National Center for Toxicological Research (NCTR)

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

Agenda Item: Welcome

DR. ASCHNER: Good morning everyone. My name is Michael Aschner, I'm chairing the Scientific Advisory Board. Welcome to the second day of the meeting. We have three presentations today by three different divisions, and after that we'll have a discussion, and then the Scientific Advisory Board will meet in a closed session, and in the afternoon one of the divisions as noted yesterday, will be reviewed by a subcommittee. So without further ado I would like to invite Dr. Steven Foley to present next. He's the Director of the Division of Microbiology.

Agenda Item: NCTR Division Directors: Overview of Research Activities, Continued

Division of Microbiology

DR. FOLEY: Thank you Dr. Aschner. So, I am Steve Foley, I'm the Acting Director right now, I've been in this role for a little over a year for the Division of Microbiology. Kind of our standard disclaimer on a lot of the talks, and here as well too. So we start to look at the Division as a whole, look at the staff and the excellent staff we've got. We've got roughly 19 research scientists; this includes staff fellows and government GS employees as well. We've got four support scientists and

four administrative staff, and that includes me in that number.

So we've got about 27 government FTEs. And right now we've got about 10 ORISE post docs, some graduate students in there, this number is fluctuating a little bit as we're bringing a couple on as they recently graduated, and then we have a couple that are just going on to permanent jobs, so that has been good for them.

When we look at the division, we have a couple of different areas of expertise in the division, and we've got several scientists in each of these areas, and several scientists that kind of cross borders if you will with these different ones. We've got a lot of work in the microbiome space, antimicrobial resistance and food borne pathogens, and there's a lot of overlap, and they used to do quite a bit of work with antimicrobial resistance and food borne pathogens.

Environmental biotechnology, this also would include things like pathogen detection and characterization, some work in nanotechnology, we'll look at some of these areas a bit later in the talk, some work specialized in women's health related areas, and then virology, and the virology has been an area over the last two years where we've had an increase in inactivity. We had Dr. Azevedo and her team kind of pre-pandemic, and we built

that up with areas of expertise as well in the area of virology, both internally and bringing on a new staff fellow.

So the mission of the division is to serve a multipurpose function with specialized expertise to perform fundamental and applied research in microbiology and areas of FDA's responsibility in toxicology and regulatory science. And we've got several kind of goals if you will to meet that message, I'm not going to into those in detail because we'll talk more about them on the next slide, but we want to make sure that we're prioritizing our research that we're doing to meet the mission of NCTR and the FDA, and communication is a big piece to help us to make sure that we're meeting that mission on the regulatory science side.

So some of the strategies, one of the things that we want to do is make sure our research is contributing to FDA guidelines and regulations, that we're providing the data that's needed for those regulatory decisions. And so we've been trying to get our scientists and our staff to understand the regulatory process better, through communication with different centers and some additional training in these areas. We want to make sure our research program is integrated well within the FDA infrastructure, that we're providing complementary or filling gaps rather

than duplicating efforts. And again a lot of that is involved with open communications and trying to improve on those.

Again, we're working to try to enhance some of the FDA interactions. We're trying to get our scientists and other staff to interact on working groups and other sorts of stuff to understand the needs and where we can fill gaps, those types of stuff, and again try to collaborate better and expand those so our research is meeting the mission.

And then strengthening program management as well. One of the key things here is establishing benchmarks for scientific excellent, whether that's publications or providing datasets, those types of things that are important. And making sure we've got the facilities and equipment that's up to date and to meet the needs so that we can adapt on the fly if you will to meet those mission critical areas.

We do a lot of outreach as well, some on the global level for different workgroups, several of our staff and scientists are members of different editorial boards on top notch microbiological journals. On the national side, some of the different societies are attending the conferences and in some cases leadership roles within the different societies. They're also active on a number of US

government panels, and several interagency workgroups on the microbiome or some food safety related areas.

And then a key thing that we're interested in too is mentorship in developing scientists, you see we had a large number of ORISE in the division, and we also have a lot of interactions with the local universities here, and we're trying to expand that out to some of the other institutions outside the state borders. This was one of the things that our subcommittee a couple years ago noted, that it's important for us not to just focus on some of the local universities being a national center to reach out to and try to interact with universities throughout the country.

We're working to do that, the pandemic has made that a little more difficult, and there's a lot of times involved physically going to giving seminars, those types of stuff, and so we're continuing to work to reach out to other universities as well. And that's important because winning a pipeline for postdocs and other sorts of stuff, and having people realize what we can do and have those interactions is important.

So when we look at our research collaborations across the agency we've got a number of different projects with different centers, with CDER and CBER, CFSAN, most of the different product centers right now, I think everybody

but tobacco products, and we're open to working with them, we have in the past. We look at the concepts that are approved or those projects or protocols that are in the development and kind of evaluation phase.

Again, we've got several, including some with CBER, a lot of those are in the virology space, CDER on some pathogen detection, and tattoo with CFSAN, and with CVM on some of the virulence piece that Dr. Tan talked a little bit about yesterday.

If we look at some division metrics, we've got 19 or so ongoing protocols, 13 approved concepts that we're working to develop protocols, and some are just recently approved concepts and very early in the development stage, and some hopefully will be approved very soon and do these approved protocols to begin the work.

Last year about 18 abstracts accepted for conferences, and this number is a bit low compared to what we normally have, and I think that again the pandemic had some of that, not being able to travel to meetings, and I think last year with the hope that we would have more in person meetings, I think people held off on abstracts and that type of stuff in there.

So we're seeing a robust rebound in that this current year. Had 25 manuscripts published, which is kind of on par, where we had been historically. A trend here

with the bar graph, it's kind of in the middle of where we had been.

Our research focus areas, the microbiome as mentioned earlier is a key one, looking at how different things like antimicrobial agents or contaminants or different xenobiotic materials impact the microbiome. Several projects in that area, looking at detection of microbial contaminants and things like tattoos or pharmaceutical products. Antimicrobial resistance and virulence, we've got several investigators working in that space, both with foodborne pathogens as well as other pathogens.

Some work in areas that are supported by the office of women's health. We have in the past done work with tobacco products, right now we have a bit of a lull in that area, and the nanotechnology where we've got multiple projects looking at nanomaterials and different compounds and how they're going to go back to the microbiome as well, how do they impact members of the microbiome.

So a lot of overlapping things coming to this last bullet where we're trying to get data to help improve risk assessments, and integrating the systems biology approaches, we've had a lot of work with genomics, transcriptomics, proteomics, trying to pull all those together to help answer questions.

So talking a little bit about some of the ongoing work that we've got, Dr. Marli Azevedo has been doing quite a bit of work with SARS-CoV-2 with the pandemic. She is an investigator who had expertise in coronavirus research prior to the pandemic, and so she was able to ramp up quite quickly having that knowledge when we had the initiation of the pandemic.

One of the projects that she's working on is trying to look at cardiotoxicity associated with nonstructural proteins of the virus. And so they've developed a number of vectors that can be inserted into the different tissue cells and tissue culture cells and that kind of stuff to mimic intracellular exposure of the viral particles, and then also they can mimic extracellular exposure by expressing the proteins and then adding those to the cell culture.

And then through these efforts they can look at cytotoxicity that would be characteristic of cardiomyopathy using different cardiomyocyte models. And then along with that is assessing immune function differences, looking at immunological and different metabolic pathways that are impacted by those exposures.

So what their research has found is that the intracellular expression of some of these nonstructural proteins, especially NSP1, does lead to an increased

cardiotoxicity and elevated LDH levels. What you can see here, they looked at this rat cardiomyocyte cell line, and exposed those to puromycin, and this is which the vector that these proteins are expressed in has a puromycin resistant gene, and so wildtype is killed by that particular antibiotic.

The nucleocapsid, when that's expressed you don't see the toxicity. With the NSP one you do, you see red is dead in this case where you see essentially no green, almost all red where you've got cell death associated with those cells that are exposed to that nonstructural protein one. And when looking at kind of the pathway analysis there are a number of different proinflammatory cytokines that are upregulated, such as TNF-alpha and IL-6. On the extracellular treatment you also see some of the different chemokines that are affected as well too.

Another area, this is a project we're working with CDER on, is trying to establish kind of a standardized methods for sporicidal efficacy assessment. Bacterial spores are a big problem in the pharmaceutical industry, especially the compounding industry, because these are ubiquitous, they're everywhere in the environment, they're very resistant to heat and desiccation, different chemical compounds as well too, and so they make it difficult to get rid of those.

And there are ways that they're being assessed, but they have limitations, because different people use different test organisms, the quality of the spores that are being utilized are not always the best. You may have some that are not as resistant as they should be, and so you may get a false result of being sporicidal even though better quality spores may be able to survive. And then you may get ineffective neutralization, which looks like it was effective. And then trying to understand exposure time for the different compounds.

And so this work that Dr. Huizhong Chen and Jinshan Jin are working on, they've got objectives to develop or evaluate a test panel of different organisms to look at the best ways to optimize those methods and then evaluate the effectiveness, and there again with the goal to feed all that into the established standardized method.

So they recently just within the last week or so had a publication accepted for this research, where they have developed and assessed the different spore qualities, different organisms, a series of bacillus in this case, and what they found was there are some strain variabilities, difference between strains. What this graph shows is that the blue is where you get significant reduction in the numbers of spores, and the lighter blue less.

And so you see it with the days of incubation of zero, kind of that very early, they're more liable to the sodium hypochlorite. The same thing on the end of these left spores state, they aren't as good of quality if you will. So they found that the optimum maturity period for these spore preparations were in the seven-to-21-day range. And so this is part of the data that's going in to develop that method.

Dr. Sangeeta Khare along with Dr. Kuppan Gokulan are doing quite a bit of work in the microbiome space and trying to understand how different xenobiotic compounds impact both the microbiome as well as the host mucosal associated immune responses. And so they've been working with a lot of different collaborators, and for example the Center for Biochem, Toxicology, and other groups that are funded through the National Toxicology Program to look at the in vivo impact of different agents on the microbiome and then the immune response.

And a goal of this is really to develop some translational approaches that can be used for the safety assessment of these different compounds on the microbiome, and extrapolating what we see in either of the rodent models to human impact. And then to build a decision tree that can be used for the toxicological evaluation of the microbiome in these assessments.

So as part of this work they've looked at the impact of different environmental pollutants, additives and different food contact surfaces, different nanomaterials, and antimicrobial residues as well, seeing how does that impact the gastrointestinal tract, both the microbiome as well as membrane permeability, those types of things I've used in collaboration with others, different disease models with different animal exposures as well as some tissue culture and ex vivo models where we have tissue punches and those types of stuff to look at membrane permeability.

And so this group has been very active and had a number of publications over the last number of years in this space and are providing a lot of good data in these, and are helping them work to develop this type of a decision tree where you can look at the data that's available, and then if there's different studies, there's data gaps, where do you go, what kind of studies do you need to do in order to fill those data gaps. And ultimately again is to try to predict that host, the microbiome, and the xenobiotic interactions and predict potential toxicity associated with those.

Dr. Jing Han and I are working on a project where we're developing a salmonella virulence gene database. This is in collaboration with the folks at the Center for Veterinary Medicine, and we've had a lot of interaction

with CFSAN on this as well too. And salmonella is a major cause of bacterial foodborne illness.

As part of outbreak investigations and those types of things, the isolates generally undergo whole genome sequencing, and so you get a whole wealth of data in there, and there's a lot of tools to analyze that, looking at predicting a salmonella serotype or the antimicrobial resistance genes of the potential resistance profiles there. One of the areas where there was a gap we noticed was in the virulence space, and we predict potentially virulence space in the genes that are present there.

And so Dr. Han did an extensive survey of the literature as well as different databases that were already out there and came up with a fairly comprehensive database with over 500 genes that are present in this. And what we can do then is upload different whole genome sequence files to that, and you get either a readout like this where you get a presence/absence profile, or you can get the looking at how similar the genes are to reference genomes.

Where this has been valuable is you can then take these presence/absence profiles and put them into programs like bionumerics or others and do phylogenetic analyses and do principal components or minimal spanning trees, those types of stuff, and you can start to tease out are certain genes important to virulence.

Or this example down here is a parsimony spanning tree, and what we saw is this group here is a type of salmonella that doesn't cause human illnesses very often, is commonly found in chickens. Well that same serotype does cause human illnesses, those that cause human illness tend to cluster out here in this kind of spanning space on these virulence factor profiles.

And so you can go in and see all right, what is the difference between this group that doesn't cause illness and those that do, and tease that out. That has been useful there, we've been working with CBM on that as well, it has been a very deep project.

We're also working with NCBI and an interagency workgroup to try to move some of this into the NCBI sequence analysis pipeline, and we're having a meeting tomorrow where we're going to be discussing this further. We're working to develop additional tools as part of this database. One is looking at plasmid transfer associated genes. And plasmids carry antimicrobial resistance genes and virulence genes and often can be transferred from one bacteria to another, they encode their own transfer genes generally.

And so one of the things we looked at with some data where we looked at the types of plasmids that transferred antimicrobial resistance following exposure to

the antibiotic tetracycline, and where resistance transferred this was a number of isolates that had this particular plasmid. This isolate 143 almost always is incompatibility group AC plasmid transfer, whereas some of these other strains that transfer tetracycline resistance, the IncAC was not as commonly transferred or was much more variable.

So when we started to look at, put these whole genome sequences through the database, one of the things that we noted was that with the 143 isolate where it transferred all the time it had the full set of transfer genes, whereas those others had lacked some of those. It may be part of why you see that variable pattern in transfer. We're working to continue this project to build that out.

Some other ongoing projects, briefly. Drs. Shen and Jin(?)22:58 are also working with CDER on a project looking at nanoscale materials that are present in sunscreens, titanium dioxides and zinc oxide, and one of the things that they noted is following UV exposure which you would expect to see in the sun, that some of these nanoparticles had antimicrobial effects, which may not be good if it's organisms that are present in the skin microbiome.

Dr. Kuppan Gokulon has been doing work with CDER on looking at nanoscale drugs and the impact of some of these formulations on drug permeability and different immune response areas. One of the things that he found with IL-10, that you saw different (inaudible) followed by the different types of drugs, different formulations, whether it's a nano versus the parental drugs, and there was some sex dependent differences as well within these findings.

Suzy Fitzpatrick mentioned yesterday a bit about some of the tattoo ink work that we're doing. One of the extensions on that is looking at anerobic microorganisms that are present in tattoos. When we did the aerobic bacteria, we saw about half of the samples had microorganisms in them. And when we looked at the anaerobes, one third in this early study. One of the organisms that we found most common was this Cutibacterium acnes which is a potential opportunistic pathogen. And so this work is continuing on, this is one of our first sampling with these anaerobes.

Dr. Kidon Sung is doing work on biofilms in antimicrobial impregnated catheters and looking at what's the impact of having these antimicrobials in these catheters on things like biofilm formation or antimicrobial resistance. And with pseudomonas aeruginosa one of the things he noted was that there was an up-regulation in the

genes that are associated with biofilms in those catheters that have the antimicrobial coating versus those that don't. Planktonic cells were also more impacted, which is not too surprising in there, because those are more metabolically active a lot of times.

Dr. Ashraf Khan with CVM is working on trying to characterize some of the efflux pumps that are associated with antimicrobial resistance in salmonella. They've been doing quite a bit of whole genome sequencing of isolates, and then looking at the gene expression of the efflux pump genes following exposure to different classes of antibiotics to try to understand the role of these efflux pumps and multi-drug resistance.

A project that my group has been working on is developing what we're calling a plasmid toolbox to try to be able to understand some of the functions of genes that are encoded on plasmid. If you look at the sequence analysis on a lot of plasmids there are a lot of hypothetical genes or hypothetical functions on the genes, and so we're trying to figure out ways to better characterize those functions.

One of the challenges is oftentimes plasmids are present in multiple copies within a strain, and so you've got to knockout that in each of the different plasmids that are present, so Dereje Gudeta a postdoc in our lab has done

a neat job developing some different color screening to allow us to look at that, blue-white screening or pink-white screening to allow us to more efficiently knock those genes out. We're also working on curing some of the plasmids from these strains as well, which is a challenge.

So Dr. Mark Hart has been trying to understand whether lactobacillus and products can be used to prevent the formation of toxic shock syndrome toxin-1 production by staph aureus or even to limit the ability of colonization by having the lactobacillus in different products, feminine hygiene products, some work initially funded by the Office of Women's Health.

Dr. Youngbeom Ahn has been continuing work looking at Burkholderia cepacian in pharmaceutical products. This has been a major challenge because these can grow in what should be sterile water and sometimes antiseptics, and those are often present in low levels which makes it hard to detect, and so they've been working on the development of different genomic methods and molecular methods to detect these pathogens at low levels.

So looking forward, we've got different projects with again some on the COVID space looking at coagulopathies as work that's being initiated by Doug Wagner. Some additional tattoo ink products, trying to develop some molecular based metagenomic based approaches

to detect the organisms. And then a couple different projects with CVM, one furthering our efforts on the plasmid side, and another with avian pathogenic e coli and trying to use that as a model system for the assessment of antivirulence drugs. And then one on developing some new microbiome models that Dr. Kristina Feye, our newest member in the Division is proposing.

So some of the challenges in the division, one of those is balancing ongoing efforts with emerging priorities. So if we have people who are working on a project, then an emerging issue comes up, how are we going to prioritize who is going to work on that, and those types of things. And that involves both personnel and space factor utilization challenges. People often don't want to pivot from something they're working on in depth. That's one of the challenges that we've got, is to try to balance those efforts.

Developing succession plans to fill vacancies over the upcoming years. Right now we're in a division leadership transition as well as the center and then the Office of Chief Scientist as well, we've all got acting people in leadership positions, so how those will all fill out, that will be a potential challenge or opportunity.

And then within the Division too, when people retire we've got several that are retirement eligible age,

and how do we fill those. Right now we've got fairly high ratio of principal investigators to support scientists. Do we hire more support scientists, or do we continue to have that high ratio of principal investigators? And that may also play into this balancing act as well.

Recruiting top talent, that is an issue. I know Fred and Bob both mentioned that, especially on the ORISE sides, some of the challenges that we've got there. That also extends to the scientific staff as well too, where you've got, if you look at the American Society of Microbiology job board, there are hundreds of jobs right now for microbiologists, and so we're competing to bring in top talent.

Our physical structures, we've got older buildings, which has created some limited flexibilities on some of the things, whether that's having electrical capacity for some of the new equipment, or being able to make some minor modifications of labs and that kind of stuff for new equipment. We're really trying to make sure we've got the equipment, and make sure our labs are maintained so that we can take on new challenges and meet the needs of the agency.

Computational biology, that's an area where we've done quite a bit of work, we're doing a lot of collaboration with Weida's group. We can generate a lot of

data in the microbiology space fairly quickly. And the bottleneck often is the analysis piece. And so we've got a working group within the division to try to understand ways to get through some of these bottlenecks. Making sure that we're engaging the product centers on that communication piece, I talked about this early in the talk.

That's something that we've got to continue to do, both at the beginning and then throughout the projects as we go through to make sure that we're getting the kinds of data that are needed. And then the final one, this goes back to that first bullet, is trying to balance NCTR developed research priorities or scientist developed research priorities with those from the different product centers.

And when we hire people we hire people a lot of times because of their scientific expertise, they come out of academic backgrounds, and we want to focus on areas where they've got expertise. However, sometimes we need to pivot in order to do that, so that sometimes can be a little bit of a challenge if you will for some of our folks.

So some feedback requested. Are we meeting the needs of the different product centers, how can we do a better job of engaging? I think over the last two years, with the pandemic and everybody getting more used to these

sort of virtual type environments, this has gotten a lot easier in there, because the distance from Jefferson Arkansas to White Oak is not much further digitally than from Rockville Maryland to White Oak. It has made it easier as people have gotten more familiar with those.

And then kind of horizon scanning as well to is another area where we can have some feedback. So I want to thank the members of the advisory board and the representatives from the different offices, and Dr. Patterson and Dr. Slikker and Dr. Mendrick as well, and then the division staff who have provided a lot of feedback on this and are doing excellent work. So thank you.

DR. ASCHNER: Thank you Steve. And we have about 10 minutes for discussion or questions from the Scientific Advisory Board.

DR. GANEY: This is Patti Ganey. Very nice presentation. This is really just a curiosity. When you're discussing the cardiomyopathy studies and using cardiomyocytes, have you given any consideration to doing similar studies with endothelial cells, or vascular tissue?

DR. FOLEY: They've worked with some of the kidney cells and some of the others. The vascular part, that's a good area to look at and to see. I know Dr. Wagner's study that is just kind of initiating may look at some of that related to the coagulopathies, except they're going to use

some animal models in that, so they'll be able to look at I think more of a whole animal and whole-body type thing, looking at the impact on that. But yes, I think looking at the vascular lines would be good.

DR. WALKER: Good morning. I really enjoyed your talk. I'm very excited about your work on the microbiome. I have a lot of questions but I'm going to limit it to just two. The easier question is are you all looking or profiling any microbiome derived metabolites in any of your studies.

DR. FOLEY: Some. A little bit. Some of the work that Sangeeta Khare's group has been doing, they've been doing a little bit of that with some of the metabolites. I know Dr. Feye, who we just brought on in November, a large chunk of her research is going to involve that. She has developed a research team that involves some of our folks from systems biology to try to pull in that aspect of looking at the metabolites, doing some of the mass spec, those types of areas.

DR. WALKER: So the larger question is here in Houston we're all very excited, I'm sure you saw the recent science papers about how the microbiome determines the response to immunotherapy, we know it's determining, it's just doing so much. So it seems to me to be a huge issue in terms of how you evaluate safety and efficacy if the

microbiome is having this huge effect. So I'd just like to hear your thoughts and where you're thinking about that.

DR. FOLEY: That is a very important question, and one of those that we've tried to look at it in a number of different ways. One is we're working with several different centers, even the Division of Neurotoxicology on some of the efforts with the gut microbiome access, and some of the work, again some of the things that Dr. Feye will work on and some of the things that Dr. Khare worked on, trying to get at what are some of the variables that come to play in that.

I know one of the projects that Dr. Khare has worked on is looking at what vehicle for different compounds, how does that impact, because you may be having a test article and you want to see what the impact of that is on the microbiome, but how that is prepared or how that is administered can play a role in how that impacts the microbiome.

And so really trying to understand some of these variabilities I think plays into that a little bit, because it is extremely complex. Understanding some of the metabolites as well, too, and how the organisms of the microbiome impact the compounds coming in, and how the compounds impact the microbiome, that's where I think your first question on the metabolites and understanding that is

really key to understanding how those systemic impacts that come in. I know I'm not answering it probably as succinctly as I should, because it's a complex area and something that we want to look at and try to understand better, and that's what the project that Sangeeta and Gokulan are working on that's funded through the National Toxicology Program, that's some of the kinds of things that they're asked to answer.

DR. WALKER: I am new, so I don't know if this is possible, this might be an incredibly great thing to bring together an expert working group to think big pictures on what this would look like, and then where are the opportunities to actually do something now. Because I think it's actually incredible in terms of where moduling the microbiome is. It's a black and white switch, you get the phenotype, or you don't, you get the response, or you don't, so it's pretty amazing. So thank you.

DR. FOLEY: We have been involved with HESI on some of these. There is a workgroup to try to look at some of these, and Sangeeta and I and our late director Carl Cerniglia have been involved in those activities to try to understand how the microbiome plays into the toxicity and what are the methods to address that, because a lot of the stuff, there's quite a bit of variability in methods, are

there basic standards that need to be taken into account for those.

So our group, we've got Sangeeta also chairs the Interagency Microbiome Working Group with the NIH and FDA and other partners, and so we are trying to engage outside as well too, because there is an area of opportunity and there is an area of kind of a black hole to some extent, because there is so much stuff there.

DR. WALKER: Not just toxicity, but even beneficial response.

DR. FOLEY: That is true.

DR. ASCHNER: Thank you Steve. We are going to move on to the Division of Neurotoxicology, and we'll hear from Dr. Talpos, please.

Agenda Item: Division of Neurotoxicology

DR. TALPOS: So, thank you for your time. I am John Talpos, the Director of the Division of Neurotoxicology. And I'm going to provide an update on activities within the Division. And we would love your input on current as well as our future activities, because a lot of the current work I'm presenting today is still very much in process.

First of course the disclaimer. This information in these materials is not a formal dissemination of

information by the FDA and does not represent agency policy or position.

The division currently has 15 PIs, comprised of research scientists and staff fellows and visiting scientists as well. We have nine support scientists and two administrative physicians. We currently have six open positions, although we've identified candidates for two of those, and one we'll be starting next week, and the other later this summer. Also we're about to put out an ad to hire two new support scientists. We have five ORISE staff for a total of 31 members of the division at this point in time.

When it comes to outreach, we're currently working with all of the NCTR divisions. We're collaborating with CDER, CDRH, and CFSAN, and we also work with the National Toxicology Program, and with the National Institute of Perinatology in Mexico. We're currently working with HESI as well as the Critical Path for Parkinson's.

From a leadership perspective we're working with local universities, including the University of Arkansas Medical Sciences and the University of Arkansas – Fayetteville. We're working with the University of Texas Health Science Center, and we also have an ongoing collaboration with researchers at the Icahn School of

Medicine, and we're also working with researchers at the University of Birmingham and the Virginia-Maryland College of Vet Medicine. And a member of the division is on the Steering Committee of SmartTots, which is a collaboration between the FDA and the International Anesthesia Research Society.

The mission of the division is to identify and quantify neurotoxicity associated with FDA regulated products, while meeting and supporting the evolving needs of the other FDA regulatory centers. We work to be a resource for the rest of the agency, whether that be through generating data or offering knowledge and experience. To generate data we use a combination of translationally valid imaging approaches, alternative preclinical models, and cross-species metrics of brain function to identify markers of neurotoxicity.

How is the division doing? Well, based on our publication rate, we look to be on pace to have as many or more publications than last year. So we've submitted quite a few, although not many have been accepted at this point in time.

Our total protocol numbers were almost unchanged from last year. We have almost 50 active protocols within the division, with about 80 percent of those being experimental protocols, while the other protocols, S

protocols, support a variety of scientific activities. Of our scientific protocols you can see our most common class of collaborator is the product centers, and most of our research projects involve some type of collaboration.

The Division does have a diverse range of projects supported by our 15 PIs, but most of these cluster around a couple of specific themes, like developmental neurotoxicity, anesthesia related neurotoxicity, barriers of the CNS, and neurodegenerative disease. And I'll touch on a couple of these projects in more detail.

I'm going to start with some of the active projects within the division. And I'd be happy to hear your input on these. These include biomarker qualification for the use of T2 MRI for nonclinical neurotoxicity safety studies, development of a blood-brain barrier chip in vitro model, and using in-vitro models to assess the developmental consequences of early life exposure to opioids as well as cannabinoids.

So first I want to talk to you about some great work being done by Dr. Serguei Liachenko. He has been developing T2 MRIs, biomarker for neurotoxicity in nonclinical studies, and this work has been done largely in conjunction with CDER, and Serguei has been working on this project for about a decade.

So Serguei performs T2 MRI both preexposure and at multiple post exposure intervals within the same animal. Through this approach he hopes to determine both NOAELs and LOAELs, but just as important he hopes to determine the time and location of greatest neurotoxicity to guide classical histopathology. While MRI might eventually be able to replace histology, the goal at this time is to use it to guide histopathology, to make better assessments in less time with fewer animals.

So for the example of hexachlorophene, which you can see here at the bottom of the slide, you can use MRI to select six-day post dose as the optimal time to perform an assessment, because you can see here this is when the largest neurotoxic signal is occurring. But if you investigate it earlier or later you potentially would miss the signal.

The fact that T2 is noninvasive means that multiple samples can be taken from the same animal, so it reduces the number of animals needed, and allows for a powerful repeated measures design. Also you can follow neurotoxicity over time to allow for the potential detection of reversible effects. Serguei can detect different mechanisms of toxicity and effects on white and grey matter. Results can be obtained just hours after a

scan, and they're more quantitative and less vulnerable to an observer bias than classical histopathology.

How this approach works is that different components of the brain relax at different rates when exposed to a strong magnetic field. As you can see in the figure here, white matter, grey matter, and cerebral spinal fluid all have different profiles of relaxation. Changes in these rates can be used to quantify the amount of toxicity that's occurring in the area.

Serguei has achieved a lot in this project. He has done extensive methods development, established an image analysis pipeline, and most importantly performed a sensitivity and specificity analysis against known neurotoxicants with different mechanisms of action.

However, he still has some work to do. For example, he needs to look at the effects of week neurotoxicants, drugs that will result in necrosis but with a much more subtle profile. He also needs to look at how MRI changes with age to see if the method can be applied to different ages. And most importantly prepare the data package for formal biomarker qualification. He hopes to have an initial submission to CDER later on this summer.

The next projects I'm going to discus both use vitro models. Personally, I think it will be several decades before we see vitro models actually replacing vivo

CNS toxicity studies in most instances. This is because to be honest we don't really fully understand how the brain works, and because it's a very heterogeneous structure.

There's so many different types of cell types, it's going to be extremely difficult to go and translate the cells to vitro models and chips. However, in the meantime I think they have real utility in modeling genetically distinct or vulnerable populations, to study comparative toxicity of related compounds, and to look at mechanisms of action, as well as potential drug/drug interactions.

Example of how we can use this technology to model genetically distinct groups is work being done by Dr. Hector Rosas-Hernandez within Alzheimer's disease on a chip model. Hector is working with two cell lines. One healthy line, with the neutral APOE three allele, and the other disease line derived from an individual with Alzheimer's disease that also had the APOE four allele. The APOE four allele is one of the most common genetic risk factors for Alzheimer's disease.

Hector is working to standardize a battery of neurovascular endpoints, and ultimately, he wants to compare the pathology developed on his AD chips to that seen in human pathology. This work is done in collaboration

with Emulate, the maker of the system, as well as CFSAN and CRDH.

Hector will be working with a 3D model composed of human IPSCs that include neurons, pericytes, astrocytes, microglia, as well as microvascular endothelial cells. And he'll be using a combination of expression based and functional endpoints.

Hector has been moving pretty quickly on this project. He has standardized the culture and expansion of his key cell types. He and a small team are training on the use of the brain chip. He has also been working towards standardizing neurovascular related endpoints, including measures of paracellular permeability, P-gp function, analysis of protein levels of tight junctions and transporters, and chip imaging using confocal microscopy.

When Hector performs his work he takes a little bit of a different approach than most. He's not planning to pool his data; he's working towards a vitro clinical trial if you will. Each chip represents an individual. So from one chip he's successfully performing three functional assays at different timepoints, as well as taking six terminal samples for western blot analysis.

Here we have some example data generated by Hector and the team. This graph was created from data generated by three different researchers, highlighting the

potential consistency of the method. Going forward Hector hopes to develop a library of cells to represent different vulnerable populations and provide genetic diversity for his future work.

Although Hector has made great progress, he still has some work to do. He needs to complete characterization of his induced pericytes and mixed cultures of neurons and astrocytes. He still needs to construct isogenic chips. And he needs to finish standardization of some AD-specific endpoints. And he also of course needs to analyze AD pathology and compare this to progression in the clinical setting.

The next study I would like to talk about is work being done by Dr. Fang Liu on the effects of methadone or buprenorphine alone and combined with cannabinoids on human neural stem cells. The use of opioids has been steadily arising over the last 30 years in America. Unfortunately, this is also the case in pregnant women. Because of this there has been a dramatic increase in neonates suffering from neonatal abstinence syndrome, or NOWS. Opioids are typically used to treat NOWS in newborns.

At the same time, we’re also seeing the rise in the use of cannabinoids and cannabis related products in pregnant women. Moreover, some women are using cannabis to treat withdrawal symptoms while they’re pregnant. As such,

children are being exposed to these drugs and their metabolites in utero and early in life.

With this project, Fang is asking two questions: Can treatment for NOWS be improved? For this she plans to compare the effects of opioids or cannabinoids on human fetal neural stem cells and differentiated stem cells. She also wants to determine the impact of using cannabinoids and opioids for the treatment of withdrawal. She will look at the effects of coadministration of these different compounds.

Along the way, Fang hopes to resolve some uncertainty about the toxic potential of some metabolites of CBD. You will have heard Fred talk about this a little bit yesterday, but a primary metabolite of CBD, seven carboxy cannabidiol, reaches much higher levels in humans than in rodents, making risk assessment difficult in some ways.

Unfortunately, these primary metabolites are very expensive. Vivo assessment of these metabolites could easily cost over $100k just for the drug, assuming you could actually get enough of it. So Fang will also consider the impact of these primary metabolites in her model, which should provide some useful data on the comparative risk of metabolites versus the parent compound.

Moving to future projects, I want to talk about a project that we're putting together to look at the developmental neurotoxicity of acetaminophen in a vitro setting, our ongoing efforts to establish the regulatory impact of damage to barriers of the CNS, and our plans to develop the mini swine as a model for developmental neurotoxicity testing and other studies.

So there's a growing concern about the potential toxicity of in utero exposure to acetaminophen, as highlighted by this 2021 consensus statement. The concerns over APAP are being driven by a series of high-quality epidemiological studies.

A recent meta-analysis of these studies showed a 20 to 30 percent increased risk of autism spectrum disorder and ADHD in boys exposed to acetaminophen in utero. The risk isn't to boys alone; however it is notably higher in boys than girls. And I'd also like to draw your attention to the cumulative sample size in these studies. They're really very big, and it's an impressive dataset highlighting this potential concern.

Within the division we're starting to research APAP-related neurotoxicity with vivo models. However, our lack of knowledge about the mechanism behind potential APAP mediated neurotoxicity makes it a little bit difficult to design assessments. And there are multiple mechanisms by

which APAP may cause neurotoxicity, as you can see here. However, one potential mechanism popped out as lending itself to evaluation in a vitro setting, and that's CYP2E1 -mediated metabolism, and this is a project that's being led by Dr. Shuliang Liu.

CYP2E1, a lot of you probably know a lot about it, is highly expressed in the liver. APAP metabolized by CYP2E1 will eventually deplete levels of glutathione, resulting in free radical formation and hepatotoxicity. While CYP2E1 is expressed on neurons in the human brain, it is at lower levels than in the liver, but it is still very much there. This raises the question if CYP2E1 mediated toxicity could occur at the brain. If so, this is potentially really problematic, as alcohol, halogenated anesthetics, and even a metabolite of caffeine are all metabolized by CYP2E1. So there is real potential to push this system too far.

And of course, the developing brain is very vulnerable to all types of different abnormal energetic demands. While this project is still in development, our starting point will be to determine if neurons die after APAP exposure as a consequence of a CYP2E1 mediated mechanism.

Next I want to talk about our efforts towards establishing the regulatory impact of damage to barriers of

the CNS. So I want to remind you that regulatory standards for neurotox evaluation is still in H&E's name, an approach that is almost 1509 years old. Our efforts in barrier of the CNS is a long-term project. We're going to need to make a lot of this up as we go along, because we're really trying to change how regulatory centers fundamentally think about neurotoxicity.

So CNS barriers involve interactions between neurons, astrocytes, and microglia. And they offer protection, maintain homeostasis, and are crucial in the transport of nutrients into the CNS and transport of waste products out of the CNS. So why are we interested in barriers of the CNS from a regulatory perspective? Well, certain drug related adverse events like seizure can damage the neurovascular unit. Treatment of a disorder could have a negative effect on long-term brain health.

Take for example Namenda, also known as Memantine, a drug used to treat Alzheimer's disease. Namenda has listed as potential side effects hypertension and seizure, both conditions that can exacerbate AD pathology via their effects on the blood-brain barrier. So you have a situation where in theory the drug you are taking to treat the symptomology of a disease could be worsening the underlying disease pathology.

The effort to generate a regular justification for barrier research is being led by Dr. Shen and Rosas-Hernandez. Realistically this is going to need to occur in a stepwise fashion. Ultimately, we envision the battery that is comprised of vitro methods, in vivo and ex vivo studies, as well as samples from brain banks. At this point in time we have an idea of what our endpoints are going to look like, but this will also require some trial and error along the way.

However, vivo studies are going to be crucial, because I believe initially it will be necessary to link barrier dysfunction to increased neurodegeneration, as well as neurobehavioral endpoints, in order for us to really gain traction with this approach. While there's a great academic literature on the importance of CNS barrier dysfunction, this is not the case from a regulatory perspective. So it's difficult to devote the resources to fully execute this approach without at least proof of principle data.

For initial proof of concept Drs. Shen and Rosas-Hernandez are proposing to focus on methylphenidate. Inappropriate use of methylphenidate and related drugs may impact neurovasculature from neurovascular function. Products that contain nitrophenolate also carry warnings

for hypertension and seizure, which can also have a negative impact on neurovascular unit integrity.

The plan is to study the effects of methylphenidate on neurovascular unit toxicity endpoints in adolescents and early adulthood. This will include ex vivo analysis as well as MRI imaging. It will also likely include rats with impaired blood-brain barrier function to model an at-risk group, perhaps something related to hypertension. This proposal is still a work in progress, and we very much value your input on this.

The last project I want to talk about today is one that I'm personally excited about, and that's the development of the miniswine, this model for developmental neurotoxicity testing and other studies. Over the last five years we've seen a reduction in requests for nonhuman primate studies. We've also had several large long-term studies coming to an end that corresponded with the scheduled renovation for our nonhuman primate area. We took this renovation as an opportunity to redesign the area to accommodate nonhuman primates as well as other species, most notably the minipig.

So why the minipig? Here's the human brain. And here is the brain of a rat. Its small size, lack of gyrations, and primitive cortex limit its ability to model some aspects of human CNS. In contrast, the brain of a

monkey. Now let's look at the brain of a pig. You can see, just visually you can see it has more of a frontal cortex, and it is very highly gyrated. It's actually not all that dissimilar to the rhesus monkey.

So why the minipig? Well, this large, gyrated brain is fantastic for imaging. Which really will allow us to make use of our large bore equipment that we have here at NCTR, which in some ways has been underutilized because we can only put so many monkeys through it at a time. The minipig also has a longer adolescence when compared to the rat, but not as long as that of the rhesus monkey, allowing plenty of time for developmental assessments, but at the same time not so long that it actually just becomes difficult to complete these studies in a timely fashion.

Also it comes with fewer ethical and practical concerns, allowing us to increase throughput in comparison to nonhuman primate studies. Also I think the minipig will be of greater utility than the rhesus monkey was for most other divisions if we judge by Fred's enthusiasm yesterday. And we are retaining NHP capacity, so I don't see this as necessarily replacing NHP, rather augmenting it, and allowing us to increase throughput in times when it's appropriate.

So where to start? Well, we need to train staff to work with them to begin with. This isn't something we

can just immediately start tomorrow. That will be the first step. From a neurotox perspective I think our first activities will include proof of concept imaging and piloting some basic cognition tests. After that, I would like to do basic developmental neurotoxicity study to show that we can do histology, imaging, and behavior in these animals. I also imagine that there will be requests to do PK and biodistribution work in the minipig. We should have some capacity in FY23, and I would expect the new unit to open in FY25, and sometime after that we'd be able to do work at scale.

This brings me to division challenges. A big one for me, and I struggle with this every day, is how to invest with alternative models. This is something that we clearly can't ignore as a division, but every hour we put on alternative model development leaves us less equipped to deal with center needs. At this point those center needs are almost completely in vivo based.

Also I think in neuro tox sometimes the expectations are unrealistic of what these models can deliver, as well as how resource intensive they are. Take for example the work Hector Rosas-Hernandez is doing with the Emulate chip. There are three FTEs working on this project, and he's still a long way off from testing compounds, and that's just one assay.

We're also having trouble ensuring we always have appropriately trained staff. It can be difficult to recruit new staff, meaning that we need to retrain our existing staff. But we are somewhat isolated here, we can't just pop over to one of the local universities to get staff trained on the new method.

Finally, targeted communications with other centers and entities is a challenge. Everything is moved online, getting rid of what in some ways was a valuable barrier to communication. There's so much happening that one can easily spend all day logging into meetings and not actually get any research done. So areas of specific feedback, if you have any ideas on how to maximize FDA relevance for neurovascular research we'd be happy to hear it. I believe this will need to be an iterative process, with each step along the way generating additional support for the approach.

I'd also be happy to hear any thoughts on specific opportunities with the minipig, and if you have any specific thoughts about how to balance the adoption of alternative approaches. Should we focus on just two or three of these areas and develop deep expertise, or develop more of a general approach? Also, Serguei is getting ready to submit his T2 MRI for biomarker qualifications, and we'd value any feedback on that.

Finally, I do need to take a moment to thank the members of the division, they are a tremendous group and ultimately responsible for the success of the division. Along those lines I'd be remiss if I did not thank our many collaborators. Listening to Suzy speak yesterday it reminded me that our best collaborations occur when we have vocal partners.

Unfortunately, I think in most instances collaborating with the NCTR is often not on someone's year-end evaluation. So this really comes out of our collaborator's extra time. And we certainly could not do the work without the input of a lot of great folks from the regulatory centers. So thank you for your time and attention, and I'd be happy to answer your questions.

DR. ASCHNER: Thank you John for your exciting presentation. I think the fact it was informative is reflected by the fact that we already have four hands up. I have a few questions, but I'll defer, we'll start with Dr. Ken Ramos.

DR. RAMOS: Thank you John, for a very stimulating presentation. I was intrigued when you made reference to the methylphenidate experiments and your interest in assessing neurovascular contributions to outcomes. At that point when I listened to you, and before you made your comments about the minipig model, and before you posed the

questions to the board, the thought that occurred to me to ask you was related to coordinated efforts that you're making, and your team is making towards translational applications of the work that's been proposed in that particular project.

I think it's a conundrum for toxicology in that obviously a lot of what we do in that space is animal based, and a lot of our designs, a lot of our thinking is actually driven towards that sort of framework, probably at the expense of recognition that in the end you really need to have translational capabilities, and that ultimately what you're trying to model is human response. And so I'd like to hear your thoughts on that, and then I'll ask you a second question if I may related to the minipig model, actually more of a comment.

DR. TALPOS: This is a challenge, because there is a huge amount of great data saying that neurovasculature is important for disease progression, there's no doubt about that. But if you go and you talk to folks at the regulatory center, we talk to folks at CDER about this most regularly, and they'll say things like this concerns us, but we don't actually have any data that we can say here's the regulatory impact, so we need you to go and do these tasks. And we're not even quite sure what these tasks are. S

o if you look at transport proteins in and out of the brain, you go and you knock one down, you're going to see that two more pop back up very frequently. So it's not something where you can just focus and say okay, we're going to go and look at this one transporter and this is what we're going to do.

I think what this is probably going to end up involving for the initial proof of concept is the fairly long-term behavioral studies, where we do some kind of manipulation, and then look for the change in behavior. I think ultimately, we're going to either have to see a change in behavior or a situation where we see an increased incidence of cell death because of that exposure.

So one example could be TBI. If after a TBI you look at different potential treatments for TBI, different anesthetics, and see if well in that situation we're now getting a neurotoxicity that wasn't there earlier, and we think that's because of damage to the barrier of the CNS, you're always going to have this confound for vulnerable populations of the thing that made them vulnerable, and how do you know that that is not what's causing the underlying neurotoxicity.

So I've tasked some of the biggest proponents within the division of us focusing on barriers of the CNS with basically saying you need to establish the relevance,

and it has got to not be something that's completely molecular based, we need to be seeing either increased levels of necrosis, or clear behavioral changes. It's hard.

DR. RAMOS: Indeed. Especially when you think of it in the context of the relative lack of sensitivity, behavioral readouts that we currently have in place and that have been validated because notoriously a lot of those readouts are extremely insensitive towards significant functional deficits in neuronal function.

And so I think it's sort of a catch 22, because you're using an insensitive readout to make predictive value over something that is going to be extremely important, so the challenge I think in that space would be to do complementary analysis in my view, maybe take omics based readouts that you can pair up with behavior and increase the interpretive capacity of the behavioral test, or do them in tandem in ways that I think allow you for maybe separate interpretation of what you are actually looking at.

So I embrace I think the comment and response that you provided, but would challenge you to perhaps think about ways that you can rock the boat and sort of put new paradigms in place that might actually be food for thought for the conventional regulators and the people who are

going to be more traditional in the way that they approach interpretation of those findings.

And so omics might be one way that you might be able to achieve that, since I think most everybody would accept the idea that a genetic change could eventually translate into some meaningful biological deficit.

So that takes us then to the minipig model. Your arguments in favor of the use of that model I think are compelling, and having worked with pigs in the past, minipigs in the past, I can tell you that you're in for a nice treat, because they're not the most cooperative of animals that you could actually use in experiments, and they run faster than you do.

That being said, I think there is tremendous value in what you're proposing. And actually, probably something of benefit to you in the neurovascular investigations that you're proposing to do, since the pig is probably the best animal model for emulating cardiovascular system functionality of humans. The parallels between pig cardiovascular structure and function and humans is remarkable.

And so to the extent that that would be applicable to the neurovasculature, I think they might actually be complementary for both the other projects that you want to do with pigs and the neurovascular studies that

you are proposing to engage in. So I applaud the thoughtfulness with which you've approached the problems that your division is facing, and look forward to hearing more down the road.

DR. ASCHNER: Thank you Ken. I see that Mary Ellen has her hand up, please go ahead.

DR. COSENZA: So, in the first project, the T2 MRI, which you're working on with CDER to somehow either enhance or augment or replace work that's being done from a regulatory perspective, I'm sort of interested in what the thought is there, because right now what's sort of done from a regulatory drug development perspective, for an IND, it's just your general one month study looking at histopath, and then we do safety pharmacology in rats, which is mostly a functional observation battery. So I'm sort of curious, are you thinking, or is CDER thinking with you that you would add MRI to one of those studies, or the MRI leads you to biomarkers that you would add to one of those studies? Where does this sort of fit in that paradigm?

DR. TALPOS: So, Serguei Liachenko the head of the bioimaging facility, would be the absolute best person to answer this. And I think we'll know more after he goes and does the submission to CDER. He has gotten to this point where he has sort of hit a dead end and just needs to get

some regulatory feedback. And so I think he's preparing to submit knowing he's going to get a whole lot of follow up work on it to begin with. But that's what you've got to do at a certain point. And I think he does envision a day potentially at some point when you could use MRIs as opposed to histopath.

But the idea right now is instead of going and doing these massive studies, you treat, and then in a relatively small number of animals, you follow up, you go and scan every couple of days. And what's the standard now, is it seven slices for a histopath study? And so we end up potentially just missing a whole lot. And you only have one shot, one time interval.

And so his idea is you treat, you scan over and over and over again, and you look for the moment when something lights up, and you look for the structure that lights up, and that's when you go in and you use your histopathology. That's when you perform your histopathology in an independent group of animals, so you're much more targeted and much more selective, and the idea being that if there is a problem then you're going to be far more likely to find it if your work is guided by the T2 MRI.

DR. COSENZA: I am just thinking of the practicality of that, all the CROs that run tox studies are going to have to get MRIs to do that, the companies that

run these studies, or would this only be triggered by an earlier signal maybe in your one month studies, and then you would add this to a smaller, more focused study. Anyway, these things obviously will be discussed.

DR. TALPOS: It is a good point. Not everyone is setup to do this kind of work. But in theory it's relatively, it could be something relatively straightforward for a CRO to take on for example, that they just regularly offer this. But I think a lot of it will have to wait for CDER feedback.

DR. COSENZA: I have other questions, but I'll let other people jump in. It was great, I love this topic.

DR. GANEY: What would you expect the concentration of acetaminophen to be in the fetal brain after a woman takes a therapeutic dose, a pregnant woman takes a therapeutic dose? And this is an important question if you're thinking about doing in vitro studies. And so the second part of my question is have you considered partnering with the modeling group in the Division of Biochemical Tox, they are developing a perinatal PPPK model. I'd like to hear your answer.

DR. TALPOS: Based off of what we know, it crosses the placenta and the blood-brain barrier quite readily. So you can use the maternal levels, at least in some species, to pretty accurately represent what's getting into the

fetal brain, because it does pretty easily cross the blood-brain barrier.

And to answer your question, some time ago I started working with Annie Lumen while she was still here on this project, and now I'm working with both Miao and Kiara to follow up on this. So I'm working with them to put together a project to really nail the fetal exposure level of this using a combination of generating vivo data specifically for them, they've got a big list of desirable endpoints that they want that I need to follow up with them on so they can go and plug that into their model.

So hopefully we eventually will have data to support all this. Because one of the challenges that we have with acetaminophen, and this comes back to figuring out what a potential mechanism of action is, is we can't push the dose, because if we do we immediately get hepatotoxicity, which causes ammonia release, which goes and buggers up the brain.

So we just can't do that 10X dose to figure out what's happening and then come back down to see if there's a hazard with the lower dose, that's not an option here. So we're putting a lot of attention on the front side of this project to figure out the PK. In fact, our overall tox evaluation kind of got put on hold when we realized the

challenge we had dealing with the PK, and so for the moment we're just focusing on getting the PK figured out.

DR. ASCHNER: I have a couple comments or questions as well. The first one may be somewhat of a naivete on my part, but when you talk about the T2 imaging and the relaxation as a measure of neurotoxicity, I know from some of the work that we've done that you actually use T2 weighted images to look at iron deposition in the brain.

So I'm wondering how do you separate frank neurotoxicity from just increased iron deposition, recognizing that obviously if there is too much iron it may be neurotoxic, but why is the T2, just the T2 a readout of toxic damage, that's what I'm asking specifically.

DR. TALPOS: I am afraid I can't answer that. I simply don't know enough about the method, I'm sure I can get Serguei to comment on that. I apologize.

DR. ASCHNER: Just one thing to consider, and probably there's a good answer for it, but for my curiosity I wasn't sure. The second question that I have is sort of conceptual, in terms of the neurovascular project that you're doing. There seems to be a lot of emphasis on the AD. I don't understand why there is so much emphasis on the AD right now rather than just basically trying to standardize the cultures in terms of the number of astrocytes and pericytes and endothelial cells and so

forth. That's going to be the critical point in getting anywhere.

And once you can do it in wildtype, I guess normal human brains, if there's anything such as a normal human brain, then you can move on to do it in APOE three, APOE four. Why not at this point just try to do it from the stem cells in a normal brain?

DR. TALPOS: That is actually more or less where Hector started, is working with the APOE three gene. That's basically normal. Everyone has an APOE gene in them, the three is the neutral risk factor. So he was originally working with that initially. His project all along was to look at different AD risk genes, and so he's starting with that first control, and then the APOE four as his comparator. But really for your initial standardization that is what you're working with.

DR. ASCHNER: I have a bunch of questions. Again, like Mary Ellen, I don't want to monopolize time. Just a word of caution, in terms of CYP2E1, it's very sensitive to food intake. So whatever drug you might be testing or using, I think it's going to be very critical to make sure that the animals have a normal food consumption.

DR. TALPOS: Standard diet and everything? Good point.

DR. ASCHNER: Does anybody have a question? Anybody else? If not I have one that relates actually, it's more a divisional kind of question, the first slide. So you mentioned that there are six open positions. Are these, is this any different from years past? I know some people have retired, Bill Slikker is one of them, Syed Ali I believe retired as well. Are these full FTEs? Are these the PIs? When you say six positions, is this postdocs? And how does it compare to previous years?

DR. TALPOS: They are full FTEs, a combination of PIs as well as support staff. So we are a little bit smaller right now than what we are historically. Part of that is just because I was in the acting position for quite a while, and I didn't feel comfortable hiring in any PIs that would have a long-term impact on the future of the division while I was in an acting position. And so we're looking to start hiring some PIs this summer, we're putting out the ads for the PIs this summer.

At the same time we also had Syed Ali who retired at Christmas time as well as another researcher that took up an academic position. So we did also just have two people leave the Division pretty recently. So yes, we are a little smaller than we have historically been, but I think we're going to get back to size soon.

DR. ASCHNER: Thank you very much John, thanks for answering all my questions. I have more, but I think in the interest of time we'll have to move on. Thank you. Okay, going back to the agenda, the next one is Dr. Rick Beger, and he represents the Division of Systems Biology. And after that we'll have a break. Thank you.

Agenda Item: Division of System Biology

DR. BEGER: My name is Richard Beger. I am the Acting Director for the Division of Systems Biology, at six weeks of my acting role out of a 12-week role. This presentation is not a formal dissemination of information by FDA, and it does not represent the Agency position or policy.

So the number of positions in our Division of Systems Biology is 20. We have 10 support scientists, three administrative, which one is actually in the process of leaving right now, so our vacancy has now moved up from eight to nine. We have three ORIS postdocs, for a total of 36 or 35 if you count that one person that is leaving right now.

The immediate office, I'm currently the Acting Director, and Jessica Hawes is the Deputy Director. In the last years we've gone from three branches down to two branches. I am the Branch Chief previously of the biomarkers and alternative models. We've changed the name

of our new branch that has been rearranged to Omics, Models, Imaging, and Chemistry, which spells out OMIC, and the other branch is Innovative Sciences and Technology, which the Branch Chief is Laura Schnackenberg. Now these two branches sort of line up with where the personnel are located. Most of the people in my branch are located in building 14, and most of the people in the Innovative Sciences and Technology are located in Building 53.

So our Division collaborates with every division at NCTR. We have collaborations with the product centers at CDER, CBER, CDRH, CVM, and CFSAN, and we've had many discussions with CTP, so we expect to have collaborations with them in the future. We have government agencies we work with, NTP, NIH, VA, USDA, and OECD. We have many collaborations with universities.

Our collaborations with other centers and offices, we have pandemic-related research with CBER and CDER. Perinatal, therapeutic and vaccine nonclinical studies on COVID. We have some biomarkers and clinical specimens from adult, eventually children. Some of this is looking at multi-inflammatory syndrome and POTS. Novel detection methods.

The division is collaborating with CDER to investigate potential neurological targets and neuropsychiatric effects of Montelukast with CDER. On CVM,

we have toxicological translation across non-clinical species. CVM and CDER were starting research on cannabinoid neuropharmacology. And with CDER we're actively discussing the MPS model for hepatotoxicity. And CBER we're been working with on a MPS model for placenta research.

Metrics, we've had 17 publications the last year, which is sort of where we've been in the past. We've had 15 abstracts, which is down as other divisions have said. People have, not being able to travel, this has lowered the amount of abstracts. We've had 23 presentations, external and inter-center. We have 40 ongoing active protocols, with over 50 percent of those with CDER, and about 20 percent with CBER. There's currently eight protocols under review and six approved concept papers. The number of active protocols has actually gone down compared to previous years, and we are closing out a few.

The Division of Systems Biology Mission, we will try to address regulatory needs, knowledge gaps, and emerging threats in regulatory science. We apply systems-biology approaches and innovative technologies to regulatory interests. So we basically look at the safety and use of medical products, safety of regulated foods and supplements, safety and detection of components and impurities in regulated products, and develop technological standards and methods.

This slide shows how all these tools in systems biology can be used to look at populations, people, nonclinical and down to cells. So we actually have metabolomics, proteomics, transcriptomics, modeling methods, and we ended up with signaling and pathways.

The Division of Systems Biology goal is to address scientific knowledge gaps and safety concerns of regulators at FDA product centers, evaluate new approach methodologies, NAMs, discover and evaluate translational and clinical biomarkers, develop models and robust technologies to assess therapy, safety, and quality, characterize pathogens, and predict toxicity to adverse events. Address regulatory concerns and knowledge gaps related to emerging threats, like infectious diseases.

Our research interests are mechanisms of toxicology and susceptibility to adverse effects. So we look at the translation between species and influence of sex, age, and other sub-populations. Identify and qualify biomarkers to predict risk and early stage of development of adverse effects. Safety of regulatory products and advanced therapeutics, we looked at some of these new classes of drugs, oligonucleotides, cell therapies.

We are heavily involved in these four areas, immunology, cardiotoxicity, hepatotoxicity, and renal toxicity. We have research in perinatal health,

reproduction. We're moving into drug addiction and psychoactive effects. And we look at methodologies, diagnostics, and models for regulatory science applications.

So our strategies are to use these systems biology tools to look in our human based new alternative methods, NAMS, in vivo disease pharmacodynamic models, and clinical research. We utilize these pharmacological tools, and mainly a lot of these drug classes are known with effects, TKI inhibitors, anthracyclines, opioids. Incorporate innovative computational and instrumental technology. Integrate the clinical data or the metadata with the systems biology informatics and also use the histopathology, the toxicology endpoints to evaluate the differences in risk and toxicology related to species, tissue, sex, and special populations.

The Montelukast Working Group, the Division has formed a Montelukast Working Group with collaborators from the CDER, Office of New Drugs, and including members of leadership and review within the Division of Pharmacology-Toxicology for Immunology and Inflammation, with the consultation of clinical colleagues and leadership in the Division of Pulmonology, Allergy, and Critical Care. These research projects are designed by the working group and were identified by the FDA multidisciplinary team,

identified mechanistic scientific knowledge gaps and proposed studies to generate data in order to understand the potential mechanism for neuropsychiatric adverse events with montelukast.

Collaboration between the FDA centers is essential for this work, in order to ensure that the focus is retained on the agency priority questions, to strengthen research study designs, most appropriately to address FDA safety concerns associated with montelukast, to ensure consensus agreement on the interpretation of the results, and to facilitate continued scientific discussion between research and regulators.

The result of these studies will contribute to the overall weight of evidence towards understanding the potential mechanisms and risk for neuropsychiatric events reported in patients with montelukast administration, which may inform regulatory recommendations.

So the projects that we're actually involved, DSB is providing research support to CDER via a set of complementary investigations to address neuro related knowledge gaps and safety concerns for potential neuropsychiatric events with montelukast administration that has been discussed at previous FDA advisory committee meetings.

The first project is investigating potential neurological targets of montelukast and includes two types of approaches to identify interacting proteins in vitro. The first approach uses a screening panel of 173 proteins to assess the capability of montelukast to interact with a variety of G protein coupled receptors, ion channels, and transporters expressed in the brain. The second use is montelukast conjugated beads to pull down the binding proteins from the rat brains. Candidate binding proteins would need to be validated, and functional changes will be assessed in cell-based assays using human cells.

The second project will conduct spatial mapping of montelukast, its metabolites, and neurotransmitter changes throughout the brain of rats treated with daily doses of the clinical granule formulation generously provided by Organon, a subsidiary of the original NDA sponsor for singular and in vivo rat pharmacokinetic model that simulates the 24-hour exposure levels first developed for the study, MALDI-IMS which I'll describe later, spatial maps of this drug metabolite and neurotransmitter changes could be overlayed with images of adjacent brain sections stained with morphological identification and proteins of interest such as candidate binding proteins. Brain, drug, and metabolite concentrations, brain to blood exposure ratios, and nucleic exposures will be confirmed in isolated

neuroanatomical regions using traditional LC-MS/MS methods. After the montelukast working group reviews the data from these studies the results will be published, and follow-up studies will be designed to address any remaining scientific gaps.

One of the first studies I'll talk about is the investigation of opioid induced neural tube defects, NTDS, in a mouse model. In 2015 the FDA released a drug safety communication regarding a possible link between opioid exposure during pregnancy and increased risk of NTDs.

So the aim of this study is to confirm that NTDs are induced by opioid exposure, and to characterize maternal toxicity, specifically hypoxia associated with the gestational day, GD day eight exposure. The study will address data gaps, provide confirmational assessment of maternal toxicity in response to opioid exposure. Information may be added to or used to modify the existing drug labels.

So on the righthand side it is measurements of the morphine, methadone, and VPA drugs that were used in this study. And we can see that methadone, especially at the high dose, had high increases on carbon dioxide measurements at 30 minutes and two and a half hours. But this did not end up after ten days with an increased amount of neural tube defects as shown on the lefthand side. But

it did have the highest mortality rate. So it's conflicting information, so we're trying to move forward.

And some of the stuff that we did in this study is going to do some MALDI IMS. And so this is a slide here which describes the MALDI IMS procedures that we have. This is being applied to many different studies besides this study.

In this particular study we take frozen mouse fetuses, and we can use, obviously sometimes we slice these by around 12 microns. These are put onto a special slide for MALDI imaging, MALDI ITO slides, and then this is put into a sprayer, and we apply a matrix. The matrix that we apply depends on what type of metabolites that we're trying to detect. This goes into a 7 Tesla Fourier transform mass spectrometer. In there we're able to actually start shooting a laser at each point across the brain or the whole fetus and collect a full spectrum.

And then you can do that across the whole body, and what you get is an image where each peak is an analyte that can be looked at across the whole spectrum. So this is a method that generates a lot of data. And obviously at the end we try to connect this to an H&E staining and anything we find as very interesting we confirm by LC-MS/MS.

So in this particular study we looked at sphingosine-1-phosphate in methadone pups. So over here we

have on the lefthand side the H&E staining with the vehicle normal brain. We're showing the intestine, the spinal cord, and the brain. And when we look at the MALDI imaging we can see it spread across, fair amounts in the brain and in the spinal cord. When we look at the methadone high normal brain we see similar amounts of the S1P, but we have high amounts of it right above the spinal cord.

When we look at the methadone high with an exencephaly with the brain coming out, we see much lower amounts of S1P in the brain, but we also still see high amounts above the spinal cord. These changes in S1P is consistent with previous research that it is heavily involved in neural tube development.

A liver on a chip system to predict individual susceptibility and adaptation to drug induced liver injury. We're trying to establish an in vitro model that could characterize transient adapted hepatic responses to multiple human cell lines to acetaminophen. We like to identify biomarkers that could distinguish between benign and serious outcomes when drug hepatotoxicity occurs. We're also adding these studies for looking at the effluent looking for PK and omics markers during these studies.

The system has been established with a quad-culture liver-chip system using human hepatocytes, NPC cells. We have evaluated cells from two human donors and

observed similar toxicity. We compare various endpoints related to acetaminophen induced liver toxicity. And we're working on optimizing the high content imaging system. The challenge is there is a lack of established effluent-based cell death assays for NPC cells.

We also have COVID effects on pregnancy and prenatal and postnatal development. Here we're looking to understand the potential adverse effects of COVID during perinatal periods. This will provide hazard risk assessment data for infection that could lead to enhanced safety for pregnant women and pediatrics. We have the virus during pregnancy and fetal organogenesis, term infants, and adolescents.

We're looking at this plus or minus for remdesivir, we're looking at the histopathology and other pathology appointments, assessment of development endpoints, and functional observational batteries. The hope is some of these things will actually correlate with some of the clinical investigations that we plan on doing in the division.

Continuing with the MALDI imaging story, we're here characterizing the effects of viral load and immune-cell infiltration of COVID-19 patient autopsy tissues. So in this particular case we get fixed tissue from patients who died of COVID, and we're looking at these using MALDI.

And since they're fixed we cannot look at the normal stuff, but what we're able to do is to use an enzyme, in this case PNGaseF, which cleaves glycogens from glycoproteins. And then we can actually use the same process, use MALDI to hit the tissues that have these and evaluate these in the tissues.

In the first study I'd like to show you is the characterization of effects for viral load in immune cell inflammation in COVID-19. In this particular case we're actually looking at a spleen, and so in this particular case the green glycans actually correspond to the white pulp that harbors immune cells, and in the blue we see the other glycans that aren't quite as branched, and these are associated with the vessel structures. We hypothesize that the increase in abundance of these highly branched glycans will be indicative of immune cell activity in the spleen. And here we have the close-up of a certain region with the H&E, IMS, CD8 staining and CD163 staining.

Here we're looking at the same, well now we're looking at a lung tissue, and preliminary data here shows the high mannose glycans track with the infiltrating immune cells which may indicate acute phase; however the study is ongoing and will also not only look at these clinical samples, but we're also going to be doing the same studies in nonclinical studies. We hypothesize that these are

indicators of immune cell activity. These are low resolution, and so our idea is we want to go into higher resolution studies that will be done in the future.

For the sake of time, I am going to skip this slide. But basically, here we are seeing sialic acids in lung tissues. And these are specific to CD11 macrophages in contrast to the CD8 cells.

Here we're looking at the distribution of sialic acids in lung tissues. Here we're looking at the alpha 2,6 versus the alpha 2,3. The alpha 2,6 is much higher in the COVID samples than the control non-COVID patients which have alpha 2,3 staining. Here we're looking at three different sialic acid distributions of alpha 2,6 versus alpha 2,3. In all cases the COVID had much higher levels.

We hypothesize that these are important actually for getting into the cell, as something that evolved with the tissue tropism so not only in the cell that we would collect for COVID that we would do the ACE-2 and TMPRSS2, but you also need the sialic acid. So this might be quite different from what previously happened 20 or so years ago with the SARS-CoV, the first one. And here's some other ones that have different architecture that might be needed to get into the cell.

We're also looking at using body fluids, real-time, using what we call SpecID in our division, this is a

patented/licensed portable mass spectrometry-based platform where you ionize the sample, in this particular case we're using saliva, and saliva that's actually spiked with different strains of viruses. In these particular cases all the different viruses that we looked at had a 95 percent homology to the human coronavirus. As we can see over in the PCA plot, they all somewhat segregate by PCA plot the different viruses. So this is a promising technology that would rapidly detect certain viruses in saliva.

Another study that we're involved in is something that was brought up by CBER yesterday, is the lipidomics and proteomic analysis of serum and macrophage cells. The goal of this study is to reveal factors influencing the newborn macrophage's phenotype and assess whether maternal obesity impacts vaccine outcomes.

Here we're showing the lipidomic data, and you can see by the staining over here that the neonates have much more oils, fatty acids, and we see a lot of different changes here in the AC plot, and the pie plots. Most of these changes are in the PC and iso PC, but all classes show some changes. Most of the changes, I won't go into detail, but are in the short or very long fatty acids.

Moving on, we have evaluation of drug-induced cardiotoxicity with patient-specific iPSC cardiomyocytes. These individual cell lines were more susceptible to DOX,

and kinase inhibitor induced cardiotoxicity. Can they be used to assess the risk of cardiotoxic toxicity in a heterogeneous population? Can they be used to predict an individual's susceptibility to drug-induced cardiotoxicity? So these studies have shown that it's very important to use a very heterogeneous population in these in vitro systems, and they also identify markers that enable patient stratification prior to drug treatment.

Here we're actually looking at one patient's response to three different TKIs that were given at three times Cmax. Patient 1102 was much more sensitive to ceritinib and nilotinib, but was not very sensitive to lapatinib. This shows that it is very important to use multiple cell lines and multiple patients.

Further analysis correlating gene expression data with acellular phenotypes of IPS cardiomyocytes. This model actually was used by another group that generated a model using the DOX-induced cardiotoxicity with gene expression data. They put that data in there, specifically the cell index of the in vitro cardiotoxicity, and showed that they had a correlation between their model and the model that was generated here in OCTR.

A clinical study here on the putative plasma biomarkers that predict DOX induced cardiac dysfunction in breast cancer patients. Here we have a total of 85

patients. We first had a discovery set of 40. We discovered those six biomarkers shown in the table below, and then validated these with another set of 45 patients. There were four cycles of DOX and cyclophosphamide. Basically we collect the blood before the first, between T0, T1, and T2, we looked at the left ventricle ejection fraction, and basically we were able to show that these biomarkers were consistent at T zero and they could predict. We're hoping to have this study go forward in other clinical places.

Below we show that the biomarker is using two different techniques, the aptamer-based proteomics for discovery, and Olink actually correlated quite heavily between the two assays.

We're also developing a pro-inflammatory model for CAR T-cell products. In this particular case we have tumor ejection. We collected the CAR T-cell, injected CAR T-cells at day 23, and looked at the CRS response. Here the CAR T treatment, we see that the body weight drops off quite readily, but in the treated animals not so much. But there is a significant increase in IL6 at 48 hours. So future research, I'm going to quickly go through this, we have some cannabinoid pharmacokinetics discussions with CDER and CVM. We're also looking at pandemic research related on the multi-system inflammatory syndrome in children.

And then we also have a modeling system that we're trying to put together to predict adverse events using what we would call drug-endogenous ligand-target networks generating from 3D similarity and machine learning methods. I won't go into this heavily, but we're hoping that this provides, we just had discussions with CDER last week and they're hopeful that this would go forward.

We also have proteomic and metabolomic looking at visible safe violet light in human stored plasma and platelets. And we have a lot of COVID research going forward, specifically coadministration studies, looking at clinical patients, looking at different variants, looking at improving vaccine effectiveness, and we're also looking at some of the kidney toxicity.

So the challenges is balancing it like other visions, developing the emerging research with ongoing research. The budgetary restrictions, when do we get our money, that's actually very difficult to deal with sometimes. Staffing, funding of the staffing, opportunities for significant others, which directions do we need to go, strategic organization, the cost of the equipment plus the maintenance is quite prohibitive, and communication with the product centers.

Some of our future collaborations would be research needs of the FDA centers, development of non-

clinical models, NAM models, in silico models, in-vivo models translating this to clinical research. Addiction and neuropsychoactive drugs, biomarker discovery, qualification, or validation, rare disease research, and therapeutic gaps and safety concerns. And anything emerging threats and diseases.

So the feedback for the approaches we are currently advancing, MALDI-IMS, MPS, iPSC cells, are there other areas we should explore other than those that have been mentioned? What are the developments, technologies, on the horizon that we are missing? What approaches in addition to our current efforts might better fit the needs for the FDA centers? We have ongoing efforts with CDER to compile and centralize these needs. And with that I'll take any questions.

DR. ASCHNER: Thank you Rich. The floor is open now, does anyone have questions for Dr. Beger, please.

DR. TROPSHA: Thanks for the presentation. I have a couple of questions, one on science, one on challenges I guess. Concerning science, I know you kind of rushed at the end. Can you tell me a little bit more about this project on drug indigenous ligand target networks, and whether this is between your Division and CDER or does it also involve Weida's division?

DR. BEGER: So, that's an extension, our modeling has been ongoing since I arrived here 24 years ago basically, and so this has been taken over by Svetlyo Slavov and this is a direction we're trying to, what we've seen is we've done all these other models previously that are nearest K and N models and our PLS models actually work quite similarly in almost all the cases, we've looked at seven or eight different cases, so now we're trying to use the K and N models and look at specific drug classes that are causing problems. We're not going to look at all different drug obviously, all 2000 drugs, we want to limit this to a few drugs, and we're discussing that with CDER right now, on which ones to look at.

We're also bringing in some of the endogenous metabolites that fit in the mass range of these things to see if they might have similar side effects, because initially all drugs were antimetabolites, so they actually mimicked endogenous metabolites, but it makes sense to actually compare there and that will give you opportunities to predict some of these after effects but actually move on to reclassification of certain drugs to other opportunities, other areas. This was recently put in as a quad chart, so it's a very new approach to our long line of research.

DR. TROPSHA: I think it's interesting and complex. Again, I've asked is there any collaboration with Weida's division, which I think will be a major help for this.

DR. BEGER: We've talked with some people in the Office of New Drugs, a bioinformatics person. They're probably not with the same group that you're talking about.

DR. TROPSHA: You mentioned some clinical studies with COVID patients. Where will we get the data? Are you involved with the N3C Initiative across NIH/NCATS which is sort of a national collaboratory?

DR. BEGER: So we have collaboration locally with UAMS and University of Tennessee Health Science. And currently that protocol is waiting on our research collaboration to be signed. Once that's signed, hopefully the protocol can be approved, and we can start analyzing the samples. We basically have all the purchases lined up ready to go, but that's our bottleneck right now.

DR. TROPSHA: And a last quick question. You mentioned at the beginning that there are eight open positions. And then you also mentioned challenges as far as starting new projects versus continuing old. Is this a challenge to recruit to this position? Because that's how you obviously could start additional research.

DR. BEGER: I think some of this is historical, we've had a lot of turnover and retirement, we have filled in quite rapidly. Some of this is we're trying to figure out exactly which directions we want to go for, because you don't always replace the person with the exact carbon copy of that person. So we're trying to figure that out, and I think we're also going to wait until there's DSB leadership assigned, the position has been advertised and I think once they put a permanent person in here that will be one of their decisions.

DR. ASCHNER: Thank you Alex. Are there any additional questions? If not, thank you Dr. Beger. And I think we're scheduled for a break. We're a few minutes ahead. Why don't we take a break, it's 11:06 AM on the east coast, let's take a half hour break, we'll be back at 11:35. Thank you.

(Break)

Agenda Item: Discussion of NCTR Research

DR. ASCHNER: Ok, for the next 45 minutes we are scheduled to have a discussion by the SAB. So I would like to open the floor for SAB members. If you don't mind we can each provide our impressions of the last day and a half. I'm happy to go first, if you would like me to do so. I've put together several notes. I will start. So first of all I want to thank the leadership of the NCTR, I want to thank

everybody for their presentations. I think we've gotten a lot of information over the last day and a half.

There are a lot of exciting things ongoing at the NCTR. I've been on the board for the last several years, and I applaud the NCTR for consistently increasing its collaborative research, I think it came across yesterday, today, when I looked back over the last several years. So that's certainly something very laudable. It's also very clear that the collaborative research efforts that cross with the FDA centers have increased, and again this trend hopefully will continue.

I find the NCTR divisions to have excellent scientists. You have state of the art equipment and staff. The quality of the research is outstanding. It's published in peer reviewed journals. And that is happening, I'll get to it a little bit later, despite the fact that you seem to have some problems with the professional recruitment, definitely challenges in this field.

I recognize that many of the things that you do are obviously driven by directives from the FDA, and I've brought it up many times, I think quite often you're sort of trying to invent the wheel. I've mentioned this a couple times over the last couple days, with NAMs, some other methodology that's available at other federal institutions that at least I'm aware of, and at times I think it would

be better in terms of consuming or freeing your time for other things, both in terms of effort, in terms of finances, if some of these technologies could be learned and you could accelerate the process of getting them to the NCTR rather than trying from point zero.

So I've mentioned it before, and I'll make the point to mention it again. I think it's impossible to be an expert in everything, we all recognize that, and at times when you need to develop something new it's much easier to go elsewhere, send the postdocs or the graduate students, and have them bring the technology back to the NCTR.

Another point that I think we've discussed in years past and I haven't heard anything about is productivity. I've asked many times, and this is more to the NCTR leadership, about how you evaluate the metrics of publications for the full FTEs. Coming from academics I think it's clear to me how you look at productivity, it's usually in terms of grant money and publications.

But I don't know if you have 30 people in your division, if you expect 20 publications or 40 publications, and how do you make the decisions that the division is I guess very productive or not. Again, this is the kind of information that I think would be helpful to me and hopefully to the advisory board.

I think another thing that I haven't heard much about, and I was a little bit disappointed because we've had a lot of discussions on this in years past, is the nanotechnology field. There was a lot of investment in it over the last few years, but we heard very little about it this time, and I'm not sure whether this has sort of fallen off the radar, and the FDA doesn't have any interest in it anymore, but I don't think we've talked about it very much.

I want to commend you on the AI. I know this is one of the recommendations that we've had over the years, you've started long before this was recommended to you, I think you're at the forefront in terms of the different kinds of things that you have developed, you've developed programs that are tailored to link large and diverse databases, which are submitted for product registration for assessing toxicology, I find this field very exciting, and it's not my area of expertise, but I think you're way ahead of many other agencies and many other institutions. And finally I will point out to the retirements and the attrition, at least for me this seems to be somewhat of concern.

I recognize that COVID19 has impacts, and no matter where we work, federal, industry, academic, I also recognize that you're under recruitment restrictions of foreign nationals, but when you have divisions where folks

are retiring and it's hard as you know and as you recognize to replace them, I think that's something that the leadership should look into it and come up with some strategies to see how this can be rectified. I don't know if you can change the FDA's rules in terms of this, I don't know at what level this is being discussed, but rather than shrinking I would like the NCTR, I would like to see it growing and programs getting bigger and bigger.

So overall as I said I'm very impressed with the science. I think you're doing a great job. I definitely feel that your collaborative efforts have increased over the last year, you have very exciting programs. The methods in most cases are state of the art, and I've enjoyed the last day and a half, and I commend the leadership, each of you, the staff, the scientists for a job well done under restrictive measures, both in terms of the COVID and the problems with the recruitments. And I'll stop here and open the microphone for anyone else who wishes to discuss the last day and a half. I think Greg has his hand up, so why don't you go ahead.

DR. LANZA: Thank you very much, Miki. That was a great summary. I only really want to make a couple points that add to what Miki brought up. First, in the case of the nanomedicine, of course I'm a nanomedicine guy and I definitely appreciated that there was very little of it,

and in some cases, it had been phased out of what the center had been doing.

So it's a little surprising to me. My background regarding this is not just my own lab, but I'm actually the coeditor in chief of WIREs, which is a nanomedicine view journal, and we have an impact factor of 10, and we're flooded with nanomedicine across infectious disease, every kind of thing, all over the world. And these are reviews. And it's surprising how much work is going in there and how diversified it is. Perhaps something needs to be done in this case, whether this is just in response to product candidates coming to the FDA in their need for your help.

In the case of AI, I wanted to say one other thing. I definitely applaud the work you've done. I remember when we first started talking about it and pushing it, and you've certainly done more than I would have imagined. The thing I have is, given the size of the group, is the fear that you might get too involved in activity and have few results. And the reason for it is you're stretched thin across a wide variety of topics, and as we were talking yesterday for instance about accuracy, the question arose well how much accuracy, or what is it being done.

The key for it is it actually makes better decisions, work faster, that you learn some things about what AI says you should be looking at through looking at

the neural network nodes that you wouldn't have thought to look at. So as you go forward on it I would try to generate these kinds of results that you can make into some metric of progress for the division before they just think it's an activity and not a result in itself.

The only other thing I did want to complement you on, but it's just really to endorse what Miki said, I remember being on the review, I don't know how many years ago it was, when we were talking about trying to collaborate within the NCTR group itself better because it was somewhat siloed as well as trying to get the centers to interact with you as well as possibly recruiting more of the interactions with outside academic agencies. And so in that regard it was eye opening again to see how it has improved, and the benefit that it has is we seem to be working much more as a team, and I want to expressly complement you for that.

One other thing, I know Dr. Slikker was pushing for it, but the fetal toxicity work that you're doing I think is particularly notable, and I know it's early, but the truth of the matter is it's side tacked mostly, whether it's by industry or academia. And so if you don't do it, who will? But yes it's critical. So I really think that's an area that you may be one of the very few who are actually putting this kind of effort into. I wanted to

emphasize that so that you'll emphasize it going forward as well.

DR. ASCHNER: Thank you very much Greg. Let's go to Mary Ellen.

DR. COSENZA: This is my fourth year on the SAB, and I have to say I'm again impressed at the quality of the science, the presentations, and appreciate the time and effort that I know goes into putting all of that together. I will echo some of what both Miki and Greg said, I was increased with the increase, or at least it seemed from the presentations and increased level of collaboration both between NCTR and the FDA divisions, that certainly came across stronger this year. And also the last point on the interactions within NCTR itself, that's clearly I see a real improvement in that over the four years that I have been participating.

I do still worry a bit about the hiring and recruiting. I know I'm repeating what others have said, this was something we talked about when I remember being in Little Rock Arkansas for my first SAB, it was a big topic of discussion then. And we talked also then about outreach to universities beyond the regional area as a way of potentially helping to build that pipeline, so I just bring that up again.

Another point, as somebody who has worked in drug development pretty much all my career, I always think about the practicality of some of this type of work, how does this actually apply to improving the safety of drug development and biologics which is an area I have focused a lot on.

So I sort of see this as another point that was made about balancing between the urgent issues, things that come up and you have to work on quickly because we have a national emergency or global emergency like COVID, versus the things that have longer term benefit in terms of improving the way we do safety assessment.

And the last point I wanted to make, which I think Greg also spoke a little bit about, is both the work on in vitro development in terms of animal alternatives, if we don't really focus on that, it's never going to get done. It's just easier to do a one-month in vivo tox study than it is to try to keep developing these.

Although I know it's something I've worked on early in my career and it hasn't been as fruitful as I would have liked to have seen, we have to keep plugging away at it, because we do have to find ways to reduce our use of animals. But that also blends with how does that become something that is helpful and not just an add-on,

that we do what we're doing but now we're adding all these extra in vitro tests or assays as well.

And I just want to note that in terms of the developmental and reproductive tox in the ICHS6 update that I think was just issued last year there's a whole section on animal alternatives, with ideas for people to think about using. Not sort of saying you can just drop the in vivo work and do this, but here are some things you can start to think about, and I think NCTR as well as HESI and other groups are well placed to try to be leaders in that area. So again, thank you for a great day and a half and for allowing me to participate.

DR. ASCHNER: Thank you Mary Ellen. I am just going based on my screen, so Alex is next. Then Cheryl.

DR. TROPSHA: Thank you. This is Alex Tropsha. So I think it was a couple very rich in science days. I've truly enjoyed every presentation. I've found really a lot of new science to be done and reported, and clearly there's a lot of intensity in the research that has been done. Also of course with every new person speaking there is less and less to say, because people speaking before me I think made truly excellent points.

So I'll try to make some additional points that hopefully will be slightly different or reinforcing what others said. Two related points, recruitment and project

initiation or project termination. So every person spoke about new science that has been done, and I think that's fantastic. I think it's important to initiate new projects.

What is not always clear is what are the criteria used to initiate a project, and especially terminate a project that for one reason or another needs to close. Because certainly it's impossible to constantly add, especially if people retire and there are challenges with recruiting new people.

So I think across divisions I think hearing more clarification on project initiation and project termination criteria, and load balancing in this regard. And sometimes maybe it's natural as people retire some projects phase out perhaps naturally, but I think that's really part of the strategy of any institution or center that constantly updates itself. And so I think these two aspects, recruitment/retirement, and criteria for project termination, I think that's strategic, and I think it would be nice to hear what the strategy is.

Considering collaborations, it's very clear that there is a lot of collaboration external to NCTR, and there are collaborations within NCTR. What was not very well articulated I think is whether there are strategic topics that are strategically distributed across divisions. So almost everybody has done something related to COVID19, but

it sounded more like each division picked up sort of something that was close to them.

It wasn't very clear to me if it was a cross-divisional strategy of responding to COVID-19, and there could be other projects that may require strategic cross-divisional collaboration. And certainly there have been instances that kind of looking from afar have been addressed by different division leaders, but I didn't see how it amounted to an NCTR-wide project.

Somewhat I think on top of what (name) said, so the output is produced by members of the division. We've looked at cumulative numbers of publications, presentations, et cetera, but it's really not always clear whether there are super producers within each division, and then some individuals who produce less. And I think it would be interesting and important to develop some metrics of individual productivity and kind of look and see the balance between individual productivity and divisional productivity. I think someone talked about this.

And last point I'd like to make, and I think it was one of the questions I asked, it's of growing importance to increase the use of what's called new approach methods, personally I don't like this term as much, as regulatory tools, not regulatory science, but regulatory tools. And so a plan for actually implementing

and transitioning these tools from research tools to practical tools used by regulators. I think that's an important objective for the center, and it would be nice next time to have a plan for actual practical implementation of such tools. Thank you.

DR. WALKER: This is Cheryl Walker.

DR. ASCHNER: I was just about to ask Greg if he has anything specific to say or if his hand is up still from before. It's you, Cheryl, sorry.

DR. WALKER: This is my first time on the committee, so obviously I have a lot to learn, and these two days I think were very fruitful for learning that. I wanted to make three comments. One of them of course is completely reiterating what was already said about the difficulty in recruiting and retaining folks.

I've spent 30 years of my 40 years in science working at satellite campuses, and so I understand how difficult that is. I also understand the sense of community that forms and solidarity that forms in these communities, these outposts, and I think that's a real strength. It seems to me that that's happening and being taken well advantage of there, which is great.

In the two areas that I paid the most attention to because they're some of the few I know a little something about, I just wanted to mention two opportunities

that I think are very apparent to me, even coming in and looking at this from the outside for the first time. One is the opportunity to move into the area of looking at single cell biology. I think you have that opportunity for sure in your epigenetic space, in lots of other spaces as well.

It's not an area to move into just because it's cool, even though it sort of is cool, but you do in fact get very specific and important insights for doing that. And I think now with our ability to do most any omic at the level of the single cell, you're very well positioned to do that, and I would use epigenomics as one example.

And I just wanted to point out that with the recruitment of Shuk-Mei Ho to the University of Arkansas Vice Chancellor for Research, I think she's still there, you have one of the world's epigenomics experts right there in your backyard, and I think that there are lots of opportunities for collaboration to really get at these next gen approaches.

And then the second area that I was very impressed about was looking at the microbiome. And I think that the microbiome as both a target and a determinant of response is very key, I was very pleased to see what's going on there. But I also feel like this is another area where you have an opportunity probably with the right external collaborations to really make quantum leaps. And

so that was another place where I was very impressed and glad to see work going on. Thank you Miki.

DR. ASCHNER: Thank you Cheryl. Patti, please go ahead.

DR. GANEY: I would like to reiterate or at least echo what everyone else has already said before me. This is my fourth year as well, and I'm always just impressed with everything that's happening in NCTR, the presentations, the interactions among the divisions, as well as with other centers is just almost overwhelming, it's too hard to track for me, there's so much of it, and I think that that's positive.

I wanted to follow up on one of Greg's comments about AI. I think this will come up again in the subcommittee meeting this afternoon, is I think it's important to make sure that the expected utility is driving the development of those methods.

So if you're developing a method to do something you have to know in advance how useful being able to do that is going to be. Like Greg said, you're not just doing it because you can and it's fun, it's not just an activity, it's actually going to end up with something that's useful to the FDA.

And then going back to the personnel, I had a question, and I was kind of surprised that no-one had

addressed this. Many of the division leaders mentioned that they were investing not insignificant amount of funds from their extramural funding into ORISE, and as part of the pipeline, but I didn't hear anything about how successful that is as a strategy for bringing people to the NCTR who will say at the NCTR.

And if it's not successful perhaps you would be better investing that money in some other type of strategy. And I know that you're limited because you're a federal agency, but there are things that other, even universities do. They have a really comprehensive vacation package, or a really off the wall promotion package, some perks that you can throw in.

And I don't know first of all whether you have that latitude, because you're a federal agency, or if you do whether you've explored those possibilities. I think there's not much you can do about Little Rock, start a performing arts center or something, but I think that there are things that you can do within NCTR that perhaps you haven't explored yet. But really I think you are all doing a wonderful job, and I commend you and wish you luck.

DR. ASCHNER: Thank you Patti.

DR. SAUER: As I said before, as we go down the list there's fewer and fewer things to bring up. Again, you guys have obviously, at NCTR, faced quite a bit of

adversity with the open staff positions, the turnovers in senior leadership. I think you guys have done a great job with it. Listening to this year's presentations, I was really excited by the way the format rolled out and the similarities between the presentations, that's something we asked for a couple years ago, and I think it really showed itself this year, which I think is a really good thing.

To highlight one other point that was spoken to, and that's the in vitro models, the alternative models, there's lots of groups out there doing this work, the question is how do we pull those all together, and does NTCR represent an opportunity for you guys to pull that together, to show that leadership? That may be an opportunity. Because we know there's groups in CDER, I've worked with them around alternative models, I know there's industry type groups that are out there, as well as nonprofit groups. So how do we pull all that together? I think that's really the secret to making something happen.

The final thing that I'm going to complement everybody on is for the first time I heard the conversation around qualification of DDTs, the ISTAND program, these are internal opportunities to FDA to be able to basically codify some of these tools that are being worked on in NCTR. So I'm really glad you're thinking along those bounds or in that area, I know it's hard and it takes a long time,

but I think it could be a really important metric to basically measure against on bringing these tools forward. So again, thanks a lot for great presentations these past couple days. And that's all I have, Miki.

DR. ASCHNER: Thank you. Tucker, I'm going to give you the floor after Ken Ramos, please.

DR. RAMOS: So, I actually missed the first half of the meeting yesterday in the morning because of some scheduling complications that we run into. But I listened obviously to the sessions in the afternoon and then this morning, and I was pleased to see progress has continued to be made, and I think there has been evidence of maturation in the way that the science has been portrayed and in the way that the studies are being conceptualized.

I also greatly value the efforts that have been made to highlight interactions across the centers, the FDA centers and the NCTR, and I think using that as a guide certainly provides an appropriate framework for us to be able to judge the quality of the science that's been going on.

On the advice side of the equation, and I realize the constant tension between regulatory programming and responsibilities and jurisdiction and state of the art science investments and state of the art scientific

activities, and so you have two NCTR things for example you have to balance both of those equations.

So I would say, I would strongly encourage the scientific community at NCTR to continue to look for opportunities to grow the portfolio of regulatory science applications and to rely as much as possible on innovation and technological investments as exemplified I think in some of the talks that we listen to, but not all of them. And so I still continue to be encouraging some of the programs to try to rejuvenate themselves and to try to be a bit more sort of on the state-of-the-art side of things.

Something that I think piqued my interest, and I asked a question yesterday about it, and I wanted to hear thoughts from the rest of the board and from both Tucker and Donna, it relates to the comments that were made about the reports that are provided by NCTR divisions to the different centers relative to manuscripts, and the way in which that comment was presented almost implied, at least I interpreted that to mean there isn't a formalized mechanism whereby deliverables of the investments that are being made at the NCTR need to be evidenced and documented.

And so that is an area where I think perhaps some attention needs to be paid to, because at the end of the day it's tax dollars that are being used to fund all of these programs. And so a very clear articulation of what

the expected deliverables are and what those deliverables actually were needs to be always taken into account.

Now that's not to say that that evidence wasn't presented in the presentations, because we've always heard about the papers that were published, and we've always heard about the ancillary activities that the scientists in the different divisions are involved in, but I think some clarity should be adjudicated to each one of the projects that I think you guys get involved in so that you know what the deliverable is at the end of that project, and as opposed to this sort of undefined sort of outcome that either a paper or a report, I wonder if there's actually maybe room for both, and how this report can actually be accounted for, perhaps in a more systematic way.

And then the last final comment that I would make is clearly the last two and a half years have been very challenging years for everybody, but obviously you being in the government I would assume that that actually is multiplied maybe a couple fold. And so I commend you for the investments that have been made and the continued activities, all of which I think might lead to important contributions on the road. So thank you Miki for that opportunity. And Tucker, nice to meet you virtually.

DR. PATTERSON: Miki, do you want me to go ahead? I was frantically taking notes, but I heard some common

comments across the SAB members, I'm going to try to go one by one and address these, and I could maybe clarify some of the issues. You probably heard over several past SABs that hiring is an issue. It is and it isn't sometimes, it just depends on the expertise that you're looking for, but I don't know if any of you have tried to hire recently, but it is definitely a seller's market right now, it is not a buyer's market, it doesn't matter if you're trying to hire a postdoctoral fellow or you're trying to hire a plumber. The market is extremely tight right now. One of the issues that we have here, again with the ORISE program, it's been a great program for us.

I think within the agency we were actually the first center to have the first ORISE participant, back in the late '80s, and that has been a very great program for us. I actually came in to NCTR on the ORISE program, so I know somebody was asking about cascading over to the government side, and if we would be able to retain them. Again, it just depends on if we had the expertise that we're looking for.

Again, that's a trainee program, we're trying to train up the next generation of scientists. And so they come in as a trainee, and hopefully they're ready to go out into the world if that's what they choose to do after they leave here, but we've had very good success at retaining

some of our ORISE, we can get them over on the staff fellow side and hopefully into an FTE later on. But it's a difficult environment right now with hiring.

The agency, and not just the agency, but the government in general, is looking at this issue especially in the science fields and trying to reclassify some of these harder to attain positions into more of a direct hiring authority, whereas you find a candidate and you can actually hire them without going through the normal USA Jobs competitive process that sometimes can take months to onboard someone. We're dealing with that right now.

This is really unprecedented at the center, but before Dr. Talpos was hired in DNT, we had four division directors out of the six research divisions we were looking for at one time. To my knowledge that has never happened here at NCTR, and a lot of that is because of course retirings, and we knew this was coming, we've been talking about succession planning for years, and when your CFO and your XO and your Center Director and Division Directors all leave within about a year of each other, that's difficult, and it takes a while to move forward and get these positions filled. But I think there are some things in place for these difficult to hire positions that are coming down that will definitely help us in the long run.

Talking about the nano, our nanotechnology group, of course it's still here, it's still going strong under the direction of Dr. Anil Patri, we had the National Nanotechnology Initiative that he sits on various workgroups, in the past year we've published three standards with ASTM, we have an Office of Women's Health project determining six differences in immune responses to nanoparticles in vitro. We've had course funding looking at immunotoxicity of cobalt chromium particles that are generated from prosthetic implants after repeated exposure to radiation. We're ramping up a collaborative project with CDER to investigate differences in doxil and generic liposomal doxorubicin formulation.

Those are just some of the things that are going on in our nanotechnology group again. We're looking at specific expertise there that has been difficult. We've been trying to hire a deputy in that group for about two years now to try to help Anil out with some of those tasks. And so it's just hard finding people with that expertise that are out there in the market looking for positions.

But no, our nano group is going strong, it's just a little bit different because it's not a separate division, it's underneath our Office of Scientific Coordination, it's looked at more as a support part here of NCTR, and that's why it's a little bit out of place in

terms of listing it with the other divisions and what's going on there. But no, it's still here, it's still going strong.

I heard about the metrics, the deliverables. Ken, you brought up a great point there, and that's something that we are now looking at lot harder at. We have been looking at that over the past year or so, with not only what is the significance to the agency here, we've always had the significance and benefits to the agency, but what are the clear cut deliverables, are you just trying to get a peer reviewed manuscript out of this, or what is this going to provide the regulatory product center to help them move forward in that regulatory decision.

And it's so important across the agency that for the last two years I've been sitting on what's called a research impact working group, how do we use metrics across the agency for our research, how does that translate into the regulatory environment.

And when we all went to the table, all the product centers all had different ideas about the types of metrics they were using to measure the research impact and its effect on the regulatory space, and now we're all kind of honing in in the same general area now, and hopefully we're going to have some points here in the near future that everybody is going to look at these metrics and

they're going to be common across all the different product centers, across NCTR, and using that research to get a sense of how that's impacting the regulatory space.

So it's not something that we're ignoring for sure. Even though the division directors didn't present that in the presentations that you saw, they were focused more on the research and what's happening there, in our performance plans we all have different endpoints of projects that are there.

I have, first as the Deputy Director for Research, and now as the Acting Center Director, my performance plan is tied to various projects, and are they going to come to fruition, and that cascades up to Jackie in the Chief Scientist Chair, all the way up to the Commissioner, and so we have different metrics and different deliverables here at NCTR that go all the way up to Commissioner.

And that's on various research projects of high impact that we're looking at. So it is definitely being looked at, it's not being ignored, you just don't hear it down at the research level a lot. We're constantly asking the PIs what's the progress on this.

And then what I have seen a great improvement in over the last couple years is our collaborative efforts with the other product centers, and not only that, but cost

at meetings. With some of the product centers, we have quarterly meetings, and we update them on the progress of the projects that they're either sponsoring financially or collaborating on. And so we get the back and forth with that, with the product centers.

And so that has been very helpful because you and I know that when there's accountability, when you're going to have to go in front of your supervisor or somebody who is funding your work, you folks that have been in the academic environment, there's going to be, what did you do, we gave you the grant money, what did you get out of that? And so there's accountability there now with that, and I've seen a lot of improvement, and I think that has helped with our back and forth with the product centers.

Back on the ORISE issue, although that program ahs been very successful, the agency is moving towards what they call a new FDA traineeship program. I think Mary Ellen brought this up about maybe trying to incentivize our hiring and being able to bring people in.

And I think that program, again that's going to be across the agency, it's going to be very flexible in the type of trainee you can bring in, this goes down from the undergraduate all the way up to a senior level scientist that may want to just come to the agency for a year or so and bring an expertise to the agency that they can learn

but they can also train our researchers on, even though it will be a traineeship program I think the perks with that program will be better than our current ORISE program.

So I think it will be an attractive mechanism that maybe will allow us to recruit a little bit easier into the agency than the ORISE program has been. And I'll stop for now Mickie, that was most of the high-level things I believe.

DR. ASCHNER: Weida, I see your hand.

DR. TONG: The question was raised about the AI and how we can tie to the AI activities. In my Division it's more towards the regulatory needs. So the question is more like we should have put the cart in front of the horse or horse in front of the cart.

And this is sort of the beauty to my daily consciousness to balance the reactive nature of our work in our division, or we needed to be proactive to develop new tools which anticipate future regulatory needs in the FDA. So I probably did not make it really clear on some of the work we do, which actually it is the reactive, the request that was directed from the other centers.

So for example the SafetAI Project was initiated by CDER, they want to have a list of the AI models to assist with the drug review process, and we have not only just developed the models, we also put these models through

the ISTAND qualification process. So we are fully aware that those are not just mere exercises, not just research. If you want to put it into the regulatory applications you really need a rigorous qualification process. So we have a lot of interactions with an ISTAND team.

And for the BERTox initiative, which literally was driven by the other centers, for example you heard about the project, and we developed with CTP and they wanted to deal with millions of documents, they need to have a certain way to put out the information from these documents. This is part of the reason we have the BERTox initiative.

The other two initiatives are more on the sort of the proactive nature, and so the question raised by the committee members is spot on because I'm struggling as well, we're going to have a division review for the next two days, I really wanted to hear the input and how to balance the reactive and proactive as a portfolio in this Division. At this point if you look at the Division portfolio we are 50/50, and 50 is purely for support, another 50 is for research. But I certainly welcome more comments through the subcommittee review.

DR. ASCHNER: Thank you. Are there any additional comments from the Scientific Advisory Board? So this will conclude the open meeting. Everybody on the Scientific

Advisory Board should have gotten a link this morning for the closed session. We will start at 12:30, so you'll have about a 6-7-minute break.

Again, I want to take this opportunity to thank the leadership of the NCTR. I also want to acknowledge Dr. Slikker, he was onboard until eight weeks ago I take it, so I want to thank him for I don't know how many years he was there, but I know he put a lot of effort and time and clearly this has shown up in the last day and a half.

Thank you to all the scientists that presented, all the Division directors, all the staff, thank you Donna and Kim for making this Zoom work along with Elly, we appreciate it, and keep up the good work, and we'll see you I guess in an open session next year. I mean I'll stop here; I don't know if you want to say anything Donna.

DR. MENDRICK: I just want to add thanks to everyone who has participated, it has been a great meeting, and I particularly thank the SAB members, I know you have other things you can do with your life, so we really do appreciate your input.

(Whereupon the meeting was adjourned at 12:24 p.m.)