talked about that you wish us to provide you with input, I think I speak for everybody on the SAB, please do so. We are here to help and we are available throughout the year, not just one and a half days during the year. With that, thank you to everybody. Enjoy your afternoon whether you are golfing or working. I'll see the SAB momentarily, and goodbye to everybody else. Bye-bye.

With that, this year's NCTR meeting has concluded. Again, take care, have a great week, have a great day.

(Whereupon, the meeting adjourned.)