U.S. FOOD AND DRUG ADMINISTRATION

National Center for Toxicological Research

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PROCEEDINGS (8:00 a.m.)

DR. ASCHNER: Good morning everyone. My name is Michael Aschner, I'm sorry I wasn't here yesterday, inclement weather in New York, but I'm here today. I heard you had a good meeting yesterday, and hopefully we'll continue on the same track today. So without further ado we'll proceed, the first presentation is by Dr. Foley, and he represents the Division of Microbiology. And we'll keep it the same way, 30-minute presentation and then 15 minutes for questions. Thank you.

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

Division of Microbiology

DR. FOLEY: Thank you everybody for coming. Dr. Cerniglia, our Division Director, was not able to make it, so I'll be giving the presentation for the Division of Microbiology. Our division as Madhu said yesterday, the people in the division make the division now what it is. We've got 27 government fulltime staff, including 19 research scientists and staff fellows that run research programs, four support scientists and four administrative folks.

As well as 12 ORISE post docs or graduate students and some undergraduate students as well too, we

do quite a bit of interaction with some of the universities, University of Arkansas Pine Bluff, University of Arkansas Little Rock, and University of Arkansas Fayetteville, we've had students or have students right now from the universities working in our labs.

We also try to be quite active in our outreach. We've got collaborations with most of the FDA centers, each of the different research divisions within NCTR, we're active with the National Toxicology Program, on different working groups and other interactions with different agencies within the federal government, some of the state health departments as well too.

And then as we mentioned earlier universities, both local, some national, international as well. We do quite a bit of work in the global and national outreach parts, so we've got Dr. Cerniglia has been very active on WHO activities with different committees there.

We've got some others on international working groups such as the HESI Microbiome Steering Committee, and different societies as well, American Society for Microbiology, the American Academy of Microbiology, the MCBIOS bioinformatics society, different editorial boards and government panels. We're trying to be quite active within the scientific community as well.

So our mission for the division is to serve a multipurpose function with specialized expertise to perform fundamental and applied research in microbiology in areas of FDA's responsibility in toxicology and regulatory science. And with the vision then we strive to be a valued resource for advancing regulatory science in the area of microbiology for FDA.

And so to do this we've really tried to over the years, tried to understand some of the regulatory processes. Being a research center, one of the things our scientists oftentimes struggle with is some of the regulatory questions and the regulatory process, and so trying to marry those two, we've been trying to help our scientists understand that better and then integrate our research into the FDA infrastructure in order to help contribute to the mission.

We're also working on trying to enhance our FDA interactions through different working groups, reaching out to scientists going to some of the FDA-wide meetings, that kind of stuff, in order to have a better idea what the needs are and to make sure that the research that we're conducting is very focused towards the regulatory mission of FDA. We're continually trying to look at and expand relationships with the centers. I think pretty much

every one of our projects in the division has some interactions with the centers or ORA with those.

And then on the management part we want to try to strengthen the program management, again prioritizing research, trying to establish benchmarks to make sure that when we are doing research that it's impactful, it meets the needs of the agency. We want to again try to communicate in plain language.

And then a key factor in order for us to be proactive is to make sure that we have facilities that are up to date, we've got good infrastructure and the like. I know Manju you talked about the Pac Bio II sequencer, that was an example of a large piece of equipment that the division is helping to contribute to to help with some of the sequencing efforts that we've got.

So our research in the divisions focus primarily in five areas, five broad areas, including evaluating the impact of different compounds on microbial agents, contaminants, additives, nanomaterials and others, and how they impact the microbiome or how the microbiome impacts them as well. We also bring in some of the host portions of that to do how do these compounds impact the epithelial barriers and that kind of stuff within the intestinal tract.

Work too in the development of methods for the detection and the characterization of microbial contaminants in there, so some validation efforts. We've gotten a little more involved in that over the last few years. As well as some specific work looking at antimicrobial resistance and virulence mechanisms of foodborne pathogens, and other pathogens as well too depending on the organism and the needs there for the agency. I know Dr. Whitehouse talked quite a bit about some of the efforts that we've got with CVM and we'll look at that in a little more detail as well.

And then working with some of the specialty programs that FDA has in women's health, some of the tobacco products we've had some interactions over the last several years, and then in nanotechnology. One of the areas we're starting to maybe look at is nanoplastics in some of these as well in the nanotechnology area.

And then trying to integrate a lot of the different types of things to improve risk assessments for FDA regulated products, integrating systems biology, bioinformatics, genomics, the proteomics, trying to integrate those to answer some of the questions.

So for our focus areas of accomplishment, we're going to look at three of those broad areas and look at a

few of the projects that fall under each of these areas. And so the first one we'll look at is some of our antimicrobial resistance and various associated accomplishments.

In this area we did quite a bit of work with CVM, as was mentioned yesterday, looking at some of the genetic basis of antimicrobial resistance and virulence mechanisms in salmonella, as well as work where we're doing developing and optimizing databases to help with the utility of whole genome sequencing. And then looking at some work with CDRH on antibiotic encoded medical devices, do they potentially contribute to the development of antimicrobial resistance as well.

So our first set of projects are the antimicrobial resistance and virulence studies with salmonella. Dr. Bijay Khajanchi in our group has been doing quite a bit of work looking at plasmids, or these mobile genetic elements that can transfer from one bacterium to another, things like antimicrobial resistance genes and virulence or pathogenicity genes.

And one of those that he's looking at are what are called incompatibility group FIB plasmids, and a number of these carry iron acquisition genes. So one of the things that the innate immune system does is it

chelates iron as part of an anti-pathogen or anti-infection process, and a number of these plasmids that salmonella and other bacteria can get are able to then compete and try to get that iron, chelate that iron, bring it into the bacterial cells. And so he has been doing quite a bit of work with some of these.

FIB plasmids in addition to iron acquisition also often encode antimicrobial resistance genes as well too, so you get increase potentially in virulence as well as resistance with one genetic element. So what he has found is that under low iron acquisition, those organisms that have these plasmids are able to survive, and that's important, especially for invasive infections where they get out of the intestinal tract.

He has also been working on the development of a three-dimensional tissue tract model that hopefully better mimics the in vivo system. It also gives the opportunity to mix cell types, so you can have epithelial cells with immune cells, and that is an upcoming project that's working its way through the approval process at the moment.

Dr. Jing Han and others in our group, too, are also looking at how antimicrobial exposures impact the transmission of these plasmids. And so some of these

efforts have shown that drugs like tetracycline as you increase the concentration of tetracycline you can have an increase in the ability of these plasmids to transfer. So that plays a big role in risk assessment and some of these sorts of things. So that's an ongoing project that we're working on.

Dr. Ashraf Khan is also evaluating what are called efflux pumps that may play a role. What they do is they pump drugs out of the bacteria before they get to a high enough concentration, and there has been a lot of work done with genomics and trying to predict the gene phenotype function. Generally, it's very efficient, you find the gene, you find the phenotype for antimicrobial resistance, but there's a small percentage of time where that's not the case. And so he's trying to evaluate whether these efflux pumps might be playing a role in that as well. Those are relatively new studies that are ongoing.

Dr. Jing Han is also working on a database for salmonella virulence genes. She has done a lot of curating of available databases going into the literature, and then come up with a database where there's about 520 virulence or putative predictive virulence genes that are in this database.

And her colleagues in the bioinformatics and biostatistics division have developed some algorithms, you take whole genome sequence data, put it in the database, and it will provide an output presence or absence of the different genes, and then we can put that into some phylogenetic programs and try to get to can we use that then to predict pathogenicity potentially.

So 520 genes is a lot of genes, and so we're right now trying to winnow those down to which ones are most impactful for virulence, and so that's an ongoing thing. We're working on an interagency group to help with this. There are members from USDA, some from CDC and FDA that are involved in this effort as well too.

And the goal for this will be to bring this to NCBI as part of the sequence analysis pipeline that we'll be able to potentially predict the virulence genes there. And so, this is ongoing efforts. We also are looking at developing a plasmid characterization database and related analysis tools too to use whole genome sequencing, there has been a huge explosion in that.

On the antimicrobial antibiotic coded medical devices work done by Dr. Kidon Sung and Saeed Khan in our division they've looked at staph aureus and pseudomonas. Pseudomonas, I'm going to talk a little bit here about

some of the work with the pseudomonas. What they found is the pseudomonas grown in conjunction with the antibiotic coded catheters versus non antibiotic you see increased growth rate, biofilm formation and invasion among the pseudomonas strains that they have when they've been exposed in the antibiotic encoded catheters.

We're doing some work to try to figure out why that is using transcriptomics and proteomics, and there are a number of genes, some associated with antimicrobial resistance and other potentially with virulence that are up-regulated in those strains that are exposed to the antibiotic encoded catheter compared to the controls. There are ongoing efforts trying to understand the antimicrobial resistance piece of this a little bit more, and then to try to help understand some of the risks associated with this in collaboration with CDRH.

On the microbiome front we're looking at the xenobiotic or the extrinsic compounds and how they interact with the microbiome or how they interact with the host cells. We have a number of projects in this area, this area is an area where we've got some expansion work with CVM on residue levels of tetracycline and azithromycin and how that impacts the gastrointestinal microbiome and gastrointestinal tract.

Some work with CDER on sunscreens and how nanomaterials and sunscreens, do they impact the microbiome, the skin microbiome or not, and if they do how. And then a number of studies that are associated with NASH Toxicology Program, Goncalo mentioned a little bit of these yesterday as well, but trying to understand, bring in the microbiome into some of these assessments of some of the toxicity assessments.

And so Sangeeta Khare and Kuppan Gokulan and Carl Cerniglia are working diligently in these areas, also trying to setup some established parameters for this, and then looking at things like how different vehicles that the compounds are delivered, how that potentially impacts. There's a lot of work kind of building the base for some of these other studies, and now they're getting into some of the work with arsenic or BPA, different compounds as well.

On the residue, we want to look at that a little more in depth. The investigators here have done quite a bit of work on tetracycline, I've reported on some of the, I worked with Dr. Cerniglia in the previous years on some of the tetracycline work. So they've now moved into we looked at azithromycin following acute or chronic exposures. And the initial work right now is looking into

how these compounds impact the colonic or the intestinal epithelial cells, which would be a model that would mimic kind of an internal surface devoid of the microbiota.

And what they found is that the higher concentrations of azithromycin for example do compromise the intestinal barrier function, which is potentially problematic. And the reasons behind this is that when they've done some gene expression work with the immune system that there are differences in some of the immune modulators that are up regulated, some down regulated. So that may be due to some of the immune regulatory activity with the exposure to the erythromycin, or it could be due to the stress from those antibiotics put on the cells, so they're continuing to assess that.

And then the next part of this will be to actually bring in the microbiome piece like they've done with tetracycline to see do these residue levels of erythromycin impact or make a shift in the microbiome following exposures at different residue concentrations.

Dr. Huizhong Chen and Dr. Jinshan Jin are looking at Nanoscale materials that are used in sunscreen to see how they impact the microbiome. So a number of sunscreens have these either titanium dioxide or zinc

oxide nanoparticles in there to help provide some protection from the UV rays.

And so they were curious what's the impact on the skin bacteria. And so these compounds, some of them can be native compounds, uncoated, others are coated with silica or aluminum, so you've got the titanium dioxide, coated zinc oxide and uncoated zinc oxide compounds that they've looked at.

And I've tried to model microbiomes at the moment where they've taken common skin bacteria and they've assessed initially with those. And so some of the work with the titanium dioxide and the coded zinc oxide nanomaterials, they don't seem to play a large role in impacting the microbiome.

However those, the uncoated zinc oxide do have a dose dependent antimicrobial effect in both their liquid and plate assays, this is a plate assay here where they've taken the different model skin bacterium that are likely present in the skin microbiome and spotted those onto the plate at different concentrations, and then there's the nanoparticle in the media.

And following exposure to UV, the uncoated zinc oxide, you see a greater killing of the bacteria. So the UV exposure enhances the antibacterial effect of these

uncoated zinc oxide strains. This is an ongoing project with CDER to try to get a better handle on some of these compounds in the sunscreens.

In the pathogen detection area, we've got work ongoing with CFSAN on tattoo inks. There were some discussions yesterday about the metals and other compounds in tattoo inks. We've been doing work on the microorganisms present in tattoo ink for the last three or four years funded through CFSAN, and we're continuing those efforts.

We've got work looking at the contamination of sterile water and different antiseptics with Burkholderia cepacia complex. This has been a big issue because there has been multiple outbreaks associated with these contaminations, it's a very difficult organism to grow, and so it's difficult to detect in these products. And then some work that's been done with CBER looking at fecal microbiota transplantation.

Tattoo ink, this project initially started with a survey of different tattoo inks in permanent makeup, the PMU's permanent makeup inks, and were looked at surveyed 85 different inks from 13 different companies that are available in the US. Almost 49 percent of them were

contaminated with microorganisms, some of these included opportunistic pathogens.

The mycobacteria is important because there have been a number of diseases or illnesses associated with tattooing where people develop these mycobacterium chelonae infections, and so they're curious about the mycobacterium, how that impacts, but also other organisms that might be present in these. So what this initial study that they did the survey about half of them had these organisms.

So they carried out a follow-up study looking at an additional 27 tattoo inks, permanent markup and ink diluents from 10 companies. Ten of those samples were positive with potentially very high levels of the 10 to the eight CFU per mil or microorganisms.

They also looked at endotoxins and found that many of these samples contain very high levels of endotoxin as well too. And this work was confirmed by some ORA labs and CFSAN, and it ultimately led to a recall of some contaminated tattoo inks.

And so this project has been recently re-reviewed and is going through the process and will continue looking at different tattoo inks, looking at country of manufacture, potentially reassessing some of

the previously recalled tattoo inks to kind of get a handle on what's going on in those areas.

On the Burkholderia work, this is work that has been done with CDER, it has been funded through some of the CDER initiatives, and a big challenge with BCC is that a number of these, they're very difficult to grow, they can be in low numbers. A lot of the times people who are getting these chemotherapeutic agents have a weakened immune system, and so there's kind of a perfect storm where it's hard to detect, and they're often times patients that are in a compromised immune system are exposed to them.

So these efforts have worked to try to identify a better media to grow these, and so one of the things that they found actually is that the more dilute media, so you take full strength, take tryptic soy broth, which is a pretty common media, you dilute it in half strength and you get a better ability to grow these organisms. So the pre-enrichment in the USP documents, you may have found that by cutting the strength of the media it helps to increase the recovery of those.

They've also found that these organisms can survive very well in disinfectants like chlorohexidine or benzoxonium chloride solutions of up to almost 200 days in

this ongoing study. They're able to survive fairly well in autoclaved distilled water that's been spiked over a range of temperatures and a range of periods. Very hearty organisms, very difficult to culture, so we're also working on the development of rapid based PCTR detection methods for these. This is work being done by Youngbeom Ahn in our division.

Bruce Erickson in our division, has done some work with trying to understand the risks associated with the contamination of fecal microbiota samples. This project has spent a lot of time on the front end trying to get it up and running, get the conditions optimized.

This is our bioreactor that we have, essentially human gut samples in a jar. So there have been some challenges on the initial front trying to get the bioreactors up to grow, finding the right media for what's now clostridiodes difficile, a recent name change from clostridium difficile. And so he's been working on that.

Now I think we've got it up to where he's got the parameters established for c diff, started an initial 14 day bioreactor study, and he's been working on the data analysis of this. The goal is to try to determine kind of standard tests, the long-term goal would be to develop

standard tests to screen fecal donations for C diff or other pathogens that may be a concern.

So switching gears kind of to our future strategies a little bit, how we want to kind of move forward with our research. We want to be as our mission, our vision said a resource to the agency. And so as part of that effort we're working to try to enhance our communication channels by again having people participate in different FDA workgroups, I know we're on some with CVM and some microbiome, what was the Office of Foods and Veterinary Medicine, now their Foods Program, kind of continuing to work in these areas to identify opportunities and needs where we can fill those. We're also continuing to try to identify opportunities for training of fellows and other sorts of things to help to train the next generation of scientists.

We're trying to work to prioritize our research efforts to best serve the needs. There are a lot of interesting things to try to chase down, but one of the things that we want to do is to make sure that our research is FDA relevant, meeting the needs of the agency. And so we try to engage our colleagues early in the research design phase, and try to get their input and put that input to practice.

We also want to work to leverage opportunities with other federal, state, and international regulatory and public health agencies. We don't necessarily want to be reinventing the wheel, using that cliché in there. And so we're working to have increased participation in interagency working groups.

I mentioned on the salmonella database project there's a Gen-FS group which involves CDC, NIH, USDA, and FDA. And so we're trying to be actively involved with that, seeking input from those groups, kind of scanning to see where are we duplicating efforts where we don't need to be, and trying to then move forward with the optimal efficiency. Also working to enhance our interactions with universities.

We've got quite a bit of work now at the University of Arkansas Pine Bluff, which is a university about 20 miles down the road from NCTR, and the nice thing about that is that students can take classes and come work in the labs. So that has been invaluable both for the students and for us as well too. And then working with some of the PhD granting universities as well too so that we can overcome some of the recruitment challenges for fellows that Goncalo had mentioned the other day.

Some focus areas, we want to continue to look at the microbiome. That area is still ripe for a number of research questions, and so we're continuing to do that. Continuing to interact with the National Toxicology Program. We've been involved with HESI in some of these areas to help move the microbiome studies forward.

Continuing to conduct safety assessments for the human and veterinary drugs and integrating these system biology approaches to do that so that we can comprehensively answer some of these questions. And this involves engaging stakeholders again and then also assessing our research equipment needs so that we can be proactive and ready to address questions that come up.

And then work on the microbial contaminants area. We've been participating more recently in some of the method validation activities, and then reaching out to the different centers to help understand microbial detection and characterization needs that we have, because we have good facilities for doing culture based methods, for doing sequencing and whole genome type assays as well.

We want to look too to some of the initiatives that FDA puts forward in the Office of Women's Health, nanotechnology as well to try to engage to develop research that will address the research gaps and funding

priorities that have been identified in each of these areas.

In the nano area one of the things that we're starting to look at is nanoplastics, Marli Azevedo in our group has kind of taken a lead on some of that with the nanocore. And so we're again trying to look at gaps that we can fill to help the agency go forward. Some of the funding initiatives, we were funded this past year from the Office of Chief Scientist for our salmonella work for example.

So feedback that we're looking for, hopefully we can get through the process. Is the division addressing the needs of the FDA centers? Are there ways to get the research results to the stakeholders in a more rapid timeframe? I think that was mentioned in some of the talks yesterday about communication, and that is a challenge, especially the distance is a big one, we've also got a number of scientists are introverts who don't necessarily want to get out and present and that kind of thing, and that's a huge challenge on some of our areas.

And so how best can we pull people out of their shells. And are there ways that we can get additional support for postdoctoral fellows? Trying to hire, I know Weida mentioned yesterday about bringing in FTEs, are

there ways that we can support postdoctoral fellows better through some of these center interactions and that kind of stuff.

And then again trying to understand the center needs and applying that there, what are emerging areas that we should pursue as we're looking forward on equipment purchases and that kind of stuff in there. Again, the last bullet kind of hits on those two, what are areas we should transition to, how best to recruit fellows to NCTR. So I'll leave it there and I'm happy to answer any questions.

DR. ASCHNER: Thank you Dr. Foley. I'll open the floor for questions.

DR. KASPAR: Chuck Kaspar. Thank you for your presentation. I have a couple questions if that's okay. The first one, involving your work with increased dosage of tetracycline and increased plasmid transfer, that's rather new. Are there any ideas of the mechanism of action, does it select for certain flora, or does it up regulate transfer mechanisms of the DNA?

DR. FOLEY: That's a good question, and actually one of the postdocs in our lab right now is doing some RNA sequencing efforts right now to try to look at the gene

expression, in fact he's in the lab today working on some of those experiments as well too.

The idea, there are some potential things. One, the tetracycline, there's some synergy to some of the signaling peptides, signaling molecules that may be impacting a signal transduction pathway. Or potentially it's creating an SOS type response where you've got stress on the bacteria, and one of the things that you see when you get that stress response is they tend to shut down some of their repair mechanisms, that kind of stuff that allows for, to potentially allow for more transfer of genes, allowing more mutations to occur in that kind of stuff.

And so we've looked, and it's interesting, where we see it, tetracycline is a big one, at chloramphenicol we see it as well too a little bit, and so those are ribosomal, the target are the ribosome of the bacteria. Whereas some of the other drugs we don't see that similar kind of thing in the strains that we've looked at. So I'm wondering is there something with the protein synthesis. That's a good question and that's something that we're trying to answer at the moment.

DR. KASPAR: Second question is I know there's a lot of work on mixtures of bacteria for fecal transplants,

but using known cultures rather than using donor based fecal transplant. Are you doing any work on defining a known mixture of organisms for fecal transplants?

DR. FOLEY: A little bit. Dr. Doug Wagner in our group has done a little bit where he's trying to identify specific organisms that may be useful in that process. We haven't done a ton of work in that area of trying to establish kind of set organisms that could be done or defined culture versus a fecal transplant sort of thing. CBER, if they're working on that or not, they probably are.

DR. WILSON: Carolyn Wilson, CBER. You make a good point, but in reality most of the clinical trials right now are still going on using donor material. So from our perspective this particular project is very important, because the concern is that the risk pathogens that you're most concerned about potentially causing disease in the recipient, most of the tests are clinical diagnostic assays, which are applied to a patient at the moment of a raging infection, so they don't necessarily have to be the most sensitive assays, and our concern is is there some low level of some bad salmonella or what have you that you don't want to make this patient worse.

And so the purpose of this is really to say can you still detect these kinds of pathogens. But your point about the consortia is important, and we do have that animal model that I mentioned, and the next step in that is to start dissecting out the microbiota in the consortium.

DR. KASPAR: My thought was because they have the bioreactor going you could look at stability and long-term persistence of that normal flora.

DR. FOLEY: And that's something that we can do, because of that bioreactor we can setup cultures, and looking at some of that. I think some of the other work that we've kind of gotten on our minds a little bit too for future reference would utilize that as well, because I know some of our plasmid transfer stuff, one of the things that we've talked about doing is trying to look at setting up a model, intestinal microbiota, and then seeing can we track plasmid transfer from say salmonella to enterobacter, some other organism that might be common in the microbiota.

DR. GANEY: Thank you for that talk. I'm new at this, so I'm still trying to get a handle on structure. One of your bullets raised a question for me, you ask is

the division addressing the needs of FDA centers, plural. So are you the only microbiology unit in the FDA?

DR. FOLEY: No, we're not. Pretty much each of the centers, except from the Center for Tobacco, they're probably the only one that doesn't have one. And they have microbiology people for reviewers and that kind of stuff. Pretty much everybody does have some, there are some limits on the numbers of people and that kind of stuff, so we're trying to fill niches that they may have, they may not have either the equipment or the expertise in an area where there's a question, and so we're trying to fill some of those gaps. That's where that communication piece is vitally important so that we're not duplicating efforts.

There have been times in the past where we've started down a path and then realized, oh, CVM is working on a similar thing, so we've said okay, let's back off on that area, provide support where we can with that. There are enough other questions in those areas, so let's take a look at one of these others and work with them on those.

DR. GANEY: I was also struck that your vision statement doesn't say anything about toxicology. And then looking through what you're doing, a lot of it, not all of it, but a lot of it is toxicology related. But I can see where with the kind of expertise you have, it would be

easy to become very diffuse and lose focus on toxicology if this really is your primary mission to be support for the NCTR.

DR. FOLEY: That's a good point, a valid point. It's in the mission statement, I think toxicology is mentioned, but in the vision statement you're right, that's not, so that's something we could take back and look at as we evaluate our divisions, the next one to go through the in depth review, and I think in that process that's something that it's a good time to take a look at these documents and vision and do some strategic planning kind of things around that. We've definitely actually moved probably more into the toxicology front over the last five years or so because of the microbiome piece and working into that. Good point.

DR. STICE: This is more a comment than a question. I see in multiple presentations the need and actually request to help with recruitment, ideas and so forth. I don't as one SAB member don't really know everything that you do. So just a suggestion maybe in the future that we could have a short section just associated with recruitment and what you're doing, and that we could then comment on that, might be helpful in the future.

DR. LANZA: Thank you, interesting presentation. I was a little interested more on the antibiotic coating of catheters, but not from that perspective, but the nanoparticle impregnated catheters, and whether they were as safe and effective, I didn’t hear that there was any discussion about them, and particularly with durability when they’re implanted for three to five days, losing the metal or losing the effectiveness. Did you have a program related to that, or has it already been done and gone?

DR. FOLEY: We haven’t with nanoparticle impregnated medical devices. We did have a study that Dr. Khare did a few years back, where they looked at nanoparticle, I think it’s silver nanoparticle impregnated food containers, looking at that, and I’m trying to remember the results of that. Anil is back there raising his hand, so he probably has a –

DR. PATRI: Anil from NCTR. Greg, yes, the Center for Devices has research projects and looked at the devices and catheters containing nanomaterial, as well as the Center for Drugs, they have studies that they have done to look at those, as well as the two aspects to it, if you have nanomaterial intravenously injected, they bind to these catheters, so there is another dimension to that, some of the hydrophobicity depending on the surface, they

can bind to the tubes. And so they have looked at and they are looking at those kinds of projects as well.

DR. LANZA: My biggest point actually is because work in the hospital and getting those catheters in and out and having to find new sites for them and so forth, and not always wanting to put a long line in patients, it's a very significant cost, and it's a health risk, particularly in patients who are debilitated, might have infections. After I saw the antibiotic coated catheters weren't working as well, it made me think back to would these other catheters be better.

And it's not uncommon frankly, yes, the nurses should have changed the catheters every three days, four days, they put a thing in, then you come in and you see your patient had theirs for a week. It's all the time. And it's a significant cost of effort. So I just wonder if maybe we should focus on it from an FDA standpoint, because if they're safer and they last and they're not shedding, then I think that might be better than many of the things we're doing.

DR. ASCHNER: I have a couple questions. One of them is perhaps more scientific. Is there any evidence that tattooed people have more infections? Any epidemiological evidence?

DR. FOLEY: They do have skin infections. There have been several reports of skin infections following the actual tattooing, especially in mycobacterium chelonae infections. So you get skin and soft tissue infections from that. On the systemic side of things, I'm not sure on that.

DR. FITZPATRICK: Suzy Fitzpatrick, CFSAN. So it's true when you get a tattoo they tell you you might get an infection, so to watch out for that. But it's also true that I don't think they've studied this enough, I don't think people realize, and probably none of you realize that the ink that we're putting on people are really for cars.

So I think now that people are becoming more aware this could be a problem, you'll probably see some epi studies. And we're hoping with our study here that Fred's group is doing, we'll not only look at if the ink crosses the placenta but also where they're going in the brain and then the kidneys and everywhere else.

And we found, we've seen a lot of PCBs, a lot of lead, a lot of contaminants besides microbiological contaminant. I think it's been another emerging area that we're going to see a lot more on when our person, when she

goes to the toxicologist the only thing that people ask about are not cosmetic but tattoos.

DR. ASCHNER: The other question is more technical. You outlined a number of collaborations which you have with the other FDA centers, but I don't think I heard anything about collaborations of your division with other divisions within the NCTR.

DR. FOLEY: That's a good point. We do have quite a few interactions. I think we have interactions with every division, everything from the gut, brain access, work on some of the microbiome stuff there, to bioinformatics on the salmonella database for example, we're working with them to help develop some of the analytic tools, systems, biochemical toxicology, the tattoo inks, it was an example there, microbiome studies there with genetic and molecular toxicology, we're doing some work with sequencing, systems biology, we've done work with rapid detection. So I apologize for not bringing that out as clearly as I probably should have, but we are interacting, we've got interaction with every division out there, some nano work with the nanocore as well too.

DR. ASCHNER: Thank you. I think it will be good to bring it out next year. I think we're out of time, so we'll more forward, thank you Dr. Foley.

DR. ASCHNER: Our next presentation is by Dr. Ferguson. And she is going to review the Division of Neurotoxicology.

Agenda Item: Division of Neurotoxicology

DR. FERGUSON: So I will give a brief overview of the Division of Neurotoxicology. And I'll follow the same format that the other division directors have given. And I want to say I will absolutely not stray from the template that I was sent by Donna Mendrick. I once heard somebody describe her as small but mighty. So it's going to be exactly like the template she sent us.

So we currently have 35 staff employees. That's two down from what I presented in March. One was a retirement, and one was a term appointment that ended. We have 19 research scientists, staff fellows, visiting scientists. We have one open position for a neuropathologist, and that was due to a retirement. We're currently interviewing for that position.

Of those 19 that are on staff, one is actually due to arrive in February, and that's our bioimaging specialist. We have 11 support scientists, two administrative personnel, and two postdocs, three open postdoc positions. Of those three open ones we have one that starts in January and one that starts in April. We

also have a part-time undergraduate student from our local University of Arkansas at Little Rock.

This slide shows our outreach. We participate in collaborating with virtually every other division at NCTR, and three other centers. We participate with a number of government agencies, CDC, EPA, NTP, and so forth. And we also collaborate with a number of different universities, including the local med school, University of Aransas for Medical Sciences, and the Arkansas Children's Hospital. The flagship university of University of Arkansas is located at Fayetteville, we have an ongoing collaboration with them as well.

Later I'll describe a little bit about our Mayo collaboration with the MASK study. And we're heavily involved in the Steering Committee of SmartTots. And that's a collaborative effort of the FDA and the International Anesthesia Research Society. We have two ongoing collaborations in Mexico, and a collaboration with the European Cooperation on Science and Technology.

This slide shows our Division Mission and Vision. I was told as Division Director, that I can create the Division's Mission and Vision, so that's what I did. I won't read it to you because I know it's in your binders.

Now I'll talk a little bit about our top three accomplishments during the previous year. Understand these are not the only accomplishments. But I chose these three because they do represent the diverse variety of work that's going on in the division.

So the first one is our new magnetic resonance imaging of nonhuman primates. So we now have a 4.7 tesla MRI that's been installed, calibrated, and verified. And this particular 4.7 tesla MRI has a wider bore, and this wider bore can accommodate adult nonhuman primates.

So this slide shows a horizontal plane of an adult nonhuman primate. And you can see that it provides great anatomical resolution. It can also do quantitative T2 mapping. The nice thing about this is that the animals do not have to be anesthetized for a long period of time. For this particular adult, it only took 32 minutes. And here you can see the sagittal and coronal planes of that same animal.

So the plans are that we will continue to use this 4.7 tesla MRI to image some of our adult nonhuman primates that have been exposed developmentally to drugs, neurotoxicants, as well as other compounds.

Now we've expanded in the last year or so, we've really expanded the work in the division in vitro. And

expanding our work in this area really helps support agency reviewers, and it validates, helps them validate and interpret the data that's been submitted by industry. It also helps inform decisions about the safety and efficacy of drug candidates.

But I do want to note that the Division will continue to use whole animals, live animal work. Much of our work is focused on developmental exposure, and in looking at the long-term effects. So whole live animal work will continue in the division.

And I'll talk about three particular in vitro projects. The first one is a stretch system to model traumatic brain injury. So we began with, let's hope this pointer works, we begin with what's called a uniaxial high speed stretch model. And this was a unique instrument developed by the Department of Biomedical Engineering at the University of Arkansas at Fayetteville.

So this particular instrument uses low levels of uniaxial stretch to stretch those cells. When it's done, it only goes up to 15 percent stretch, however. So when this is done it increases the number of dead cells, and it increases the number of apoptotic cells. We've had one publication. I'm sorry, these cells were primary isolated rat brain endothelial cells.

Now we've moved on from that work to use a commercially available biaxial stretch model of TBI. Here the cells are plated on a flexible bottom plate and pressurized medicinal air is infused up to levels of 50 percent to stretch those cells. Two publications thus far have resulted from this, one using rat cells, and one using human primary dopaminergic cells.

And to give you an idea of what types of result we've gotten from this instrument, high biaxial levels of stretch will induce necrosis, high levels being 50 percent, and apoptosis can be induced by 25 and 50 percent. Mid to high levels of biaxial stretch will decrease intracellular levels of dopamine, shown in the graft on the left. Decreasing levels of intracellular dopamine with increasing levels of stretch.

Increasing levels of stretch also decrease TH expression. But extracellular levels of dopamine and DAT expression were unaffected.

So now that we have these two models of TBI, the better one being of course the biaxial stretch model, and we have some results from this, we can now move on to a live animal model of traumatic brain injury.

And the third example is the MEA, or Microelectrode Array. So MEA is a device that contains

multiple electrodes, they're embedded on a substrate, and you can see those electrodes are shown in black here. And they're organized in a specific arrangement through which biological, mostly neural signals are obtained. So essentially this device represents an interface between the computer and the cells. And the instrument itself consists of a computer of course, an interface aboard, the headstage, and then a 24 well plate. And this shows a blowup of one of those wells, so you can see the electrodes in there.

So this MEA assay, once the cells are plated they will begin to exhibit spontaneous action potentials, and those of course are collected and measured. The cells will then exhibit excitability or bursts of action potential, and again can measure those. And those cells are assumed to make synaptic connections with one another. One those synaptic connections are made, synchrony or connectivity can be measured. The software generates waveforms for us, and both inhibitory and excitatory neurons can be measured.

This slide shows an example of our results. So these are the wells, you can see the dark black represents the electrodes. This is a well, a control well. This is a well that's been treated with MPP plus.

Now, most people know that MPP plus is a known neurotoxin, it's used to create models of Parkinson's disease. But at this particular concentration the cells are still alive and they're still functional. And here we use nicotine as simply a prototypic stimulant. So shown on the bottom row here are action potentials. Each dash there represents a single action potential. And because action potentials are stereotyped events, the most important thing here is the timing of those.

So you can see the control action potentials, you can see the well that's been treated with MPP plus exhibiting fewer action potentials, and that's shown in a decreased spike count as well as a decreased spike rate. The well that's been treated with nicotine however exhibits increased spike action potentials, shown by an increased spike count and spike rate.

SO the MEA is a really useful device, and by doing this with compounds that we assume will decrease action potentials as well as those that will stimulate action potentials, we've verified that we can use this instrument.

So now the only thing is to move forward with this, and I guess most of you won't be here during the subcommittee to hear Dr. Syed Imam talk about further use

of this, but one of the cautions that we have to do with this, because it is a comparatively high throughput model, we have to be careful that any compound drug or toxicant that we use in this does cross the blood-brain barrier before we actually translate results to what might be happening in whole animals or humans.

Dr. Syed Ali has a recently approved protocol to look at blood-brain-barrier on-a-chip technology as a tox screen, and I forgot to mention this to you yesterday Suzy. Here he's going to compare blood-brain-barrier properties, that is things such as TEER or trans endothelial electrical resistance, paracellular permeability and expression of tight junction proteins, with those in a static trans-well model.

Now, that static trans-well model is very cost effective, and it's very user friendly. But it just can't replicate the key characteristics of the blood-brain barrier. Specifically, the endothelial cells are not subjected to the dynamic mechanical stimuli that can cause subtle differences in cell morphology and barrier permeability. Those trans-well models are simplified co-culture systems that just don't capture the complex architecture of the blood-brain barrier.

He'll analyze blood-brain barrier changes after treatment with known disruptors, such as methamphetamine, TNF alpha, and LPS, and compare those to the static trans-well model.

And to give you an idea of what types of data we hope to generate, this is from a 2016 publication showing TEER in cells that were not exposed to different levels of oxygen, cells that were exposed to physical hypoxia, which exhibited decreased TEER, and cells that were exposed to hydroxyzine, also exhibiting decreased TEER.

And our third accomplishment is that in the last couple of years we've become increasingly responsive to the agency specific needs, and I'll give you examples of this. We collaborate most heavily with CDER, we've been doing our pediatric anesthetic work since 2004 for a number of years, and virtually the entire portfolio of that work has either been supported by or requested by CDER.

Gadolinium is a contrast-based agent. We've looked at deposition and retention of gadolinium in the rat brain. We have a cohort of nonhuman primates that were exposed as adolescents to methylphenidate and you saw one of those primates in the MRI images.

And you might be aware that FDA recently approved S ketamine for the treatment of depression. So S ketamine is approved for patients that are 16 years or older only, but at some point of course, it will likely be used in teenagers and adolescents. So we're looking at adolescent exposure to ketamine in rats, and this study will ensure that adequate safety margins are in place for efficacy testing of ketamine in trial in children.

I've listed this as under review, but since this time it's an approved protocol, Dr. Cuevaswill look at sex differences in amyloid beta transporters in Alzheimer's disease. And I'll talk about this one a little bit more later, using acetaminophen during pregnancy.

My lab has been in collaboration with CFSAN to look at developmental exposure to bisphenyl A, and we're currently doing a study looking at developmental exposure to inorganic arsenic. Both of those studies were NTP supported and done in collaboration with CFSAN. We had funding from the FDA's Office of Women's Health to look at gender differences in nicotine using MRS.

And then in collaboration with multiple centers, CFSAN and CDER, we have a protocol currently under review to look at developmental exposure to cannabinol or CBD. Last Monday, which was November 25th, FDA issued a

statement on their public website citing a potential warning about using CBD. And it specifically cited that we don't know the data regarding exposure to CBD in children or infants. If you think CBD is not used rarely, 15 percent of US adults use a CBD product. It's in virtually everything. It's in nail polish, for Pete sake. It's in pet products.

The one center we did not contact when we were putting together this protocol is CBM, but it's in a number of pet products as well. So this particular protocol if approved will look at developmental exposure. It will give us the answers we need to know what's happening in pregnancy and infants. The label for epidiolex does list developmental and reproductive abnormalities.

So let me give you some details about the acetaminophen project, because it really does capture how these cross-center collaborations come about. Acetaminophen has been marketed for pain and fever relief since 1950, and it really is the treatment of choice for pregnant women. But comprehensive datasets for its use during pregnancy simply don't exist. The label says if you're pregnant or breastfeeding ask a health professional before use.

In 2015, FDA issued a safety announcement which noted recent critical findings of in utero acetaminophen exposure and adverse developmental outcomes. And that particular adverse outcome was a higher risk of attention deficit hyperactivity disorder.

About six weeks ago an NIH funded study was published linking acetaminophen exposure during pregnancy to not only a higher risk of ADHD, but also autism.

So CDER's Medical Policy and Program Review Council contacted NCTR, recommended that we conduct a nonclinical study to characterize those potential risks. We've had many discussions with CDER to determine exactly what type of experimental design would generate the data they need. That concept paper was approved this past summer, and the full protocol is currently under review by our CDER colleagues.

Now some of our future directions, these are very recently approved protocols. Dr. Sarkar has a protocol in collaboration with the Division of Systems Biology for lipidomic analyses of the Alzheimer disease transgenic rat. He's also collaborating with the Division of Microbiology to analyze intestinal and neuronal pathology in those same transgenic rats, as well as human

postmortem tissue. And that postmortem tissue will consist not only of brain tissue, but also intestinal tissue.

Dr. Syed Imam has a collaboration with systems biology for assays of Parkinson’s disease, Lipidome, and peripheral biomarkers.

Now I want to spend just a minute talking about our collaboration with the mayo clinic. This is with the Mayo Anesthesia Safety in Kids or MASK study. So this is a study of almost 1000 children that prior to three years of age received either no exposure to anesthesia, a single exposure to anesthesia, or multiple exposures.

And those children have been followed for many years, and this slide shows three of our recent publications with them. We sent the Mayo Clinic several of our operant test panels. These are operant test panels that are used at Arkansas Children’s Hospital to assess cognitive abilities of children. So these children have been assessed not only on neuropsychological tests, but also our OTB test battery.

So, future directions, we have a new postdoc at NCTR now, Dr. Andrew Shen who is teaching us how to isolate capillaries and look at the activity of the transporters. We’ve always been able to measure levels of tight junction proteins, but now we’re going to be able to

measure the actual activity of those tight junction proteins. And we're going to increase our use of that 4.7 tesla in our adult nonhuman primates, and Dr. Liachenko has a concept under review to validate the T2 MRI biomarker of neurotoxicity.

We have a couple of new instruments in the division. One is the Cytation 5. This is a combination plate reader and high content imaging system. So this instrument can do live imaging, fixed samples, cells, zebrafish, as well as slides. The MEA system that I talked about earlier also has another capability that we hope to have someone come and train us on, it can look at LTP induction and synaptic excitability in hippocampal slices, that's important.

And like all divisions we have challenges. These high tech instruments. We have two MRIs, we have a microPET. Their maintenance contracts are incredibly expensive. And if a maintenance contract lapses then you have to have the tech come out and recertify, recalibrate, do something that costs a lot of money, and then you can put the maintenance contract back into effect. So we're looking at creative ways to fund those maintenance contracts.

And then better hiring techniques. I know I sound like a broken record there. That's actually a very old analogy. I probably sound like a broken CD. That's old too. Mp3. I sound like a broken MP3. We're living in a new, in terms of recruiting for postdocs or FTEs, we're living in a new normal. Our new rule is that an applicant has to have lived in the US for at least three of the last five years, if they're not a US citizen. So that really hampers our ability to search globally for postdocs and government staff.

And of course, we always want to enhance our interactions with other centers. This has come up more and more. And I think a lot of it is the lack of proximity. Two of our best resources here that I have found for Neurotoxicology have been Donna Mendrick and Bill Slikker. They are at White Oak much of the time, they know the folks there. When I have someone in my division that says I'm interested in rodent stroke models, who can I talk to, I call Donna, she puts me in touch with somebody. So they've been incredible resources to me as a new division director.

And we always ask for feedback from the SAV. Are there pressing neurotox issues that we should be looking at? What emerging technologies might we be examining? And

how can we best verify those newer technologies? Now before I end I forgot to put in an acknowledgment slide, that's my bad. I was Acting Division Director for a year, and I've been permanent Division Director now for one year.

And you might imagine that transitioning from research scientist to division director was incredibly challenging. It is very challenging. Any successes that the division has had in those last two years have been absolutely due to the patience and the support of those neurotox employees with me. Any failures, they're mine. Thank you.

DR. ASCHNER: Thank you Dr. Ferguson. I'll open the floor, let's start from right to left. Greg?

DR. LANZA: I was looking at the scan times in the MR. I'm not sure, I didn't catch what the instrument is, but those are long compared to human, and I'm wondering if it's not worth getting someone in here that can help you, because you can accelerate, for instance you can scan the human brain T2 map in under two minutes, the whole thing. It's accelerated by under-sampling case base. So someone who can do some of that PULSE program might help you. And the other thing is, the reason I'm assuming

you're not using, or not, I'm asking, diffusion weighted imaging, rather than just T2 mapping –

DR. FERGUSON: Actually, I don't know if Sergei is in the audience, can you answer Sergei?

DR. LIACHENKO: I'm Serguei Liachenko, I'm running MRI at NCTR. Yes, we have all those options. Unfortunately, we have this research grade Brooker scanner which does not have all the capabilities of the clinical scanners, so we cannot do T2 mapping in two minutes. We don't have that. But we were working in that direction, we have some ways of improving this. Maybe we'll hear this a little bit later in our next –

DR. LANZA: Can you use diffusion weighted imaging?

DR. LIACHENKO: Yes. We have all the capabilities, we have diffusion weighted sequences, we have perfusion –

DR. LANZA: You have planar.

DR.LIACHENKO: Yes. I've done this kind of work on rats, and I found that T2 gives better actually signal result in case of neurotoxicity, but this has to be through monkey imaging. So we will use all of those.

DR. LANZA: For those who aren't familiar, diffusion weighted imaging is also a type of white matter

imaging, but it tells you whether the water is in the cells or outside the cells. And so rather than just you have single, it helps you to partition it. And that's in the brain and trauma type things it seems like it would be another advantage for you, that's why I ask.

DR. LIACHENKO: We just started using the big bore MRI on nonhuman primates, and there's a lot of work ahead of us.

DR. LANZA: maybe the next thing you've got to pony up for a clinical scanner.??

DR. COSENZA: I'm interested in the cell stretching model. So do you know how 15 percent cell stretch, et cetera, relate to actual human trauma, concussion, or whatever?

DR. FERGUSON: We have an expert on that in the audience. Can Hector answer that?

DR. COSENZA: It's very difficult to measure the actual deformation in human brain, but there have been some computer simulations, some blood recreated events, and I'll present some of these tomorrow, but up to 60 percent stretch has been shown in humans after a severe TBA.

DR. SAUER: That was a great presentation. I just have a couple quick questions actually relating to the in

vitro models as well. I think you've developed, I think your team has developed some really great models. But what's the next step there? What I didn't see is how those are going to be applied. I see within your mission statement biomarkers weigh heavy. Were you thinking about looking for soluble biomarkers there, or how are you going to leverage those models?

DR. FERGUSON: At least for the MEA system Dr. Syed Imam has a recent patent with nicotine and nanoceria as a potential treatment for Parkinson's disease. So intends to look at the nic/nano effects in the MEA system. And again we hope to gain the capabilities of looking at hippocampal slices in those. I'm not sure how much further we'll take the stretch models of TBI, given that we now have an approved protocol to look at a whole animal model of TBI. So I'm sure there's more work to do.

The blood-brain barrier chip though, I need to talk with Suzy a little bit more because she's interested in some collaborations on organs on a chip.

DR. SAUER: So also are you aware of the activities in Europe for the Innovative Medicines Initiative Trans Bio Line? They have actually a neurotoxicity working package. I think there they're looking specifically for fluid biomarkers. I mean I think

imaging biomarkers are definitely important, and they're going to be a mainstay, but drive safety evaluation, fluid biomarkers would be very useful.

DR. FERGUSON: Dr. Syed Imam has a collaboration with HESI specifically to look at fluid biomarkers of neurotoxicity. And we're looking at known neurotoxicants. The first one was cyanic acid. And he's collected not only tissue samples but also CSF and blood and urine to look at potential biomarkers. And he's moving into phase two of that project now.

DR. STICE: You probably know this already, but I'll just mention this, Tim Schafer obviously at EPA is a big expert, and knows MEA systems. And so hopefully you're bringing in people that have been there, done this before, screened chemicals extensively with MEA systems for neurotox. So if you could get a comment on that.

DR. FERGUSON: And I would like to get in touch with that organization you just mentioned it on.

DR. SAUER: I can provide contact information for TransBioline.

DR. ASCHNER: We've talked a little bit about the TBI. The one thing that I'd like you to describe is what I'm missing here is the relevance of this model to the FDA.

DR. FERGUSON: That’s something that when we were putting together SAB presentations, we actually talked about that. The idea there is it’s a whole animal model, to validate the whole animal model and get it up and working in our division, will allow us to look at potentially vulnerable populations, to look at sex differences, to look at age differences, genetic differences and that sort of thing, such that when potential drugs are submitted for approval for TBI, because right now there are no drugs approved to treat TBI, there’s a new test to detect TBI, but no drugs to treat it.

So that when those drugs are submitted for approval we can particularly if they’re submitted for particular populations we’ll know exactly what’s happening in TBI so that we can inform the reviewers as to whether it’s going in the right direction.

DR. ASCHNER: The other question I have related to some future research. Are there any thoughts about doing any research on vaping, e-cigarettes?

DR. FERGUSON: You’re asking for pressing issues, and I recognize most of the people who died from smoking, it’s lung related, but there’s clearly neurotox effects.

DR. FERGUSON: We would be interested in potentially the developmental neurotoxicity of those. I'm sure the general public believes that they are more safe than cigarettes. If a pregnant women was smoking cigarettes she's more likely to trade to e-cigarettes, we'd be interested in something like that.

DR. ASCHNER: The last thing I have is sort of a word of caution with MEAs. I see the relevance of tissue culture, and I've always been a strong proponent of tissue culture, but how you extrapolate the data from the dish to humans is always a problem.

So you gave an example with the MPP plus. If you treat the neurons with MPP plus you'll see a lot of changes in spikes, rates, count. But if you do it in the brain with MPTP or in a dish with MPTP you won't see anything because you don't have the astrocytes which are the main site where the MPTP is going to be converted to the MPP plus.

So I think one has to be careful. And I'm wondering if you have any plans to carry over or carry forward the findings that you'll be getting from the MEAs, tissue culture to whole animal studies.

DR. FERGUSON: Absolutely. The in vitro systems, in my opinion they're a start. They point us in a

direction where we can then move with whole animal work. I don't want to sound like I'm on a soap box or anything. We still need the whole animal. There are drugs that are metabolized where the metabolites are neurotoxic, but not the original drug. You need the liver and you need the brain, you need the whole animal. I think there was a question behind you too.

DR. LANZA: I just, we talked about TBI, but I'm not sure I picked up on what kind of TBI you're talking about, like mild TBI repetitively or something moderate that's quite significant or profound TBI. What are you doing?

DR. FERGUSON: So the approved protocol is to look at a closed head impact in the rat, and initially to look at a single impact, and at various weights collect the data, hopefully we can get a weight and a drop height that will qualify as mild, moderate, and severe. And we'll qualify that mild, moderate, and severe using behavioral assays and blood assays.

And so once we know height/weight that causes mild, height/weight that causes moderate and so forth for severe, then we can move forward and look at the potential sex differences and age differences and so on.

DR. ASCHNER: Thank you. We have some more time, so are there any additional questions? Okay. Thank you, Dr. Ferguson.

Agenda Item: Division of Systems Biology

DR. ASCHNER: The next presentation will be by Dr. William Mattes. He represents the Division of Systems Biology.

DR. MATTES: First off, I want to say Donna's template is a good one. I would like to point out by saying that again our division is different from everyone else's. Actually it's different for one item on this slide. In the back of the room we have a first, a product center detailee. Jess Hawes in the back of the room is a detailee for CDER, and all I can say is I would like to see NCTR become vigorously involved in a program where we could get detailees from the product centers, and in fact perhaps detail our scientists into the product centers. Aspirations.

Anyway, you can see the numbers there for the number of scientists, support scientists, administrative staff. Right now we don't have any commissioner fellows, and we have three ORISE post docs.

We have collaborations really with all of the NCTR divisions, and I think you've heard, and thankfully I

don’t have to enumerate them because you’ve heard about many of them from the previous talks. We have collaborations with a number of the regulatory centers, the product centers. We have collaborations with a number of government agencies as I’ve listed there. And the number of universities that we have for different collaborations is really quite mind boggling I would say for me at least.

I’d like to note certain collaborations. In with CDER we have one in terms of refining the iPSC cardiomyocyte model for cardiotoxicity prediction. We have another collaboration looking at in vitro toxicity assessment of opioids on neural precursor cell development. We also are working on development of tools to rapidly detect contaminants and adulterants in crude pharmaceutical products.

I think you’ve already heard about some of our interactions with CBER in terms of the MPS model of testis function and metabolomics in the MAIT knockout mice. And we also have a collaboration with USDA in terms of e. coli detection.

Systems biology. We consider our mission is to address problems of food, drug, and medical product safety using systems biology approaches and innovative

technology. Why systems biology? I see it in terms of the fantasy of vision as tools and approaches that can bridge the nonclinical models in terms of their adverse events, individual responses, with the clinical settings. And that's really the end game, the clinical settings. I use a term, translational toxicology and precision safety assessment, but everybody has got to have some buzzwords.

So how might one look at this? One can look at this as the model systems on the left and the targets on the right in terms of the human individual and the human population. That's kind of what you see there is the A and PK pathway, which is a crude way of looking at I would say at life, but nonetheless it's one way to try to evaluate different systems.

And the tools you use are your usual omics panoply. Transcriptomics, proteomics, and metabolomics. The last two I would note have particular value in terms of being able to be translational. I'm quite excited about the proteomics and metabolomics in terms of being able to make the same assessments in an in vitro, in vivo animal model, and in a clinical setting.

So our goals are to develop and look at translational prognostic or predictive biomarkers for improving pharmaceutical product safety, delineate

mechanisms for species, tissues, sex and sub-population specificity in terms of drug toxicity. We're interested in looking at mechanisms of opioid addiction, and the mechanisms for next generation pharmaceutical toxicity, such as the oligonucleotide therapeutics.

We're also interested in developing the in vitro models for better evaluation of reproductive, developmental, and clinical toxicity, in silico models for predicting relevant toxicity, such as the next gen pharmaceuticals, and also leveraging some of our expertise and technology for developing technologies, such as detecting drug alteration.

The strategies are to use tool compounds. The usual suspect as they say is anthracycline, tyrosine kinase inhibitors, where you know the mechanisms and then you can look at the model system and correlate it with the clinical system. You want to characterize these systems biology effects such as using MRNA, the transcriptomics, all the omics. You can.

And integrate this data with the informatics, accounting, and what I think is important is to always be keeping in the back of your head what are the species, tissues, sex and subpopulation differences. And ideally,

we also incorporate innovative technologies to address these questions. Technologies such as MALDI imaging.

The general themes, again translational safety biomarkers and examining mechanisms. Looking at developing alternative models. And innovative technologies such as computer modeling, ultimately to drive to this concept of cross-species prediction and translation.

But again, our integrating focus is how can our research be used in FDA regulated products, such as the technical use, how can we develop and improve on the tools and methods and investigate biomarkers. How can we look at hazard identification and understand mechanisms of action? And eventually be looking at how can we impact concepts such as clinical dosing limits and clinical monitoring.

Now, in terms of accomplishments, I'm going to point to a few here. But rather than read through them I want to point out the concepts that are behind them. In one case it's evaluating the tools such as this IPSC stem cell cardiomyocyte model. This particular publication involved a number of groups, importantly, asking the question how does the data compare across multiple sites.

Then there's another question is if you are using analytical tools such as metabolomics and proteomics, how do you handle samples from nonclinical and

clinical settings, and that was the second publication you see there. There's also the important question about when you are using new tools, such as metabolomics, what kind of standards do you have to document your work.

Similarly, when we're developing new tools such as this lipidyzer, which you've heard about, and I'll give more data on, what kind of performance standards can one look at. And as I mentioned about biomarkers, there's this question about what can one investigate, both in a preclinical and clinical setting, and that you'll see in one publication.

And finally, in terms of in vitro models, you've heard a little bit about the testicular models, and in fact this is describing one. IN fact, what you can see in that model, and this is not an MPS model, this is a more traditional I would say organ fragment or tissue fragment model, but you can see then it has promise in that you're seeing testosterone production after 48 days of culture. Not quite there in terms of where you are with the in vivo setting, but in the right direction.

I mentioned the lipidyzer platform, which is a new technology which really expands our metabolomics portfolio. You can see here looking at 787 individual lipid species, and 13 different lipid classes. And what

you can see is on the left the levels in different individuals in their liver, and you can see the individual differences.

And then you can also see in comparison with the three groups on the right, which are the lipid classes in heart, clearly indicating the differences between heart and liver, simply in lipid classes. That shows up here also as you can note the lipid profile of phosphatidylcholine and phosphatidylethanolamine species, and you can see the higher level in heart of docasahexaenoic acid. So this tool is as I say expanding our metabolomics portfolio, giving us a better look at both clinical and preclinical experiments.

In terms of plasma markers of cardiotoxicity, this is a mouse model, and what are highlighted in green are markers that were elevated that is compared to controls. And you can see even at the very early phases, even after the first dose you can see an elevation of certain markers, which have the potential to be early injury markers of toxicity. There’s an effort ongoing to expand to take these putative markers and look at them in a clinical setting.

So in terms of current projects that I’d like to talk about, there’s a variety here, one in which we’re

looking at one particular tyrosine kinase inhibitor and exploring the toxicity of it as well as comparative toxicity of its metabolites. Also, in terms of tyrosine kinase inhibitors we've looked at cardiotoxicity in the cardiomyocyte model, we're continuing development of mouse models of cardiotoxicity, in this case delayed cardiotoxicity, which you see in anthracycline treatment.

We're also looking at going to verify some of these novel biomarkers of doxorubicin cardiotoxicity in breast cancer patients. We're expanding or I would say trying to mature this analysis of organic chemical contamination of drugs, and we're also looking at molecular modeling of opioids and mechanisms of opioid addiction. And I'll show some examples of how that looks.

First, this comes out of looking at a whole series of tyrosine kinase inhibitors in terms of exploring which are mechanisms of hepatotoxicity and mechanisms of cardiotoxicity. In the course of that investigation Zhong Shi(?)14:18 in my division noted that regorafenib, which is a famously hepatotoxic has two metabolites which are not hepatotoxic, at least in vitro. And what you can see is in primary rat hepatocytes the M5 metabolite, even at high doses, does not seem to have a cytotoxic effect.

This is also seen in primary human hepatocytes. He has actually expanded this to looking at the effects in induced pluripotent stem cell cardiomyocytes and seeing exactly the same type of pattern. It’s very exciting because the data submitted for the IND package for these clearly says that these metabolites are pharmacologically active. So we’re very interested in exploring these, perhaps as indeed a new or potentially effective and nontoxic or reduced toxicity compound.

Now I mentioned about our exploration of induced pluripotent stem cell derived cardiomyocytes. We have a collaboration as I noted with the Medical College of Wisconsin. They have received a grant to work with Cellular Dynamics to generate 250 stem cell lines and cardiomyocytes from a whole cohort of this HyperGEN family blood pressure program, where you’ve got both ethnicities in terms of the population and complete phenotype and clinical background for these cells. We’re going through a variety of cell lines and a variety of tyrosine kinase inhibitors to explore the effects of individual cell lines on the sensitivity to TKIs.

What is actually I’d say pretty phenomenal is in fact that you see differences between different cell lines, and it depends upon the drug, such that with

vandetanib you see that of these six lines there's a pretty consistent response of all the cell lines. Not so much with nilotinib. You can see the three cell lines are far more sensitive to nilotinib than the other two. This actually highlights I think an incredibly important question as we move forward in moving to in vitro human based systems, which human do you use.

We perhaps don't always think about that when we use animal based in vitro systems, and it probably is true even there, but it drives to this question, when we consider that we do animal experiments, usually there are a certain number of animals, and you look at them as a group and as individuals.

When we use in vitro systems rarely do we address this very important question of are we dealing with one individual or are we dealing with a representative population. So I'm very excited about this work and how it's progressing, because I think it has great impact on our move to exploring in vitro systems.

I mentioned about the model for delayed onset cardiotoxicity. And this is a case where in fact it's well known you can have patients treated with anthracyclines, get complete remission of the cancer, and then months,

years later, start to show elements of heart failure or reduced cardiac function.

So in this case what you're seeing is we're stopping treatment but then examining the animals at time points afterward. And here is an example where in fact by using ultrasound to measure left ventricular fractional shortening, you can see the effect after the exposure is stopped. We're exploring that so that we can look for biomarkers and/or individual sensitivities to that effect.

Along those lines, let me point out we are also working with clinicians at the University of Arkansas Medical Sciences to look for biomarkers predictive of treatment induced cardiac dysfunction, and this is in the clinical setting.

Here you had 100 patients with four cycles of cyclophosphamide, and a blood draw before treatment and also after cycles two and three. And the left ventricular ejection fraction was mentioned two to three weeks after the last cycle, and then you group in terms of the patients by whether they had normal or abnormal LDEF.

What was used to look for putative biomarkers was an apatamer based proteomic approach, and what you're looking at here in terms of potential putative plasma protein biomarkers are proteins where their levels were

different before treatment. So this is actually an interesting aspect of not just what happens early in treatment, but are there patients predisposed to the cardiac effects of the anthracycline.

Now, I mentioned about the technology to pick up adulterated drugs, and this just came out of some of the technology development within the division, and this is a collaboration actually with the St. Louis CDER Office of Testing and Research.

In this case, let me point out that what you have here is sibutramine, which is an appetite depressant, it creates a decrease in calorie intake. It's found in weight loss products, but it is not approved by basis of its cardiovascular side effects. Nonetheless you see it in things such as herbal products. In this case it's an all-natural herbal supplement.

The important piece of this technology is that it's a small mass spec. It would fit on the top of this podium, it would effectively be portable in the back of a truck, and the second piece of it is it does not require sample purification. You can put on creams, you can put on crushed pills, in this case. So it really does represent a way to look at crude substances. In this case you can see it picking up the sibutramine in the pills.

And we also have been developing a novel molecular modeling approach that was basically invented in the division, and applying it in this case to a whole library of opioid ligands. Now, in this case what we're doing is we're modeling the binding, high affinity binding, to all three opioid receptors.

There's a lot of work done on the mu receptor, which is MOR, but we're also looking at the kappa and delta opioid receptors. This is the first model to look at affinity to all three receptors, and it shows some characteristics for both individual receptors and for the group of the receptors.

Also in terms of the opioid work we have plans to look at the effects of opioids in brains of rats that are addicted and those that are not, using this rather novel tissue mass spec imaging approach, the multi-imaging that I think other presentations have referred to. So the really cool piece of this technology is you can take a tissue slice, and by basically using a laser as you raster across the tissue and blast the tissue, you can look at a whole variety of metabolites in the same tissue slice.

You get a mass spec spectrum, and what you pick out then lets you look at the different metabolites. So in this case we're looking at different endogenous

neurotransmitters, and our goal is to examine the distribution of those neurotransmitters and the opioid metabolites in different animals. Now you can also apply it as you can see here into zebrafish, the whole zebra fish, and in terms of evaluating PK/PD in lung tissue.

So our future directions, we want to continue this investigation as I noted about the mechanisms of opioid addiction using the technologies I discussed. We want to continue moving what we've seen in vivo, that is with the doxorubicin cardiotoxicity biomarkers, and verify that in a clinical setting.

We want to expand the application of the technologies that I noted, the spec-ID, the rapid mass spec identification of product adulteration, the MALDI-imaging I just showed, and the metabolomics and lipidomics platform. We want to further explore the in vitro technologies I've also referred to, the human and animal IPSC derived cell models.

And I've noticed our interest in moving into microphysiological systems, and our interest in investigating some of the more cutting edge therapeutics such as the oligonucleotide associated compounds, in this case thrombocytopenia is seen with many of the oligonucleotide therapeutics.

So my questions for you are for the approaches we are currently advancing, are there other areas you might suggest? What developments that might impact FDA are we missing? And in addition to our current efforts, what approaches might better inform FDA research needs at other centers? And we continue to try to reach out to other centers to try to I would say identify, compile, and centralize the research needs of FDA reviewers. So with that I'll open for questions.

DR. ASCHNER: Any questions from the SAB? ??

DR.STICE: Very nice presentation, thank you. I guess a comment on the systems biology in general, and I think you're taking this approach, and I guess it relates back to one of the things that was mentioned earlier, like the TBI model.

If you know the mechanism and when it's occurring in the clinical setting, and you can translate that back to an animal model with the same mechanism, you can then potentially translate it back to a microphysiological system or a cell based system. So if you have that continuum all the way through, those are going to be where you have the most benefit and most impact I think in the future. So I think you're thinking

that way, but I just wanted to reiterate that type of thought process.

DR. MATTES: that always is I would say the important question, how do you take one model and connect it with the ultimate end, or back connect it, because there is that opportunity to say well you see this in the clinic, what do you go back to in terms of your model system.

DR. STICE: And the other comment I had on the donor variability, or question more so than a comment now. So are those multiple lines from those donors that were derived on different days? Because I always fear drawing too much conclusion from one cell line, from one donor.

DR. MATTES: Is it derivation or is it actual individuals, absolutely. In this case there were single lines. You'll note that cellular dynamics, it was the organization deriving them, and I'd have to look back as to what criteria they used to decide which of those, which line they provided from each individual.

But it is I will say it's an open question we have not addressed. What is interesting is that you see a whole series of cell lines respond similarly to one drug but not to another. So it's still open to the question, but it says that there are some similarities still there.

DR. SAUER: Bill, you showed a lot of great research, a lot of great science there. How are you going to transform, what's your strategy for transforming that great science into tools for regulatory decision making? Because that's what you're on the cusp of, right?

DR. MATTES: In some respects some of this is. I think this has been discussed in the past, it's not entirely we develop the tools. For instance, microphysiological systems. Are we developing that for use in regulatory decision making? Not necessarily. What we are doing, and NCTR and actually throughout the agency, is become familiar with it so that when we see a submission we know what's going on. So there's the dynamic between developing tools adding to the understanding of how a tool works and also maintaining the internal knowledge base to be able to help review when those tools come onboard.

DR. LANZA: Very nice presentation. I wanted to go back to the cardiotoxicity issues, because internationally this is becoming greater and greater an issue of concern because we want to manipulate the therapy as you mentioned while we can, not after it's too late. And also it's an opportunity from an imaging standpoint potentially but also with biomarkers to try to even use

them as endpoints, whether it’s for clinical trials or for making judgments on drugs and so forth.

So in that context, one of the things I wanted to bring up is that of course there’s a wide group of drugs you should consider besides anthracycline, but even in the case of anthracycline, have you considered looking at this with iron chelation therapy? Because now we don’t really give anthracycline, even particularly in sarcoma, without support of iron chelation therapy, which is mollifying much of the cardiotoxicity. Its iron is critical in the metabolic.

And the second question is the doses, in the normal situation with cardiotoxicity the changes in the heart, the biochemical and so forth changes in the heart occur first, and then you might get strain, but then it starts going down. By the time you get an elevation in cardiac enzymes like BMP or pro BMP, it’s already down to function, and then you get to the functional changes you’re talking about.

So one of the things I wondered if you considered going earlier in the timeframe so that you’re not treating them and getting already a fractional, but using strain imaging, and/or adding cardiac MR at high field to look at changes in how much inflammation there is

going on and so forth. Because I think these are going to be regulatory endpoints decision points, clinical endpoints in the future, and I wonder if you can address it. And I didn't see it on your futures list.

DR. MATTES: When I think about your question and I think about the big picture of how sort of the experiments have developed, and I'll just say this classic case of moving backwards from clinical understandings to look at what's going on with your nonclinical model. So the table I showed of putative biomarkers were biomarkers, quote proteins, whose levels were different before treatment.

So they were presumably identifying sensitive patients, and while I didn't put it up there, there was a publication, we have a publication looking at cytokine levels, and there are three cytokines that are quite different before treatment in those patients that end up having a reduced LDEF later on. The really intriguing concept is that in fact there may be perhaps inflammation differences prior to treatment that predispose the patients to problems.

And what I mentioned about going backwards from the clinical to the nonclinical, so our problem is if you've got a mouse model, nine times out of ten folks are

not going oh let's see if we can get a little blood before you treat them, but that's exactly what we need to do.

But we're following up with a larger study in terms of the biomarkers I showed as well as the cytokines. But it's getting to, I'm not sure if this is completely addressing your question, but I'm looking at the data and thinking more along the lines of that there are groups sensitive to treatment before it even starts.

DR. LANZA: I appreciate that, that's risk stratification for treatment. But what I'm talking about is patients who are coming, they're getting standard of care, and there are two things about it, is that from a regulatory standpoint people don't want to have to wait for survival or heart failure long after.

So looking for endpoints for two reasons, one is to determine if there's efficacy in the treatment or toxicity and make decisions based on it. And I think from the regulatory standpoint this will come forward. Now in the past this was done with cardiac echo in patients, and the truth is that it's insensitive due to variability. So they really don't show, even though in the great case you can show it doesn't work.

So now it's shifting to cardiac MR. But I'm just thinking along the echo data you were showing that even if

you restratify the question I have is the reason you have these cytokines because they already have inherent strain differences, and so that now these are vulnerable hearts, you're measuring a blood thing, which is good, what's the mechanism from the cardiac mechanical standpoint, that's all. I just want to endorse doing more work on this because this is really from the clinical side, but also from a regulatory standpoint, is becoming more important.

And the simple reason is the therapies are getting better. Even the anthracycline, I mentioned, iron chelation therapy allows them to use 1000 milligrams per meter squared of doxorubicin. That would be unheard of. So they're surviving better, although not forever with sarcoma.

The point is that now we have to start thinking about the outcomes, not do they survive, but look did I send home someone who we killed your heart, or now you have cisplatin neuropathy and you're shaking like a leaf and you can't do your job anymore. It's incumbent upon us and the FDA to help to work towards now dealing with people who are surviving as cancer moves from the acute and I survive to chronic disease management. This is what I'm doing, I think you're on track with it, but I just want to endorse it more along that line.

DR. MATTES: And from a regulatory standpoint I think there now is that ongoing discussion about okay, something comes in, we've got a really low bar for approval based on toxicity, and then you get it into the clinic and you see what's going on. But at what point do you start to say well maybe I don't need to dose at NTD, maybe I actually want to take into account the toxicities and how do I monitor that in a clinical setting, which is exactly what we're trying to come up with the biomarkers to better assess that.

DR. LANZA: One of the big questions is in many cases I see the change, how chemically did it happen? Looking at phenomena. But you're able to look at in detail what's the underlying mechanisms in the systems biology from the small to the end, even in a mouse, that's my point.

DR. GANEY: Thanks for that Bill. I have a follow-up to John Michael's question because I noticed that the titles of one of your most recent papers, it's not a bullet in case you're wondering, read something about reporting standards.

So in the event that your research leads you to, because you have a lot about different in vitro models, predictive models, in silico models, if you come up with a

model that you think would be useful for regulatory decisions, is there a process prescribed for validation and everything else you would need to be able to make that useful to people?

DR. MATTES: Such as if you have a model or a model system or even a biomarker, right? Suffice it to say as john Michael knows I'm a little familiar with the biomarker program, and 21st Century Cures Act really codified exactly how that would approach.

And people hear about biomarkers going through that system, but in truth animal models, and perhaps even, I mean it hasn't been discussed, but my bet is a computational model will probably go through the same approach. In terms of the stuff that we're working on, I sort of had in the back of my mind at what point do you actually put something together and start cranking it through the system. The precedent is actually out there before.

An FDA employee, Jim Weaver, who was working on the vascular injury biomarker working group, and he had all kinds of data on it, and when it came to putting that through the biomarker qualification system, he was firewalled out of that. So it can happen, we can put something through that process.

DR. SAUER: Just for a point of clarification, Bill, qualifications evolved as you know very well. When we put computational models forward we actually use a system called Fit for Purpose, which is still with OTS through Office of Clinical Pharmacology, and so that seems to be the simpler path and the desired path, at least for CDER at this point.

DR. FELTER: So my question slash comment has to do with your last question to us about possible opportunities for expansion beyond the very strong connections you have with CDER. And it comes in part because of the example you gave looking at the contamination of the Chinese herbal medicine with the appetite suppressing drug.

So I think it was earlier this year FDA announced the formation of, I'm not sure if it's called the Botanical Safety Consortium, and it seems like some of your techniques may be highly applicable to the goals of that group as well. And I don't know if you've established connections in that space.

DR. MATTES: The simple answer, we haven't yet. The context is this is relatively new, this data has come up over the last six to eight months, so there's lots of opportunities.

DR. STROMGREN: I just wanted to add on to that question. We do test for illegally spiked APIs in various dietary supplements, natural botanical products. We have some portable instrument platforms, ion mobility spectrometers specifically, have been placed at some of these points of entry. So we are screening for those.

Essentially, we call it the Health Fraud Program. We are finding a lot of illegally spiked APIs, the violation rate runs around 40 percent, which is one of the highest we see. So we already have these efforts, working with CDER as well. I've talked with Bill yesterday, I'd like to learn more about the SPARC MS as well, see if we can expand those efforts. But that's one of our programs that has been very successful when deployed at point of entry basically.

DR. FITZPATRICK: Yes I think Gonzalo has been attending all of the botanical consortium or many of their meetings, and as you know PMG is really involved in it, and just announced, with HESI as a convener, we're all really excited about it. And of course, we would have NCTR because CFSAN looks very strongly to NCTR for most of our research, and they're very good partners.

DR. ASCHNER: Any other questions or comments? Okay, we're slightly ahead of schedule, so I think we'll

just take half an hour and convene early. So please come back at 10:35.

(Break)

Agenda Item: Discussion of NCTR Research

DR. ASCHNER: Okay. So I guess over the last couple days we've heard all the presentations. We're at the point now where we'll ask the Scientific Advisory Board to provide some feedback. This is scheduled to finish at 11:30, and then the SAB will stay here with Dr. Slikker and Dr. Mendrick for closed session.

So the purpose of this is just to provide I guess some feedback to Bill and everybody else who has attended the meeting over the last couple days, and I'll ask the Scientific Advisory Board, we'll go down the line, so I'll start with Greg, just please provide us with your impression of what you heard, suggestions for the future.

DR. LANZA: I'm going to really make two. One of them is a comment, because I've been fortunate enough to come here a few times in the past, and I wanted to know how this meeting showed so much more collaboration coming from the different centers and within the NCTR than was the case it would have been three years ago. And so I wanted to formally congratulate Dr. Slikker, but he can't

do it on his own wishing it, and you are engaging this incredible facility and research capability.

And the only other point I wanted to make is one that was made in several of the talks yesterday. It involved Dr. Tong as a great resource, tremendous productivity. I think that it has a major role in many different things, and not just streamline, not just patent recognition, not just allowing you to have better workflows or so forth, but also in toxicology, in being, if you can get the full truth between those that went in, what their treatments were and what their outcomes were, and not on the good side but also on the adverse, then with AI and a way that you can't do with statistics you can start to predict what were the early markers that predicted that.

And I think that if the NCTR and the different agencies can pick a few areas to start in and work on then the potential of AI to enhance toxicological assessments and predict outcomes, because as we just heard from Bill the drugs you've given them and now the problem, the problem is the patients are living, and before they just died and your problem died with them, and AI I think is one of the tools that needs to be exploited.

And I have a document from CDRH if anyone wants it, but it talks about the change in the perspective about AI because it can be adaptive, and I recommend everybody to look at that. I already talked to Donna, she has it. So if anyone wants it she can give it to you. But it's a situation where you've got a program that you're going to approve or you're going to use, and it's going to get better and it's going to change, but that can be good.

So the question is how to embrace it rather than waiting for it to progress way down and being well behind I think. NCTR is in center where they interact with all of you, and I think from that standpoint it's an opportunity to become leaders, not only in the US by bringing the right people in to help you from a regulatory standpoint, but leading worldwide. That's all I've got to say.

DR. KASPAR: I'll primarily limit my comments to the microbiology section. I think they're focused on key areas within microbiology, both at the present and in the future, namely antibiotic resistance and bacteria. This is a very important problem, we were discussing it at dinner last night.

The second is microbiome, I'm not sure we all know all the things, the potential of understanding the microbiome and working out those networks and the

organisms or the absence of organisms, what all the potential impacts they can have. So I think that's a central and very key area as well as the CDIF and the fecal transplants, these are all things a lot of people are working on, I think these are our key areas.

My other general comment would relate to recruitment. We now have a number of industries that are coming to UW Madison to seek our graduates, and I think maybe that would be something a select few employees would start identifying key universities that are strong in the potential areas you're looking for, and then start recruiting at an early level. We have both recruitment taking place at the undergraduate level, as well as our graduate students and professional schools, as well as at professional meetings.

DR. FELTER: I'll start by reiterating what Greg said, I found the scope, the expertise, the impact of the research, the collaboration to be really outstanding, and I really appreciate the opportunity that we've had to learn more about the research ongoing here. I will take a minute to mention something I heard yesterday that we didn't have a chance to follow up on, and I suspect NCTR is already working on collaborations here, but there was a comment that change is in the direction that NTP is going

in and their research may have some impact on NCTR, and it may impact different divisions differently.

There may be a negative impact on research that was done in the past, but new opportunities for new research streams, and to the extent that that collaboration can be made really strong I think would be very helpful, because we see a lot of things changing. I mean EPA also just announced that their goals of moving away from animal testing, and I think much of the research we've heard has applicability in moving away from animals as well and using more in vitro and organoid technologies and things like that.

The one area that worries me a little bit was some of the new alternative methods that are not animal based methods has to do with, and I mentioned this yesterday, the challenges that we have in understanding appropriate dosing and how that relates to human exposures and/or rodent studies that we're trying to link it back to.

So I see that as a really significant challenge but I know NCTR has really led the way for some of the research showing the importance of non-linearities and toxicokinetics with rodent studies, and I think we're

going to see the same challenges with non-animal methods, and there's certainly a lot of work and opportunity there.

DR. STICE: I thought the format this year was really great to have the centers present first. And I know a question that we always have had is how much impact there has been on the centers from the research that's being done here at NCTR.

So I think that was covered really well, and I see a really strong connection with all the interaction between the centers and the research that's going on, so I appreciated that. From yesterday it helped me in not having to ask certain questions that were obvious to many of you here and helpful. So I think that was really great, and I encourage you to do that in the future as well.

The other thing that I think stuck out for me in just a general way is this whole thing on metrics. I know publications and presentations you can count and they're really important, but I really liked the presentation from ORA on the impact factors, and having those five impact factors, something that maybe in the future we can get more reporting around the actual impact beyond publications and presentations and what might happen as far as validated methods that might go through the FDA or so forth. I think that's an area that maybe in the area we

can use more information around, but really stellar research, great work that's going on here, and a lot of collaboration and cooperation between the groups.

DR. COSENZA: So this was my first SAB. So I will first say that I'm really impressed with the presentations both from the different divisions of FDA as well as the NCTR presentations. The science is impressive, and their collaboration is much more extensive than I expected, so I'm very impressed with that as well. I have a couple comments which may come from my naiveté being that this is the first time that I'm here.

So I'm struck by, I don't really completely understand how work is prioritized across the center in general or across the FDA overall. So a couple of key topics that came up over the two days, tattoo ink, CBD, vaping, these are all obviously big important public health issues, but it's not really clear to me how they get prioritized across the different groups here at NCTR or overall. So again, that may be something just that I'm still learning the process, but maybe that would be helpful we could discuss.

And the other thing was I think it was in September I watched remotely from home the FDA roadmap day, I didn't catch all of it just because it's hard to

sit eight hours in front of your computer remotely, especially when I'm in California, so it started at like five in the morning for me. But I was really impressed with that as well, and I guess Bill you mentioned it a little bit, some of that during your presentation, but it would be nice to see how some of that relates to some of the other work done at NCTR maybe in future presentations. It was great overall, thank you.

DR. SAUER: So first of all the center talks this year were great. I really loved that you guys laid out really what your objectives were and your needs, and then what I loved was that the NCTR divisions were able to also capitalize on those needs and show where they were filling in the gaps.

So as was said, incredible science going on here, there's no doubt about it. Really exciting, really quality science. I think during some of the presentations I thought great science, but where's the regulatory outcome, what are you trying to do with the science? I think drawing that line across every example is really important.

I know that many of you went ahead and did that across your divisions, and I thought that was really good, because then I could go okay, here goes the applied nature

to the science. Sometimes that was lacking, but I know that answer is there, you just didn't draw the line for me.

The other thing that I was thinking about during the conversations over the past day and a half is great research group, but where are you guys differentiating yourself from other great research groups? Where are you taking advantage of the fact that you're the FDA, and you have information, access to data that other research groups really don't have? And so how do you take advantage of that? I think that's just an overall question.

Again, I know there's limitations and I know there's rules, but I think there is an opportunity here that you may be able to seize on that would really differentiate what NCTR does compared to some high quality academic institutes and other research institutes.

One thing that I was thinking again, and this is a little bit drug centric, but I think there's incredible opportunity with different therapeutic modalities coming out such as gene therapy, such as cell therapy, where right now to be truthful the safety assessment landscape is kind of the wild west.

And so a group like this, being so close to CDER and CBER, should be able to help to, if you will, begin to

lay out a roadmap for scientists to be able to follow to get through some of these yet undefined regulatory hurdles, and to really define those. Because you think about it, these therapies, supposedly lifelong durable therapies, lifelong durable safety issues as well that we don't understand right now. So how do we get our hands around that? So just a couple ideas but I think overall a great day and a half.

DR. GANEY: Since I'm at the end of the table I could just say I agree with everything that's been said so far, and in principle I do. I too am new to this board, and I really appreciated the opportunity to be here to hear everything that is going on, it is amazing, it really is quite amazing and impressive, the projects you've chosen, they're contemporary, they're important, and you're really applying excellent expertise to them.

Like John Michael there were a few times when I thought what's the relevance to toxicology, it didn't come through, I was sure it was there, and it might just have been a matter of presentation, or it could have been in several of you said we've talked about this before, this is my first time, I hadn't heard that. SO it could have been in presentation, but I think it's important to always bear in mind you're at NCTR and this is your mission.

The other thing that kind of came through from several people is since you have this immense network of collaboration, the communication difficulty, and I was trying to draw the matrix in my head of all the groups and all the projects, and I didn't get very far, I'll just tell you that.

But perhaps that's what you need, sort of a living matrix that's web based that when you come up with an idea for a new project you can ask yourself let's go to that matrix and see if someone is working on anything relevant to that, or using techniques that would help me address that question.

So I think that maybe trying to capture that communication aspect could really help you be more cohesive as a unit and capitalize on everything that you have around here, which are amazing resources. And other than that, I do want to say thank you for letting me be part of this effort, it's been a lot of fun.

DR. ASCHNER: So I'm in the middle of the table, and I can say the same thing. But I have a few comments. I've been on this committee actually I think about 10 years ago, and I was recycled back a couple years ago, and clearly there's a huge difference as Greg mentioned from two years ago, and it's day and night compared to 10 years

or so. So I think you're on the right track. And I enjoyed hearing about the research that was described today.

One comment that I have is I think it would be better to have more clarity on how you pick some specific studies, and base it more on the questions that you want to ask, rather than necessarily on the methods. There's a lot of new emerging methodologies, and we've heard about some of them today and I'm sure you heard about them yesterday, but I think the methods should be more in support of the question that you have rather than to drive the science. So that's one comment that I would like you to consider.

The other one is what I mentioned earlier, I think there were some emergent issues in terms of public health, vaping, and probably we could come up with some others which we're probably not allowed to talk about, but I didn't get a sense over just today, this morning, about how these issues are being dealt with and how they're being prioritized in terms of are we going to move forward with studying vaping or not. Other than that, considering the size of the NCTR is actually pretty amazing how broad the research is, I think it's quite amazing.

You can do everything with the personnel and the facility that you have, so I think it's very important

obviously to focus on important issues, and I think with the leadership of Bill you've certainly been able to do that. So that's basically what I have to say. I don't know if we open the floor for comments or not. So we go down the table the other side.

DR. LAL-NAG: I'd like to sort of reiterate what Mary Ellen, John Michael, and Patty, you were saying. From CDER's perspective I think the focus on extrapolating from in vitro based models, so focusing NCTR's huge focus and building physiologically relevant in vitro models, and that speaks to the point you were making earlier Greg on extrapolating that to in vivo, so that we're able to in the data coming in see what phenotypes actually are recreated in vivo so you can actually develop those predictive algorithms to make those predictions.

I think that's tremendous,and seeing that in the context of regulatory science research, and not just from a basic research perspective, I think is tremendous, so I applaud that initiative, and I look forward to collaborating with CDER more in that space.

DR. MELLON: I also appreciate the breadth of work that NCTR has been doing, and the group that I work in, which is in association predominantly with the

neurotox division, has benefited tremendously from the work that NCTR has done.

We have leveraged the information to impact labeling, we have leveraged the information to help direct additional resources in trying to find ways of solving very challenging problems that we cannot necessarily obtain from some of the sponsors who may be coming in with applications to CDER. The unique aspect of NCTR is that they have this wonderful resource, this incredible amount of knowledge and information that they can apply, that really can help address questions that we cannot obtain in other manners.

And because of that they play a very vital and critical resource to CDER. The work that it has done, I do agree, one of the biggest challenges probably, and listening over the last two days that I think everybody has heard very clearly, is trying to make sure that we have that opportunity to really leverage and make those connections between the various centers with the expertise in NCTR.

I think we've done a pretty good job of that, but I do agree that there's a lot more that can be done, and we've been having some sidebar conversations in the hallway about trying to strengthen those ties and gain

greater knowledge and awareness of the resources that exist, so that individuals either in White Oak or in College Park, wherever they may be, know where resources exist so that we can understand that potential for even greater connections and trying to leverage the information that's available.

And I think that hearing that, hearing the understanding of how it's very important to be able to tie in the research to help inform the needs of the various centers, when you think about it it really is a bit of a monstrous task for NCTR. They have a large number of other centers with a vast amount of interests, and yet they do seem quite capable pulling together and trying to prioritize and come up with ways of helping to solve the various centers' needs. So certainly, from a CDER perspective, from somebody down in the trenches, it's a tremendous resource, and we can't thank them enough for all the efforts that they do.

DR. STROMGREN: Likewise, I echo my colleagues, it's been a very valuable day and a half for me, getting to know how the Science Advisory Board operates, also getting to know NCTR colleagues. I'm based in Rockville, Maryland, I don't really get to see all the NCTR folks.

My NCTR sphere is usually just Donna, Dr. Slikker, and Anil, and I've made a lot more connections here by just being here a day and a half, and that has been very valuable. I did want to comment on a couple of very good points raised by our advisory board members. The first one is how do you connect it to a regulatory outcome. That's always the first question we are ORA ask when we look at our research proposals.

Again, our research is very different than center research in that it's very applied. But that's what it's driven by, precisely what regulatory need are you addressing by performing this research. For us, and I tried to present that, we developed some questions we ask to the submitters to make sure whatever it is they're going to work on we can use it to advance the agency mission.

Regarding the question on priorities and how the agency grapples when we have equally important things happening that's under FDA preview, vaping and opioids, right now those are the two biggest ones at the agency level. This was the main impetus really behind the ORA recent reorganization which I alluded to a little bit during my presentation.

So in 2017 we reorganized our laboratories to be specialized along product lines. So prior to that they weren't. So we could have the same lab, for instance our Arkansas laboratory performing testing on food. Again, our testing and assignments, they come from the product centers, because that's where the expertise resides. They know what risk aspects of their products they want to look at. So we get those work plans and assignments from the centers. But it would be the same laboratory performing tests in foods, looking for maybe pesticides in foods or microtoxins.

Also the same lab performing CDER assignments, looking for impurities in drugs maybe. But again, this sort of created this strange situation sometimes, two different centers would issue equally high priority assignments, and we would run into issues of how we dedicate our limited resources, do we do this assignment first or that.

So by specializing our laboratories around product lines we've sort of separated the workforce, so now we have dedicated lab personnel dedicated to drug testing, food testing, and the same on the inspectional side, dedicated inspectors that are drug cadre, tobacco cadre, so we don't ever have to make that decision when

centers consider something equally important and they issue that work we have the dedicated cadres who can work on that simultaneously. So at least that's one approach at the ORA where we are sort of carrying out that end mission, how we've tried to address that. Thank you.

PARTICIPANTDR.VAN BEMMEL I will echo just the comments from my other colleagues here from FDA that I do always enjoy coming down for the advisory board meeting, I enjoy my time here at NCTR talking with our colleagues on the current projects that we have, brainstorming about projects that we might be able to start working on for the future, and also learning from my colleagues at other centers on the research programs and resources and how they're not just, the types of research that they're doing, but how they're implementing their research and tracking the research, I think there's lots of opportunity for learning there, and I always appreciate that.

Micki, I will just take a minute to address your comment about vaping. I know you weren't able to see our presentations yesterday, but as part of those presentations I did include some future discussions. We do have what we consider three buckets of research with three facilities of research here with NCTR, one is inhalation work that we do in animal models, one is the ALI work that

we're doing, and then the risk modeling work. And we have started discussions well before the immediate response activities that were happening actually to be looking at electronic device and aerosolization toxicity as a whole byproduct and also specific constituents.

And so we'd been having those conversations before, and then over the last several months there have been definitely increased conversations across the agency obviously with the immediate response that's happening across the agency, across the federal government and with our state and local partners. So that's been an ongoing conversation that I didn't dive too deeply in here, but it's definitely something that we're looking at.

DR. WILSON: I want to also say likewise, this annual visit is a great opportunity to learn more about what is going on in NCTR and to network with NCTR and other center colleagues. It's kind of sad to say that there are certain individuals that we actually only see when we're down here, even though we're all part of FDA.

It is kind of nice to reconnect with our center colleagues as well as NCTR. I wanted to acknowledge also the work that Donna does on a daily basis to try to network with the centers and create awareness about NCTR

capabilities and hear from the centers about their needs, that that's a great forum for dialogue.

But I do think that coming down here once a year and doing a deep dive and hearing from the division directors is a different flavor, and you get a lot more granularity obviously. I will say I've also been coming here for I think at least ten years now, and that I've seen a huge shift in NCTR's willingness and capabilities to help address the Center for Biologics needs.

As some of you noted even just in the last several years that the number of collaborations with CBER have really increased, and I'm grateful for that. I think as noted there are probably more opportunities for expanding some of those collaborations. I'll just mention one that actually thanks to Bill's presentation I didn't realize the really interesting study he's done on plasma, done a baseline of looking at a whole variety of different analytics and getting baseline metrics on that.

For us that's a really important baseline, because we're now looking at pathogen reduction technologies and how to improve them for improving the safety of blood. This allows us to have some additional multi-parametric evaluation of plasma before and after

PRT. So just hearing that is one example, it's really exciting.

The last thing I will just finish on saying is that I think for us a challenge continues to be the communication piece. Aside from sort of the overall identifying opportunities for collaboration, but even then once collaborations are started to make sure that there is communication on a regular basis between the NCTR subject matter experts and the center subject matter experts to make sure that the projects are going in the right direction, that the design is really addressing our needs, and so on. So thank you.

DR. BRAUNSTEIN: I don't have too much more to add other than what's been said by Carolyn Wilson from CBER, and my other colleagues. This is the first time I've been down to NCTR, and I've really enjoyed getting to know more about how NCTR works and how it interacts with the different centers. I loved hearing about all the different projects that we have going on, and it has been really helpful for me to put faces to names of various people that I've worked with. So I have really enjoyed my time here, and I just wanted to say thank you.

DR. FITZPATRICK: I deal with all the research that comes down to NCTR from CFSAN, and we do most of our

research here. In fact, I know more about how the research is going here than I do in my own lab in Laurel Maryland, which is not a bad commentary about our lab.

But we just got a note from our people here that the research that NCTR did helped us work on a brominated vegetable oil issue that's been an issue for like 30 years at CFSAN, and because of the research that they did, and sponsored by the National Toxicology Program, we're able to do something about that issue. So I think that everything that we do is great. They keep us well informed, we talk about all the protocols, it's just a really good organization and a really good treasure for FDA.

DR.WHITEHOUSE: This is my first time here as well, and I really appreciate the opportunity to come and meet folks and see what everyone is doing here. I think for NCTR, awareness is key, and meetings like this are really critical to foster that interaction among folks.

I mostly come from a microbiology background, and I know we've had a very strong interaction with folks here in the microbiology area, and I anticipate that continuing, but I have got some other ideas for other areas within veterinary medicine that I think we can pursue and try to make those collaborations as strong as

the microbiology area. And just a couple things I've written down here, areas of shared interest, stem cells, nanoplastics and fish, and possibly the issue of CBD in pet food and other animal products. So I look forward to continuing to work with folks here on those issues. Thank you.

DR. SLIKKER: The main thing that I want to say is really thank you, because what I've seen here is a dedicated group, not only of representatives from the various centers of FDA and ORA, but also of course our staff that come here to help us make these important decisions because of their knowledge and background. I mean having the SAB members here obviously is a critical part of this. And also support from our Chief Scientist Denise, the leadership here has been fantastic to bring people together.

I think the main thing that I'm learning, I made copious notes from your comment and from earlier comments as well, is that we're making progress, there are still things that we can improve upon, but I think the ability of NCTR scientists to interact with scientists throughout the agency has ever increased because there is an openness, and there's more trust, there's more

opportunity. These things have all made this possible, that we can build this together.

And I really appreciate the leadership from Greg and from Micki to move this program forward. And also to our own staff here, Karen, Kim, and Jeff, who have been taking care of details at all the steps of the way, and then of course to Donna who has organized this as you know as part of her position description, but she takes it well beyond just the job, she takes it to a whole new level of wanting to make sure that things work very smoothly and that everyone has a chance to participate.

So I think all in all I really just want to talk about thank-yous, and some of you folks, I'm thinking about Carolyn, have been here for many years, we really appreciate that, because we know we can go to Carolyn to get information, not only dealing with CBER but with the FDA in general, and we've done that multiple times, and we really appreciate your efforts at making that happen. And many others that are returning, and then new individuals.

It's really great to see that individuals that haven't been here before were trying to range opportunities, we actually visit the site, the NCTR itself, and Jefferson Labs, and see the ORA labs right next to the NCTR labs, that also will bring more

understanding about how we interact and what we can do with each other, because you'll see the actual facilities along with meeting more of the staff.

And finally, I want to thank the staff from NCTR. I think the presentations were crisp, they were on time, they were full of content, and they were able to answer a lot of your questions. Those that we weren't able to answer we will get back to you with, we will get back with answers to you, but you raised some really good interest areas, not only for concerns of making progress, but also in congratulating those folks that have made good progress in the past.

And then finally, I want to end with this whole idea of the pipeline of regulatory scientists. I think that FDA really depends on well trained regulatory scientists, each center helps contribute to the training, but this has obviously been a real key issue for NCTR over the years, is training a regulatory scientist for FDA and for other groups that also require that kind of trained individual.

We want to find out more ways in which we can recruit individuals and make sure that we can train the best and brightest and move forward and be an asset to FDA and other federal agencies, industry as well as

universities. So this is a big issue, and I think we should have more discussions about this so that we can learn from all of you who are also recruiting in many cases how we can improve that particular opportunity.

So those are my comments, and again thank you to everybody, and for those that are traveling back I wish you safe travels, for those that are visiting NCTR I hope you enjoy that, and I know we're going to have of course a continuation of an in depth look at Neurotoxicology next, but I really appreciate all those that have helped prepare for this meeting, so thank you all very much.

DR. ASCHNER: Thank you. I'll just reiterate, thank you to the advisory board, Donna and Kim for putting the meeting together, to all the staff at the NCTR and the representatives from the different FDA centers. I'll stop with that, there's no sense in repeating some of the things that Bill just mentioned. So safe travel and look forward to seeing everybody next year.

(Whereupon, the meeting was adjourned.)