

National Center for Toxicological Research

Science Advisory Board Meeting

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P R O C E E D I N G S

Agenda Item: Welcome and Overview

DR. LEIN: Good morning everybody. My name is Pam Lein from University of California Davis. I will be chairing this session today. We are officially starting.

Before we get into the deliberations for the day, I would like to just have everyone go around the room briefly, introduce yourself, and tell us where you are from. Bill, do you want to start? We will go around the table and then to the back of the room.

DR. SLIKKER: I am Bill Slikker, director of the NCTR FDA.

DR. ACOSTA: Dan Acosta, deputy director for NCTR.

DR. BACKINGER: Good morning. I am Cathy Backinger. I am with the Center for Tobacco Products at FDA.

DR. DORSAM: Morning. I am Bob Dorsam from CDER FDA.

DR. WILSON: Carolyn Wilson from Center for Biologics.

DR. MARGERRISON: Ed Margerrison from CDRH FDA.

DR. LANIYONU: Adebayo Laniyonu from CDER FDA.

DR. GRAHAM: John Graham from the Center for

Veterinary Medicine.

DR. HATWELL: Karen Hatwell, Center for Food Safety and Applied Nutrition, FDA.

DR. LINDER: Sean Linder, Office of Regulatory Affairs, FDA.

DR. SAUER: John-Michael Sauer, the Critical Path Institute. I am the executive director of the Predictive Safety Testing Consortium.

DR. JAIN: Diwakar Jain. I am a cardiologist at Westchester Medical Center, New York Medical College in New York.

DR. ASCHNER: Michael Aschner. I am at Albert Einstein College of Medicine in the Department of Molecular Pharmacology.

DR. LANZA: Greg Lanza, Washington University Med School, Cardiology, Physician Scientist.

DR. FELTER: Susan Felter, Research Fellow in Corporate Product Safety at Procter & Gamble.

DR. PILLAI: Suresh Pillai, Professor of Microbiology at Texas A&M University.

DR. STICE: Steve Stice, University of Georgia.

DR. MENDRICK: Donna Mendrick, Associate Director of Regulatory Activities and DFO NCTR.

(off mic introductions)

DR. LEIN: Thank you everybody.

DR. FUENTES-AFFLICK: May I introduce myself? I am Elena Fuentes-Afflick from the University of California San Francisco.

DR. LEIN: Hello. Anybody else on the phone? All right.

Just to remind everybody especially the members of the Scientific Advisory Board and thank you all for spending your time to advise the FDA and thank you to the members of the FDA who are here to inform the Scientific Advisory Board as we hear the presentations that we are going to be presented with today and tomorrow.

Our basic charge is really to provide feedback to the FDA and particularly the NCTR regarding our evaluation of the research that is being conducted. It does it really - is it cutting edge research that is worthy of publication? That is one of our major charges as a Scientific Advisory Board.

The other charge that we have is to provide advice to the NCTR and how they may improve their horizon scanning for emerging sciences and comprehensive safety assessment approaches.

Again, we must keep in mind, as we are deliberating what we hear today and think about how we

might best advise the NCTR, that really these are projects that they are undertaking to inform the mission of the FDA. It is not typical of what many of us are used to in an academic environment, but we do need to keep in mind what the purpose of today's and tomorrow's deliberations are.

With that, I will turn this over then to Donna.

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

DR. MENDRICK: One reminder. Whenever you speak, please give your name so the transcriber knows who you are.

Good morning. I am Donna Mendrick, the Designated Federal Official. I would like to welcome everyone to the NCTR Science Advisory Board meeting. We truly appreciate the time and diligent work of our board members in preparing for this meeting and for the forthcoming deliberations.

I and the Board wish to thank the FDA Regulatory Centers and NIHS for their participation in the meeting and my NCTR colleagues for all their efforts preparing for this meeting.

Let me say a word about my role. As a DFO for this meeting, I serve as a liaison between the Board and

the Agency. I am responsible for ensuring all provisions of the Federal Advisory Committee Act, FACA, are met regarding the operations of the Board.

Also, in my role, a critical responsibility is to work with appropriate agency officials to ensure that all appropriate ethics regulations are satisfied. In that capacity, Board members are briefed on the provisions of the federal conflict of interest laws. In addition, each SAB participant has filed a standard government financial disclosure report.

We have a full agenda; yet, we strive to ensure adequate time for the presentations, public comments and the board's thorough deliberations. This special note for all presenters, board members and participants. Please speak into your microphone and identify yourself and please turn off your microphone when you are finished.

Pursuant to FACA, we will have a public comment period from 1:15 to 2:15 p.m. today, offering the public the opportunity to provide comments about the topics being discussed before the board today. For members of the public requesting time to make a public comment, your remarks need to be limited to five minutes. For those public commenters who have not pre-registered, please notify me if you are interested in making a comment. As of

now, we have no registered public commenters.

I would like to add that during presentations discussion if the board members require greater qualification on issues requiring participation with attendees in the audience, they may request information during the meeting to the chair or myself.

In accordance with FACA, minutes of this meeting will be prepared as a transcript - both will be posted to the website. Remember that this is a public meeting. I wish to thank the Board for participation in today's meeting.

Two comments about the agenda. One, I made a mistake in doing it. I left Sean Linder out. He is going to speak tomorrow. And on the agenda, we have the commissioner calling in at 10:30. He is actually going to call in at 9:30 tomorrow. Just two changes to the agenda. Thank you.

DR. LEIN: This is Pam Lein, chair. We will now have a presentation by Bill Slikker.

Agenda Item: State of the Center

DR. SLIKKER: First of all, I would like to welcome everyone here. This is really a great opportunity for esteemed members of our Science Board to give us insight into how NCTR and FDA can move forward. It is

wonderful to have you here. It is also fantastic to have representatives from each of the other centers and ORA here. This is the first year in several years that we have had representation across the entire FDA. We are very grateful for everyone coming today to this activity and for tomorrow's activities and beyond. It is also wonderful to have the staff here from NCTR to provide presentations to you. We are looking forward to this day and a half of a good look at NCTR and for your input and also for appraisal of past presentations that were made so that we can get insights as to how to improve the activities of NCTR to support FDA.

What I would like to do is just go over briefly the NCTR strategy. And of course we begin with the idea that we are getting to be 40 plus years old and we are very happy about that. And from the very get go that we have been focused on bringing together individuals from academics, from other government agencies, from industry to move the efforts of NCTR and FDA forward to support public health.

The vision of course fits along that same line. Not only do we have a national interest, but also because FDA, as you know, has a global perspective. We also have a great deal interest in interacting with individuals from

around the world and government organizations that do the same kind of work as FDA does and to not only exchange ideas with them, but to build partnerships with those abroad and to provide training and a common understanding of the general principles of regulatory science and how it applies to FDA's work.

Within that of course, we do it by not only developing data sets for FDA review and use and decision making, but we also do it by looking at newer technologies and try to understand whether those would be appropriate for FDA decision-making tools in the future.

This is our structure. As you can see here, we do have a number of individuals here in this management arena. Although it looks like a lot of boxes and it is, it really is only one-seventh of the total population of NCTR. The great majority of folks are down here in the research divisions, which would be appropriate since our goal is in research and fundamental science.

As you can see here, you will be hearing from each one of these divisions throughout the next day and a half. I will not spend any time discussing them. They are going to provide a really good overview for you. But the idea is that these groups are what really makes NCTR move forward and we are appreciative of the management and

support that they all get. The real goal here is to do this fundamental research that is so supportive of FDA, not only generating data sets that we need for decision making, but also as I mentioned looking at new technology and developing new technology is necessary.

Just to give an overview of our staff. We are very blessed to have about 166 FTEs that are really involved in the science part of this, the research part, including visiting scientists. We also have a really good support staff of about 45 and administration of about 95. And then we also have commissioner fellows.

The area of postdocs is really important to us. We do a lot of training, not only individuals here within the United States, but also training around the world. Over the last 20 or 30 years, we have trained over 1300 individuals from 54 different countries. We are very proud about that training record.

We feel this expresses then the goals and the processes of which FDA functions and also builds those standard principles in which we can all respond. We do have a fairly large number of on-site contractors. This has been the way it has been at NCTR from its inception. Many of these are within the areas of facilities, also in pathology, animal care, et cetera. This is our total

number of staff.

Now what about the NCTR research goals? Each year of course we update this list. Oftentimes it is very much in conjunction with the FDA, our goals and strategic plan. Certainly the idea is for us to advance the basic science approaches and tools to support regulatory decision making both personal and public health of course. We try to do this by aligning ourselves with the rest of FDA and with various centers that are represented here and ORA as well.

The other area of course I mentioned already is the idea of collaboration with the other centers. We do this not only in terms of developing data sets that will be useful for the other centers, but also looking at new technologies. We do this to a very systematic review process for each one of our concepts, which is usually a two-page document just outlining the significance and the importance of the work as well as a general outline about what we have conducted and its impact on FDA. Then it is reviewed by the other centers and only if the other centers feel like this is a worthwhile project - forward.

Once that is done then a full protocol is developed, which also goes through FDA and outside review to get the maximum input to its scientific confidence. If

that is approved then finally the funding is available to do that work. We have a very strategic way of dealing with the interaction with the other centers and ORA.

And the idea is that we are also looking at building partnerships through virtual centers and doing this in such a way that helps move NCTR and FDA forward.

As I also mentioned, Goal 3 is really interacting in a global sense. This is especially true for supporting regulatory science around the world. We have the global coalition of regulatory science research that is populated by nine different countries, including the EU, as well as strong interactions with the other centers of FDA.

Now some of the accomplishments I will just briefly mention because you are going to be hearing about these accomplishments from each one of the centers in particular. I just want to mention a few along the way. Part of it is really to find ways to improve this partnership within FDA and with other collaborators. We do this by having these kinds of meetings once a year so we can get input from the representatives from the various centers. We also do it through our concept, a protocol system that I mentioned, and other kinds of interactions that we have throughout the year.

Also the idea of advancing regulatory science is key. And the entire FDA has a strategy on this and we are certainly part of that strategy.

And then of course the global outreach that I mentioned.

Now one of those partnerships that has really been important to us is working with the other centers on particularly high interest areas. This includes of course some of the work we are doing in the opioid area, which is really using a stem cell kind of approach to evaluate the effects of opioids during development.

We also work very closely with the Center for Tobacco Products, looking for analysis of nicotine and other agents found in tobacco smoke.

And then of course in the area of antimicrobial resistance. There is certainly a close relationship with CVM and we will examine the human biome and how this interaction occurs and how things can be improved along that line of thinking.

And then close coordination with CDER when it comes to looking at the safe use of anesthetics in children. This work continues and has continued for almost a decade trying to really understand how anesthesia could be best used in children in a safe and effective way.

And then finally on this list is precision medicine with OWH. This is really important to understand how we can more carefully analyze and determine which individuals are most sensitive and then how to - therapy that would be most effective. The Office of Women's Health has a strong program in this area that we are a part of.

Another area that I wanted to mention was the idea of progress in FDA Drug Safety Communication. This has to do with various kinds of MRI agents that can be used to enhance MRI images. We are working closely with CDER on this and have a project in force now that is moving forward very nicely to try to examine this kind of issue.

We also of course have a lot of work going on in pediatrics that I have already mentioned as far as anesthetics are concerned. And then also the idea of working in a bioinformatics mode on Sequencing Quality Control Phase 2. Here, we are looking at next gen sequencing and trying to understand both the strengths and limitations of those approaches and how reproducibility can be improved by understanding them fully.

And then finally, we have an MOU with CDER, which we will continue down its third year. This is really looking at monograph systems on sunscreen ingredients, but

also other non-prescription drugs. We are very happy with this understanding with CDER to work with them, not only generating review of the literature and appraisal of studies, but also in the future, we are hoping to do some laboratory work.

Now, one of the areas that we have invested in heavily with the help of Center for Tobacco Products is really looking at the tobacco research capacity and building that within FDA. You can see the various areas that we have developed approaches in: addiction, inhalation toxicology, which meant that we had to have an inhalation facility to do that, looking at alternative animal models, using an in vitro air-liquid-interface approach - know more about that from our group a little bit later. And then bioinformatics approaches. It is really important here to understand how to deal with the tremendous data sets that are available in the literature and also help move forward a more precise way of thinking about this in the future. And then of course general adverse health outcomes including looking at some of the tobacco-specific ingredients as well as looking at contamination of any sort of microbial contamination of various kinds of snuffs and other kinds of tobacco products.

This sort of gives a breakdown of how we are interacting with the other symptoms. This is just a snapshot in time. It was different last year. It will be different next month because new projects come and other projects completed. But at this point in time, you can see that the largest amount of work that we are doing currently is with our colleagues at CDER followed by CDRH and CFSAN, et cetera. Like I said, it will change at any one time as we have new projects that are added.

I also would like to mention that between 40 and 50 percent of our projects again depending on the time that you take that particular view are certainly in tight conjunction with the other centers. There is either funding and/or personnel that are working together as co-collaborators and then that means that we have about 45th percent of our work that we do in developing new strategies, new methodologies that then can be preliminary and can be used as we interact with the other centers.

Just to go on a little bit with some of our scientific partnerships. There has been a great deal of interest and we are happy to have Nigel Walker in the room from the National Toxicology Program at NIEHS because we have had a lot of interest in areas such as Bisphenol A that you probably have heard about. Furans, Melamine,

Cyanuric Acid, and arsenic studies are developing now.

These are in tight coordination with CFSAN and CVM.

Also, we have been working on dermal carcinogenicity bioassay of triclosan with CDER and also with CFSAN and others, we have looked at aloe and its impact on the GI tract.

But you can see here that from brominated vegetable oil with CFSAN, looking at the microbiome in terms of toxicities of xenobiotics. What we are trying to do is understand how do these microbiome influence toxicology studies with a variety of agents. Our Division of Microbiology and others are working closely with NTP and other features within FDA to try to really understand that impact and how maybe a microbiome could be a more consistent component of toxicological assessments.

And then finally looking at the many in vitro systems to understand how they can be used more clearly and understanding the effects of various agents. This particularly one is the airway tissue model.

Just to summarize some of the accomplishments in the area of advancing science. What we have here is a listing of those areas that have been expansion at this point in time. Many of these areas require new technology which we have been able to accumulate at NCTR FDA of bio-

imaging; for example, both CT and MRI and PET imaging are now available. Preclinical studies. Using 3D models and stem cells. Microbiome. I have already mentioned.

Certainly looking at precision medicine and understanding how the biomarkers are a key part of that. Nanotechnology is an area that has undergone rapid expansion in the last ten years. You will be hearing about that as well.

And then of course inhalation toxicology has been brought on by the interaction with CTP and the tobacco - modeling has really been an area that has picked up during the last several years and it goes well beyond PK modeling. It really fits in with the idea of how to use modeling to be more predictive about the actual effects of an agent and using that to be predictive for - agents. And then bioinformatics. You will hear about. It has been a key feature of our expansion. We actually have a relatively new division, only four or five years old now that focuses on bioinformatics and biostatistics to really extend this area. And then the training of regulatory sciences, I have already mentioned, is very key to what NCTR does in conjunction with - FDA and with colleagues throughout the nation and around the globe.

I just want to move on to a couple more items. That includes this relatively new area, which is what we

call R2R, which is review-to-research and return program. The idea here is really to look at bioinformatics approaches that could be useful to the rest of the agency. And much work is going on in this area, especially interactions with CDER and the DASH system that they have there. We find this interaction to be very positive. In fact, there was a recent survey done about some of the work we are doing with one of these kinds of approaches of FDA label and the survey indicated that there was 93 percent enjoyment of this process, which is pretty amazing that we get that many people that are exciting using this new tool that was developed here in conjunction with CDER.

Also, certainly focused on precision medicine - as well as just allowing us to strengthen the NCTR linkages with other product centers.

Finally to finish up with our global outreach, we have had a series of global summits over the years, dating back now to 2011 when regulatory science burst onto the scene as the new energy within FDA and within the general force of science actually around the world. It has been remarkable that regulatory science has been picked up now as a discipline within many universities. There are over 20 that have programs of regulatory science, including our own university right here, UAMS. It has a

very active program that is now available online - regulatory science.

But I think the part that NCTR has been really pushing on is this global interaction with other countries and especially regulatory aspects of those other countries. We have now built this nine-nation plus the EU interaction, which then allows us to not only look at interactions and how we can build on those, but also how we can really focus and push forward our global summits on regulatory science, which happens on an annual basis.

And just most recently, our last global summit in 2017, was on emerging technologies in food and drug safety. This was held in Brasilia where we had over 20 countries represented with a record turnout of over 150 individuals. We are quite happy with the way this is building. We also find that the interaction with the other countries allows us to really focus on those things that we have in common that we can build together.

I just want to close by talking about some of our issues for the future, some of the fine tuning we are doing within the divisions. One of the things that we are really looking at is how we - branch instructors or deputy directors within each one of our research divisions. That has moved along very nicely with another two divisions,

looking at branch instructors. One is nearly completed. The other one is just beginning that process. But this really allows training for more individuals within the administrative range. It also allows for individuals to find a more balanced workload for the division directors because it could be shared then with the branch chiefs. This process seems to be moving forward quite well. And we have been able to actually hire a few people into these kinds of roles even during the freeze of hiring that was blocking some of that for a while. We are happy with the progress there.

There are some transitions occurring. Of course, as I mentioned earlier, we have the new Division of Bioinformatics and Biostatistics and their transition just going into a branch structure. They have four branches, which we are really happy about that. That has gone through all the different steps required to accomplish that.

The Division of Genetic and Molecular Toxicology has now a deputy director. That has been secured. At least we have more management capability there.

And the Division of Microbiology - does now have a deputy director, which we are very happy about.

And also the Office of Scientific Coordination.

We now have a new coordinator and director for that. We are very happy to have new office director and Schnackenberg is doing a great job of leading that area forward.

Now new proposals just briefly. We are going to be looking actually in the second half of these meetings to a focus group on analytical/imaging quantification. And the idea of this is that we all know that data sets become larger and larger and that we need better ways of handling those massive data sets.

We also realized that we have some very advanced technology including imaging of all types nano(?) activities, new mass spectrometry approaches. All these kinds of approaches require and generate huge data sets and actually a lot of quantification after the data has been collected. We are trying to find better ways to not only collect and to analyze and also visualize our data. We are having the Subcommittee really look at this in more detail and try to give us advice on how we can move that area forward within the NCTR and FDA.

And also we are looking at a Virtual Center on Maternal and Perinatal Medicine. This really involves a lot of modeling as well as looking at not only animal models, but also statistical and analytical kinds of

modeling approaches to utilize the data most successfully. You can imagine within this perinatal area that the ability to collect data in humans is very limited. Oftentimes, animal models are necessary in modeling that data and checking some of the key features with the human will allow one to more comfortable with the predictions especially like first dose in the neonates and that sort of thing - to this area. Looking to move forward.

There is also a great demand for this within the food area. As you are well aware, children tend to eat more food milligram per kilogram basis in adults get more exposure. The idea there would be to really understand that more fully again using modeling -

What is the idea of this? First of all, the maternal/fetal pairs represent a unique regulatory responsibility. And oftentimes we get into not only newborns but preemies. As you know, preemies are certainly more likely to occur now because we have a greater way of being able to maintain preemies and to get them to those initial few months and on schedule for normal development. All that area is one in which more kinds of focus could be drawn.

The idea is that we are really trying to create this opportunity to work between the various centers in

this area of developing something in the perinatal and early postnatal period as well as maternal. That is why we want to go at it now. We have new technologies that allow us to do this. We have new modeling approaches that are very powerful and also the in silico approaches can really drive this forward especially if you have limited data and you want to extrapolate when you can from animal models.

I just want to say our approach and effort to work across centers really has to do with a variety of different approaches. And the skill areas are listed below. We have already talked about cell systems, 3D and 2D stem cells, for example. Mathematical modeling is a large area of what we are looking at. And of course incorporating that with animal data where we can as well as various omics and other approaches. Really, we are seeking support for this and looking forward to that in the future.

I want to close with asking a few questions. We have asked all of our presenters to consider this. One of those things is can animal models be better utilized for preclinical decision making. What tools would help that process? It is a certainly a question that we want to have for the group.

What are some of the examples of current

regulatory approaches that can be replaced with alternative approaches? Always an important area to think about is how we can get our data in a more inexpensive, faster, and better way.

What alternative models really have promise and can be used to move forward and show those that need a lot more work?

And then working in silico approaches really help in this situation. Is there a need for additional in vitro to in vivo extrapolation approaches to make this happen?

With that, I will close. I would like to thank all of you for your attention. I will be happy to answer any questions you may have. Also, these questions that we outlined here is something that can be used perhaps for you to consider when you are looking at your report. Thank you for this opportunity.

(Applause)

DR. LEIN: It is the rare meeting that we are actually way ahead of time. We actually do have quite a bit of time for questions if anybody would like to ask Dr. Slikker anything.

DR. PILLAI: Bill, thank you. I have a question. Suresh Pillai, Texas A&M University. Bill, thanks for that

overview. I wanted to get your thoughts on whether any work is being done with the interaction between marijuana, opioid and other prescription drugs. I know it falls right in the realm of both drugs and food because - marijuana and some THCs are being consumed as food as well in some states. Brownies, et cetera. Any type of work in that area that NCTR is involved in?

DR. SLIKKER: Do you mean in terms of the opioid issues?

DR. PILLAI: The opioid issues and potential strategies to overcome that.

DR. SLIKKER: We do have two approaches that we think could be useful in this regard. One of them of course is the studies that we have done with self-administration and other approaches where you are looking at addiction potential and trying to understand if new agents or existing agents have a particular potential to add to addiction. We do have that capability to assess agents.

And we also have the capability of using now some in vitro approaches where we are able to look at opioid exposure during development and understand the influence on cells and culture that are going through the exposure to opioids. Both of those areas are some that we

can expand upon and are working on currently to see if we could do more in that area. This is an important area for the future for us to consider.

DR. LEIN: Dr. Lanza.

DR. LANZA: Greg Lanza. Bill, do you look at the in vitro to model to human translation, the comparative differences in biology between these models? So many failures because mice have total different clearance in the case of particles than humans do. The role of macrophage clearance in the lungs in many species is not the same as humans. The immune system itself, which is now a major area of research - is completely different. I think the impression in the field too often is that if it works in a mouse, it will work in a man - biology, but in fact, they are not. I think the comparative biology directed to the questions being asked is something that maybe you are doing or if you are not, maybe you should be doing so that models are more appropriate to the end result you are trying to scale up to.

DR. SLIKKER: That is a good point, Greg. I will say that one of our approaches to that is to not only evaluate several different animal models, but also obtain human samples where we can to confirm those models. This has been done, for example, in the BPA arena where we have

used mice, rats, non-human primates and then a few samples from the human situation to be able to understand if the model is predictive and how to use it most appropriately. There are some really important findings that have occurred as you move to the non-human primate and the human, the exposure to the active agent. BPA itself is much more limited than it was in the mice and rodents and rats. I think that is just one example of how you can use modeling to make sure that you are predictive is by checking against where you can get human samples.

The way of course that we are investing in is using in vitro systems where you have stem cells that are derived from humans. You get around some of the issues of species extrapolation. Of course, you invite other uncertainties and issues that have to be evaluated. That is one thing that we are doing very closely now is looking at the advantages of some of the 2D and 3D and stem cell models to see how predictive they may be, which ones of those systems may have promise for future use in regulatory decision making. And we are doing this in conjunction with the other centers and with other researchers around the world.

We think this is really important to make sure that not only do we have additional technology available,

but that we really feel comfortable using that technology in a predictive way.

DR. LEIN: Steve.

DR. STICE: Steve Stice, University of Georgia. Bill, you outlined on your goal to enhancing collaborations that you have a very systematic way of going about these proposals. Can we understand a little bit more about how they initiate? Is it at different centers and FDA initiating them or is it scientists within your center that initiate them, mix of both? Can you give us some more flavor for how that goes?

DR. SLIKKER: Certainly Steve. Good question. We use all of the above that you mentioned. Sometimes the interest and excitement comes from another center where they have a question that is really important that we want to be able to help them with.

It also occurs the other way where we have an idea of how to improve a system or how to maybe generate an approach that would be useful. We believe to FDA decision making and we get input from the other centers. It happens both those ways.

It also happens from groups like the one that you are a member of and that often times suggestions come in from our Science Advisory Board or from other parts of

the scientific groupings around the world. We use that input from a variety of sources.

Just recently, we have been talking with some of our colleagues in Japan, the Food Safety Commission of Japan, about some possible interactions. We use with those kinds of opportunities an approach where we deal with a particular research question, develop it and see whether or not we both can support it both from another country and from the NCTR FDA. There is input from all those different sources.

But the most important thing is that we use points of contact within the other centers and ORA to send these concepts to so that we know that they are getting disseminated and reviewed appropriately. We listen closely to their feedback and determine what next steps will occur. That happens with any protocol that goes through our system. Even though the sources of inspiration may come from a variety of areas including other centers and FDA scientists and NCTR scientists that they all get the same kind of review process to make sure it is pertinent and it is going to be most valuable to FDA.

DR. STICE: Maybe more specifically. So somebody comes up with an idea that research scientists here in the center. Would they go out to a specific contact within the

other centers or would they send that out to all the centers and say look for input on a particular project?

DR. SLIKKER: Usually it is somewhat focused because if the area happens to be let's say a food entity, a food contaminant, then we are going to be looking at CFSAN and CVM for that. If it is more of a drug-related issue then we may focus it more to those two or three centers that are especially focused on that. But we try to get input as broadly as possible.

The important thing is that we need a signal that is going to be something significant and important to FDA to move forward.

DR. LEIN: Pam Lein. That actually brings up one of my questions, Bill. Perhaps you could provide the SAB with a little more insight on how it is that you prioritize which projects to actually do because like all of us, you are resource limited and question rich.

DR. SLIKKER: First of all, this process that I have been talking about is one that gives a great deal of priority because there are some projects that are not going to meet the line and therefore not be completed or even started. There are other ones that will have a great deal of enthusiasm. Those are certainly going to move up to the top of the list.

Each year, it is rather complicated, but each year, we do have a budget allocation to the various divisions. We rely on the division directors to make the best use of those funding where they can. And that really means that often times within input from the management and for other centers, they will select certain projects and more forward at a more rapid pace or other ones may be delayed a bit or come on a little bit later. But the idea is that priority is really set by the impact of that particular project on the FDA.

DR. LEIN: Any other questions?

DR. PILLAI: Bill, I just want to ask your opinion. How do you deal with public questions that have hard topic issues? Something happened a couple of months ago with the monkey story in NCTR. How do you deal with that as an agency and how do you deal with that as your center? How do you put to rest some of these public concerns?

DR. SLIKKER: I think you are always going to have issues that occur. I think the thing is that there is a unified approach in that. Of course, we involve upper management of FDA and also as you know, there are attorneys that work within the health and human services department that represent FDA. Therefore, we have a group

effort to evaluate this and to move forward as appropriate. It really is a group activity between management of FDA, NCTR staff as well as when we need to reaching out to others within the department.

DR. LEIN: Any other questions from the Scientific Advisory Board? Thank you, Dr. Slikker.

The next item on the agenda is the Subcommittee review.

Agenda Item: Subcommittee Review, Division of Systems Biology

DR. PILLAI: Thank you. I was co-chairing this review along with Dr. Diwakar Jain, a member of the SAB. We were tasked - the review is on your - I think Tab 7. I am not going to go through the entire report, rather just highlight some key points.

We were asked to review the original systems biology on a couple of criteria. The criterion was the quality of the research being conducted within the division. How did the Division of Systems Biology improve or how can it improve its horizon scanning related to emerging technologies and new safety assessment approaches? And also the critical emerging regulatory research scientific issues and trends and needs that have relevance to the FDA.

The review team consisted of myself, Dr. Jain, Dr. Nigel Greene from AstraZeneca, Dr. Frank Barile from St. John's University in New York, as well as Dr. Tim Ryan from Sano Informed Prescribing.

We approached this on - there were a couple of thematic areas within the division. The divisions - I am not going to read out all the thematic areas, but needless to say, there were about six - let me mention them. They were clinical and translational metabolomics and proteomics, doxorubicin/cardiotoxicity markers, TKI markers and mechanisms, alternative drug safety models, food safety technology, computational modeling, and precision medicine.

We did this review by splitting each of these thematic areas into primary reviewer and secondary reviewer. Then we had a discussion right after this. The Subcommittee review - we were together for almost a day probably, discussing all these. Also, we did this remotely via telephone or email.

What we found was that the division focuses heavily on developing and evaluating biomarkers with hepatotoxicity, cardiotoxicity, studying the mechanistic aspects of organ tissue and cellular and drug toxicity.

We felt that the organizational structure may be

coming up short in terms of capturing the scientific potential that exists within NCTR. In today's big data driven world, we felt that there is a need to integrate many of these areas. We felt that the Division of Systems Biology must continuously strive to integrate diverse research capabilities that exist within the center.

Dr. Jain, please step in if you have any specific comments.

In the clinical and translational metabolomics and proteomics area, there was a tremendous focus on technology-based novel biomarker identification using well-characterized models for organ damage, primarily using Acetaminophen and Doxorubicin induced toxicity.

And then what is also interesting from the presentations from this division was that investigators posed a number of research questions to the review team, very specific questions. We felt that the review team needed to answer those questions to the best of their abilities, trying to be as granular as possible.

There were questions such as should there be a validation of the candidate biomarkers. A typical answer was yes. The experiments obviously needed to address the validation tenants. And then should candidate biomarkers be integrated? The answer was yes and no. How can it be

changed?

We went through the same things for all of the - the review felt that the proven capability in biomarker discovery and the quality of science to be extremely strong. And then it was also - the review team felt it was prudent to develop a strategic plan for biomarker validation and to temper the urge to identify more candidate biomarkers rather than closing out on the existing work.

We felt that the scientific potential was very strong within the division, but we also suggested that it may be prudent to be strategic in its choice and how to finish off such invalidations.

Dr. Jain, I was just mentioning while you stepped out that please step in if you want to add any of your comments here. And then do you want to talk about anything about the dox/cardiotoxicity markers review section?

DR. JAIN: -- interesting presentations on dox/cardiotoxicity as well as TKI cardiotoxicity program. The only comments we made, which were very kind of generic, was it is good to start with the animal studies. But the animal studies may not be able to answer the contribution of genomic or genetic variation in the

population, which confers very wide spectrum susceptibility to the cardiotoxicity of these local(?) medication in the clinical context.

With dox cardiotoxicity now that some patients can tolerate extremely high doses with very little cardiotoxic effect whereas other patients develop cardiotoxicity at relatively lower doses. And it is very difficult to differentiate what confers in presusceptibility to these. A few factors are known, but a big contribution comes probably from genomic variation in the population.

Starting with the animal study, I think the ultimate answer what confers or what genomic or genetic or molecular biomarkers predict cardiotoxicity could come from the clinical studies. All these studies from the animal models, which were presented, need to be followed up by the clinical studies to understand, to translate them to the human context and the same was true for studies involving the tyrosine kinase inhibitors, which also has a pretty wide range of susceptibility to cardiotoxicity.

Otherwise the program is very well conceived and the programs and the protocols, which were designed in the animals, were very interesting as well as likely to give

huge amount of information.

DR. PILLAI: In those lines, there was a particular study that actually raised a lot of enthusiasm on the review team. That was Dr. Varmus project on the question of specific interaction between obesity and pharmacokinetics and alterations in drug toxicity. We felt that was a tremendously under studied area and we felt that this really has a lot of merit and there is a strong sense of urgency in getting the data sets done because one of the review team members was mentioning that in his work, he is identifying on an average an American individual would have something about 17 different drug residues circulating in his bloodstream that nobody is aware of and then couple that with obesity issues. The toxicity challenges become very acute. There was a lot of strong encouragement that this needs to be really focused on. And also felt that the different organ systems, particularly the liver, should be studied for susceptibility of drug toxicity and drug metabolism.

We went down the line with looking at different thematic areas like alternative drug safety models and then also the computational. There was a lot of fantastic research being done. For example, the review field felt that the objectives in some of the areas related to

alternative drug safety models was that - the review team felt that some of the objectives in those drug safety studies needed better definition.

Overall, the review team noted that the different efforts have resulted in specialized laboratories with deep expertise in specific areas. However, the review team felt that though the labs are highly engaged, the overall directions and applications may need some fine tuning. This is a classic example of a very strong, deep interest and a question of integration of activities.

Similarly, for the food safety technology, we felt that this was an area that NCTR has a tremendous head start in terms of some of the - two technologies. One is RAPID-B, a platform for real time detection of pathogens. And the other one was on differentiation. We felt that they needed to integrate their activities with FDA's whole genome sequencing efforts that are going on in this area.

Nevertheless, we felt that NCTR's technology can actually be used, coupled with current ongoing FDA techniques in the whole genome either as a pre-whole genome sequencing tool or maybe as part of a dovetail approach.

We felt that the science within the Division of

Systems Biology and the food safety technology of high caliber. We also felt that there was tremendous deep expertise in chemistry in this particular division and that they needed to probably integrate their activities a little bit more with the microbiology division.

Overall, the review team felt that the science in this division was outstanding. Similarly, there were things that were computational modeling as well as on mutagenicity prediction. There were some very granular comments about the particular type of algorithms that may need to be used with the statistical modeling, whether it should be used traditional - what is it - partial least squares approaches or maybe used some more machine learning approaches in terms of trying to understand these models.

Overall, the committee felt that the science in this division was very strong. The horizon scanning or technology scanning was appropriate. But we felt that with a lot more integration it can be - significant potential could be there.

DR. JAIN: You summarized very well. I think that was excellent.

DR. LEIN: Does the Scientific Advisory Board committee members have any questions to clarify anything

in the report before we approve it?

I did read through this, but I did not see a summary paragraph that provided all that information you just summarized. Is that somewhere and I missed it in the report?

DR. PILLAI: That is a good question. I thought we had an executive summary. Didn't we?

DR. LEIN: I did not see it in the materials I have.

DR. PILLAI: I wish there was a summary. I thought there was one, but I do not see it here.

DR. LEIN: As chair of the committee, I would recommend that we approve the report with the modification that a summary paragraph that basically incorporates what you just summarized for us very nicely in your oral report is included in the written document. Does the Scientific Advisory Board approve that modification and move to accept the report? Are there any other questions? Everyone in favor of approving with the modification, please raise your hand. It looks like we have a unanimous decision on that. Thank you.

I am going to turn us over to Donna for a minute or to Bill because we are well ahead of schedule, about a half an hour ahead of schedule if I am looking at this

correctly. What would you like to do? Would you like to take our break now and just continue moving ahead by 30 minutes? We will break now for 30 minutes and come back to the table at 9:30.

(Break)

DR. LEIN: We are now at half past the hour. Could we all maybe re-assemble to get started?

DR. MENDRICK: This is Donna Mendrick. We need people to sign in. If you have not signed in, please do so by the end of today and that is everybody, NCTR and everyone. We need a record of everyone who attended. Thank you.

DR. LEIN: We are still missing a few SAB members. Did somebody want to go out to the hallway and round them up?

In the interest of not falling too far behind, this is Pam Lein, the chair. I will get going on the introductions and hopefully our last SAB board member will join us shortly. I just wanted to say that we are about to hear the response to the Subcommittee to the division review. And the director of the division is actually Dr. Bill Mattes, but he will not be here today to deliver the response. He is actually taking the advice of the SAB to go out and recruit people to come. He is at the ACT

meeting right now.

Instead, Dr. Laura Schnackenberg from the division will be providing the response to the review.

Thank you.

Agenda Item: Response to Review

DR. SCHNACKENBERG: I will keep this on schedule too. I promise. Maybe even ahead of schedule. I am glad to be here this morning to be able to provide the response to our Subcommittee review from last November.

Before I get into our response, I just want to say thank you to the Subcommittee. We are very grateful for the positive and constructive comments that they provided in the recommendations in the draft report. The comments are very valuable to us as we determine the best ways to address the critical needs of the FDA and especially as we move forward over the next five years.

And hopefully, within this response, I will respond to a couple of questions already that we have heard this morning from Dr. Lanza who had a question for Dr. Slikker about clinical translate ability of our mouse and in vitro models and also regarding the genetic susceptibility and the use of homogeneous mouse models in many of our studies and how we might look at this genetic

diversity in future studies.

As Dr. Pillai mentioned, there were multiple thematic areas that were reviewed by the Subcommittee. The way I am going to give this response is I am going to look at each of these different thematic areas, a few key points from each of those thematic areas, and then our response to those points from the Subcommittee's review.

Just to review again, the thematic areas that were reviewed were our division organization, clinical/translational metabolomics and proteomics, doxorubicin/cardiotoxicity markers, tyrosine kinase inhibitor markers and mechanisms, alternative drug safety models, food safety technology, computational modeling, and precision medicine.

Since each of these thematic areas is fairly diverse, if you have any questions throughout my response, please feel free to interrupt me. There should be also plenty of time for questions at the end of my response.

The first thematic area was the division organization. Dr. Pillai did touch upon this earlier when he gave his overview of the Subcommittee review. One question that was raised in the draft report was whether our organization in our division was optimal for utilizing the staff expertise. We are currently organized into three

different branches. But one of the issues is that we are fairly spread out throughout the campus. As noted, we do have deep expertise in each of those different areas. We may not be as interacting as much as we possibly could. To that end, we are reviewing the divisional structure.

DR. MENDRICK: This is Donna Mendrick. They are having trouble - Bill Mattes is trying to call in and they are having trouble calling in. Jeff, do you know how to work this phone? This is actually an audio recorder. I guess Bill will not be able to call in then because I do not know how to use this system. Okay. Sorry Laura.

DR. SCHNACKENBERG: No. You are fine. Just again, we are reviewing the divisional structure to see if there is a better way that we can utilize the staff expertise.

Another question was how might computational technologies be integrated. Throughout our division, we do have a lot of analytical technologies, which generate a lot of data and a lot of large data sets. How are we going to integrate the computational needs for each of those technologies, especially when we are considering that we are looking at various different omics endpoints, other clinical type endpoints? How do we integrate that all?

We are developing collaboration opportunities within NCTR, FDA, and other HHS resources that would

address our data integration and computational needs.

The next thematic area that was reviewed was clinical and translational metabolomics and proteomics. Historically, our group has been focused on identifying new biomarkers of drug toxicity or disease. The Subcommittee did note that for omics approaches, there should be validation of candidate biomarkers and this should be a clear, strategic focus moving forward.

This involves not only partnering with our clinical collaborators, but at the front end, determining who is going to be responsible for doing the validation, whether the validation work will be done at NCTR, whether our clinical collaborators will validate those biomarkers.

In moving forward and looking at this focus of validating candidate biomarkers, the division has engaged a dialogue with the newly formed FDA Drug Development Tool Qualification team to help identify biomarkers that may be of sufficient high priority to warrant a development or validation effort.

We have also begun developing putative Context of Use statements that would guide biomarker qualification. These Context of Use statements would tell us what the biomarker would be used for in either regulatory clinical or regulatory and clinical type

settings. These Context of Use statements may also help us prioritize which biomarkers we need to move forward with validation efforts.

Moving along with that, we have considered steps toward the validation of biomarkers of drug-induced mitochondrial injury, including palmitoyl carnitine and cytochrome C.

And then we are also engaging the Predictive Safety Testing Consortium of the Critical Path Institute, and HESI in this and other biomarker work moving forward in the future.

The Subcommittee in their draft report also noted that in the case of MALDI imaging, the group could quickly become field leading. We do appreciate the encouragement in our MALDI imaging studies and do intend to leverage this technology and expertise within the FDA and greater biomedical research community. In fact, within the last year, since our review, we have engaged not only partners here locally at UAMS and Children's Hospital, but within FDA to develop new protocols and studies moving forward with the MALDI imaging.

The Subcommittee also heard a talk regarding sample and data quality both in metabolomics and proteomics, especially in the field of metabolomics. This

is a major issue. There are no standards really from the pipeline from sample collection through reporting of the data. This really needs to be done as we are moving forward especially as companies start bringing metabolomics and other omics type data forward for when they put in their drug submission.

The committee did note that these activities support the overall FDA mission especially that pertains to data submission. We appreciate the affirmation and will continue to support and further these efforts. In fact, just recently, there was a data quality workshop held on the NIH campus and the division was one of the leading people involved in that data quality workshop, which had participation from both NIH and NIST partners as well.

For the doxorubicin and cardiotoxicity markers theme, the Subcommittee noted a need for more specific omics-based biomarkers, based on the specific injury to the anthracyclines. Currently, clinical monitoring of the anthracyclines, the cardiotoxicity markers such as the troponin T's are not necessarily specific to the anthracyclines. It was noted that we should move forward in these efforts and try and find markers that are specific to the anthracyclines. We do appreciate the affirmation of our efforts in this area.

The Subcommittee also noted the need to evaluate the mechanism of sunitinib cardiotoxicity and potentially other tyrosine kinase inhibitors. There are a number of tyrosine kinase inhibitors already being used clinically with others in development. But meanwhile, the mechanisms of cardiotoxicity are not really fully understood and again this may be - there maybe genetic components that confer susceptibility to various individuals to the TKIs. We do appreciate the encouragement in moving forward with these tyrosine kinase inhibitor projects.

We have developed the project that was presented to the Subcommittee and are developing other studies in collaboration with colleagues in the FDA review divisions who are familiar with the issues of TKI-induced cardiotoxicity. In that way, we can answer questions that are of specific interest to those FDA reviewers. We are well set up with the technologies to do so.

This goes back then to what Dr. Lanza was mentioning earlier. The Subcommittee did recommend an increased focus on clinical studies and clinical context. Again, this is an important point that we take very seriously. We have already partnered with the collaborator at UAMS and have proteomics results from a clinical study of doxorubicin-treated breast cancer patients.

We have recently engaged a group at Arkansas Children's Hospital to establish a collaboration with a pediatric oncology group to look for anthracycline-based biomarkers of injury in those children receiving cancer treatments.

And then as noted earlier, developing these draft Context of Use statements promptly after a putative biomarker discovery will provide the groundwork for subsequent development strategy, including our clinical studies moving forward.

The Subcommittee also heard about another study that was being developed at the time. In this case, this was looking at a delayed onset cardiotoxicity due to doxorubicin. Our previous work had been looking more at an acute model. The doses that the mice were given in our previous studies were such that after six or eight weeks, we did see a change in cardiac troponin T levels in the serum as well as cardiac lesions.

In this case, the hope is that these animals will be given lower cumulative doses of doxorubicin and then a longer follow-up time and then we will use the blood samples from those early time points to correlate with those animals that developed heart failure. In this case, we are again using homogeneous mouse model in this

study.

One of the issues that the Subcommittee noted not only in this study, but in other studies that were presented was the closer relevance of genetically diverse animals to the human population and the issue associated with using an inbred animal model. We do agree and can point to studies where using outbred animals result in variable responses to treatment.

But we do believe that you need a homogeneous population to identify biomarkers, which can then be tested for individual responses in genetically diverse strains. In this case, a lot of times what we are doing with our technologies is looking for as many different biomarkers in trying to identify as much as we can transcriptomics, metabolomics, and proteomics. If we can use this homogeneous population to kind of narrow down the scope then we can move into either the clinical samples or genetically-diverse strain and look for those specific biomarkers that we have developed Context of Use statements for rather than trying to find the needle in a haystack, which coupled with the genetic diversity would probably be almost impossible to find.

The next thematic area was tyrosine kinase inhibitor markers and mechanisms. The Subcommittee

encouraged data mining as a means of developing hypothesis of TKI adverse effects. In this case, this project was related to a data mining project, looking for differential gene expression potentially related to TKI adverse effects.

The Subcommittee did note the value of including drug properties when analyzing human data. In terms of both of these comments, we do appreciate the encouragement and the advice for our future work.

In the alternative drug safety models theme, the committee did hear three different studies that we are currently working on. The first was the use of iPSC-derived cardiomyocytes as models for mechanistic studies of cardiotoxicity. We are using the iPSC-derived cardiomyocytes rather than human embryonic cells or mouse embryonic cells.

The Subcommittee did note both the advantages and the caveats of this type of model. We do appreciate the encouragement of these efforts as well as the critical insights in terms of the model that we have chosen.

Obviously, it is very difficult to mimic the complexity of the heart in an in vitro model. We do recognize that this model can address only certain questions and any results would need to be validated to in

vivo or clinical studies down the road.

The next study was related to opioid exposure on neurodevelopment. The Subcommittee was very positive about this project and affirmed our in vitro efforts. We did note some of the caveats again with the in vitro system that we are using. We do appreciate the comments and will closely monitor our initial studies as the study moves forward.

The final study that they heard about was an in vitro model of male reproductive toxicity. In the draft report, it was noted that the overall direction of this study may need to be more clearly defined. We do thank the Subcommittee for their comments and insight. We are working to establish collaborators within FDA and outside of FDA that would help strengthen the research and to define those goals better as we move forward.

In the food safety technology theme, this was mentioned earlier, the RAPID-B technology. This is for a rapid detection of pathogens and the Subcommittee was very positive about this technology and its potential application in many areas.

It was encouraged that we should explore the integration of RAPID-B with genomic platforms used in pathogen monitoring. And typically, these are real-time

PCR and whole genome sequencing.

To that end, earlier in 2017, we did place an ORISE fellow who had been working at NCTR, developing this technology, into a lab at CFSAN as a means of seeing how we can integrate the RAPID-B technology with their other genomic platforms and how they might work together in the future.

We feel that this collaboration will provide feedback on the complementarity of RAPID-B with established CFSAN approaches.

The next thematic area was computational modeling. Within this theme as well as with the rest of our talks, but we did pose multiple questions to the Subcommittee in terms of our computational modeling and how we are going about it currently. We do appreciate the detailed comments that were provided to us in the draft report. These will be very helpful in moving forward in the field. It was mentioned what algorithm we should use. These are things that we do need to consider moving forward.

Along with the algorithms, the Subcommittee did note the importance of a quantitative description at the applicability domain. We do agree that a careful approach to developing an applicability domain is essential and we

are exploring several avenues to do this. Obviously, when developing that applicability domain, we do not want it to be too narrow, but it also cannot be too broad. But we wanted to make sure it encompasses all of those compounds that should have a positive hit in the model. We are taking this under consideration.

Another comment within the computational model theme was that the Subcommittee strongly favored the use of dose or concentration when modeling toxicity endpoints. Historically, a binary answer has been associated with mutagenicity data so either a yes/no.

We do agree that modeling toxicity data in an only binary fashion does impact the computational model. However, in the case of the phospholipidosis model that was presented to the Subcommittee, we were only provided binary data to work with. It was stressed that we should understand if we do any kind of conversion between binary data or to another type of endpoint that we fully understand the data set that we have on hand.

Our intent in this area is to move forward with endpoints based on dose or continuous data as recommended wherever possible. Obviously, this is not always the case, but we do take this point very seriously and will consider this in our future modeling.

The final thematic area that the Subcommittee review in the report was precision medicine. Within the precision medicine theme, there were two studies that were presented to the Subcommittee. The first involved looking at age and sex-related differences in gene expression and how they affect the potential for adverse events due to drug toxicity. And the second was related to the effects of obesity on drug pharmacokinetics and excretion, toxicity, and efficacy.

For both of these projects, the Subcommittee strongly endorses NCTR's unique, non-genetic approach to precision medicine, with research on the effects of gender, age, and obesity, using animal models, data mining and omics technologies.

As was mentioned earlier by Dr. Pillai, both of these areas are fairly understudied. They do correlate with precision medicine initiatives. They were very supportive of both of these projects.

The division greatly appreciates the Subcommittee's affirmation and support of the direction that we are taking.

In terms of the first project, looking at sex-related differences in gene expression and how these might predict adverse events, the Subcommittee did express

concerns about the use of an in vitro model to test hypotheses derived from mining of in vivo data.

With this study, initially we are mining the gene expression data to determine sexually dimorphic genes and how they might cause different toxicity in males versus females. Once we did the data mining then we did a literature search and came up with 41 different drugs that had differential gene expression in those CYP450s.

The reason that we are using this in vitro model to begin with is to narrow down that list of drugs. We had 41 drugs. We are using the in vitro rat hepatocyte model as a practical way of narrowing down the number of drugs that we would then use in the in vivo studies. Obviously, 41 drugs is a lot to think about doing in vivo studies. If we can use the hepatocyte model to narrow that down, that is ideal.

Once we have the results from this study, which the central hypothesis is that gene expression data can predict age and in this case sex-specific differences in pharmacokinetics and pharmacodynamics. If our results support this, we will then tackle mining the growing human gene-expression data to extent our investigations and translate this into the human situation.

In terms of looking at obesity and its effects

on drug toxicity and efficacy, the Subcommittee was uniformly supportive and enthusiastic about our efforts to evaluate these effects. We greatly appreciate the Subcommittee's support for this effort.

This was touched upon earlier. There was some attention brought to our selection of obesity model, the organ systems examined, and the pharmaceutical interventions used. The model that we chose has been supported by the literature. Obviously, the model you choose is critical for getting successful results.

For this study, we did initially select to use doxorubicin for our drug challenge. It was noted by the Subcommittee or there was a concern that this was driven by historical comparators. That is indeed the case. Dr. Desai has done a lot of work in the lean mouse model looking at doxorubicin-induced cardiotoxicity. We have a wide breadth of data, omics data as well, looking at that lean mouse that we can then compare the results of obesity on the doxorubicin-induced cardiotoxicity.

We do appreciate the Subcommittee's advice certainly. We will include your suggestions in expanding our focus in the future, including a consideration of efficacy and not just toxicity as with the case of choosing doxorubicin.

In addition, we are collaborating with several CDER laboratories to address a wide variety of obesity-dependent endpoints that are of interest.

With that, I have come to the end. Thank you all very much for your attention. If there are any questions, I am happy to entertain them.

(Applause)

Agenda Item: Discussion

DR. LEIN: Pam Lein. We do have plenty of time for questions. Any questions from the Scientific Advisory Board? The chairs of the Subcommittee review are satisfied with the response?

DR. PILLAI: Suresh Pillai, Texas A&M. I have a specific question about the food safety technology one. You said there is a postdoc being placed in CFSAN from NCTR. Since last year?

DR. SCHNACKENBERG: I believe he moved over there in January or February of 2017. He had been working at NCTR for at least two years prior to that on the technology.

DR. PILLAI: And that is to integrate the RAPID-B and the SpecID

DR. SCHNACKENBERG: He is working to integrate the RAPID-B with the technologies that they are using over

there and hopefully determine how they may work together in the future.

One of the advantages of the RAPID-B is that it does tell the difference between live and dead cells whereas the other technologies are not able to tell you that. Perhaps there is room for that to give that additional information in addition to the other genomic platforms.

DR. PILLAI: And also glad to see CFSAN at the table this year. I am glad to see that integration taking place. Thank you.

DR. JAIN: This is a very good response of all the questions, which came up during the discussion have been pretty adequately discussed. That is good. Thank you.

DR. STICE: Steve Stice. Nice presentation. Thank you. I am thinking about the computational modeling and you said the issue is around binary responses versus dose response. I think of computational modeling in the sense that it is an iterative process between the computational side and the experimental side. Are you having an influence on the experimental side based on your computational modeling?

DR. SCHNACKENBERG: At this point, no. But that is the idea moving forward is that you would use these

models to get either your yes/no answer or determine perhaps what concentrations you need to look at. The idea obviously is to take - not just to use the model as the final say, but then you would look for those answers in other models. I do not have all the details of all the computational projects we are working on. But yes. That is certainly the idea moving forward is to move those down the road.

DR. LEIN: Are there any questions or input from the representatives of the FDA?

DR. MARGERRISON: This is Ed Margerrison from CDRH. Laura, thank you. Could you help me understand the scope of the PSTC a little more? I do not know if you worked with CDRH at this point, but I think there may be quite a lot of overlap in interest. Maybe something we could talk about outside the formal session as well.

DR. SCHNACKENBERG: I am probably not the best one to answer the scope of the PSTC. I do not know if there is somebody else here who can -

DR. SAUER: I can tell you a little bit about the predictive safety testing. Our objective is really to go ahead and qualify safety biomarkers and this includes biomarkers for liver, kidney, pancreas, vascular injury, testicular injury. The real objective here is to have

those used within drug development clinical trials that would first be confirmed in animals for a given NCE and then they would be utilized within clinical trials.

Our leading biomarkers for qualification are a set of panel of kidney biomarkers as well as a liver biomarker, GLDH, which seems to be much more specific to liver than ALT does because ALT has the specificity issue with muscle injury.

The PSTC is composed of 20 different industry members that are all brought together under the Critical Path Institute. Basically, the work is done in the different laboratories of the different industry members. That data is then shared. In some cases, actually it is pretty fantastic the way it works. Sometimes samples will be run in one company or the animal studies we run in one company. Samples will be sent to another company and then ultimately analyzed by another. It really is a pre-competitive collaboration.

The way we go about choosing which organs we work on and which biomarkers are ultimately brought forward is really a consensus between the industry members and members involved in the biomarker qualification program at CDER.

DR. MARGERRISON: It might be something we can

talk about - we have been doing quite a lot internally at CDRH on biomarkers for traumatic brain injury as well. There may be some crossover things we can learn from each other as well.

DR. SAUER: Absolutely. I think the exciting part is that once these drug development tools are qualified, clearly they have applicability then to practice medicine. That is what we are looking at. How do we then bridge qualification to an in vitro diagnostic or an assay that is then approved declared by the FDA? I think we are on the same page.

I think GLDH is actually a really good opportunity for that because we can see applicability for that in disease areas such as Duchenne's where a lot of those kids get sent to the hospital because it looks like they have liver injury when it is really just the progression of the disease.

DR. MARGERRISON: Thank you.

DR. HATWELL: This is Karen Hatwell from CFSAN at the FDA. I wanted to compliment the Advisory Board's response at the use of dose responding. I think that is something that at CFSAN we find very important. We are glad to hear that something is being taken into account.

The other thing that I found very interesting

and exciting was the ORISE fellow that will be working in exchange. I would like to see more exchanges between NCTR and CFSAN or the FDA in general. I think that especially in this tight hiring cycle, this will be a wonderful way for us to continue connections and develop more collaborations in the future. I hope that you and your colleagues will talk to us at FDA and we can see more of that happening. Thank you very much.

DR. LEIN: Any other comments/questions from anybody around the table?

DR. SAUER: John-Michael Sauer, Critical Path Institute. Your clinical strategy for your biomarkers. What are you thinking there? We have gone down some roads, some much more expensive than others. What are you thinking?

DR. SCHNACKENBERG: I think for us the strength is the ability to do those animal studies and in vitro studies and kind of narrow down what we are working on. We are lucky enough where we are situated that we have great clinical collaborators here locally in Little Rock at UAMS, Children's Hospital, working on a wide variety of problems.

The idea then would be to narrow down and use those Context of Use statements that I mentioned so that

we can look for - we know exactly what biomarkers we are looking for. In one case, we have done that. I did not talk about this today and this project is pretty much finished up, but looking at acetaminophen hepatotoxicity. These were in children that presented with overdose in the clinic. We compared those to children that were receiving therapeutic doses of acetaminophen and children who had not acetaminophen for two weeks.

From the previous mouse studies done with the same clinical lab, we determined that there were some acyl carnitines, which may be related to acetaminophen-induced toxicity.

Then we followed up looking at those clinical samples, specifically looking for the carnitines. We also did look at bile acids. We are using the animal studies to narrow down the scope of what we are looking for. As we have mentioned, the genetic diversity in the population is so large. It is not just the genetic diversity, but it is going to be the phenotype. It is going to be everything that we are eating, how much exercise we have. There are a number of different reasons that people are going to have differential responses. If we can narrow the scope and hopefully these biomarkers will not necessarily be - they will translate from the animal to the humans. That is how

we are approaching things is kind of narrowing them down, looking at these Context of Use statements and then moving forward trying to validate them in the clinical studies.

If we can partner with our clinical collaborators ahead of time before we even develop the animal studies and just work with them from step A through the validation so that we are all completely on the same page with that. I think that is the most important part is engaging them early on in the studies.

DR. LEIN: Any other questions/comments from around the table? Behind me? Thank you very much. I think this was a very fruitful discussion. I appreciate the review that was done by the Scientific Advisory Board and the really thoughtful response to that. It was much appreciated. And the input from the center representatives. I think this is a really important area in moving forward that is going to be strategic to the mission of the FDA. Thank you for your efforts.

We will move on now to reports from the NCTR division directors. We will be starting with a presentation by Dr. Fred Beland from the Division of Biochemical Toxicology.

Agenda Item: NCTR Division Directors: Overview of Research Activities

Agenda Item: Division of Biochemical Toxicology

DR. BELAND: Hello and good morning. I can go a long time. Donna always gets me to stop early. I am Fred Beland. Donna Mendrick suggested that we use a particular format. I am going to try and follow that. You will see these types of slides over and over -

This gives the distribution within the Division of Biochemical Toxicology. What I would like to point out is since last year, we have decreased by about seven individuals. This is not government. These are primarily postdoctoral fellows who have come to the end of their appointments and have moved on. Given the budgetary uncertainty at the moment, we are holding off on adding people. There is also a lot of uncertainty regarding recruiting foreign scientists. If you want to discuss, we can talk about later. I think we are adequately staffed at the moment.

Within the division, we collaborate extensively with all the other divisions at NCTR. I have listed all the divisions here. I will not go through them. It is also important to point out that we collaborate very extensively with all the product centers. I think that will become apparent as I go through the rest of this talk.

We have received extensive funding from the National Toxicology Program. Again, that will become apparent. We received funds also from the National Cancer Institute. We have collaborations with the EPA, CDC, universities both within the United States and abroad. As far as what is called global leadership and outreach, division members have worked very extensively with the International Agencies for Research on Cancer, with the World Health Organization, European Food Safety Authority, and with the Organization for Economic Cooperation and Development. We have both in the FDA and also external.

The mission of the division is really to conduct fundamental and applied research, as stated on the slide. I would like to focus more on the goals and our strategy. What we do is we characterize toxicities and carcinogenic risks. Other divisions develop assays and so forth. We do that to some extent. But I really think what we tend to focus on is doing toxicological assessments. Historically, it has been cancer, but we have migrated into other things.

To do this work, we conduct bioassays. We do large-scale, rodent bioassays primarily with mice and rats. We do mechanistic studies associated with the bioassays to determine the mechanism of action and whether

or not it is pertinent to humans. And then as Bill Slikker pointed out, within I would say the last five to ten years, we have moved heavily into computational modeling. We recruited Jeff Fisher more than five years ago. For those of you who are staying through Wednesday, he will give a presentation as to where we are in that area.

We were asked to present our major accomplishments during the last five years. I think we were supposed to be restricted to three. I took the liberty of moving to five. The focus of the division is doing toxicological assessments. I think it is important to point out that the data we generate is used by the product center that is listed here. We have food safety, CDRH. We have Center for Veterinary Medicine. But I think it is important to note that the data are used worldwide. These data are not used just within the FDA, but they are used by others.

For instance, we finished - it actually has been probably close to five years now - acrylamide and glycidamide. These data have been used by CFSAN. They also have been used by EFSA in Europe. Aloe vera. Bill Slikker mentioned earlier. This is a dietary supplement. People think of it as putting on lotions and so forth. People drink this stuff.

We demonstrated a few years ago that aloe vera causes intestinal tumors in rats. At that time, we hypothesized that it was due to a component within that is called aloin. It is an anthroquinone. In the last year, we have demonstrated that aloin indeed gives the same response that you see from the whole mixture. From our point of view CFSAN then go to the industry and say the aloin content has to be this level or lower before you can market the product.

In the last year, we finished furan. It was just published this year. It is a very extensive - as we do for all these. We do dose response. We consider that absolutely critical. The data that we produced and furnished to CFSAN has just been used by the European Food Safety Authority and their assessment of furan. Again, these data are used worldwide.

Bisphenol A. We may be getting to the end perhaps. We are supposed to have the data released on Bisphenol A right after the first of the year. It is a very elaborate process to release the data because this is such a controversial compound.

And then the last thing I would like to mention is melamine and cyanuric acid. These experiments were directed by Goncalo Gamboa da Costa. We have demonstrated

I think very nicely as well. Melamine by itself is relatively innocuous. Cyanuric acid, the same way. You put the two of these together and you end up with a very toxic crystal structure that causes kidney toxicity. Really the data that Goncalo has generated says that safety factors should probably be decreased by a factor of ten if people are exposed to it.

What I would like to do now is I want to talk about three ongoing projects to give you a flavor for what we do. And then I would like to talk about three projects that are in the process of being developed such that if you want to look at the protocols, offer your input, this is the time to do it.

I talked about arsenic last year. At that time, I outlined the bioassay that we intended to conduct. If you are not familiar with arsenic, there is a lot of arsenic in the environment. EPA and the World Health Organization have established a drinking water limit of 10 parts per billion. Our interest in this was triggered by the fact that a bioassay was published - they were getting increased incidences of lung cancer in mice at 50 parts per billion. If that indeed was true, these 10 parts per billion may not be an appropriate level.

I am showing what people are exposed to in the

United States. Let's say up to .2 micrograms per kilogram for body weight per day. There are places where it is higher in other places in the world. You can be up to 50 micrograms per kilogram per body weight per day.

My interest and also Dan Doerge who I work with on this interest is really cancer, but also arsenic is a neurotoxic. It causes cardiovascular toxicity and so forth.

The interesting thing from a cancer perspective is that to get tumors in experimental animals, it has to be perinatal exposure. If you treat an adult animal with arsenic, the animals will not develop cancer. It has to be transplacental or neonatal exposure. This led to this hypothesis that there must be - there are at least two hypotheses. One is that there may be something - the susceptibility of these young animals may have to do with some metabolic inability to detoxify or increase deep metal detoxification. Alternatively or maybe not alternatively, in addition, you have a rapidly growing organism and you have stem cells and so forth. This may play into it.

Our approach was - Dan Doerge was going to look in at the pharmacokinetics. Igor Progribny is looking at epigenetic components. I was going to do a bioassay, which

I have yet to get funded. If anyone wants to help me get this funded, I would greatly appreciate it. This was going to be the whole - we were going to have this entire package like we have done with other things like we have done with acrylamide, like we have done with furan, like we are doing with Bisphenol A. To date, I have not been able to secure the funds.

I would like to just tell you a little bit about Dan Doerge's pharmacokinetic results because I think it is very interesting. I think it really points to the importance of doing dose response at levels as low as you can possibly get in order to be able to translate to humans.

This was done in adult CD-1 mice. CD-1 mice are the strain that the cancer bioassay was conducted. That is why we are using that. This is a preliminary study, but I still think the data are very interesting. It was a single gavage dosing. The doses are indicated here: 50, 100, and 200 micrograms of arsenic III, arsenite. Fifty micrograms is equivalent to about 300 parts per billion of their drinking water. Fifty micrograms is what a person in Bangladesh is getting in their drinking water.

We collected the tissues here - plasma, red blood cells. Tissues of the liver/lung are the target

organs. The way we have conducted this is by LC-ICP mass spec. We are doing speciation. We know arsenic III, arsenic V, dimethyl, monomethyl, inorganic arsenic. We can tell you each of those.

There is a lot of data here, but I am just going to present one slide. This shows the binding of dimethyl arsenic III. Arsenic III is what binds to proteins primarily. It binds to glutathione so forth, but we are talking about protein binding here in the erythrocyte. It is dimethyl arsenic III, monomethyl arsenic III and inorganic arsenic.

The important thing to see here is it is not linear. In fact, as the dose increases, the curve goes upward. The point here - this supposedly protein binding is somehow involved in the toxicity. The point is if you are using high doses of arsenic, you are going to overemphasize, over predict the toxicity. You need to be down at 50 and below. We did not even know what is going on below 50 yet. We will work that out later or Dan will work that out later on.

This is a second project that was proposed last year. What we want to do - this is a way of measuring what exposure causes cancer. And the way we are doing this or the way it is being done is by looking at the mutational

signature across the entire coding sequence. We are not talking about tumor suppressor genes. We are not talking about oncogenes. We are talking about the entire protein sequence.

In this example that I showed last year, which is done by this group in Asia, what they wanted to know is - some individuals got lung cancer. Some of the people smoked and some of the people did not smoke. And the question is are the people who got lung cancer who are not smokers, did they have the same mutational signature pattern of the people who smoked. If they did then you could argue that these people got lung cancer through exposure to secondhand smoke or something like this.

What they did is they sequenced and - along the bottom, I am showing the mutations that were observed. The first column is G to A. The second column A to G and so forth. They did whole exome sequencing and then they did a clustering analysis. And then you can see the very intense pattern on the left hand side is G to A and a very intense pattern here with the G to T. You can see that the way it is segregated is the people who are not smokers have a high incidence of G to A mutations. The people who were smokers have G to T. It is a very nice, powerful technique.

What you can say is the people who did not smoke and got lung cancer was not due to exposure to secondhand smoke. Unfortunately at the moment, you cannot say what it is, but you can say what it is not.

This leads me back to acrylamide. Acrylamide is metabolized to glycidamide, which is an epoxide and reacts with DNA. This is work that Dan Doerge and Goncalo Gamboa de Costa did.

These two compounds caused - the same spectrum of tumors and the same tumor incidents, looking at dose response in experimental animals. We did B6C3F1 mice. We did F344 on rats. You get tumors in a lot of different organs. You get absolutely spectacular dose response. From my point of view, this compound should probably cause - be involved in the etiology of cancer in humans.

If you talk to epidemiologists, they look at it. And the results are absolutely either negative or equivocal. But the reason is as far as I am concerned is because we are all exposed. The difference between a high exposed person and a low exposed person is only a factor of three. And epidemiology does not work with that narrow. And the other thing is a lot of exposure misclassifications based upon dietary recall and so forth.

What we want to do is to take these tumor

tissues that we have, do whole exome sequencing, and then compare the results we get for published data bases in humans to see whether or not the signals that we see in our experimental animals might correspond to what we have observed for certain tissues in humans.

We are working out the techniques in the animals now, but I would like to show you some data that we have acquired in the last year. This is done in conjunction with investigators at IARC. I know the numbers are very - the print is very tiny. I apologize for this. I was working with a PDF file and there was not much I could do. But I just want you to look at the pattern. The top pattern - what we are talking about are the specific mutations. The first blue line is C to A. Then we have C to G and so forth. And then we are talking about triplets so each one - there are 16 different possibilities with each one. The top one is spontaneous. And then I have acrylamide treated. Then I have glycidamide treated.

And what I want you to see is if you look on the spontaneous, you can see G to T. There is a spike there. There is a spike on the T to G and so forth. The acrylamide gives the same pattern. If you just look at the pattern, you can see there is a spike in the same position. If you look at glycidamide on the bottom, you

see T to A. The triplet is - I think it is C-T-G triplet. You do not need to see the numbers. Just look at the patterns. The pattern is very clear.

We have also looked at the DNA adducts from these cells. These are mouse fibroblasts - mouse embryo fibroblast - again, you cannot see the numbers. I was working with a PDF file and I could not do anything with it. But what I want to see - the left hand side is acrylamide. The right hand side is glycidamide. The top chromatogram - this is LC 10 in mass spectrometry. It shows our internal standard. You can see in the acrylamide and the glycidamide that the signal is exactly the same.

The next panel down shows a formation of DNA adducts from acrylamide or glycidamide in these mouse embryo fibroblasts. There is a little bitty signal on the acrylamide, but that is simply due to residual non-label internal standard. There is a very strong signal on the glycidamide.

Likewise the third panel down is n3-adenosine adduct. You can see that there is a very strong signal here in the glycidamide. It is totally missing. The point is these mouse embryo fibroblasts cannot metabolize acrylamide. But if you give them glycidamide, you get DNA adducts. Then you get a very specific mutational spectrum.

What we are hoping is that when we go to the experimental animals, we will see something similar to this.

The third project I would like to say - I want to just give you a flavor of what we do for modeling and then those of you who continue to stay until Wednesday will see a more extensive presentation given by Jeff Fisher. This project that I am describing here is being conducted by Annie Fisher in collaboration with Nysia George, who is in biometry division.

And the idea behind this - I should point out that this work is being funded by the Office of Women's Health - is to look at the risk from exposure to thyroid active compounds. That is just perchlorate during pregnancy. How does this affect the woman? How does this affect the fetus?

In earlier work, Annie Lumen did deterministic modeling. She came up with an average person. And now what she and Nysia George want to do is to look at a probabilistic model, which will again give you the population. They are interested in thyroid active compounds, but in order to do this, you need to know to begin with what the level of iodine that the pregnant woman receives and then you can look at the interaction between perchlorate and -

And what they have done is what is called reverse dosimetry modeling or method. And what this does is they have taken NHANES urinary iodine data and work back to what they think the individual - and they have used three models. The red line shows a model they have developed called Iterative Forward. Then there is another model called ECF. There is a third model called Secant Root Finding.

You can see the Iterative Forward is a nice bell-shaped curve, very normal distribution. The heavy dashed line refers to the median. The light dashed lines refer to the mean. You can see that all three methods give a similar median. The mean, however, are the ones that have less than the normal distribution to the right. The median intake or estimated intake for all three models is pretty similar between 197 to 220 micrograms per day.

Having this information, the question was what percentage of the population of pregnant women is not receiving a sufficient iodine intake. This has shown here the - an estimated average requirement is what a person should take in. The recommended value is actually about 250. But 160 is considered the minimum amount a pregnant woman should get. Using their method, they can show that 20 percent of the population is not receiving a sufficient

amount of iodine. The other two methods gave 40 percent.

They are in a position now - they now know what the iodine intake is in modeling. Now they can then go on and look at what happens when you start increasing the amount of perchlorate and how does that affect it.

Three new initiatives. The first one - I should point out that each - the first two initiatives I am going to talk about are being funded by the National Toxicology Program. What I will indicate who they are done in collaboration with up at top. This project really originated from Center for Drugs and Center for Biologics. The concern here is there is a lot of proteins and peptides that are PEGylated. And the reason they are PEGylated is it improves drug stability or solubility. It can decrease the dosage frequency because the half-life is increased. You get less degradation so you have better drug stability.

From the point of view - there are some potential toxicities that have crept up. And one of them is - you get cellular vacuolization in various tissues, including the choroid plexus. This has been observed in preclinical studies.

There is also concern that these drugs now are not being given on just a one-time basis, but they are

being given chronically and they are also being given to very young children. There is a potential that you will have life-time exposure. The issues are what are the tissue levels over time and then are there any potential toxicities.

I should point out that these - first of all, these slides were done a month ago because I had to be what is called 508 compliant. There has been a change in a couple of these slides because we met two weeks ago with the NTP. We meet twice a year. We discuss design. People from Center for Drugs were on the discussion. People from Center for Biologics were on the discussion. There has been a slight modification.

The way it is set up right now and again these protocols are in the process of being finalized. We are going to have three components. This is going to be a single dose pharmacokinetic study where we will give - just polyethylene glycol, not bound to a protein, just polyethylene glycol. The glycol weights are at 20, 40, and 60 kilodaltons. It says we are going to subcutaneous injection. We are going to give subcutaneous injection, but it was very important to biologics that we do IV because biologics - PEGylated products for biologics tend to be given IV. We are going to give both subcutaneous and

IV.

Then we will do the pharmacokinetics. Once that has been established then we will come back with a repeated dose study. At this time, we are saying subcutaneous, but again that is subject to change. We do not do this in isolation. We will have repeated discussions with the product centers because it is important that the final product that we produce is somehow useful to them.

And then the third component of this is we will be looking toward toxicities. I should point out that these studies are being conducted or directed by Jia-Long Fang.

The second new initiative. Again, this is being funded by the National Toxicology Program and is done in collaboration with investigators at CFSAN. This focuses on the dietary supplement Nattokinase and Lumbrokinase. These are serine proteases. The Nattokinase is relatively pure. It comes from *Bacillus subtilis*. The second one comes from earthworms. These are marketed to support cardiovascular and circulatory health.

Nattokinase and Lumbrokinase have fibrinolytic activity in vitro. They have thrombolytic activity in vivo in experimental animals. They may interfere with platelet

aggregation. There is a case report of a woman who was taking Nattokinase and aspirin and developed bleeding in her brain. She had hemorrhages. She had micro-bleeding.

The issue here is - this is a concern to CFSAN. Is there a possibility of people taking these drugs, having bleeding and especially if they are taking aspirin already for cardiovascular reasons?

I should point out. This study is being directed by Luisa Camacho. Again, this is a slide that got changed when we had discussions two weeks ago. What is being proposed to do is to look at the effects of Nattokinase and Lumbrokinase individually or in combination with aspirin. At least the initial studies will be conducted in Sprague Dawley rats and there will be a 28-day gavage exposure.

The levels of Nattokinase and Lumbrokinase. These are about 100 fold over what human use is. Aspirin. There is going to be an initial study where we will use a variety of doses of aspirin to try to pick the appropriate doses of aspirin to use. And then we will come back in subsequent studies when that will be added to - given at the same time as the Nattokinase and Lumbrokinase.

Here, I am showing the endpoints that are going to be measured. A lot of blood parameters have to do with

bleeding. We will do histopathology. We are also at the request of CFSAN are going to do some motor coordination and grip strength. These phases of the study will be done by Sherry Ferguson, who is part of our neurotoxicology group.

The third area. This is funded in-house to some extent. It has to do with the photo-mutagenesis assay. This is being conducted in conjunction with people at CFSAN because CFSAN is responsible for cosmetics. At NCTR, we have a photo-tox facility. There are only two in the country. There is one in private industry and there is one here at NCTR. This was put together by Paul Howard some years ago and then subsequently I would say the last five to ten years has been directed by Mary Boudreau.

We do one-year photo-co-carcinogenesis studies. The idea behind this is we have a light source that models exposure to sunlight and the question is when you put on a test agent, does it increase or decrease the incidence of cancer caused by the exposure to someone.

This is a very labor intensive because you have to monitor these animals in the formation of lesions with time. I can assure you. It is very tedious.

The idea behind the photo-mutagenesis assay is we could decrease the number of animals. In bioassay, we

normally use 36 animals per group. We could really drop the number of animals. We could do a much shorter time period. The bioassay is typically one year in length.

Investigators in our genetic and molecular toxicology division developed - the mouse that is used for these assays is the SKH-1 hairless mouse. They inserted a gpt delta gene into this. We can now do mutagenesis in the same mouse that we do the cancer studies. The advantages are we would use fewer animals. It would be shorter length of time.

Again, this protocol is in the review process right now. The idea is to establish the correlation between the UV dose and duration with the mutation frequency and the mutation spectral patterns. The hypothesis is the mutation frequency should increase with dose and duration of UV. And the spectral patterns should correspond to what you observed from UVR-induced damage.

And then the second phase would be if you put on a compound such as retinyl palmitate, which we know the cancer incidence, how does this affect the mutagenic response?

As far as experimental design, does it exist at the present time? We will measure mutation frequencies at one, two, and four weeks. It is much shorter as opposed to

52 weeks. We will use - the doses of these SEDs are pretty much the standard SED that we use in a bioassay. And then we will determine the mutational signature. It should correspond to what you would expect from ultraviolet light.

With that, I will take any questions, comments, or suggestions that you may have.

(Applause)

DR. LEIN: The floor is open for questions.

DR. LANZA: Greg Lanza. I wanted to go back to the PEG. And the reason is is that first of all, one of the areas you did not consider was nano and of course liposomes and others are being PEGylated. That presents in a totally different way. And the PEGs are smaller than 20,000. They are more like two to five arrayed.

The other thing though is that these were done in rodents. One of the problems that I understand is a problem for the nano side is that women, for instance, who have been exposed to cosmetics that have PEG, are now having antibodies when they potentially get exposed to PEG particles or PEGylated particles. I am just wondering if in the case of all of these drugs is that not also potentially part of the problem so that you do in animals that are naïve and then you do it in patients. And then

some of whom may have been exposed to PEG before have preexisting immune response. That then changes their safety profile. Is that too complicated a question?

DR. BELAND: To a great extent - we have been through close to a year's discussion. First of all, this project did not originate at NCTR. This project came from Center for Drugs and Center for Biologics. To a large extent, it is being driven by their needs. We have had discussions of should we have the size of the PEGylated - the PEG we are using - we have gone through extensive discussions with them.

As far as the immunologic response, it was my understanding that PEGylation tends to diminish -

DR. LANZA: So it reduces complement. It protects against q complement activation. It definitely does. It also reduces the ability for particles in particular to be taken up. That is why they last longer just like for the protein.

There have been clinical trials that have been conducted on liposomal and others that have been shut down because of immune response. I am going to say it is even ITE type antibodies, but it may be others.

I am just thinking that - I realize it is collaboration, but maybe you should think about expanding

it. I think it is a very important area that you are going down. I think it has two issues. One, it expands to other kinds other than PEGylated proteins and drugs. It also affects nano and also the problem that I have been seeing or hearing about in a variety of meetings is that it is the fact that studies that are done in rodents that do not have pre-prior exposure. Now, you put it down. Apparently, a lot of cosmetics and other products that people get exposed to especially cosmetics I think have PEG. And then they have an immune reaction to the PEG that is already latent. And then you give something like a particle systemic - and now they are start building reaction.

DR. BELAND: I guess the way I think about this is this is the first step. The first step will be to do this subcutaneous IV single injection with radio labeled and then we will have discussions with biologics and drugs as to is this what you want. We have a tentative outline of a multi-dose study. All of this goes into the multi-dose study, toxicity study, which is the third thing, which could be conducted at the same time as a multi-dose radiolabeled study. We will have a complete histopathological evaluation. And then based upon that, I do not think we will stop.

DR. LANZA: I do not want to dominate, but one

thing you could do that would not be pretty low is to take serum samples from 100 to 200 patients, blood serum plasma samples, and put your drugs that you have into those and screen to see if they have antibodies and just to get an assessment of what the potential for that is.

DR. BELAND: Isn't that part of what is being - I guess it was not the humans. There is a woman at Center for Drugs who is - this is Goncalo Gamboa de Costa.

DR. GAMBOA DE COSTA: I appreciate your concerns. The immunologic response was something that concerned us from the get go and why we decided - let's start with PEG to start with rather than a biologic that was developed for humans and that the rats will certainly recognize as foreign entity.

Even throughout these initial studies, we are going to assess information of the anti-PEG antibodies. We are going to have an assessment of that element. Yes, Fred is saying there are a lot of unknowns here. But to address the question of your concern about testing a naïve model versus a human who has been exposed to PEG cosmetics and for that matter even in laxatives, et cetera. We will assess when we do the multiple dose studies. The animals will be dosed for a reasonable longer period. There will be an opportunity to assess whether immunologic response

starts developing and starts changing the toxicity -

DR. PILLAI: Fred, thank you for your presentation. I have two questions. One is more specific and one is philosophical. The specific question I have is about furan. You said it has been published. The data from NCTR has been published. Because I am just looking at the literature. I do not see it at least using PubMed.

DR. BELAND: It is in Food and Chemical Toxicology.

DR. PILLAI: This year or last year?

DR. BELAND: This year.

DR. PILLAI: And the answer was that it is a non-issue. Is that the take-home message?

DR. BELAND: According to EFSA, there is a potential for liver toxicity. You are talking about furan.

DR. PILLAI: Yes, I am talking specifically furan.

DR. BELAND: Their call was that dietary exposure to furan could potentially cause liver toxicity. That came out last week or this week. I do not know what week it is any longer. And the FDA has prepared a statement for the public regarding EFSA's call on this.

DR. PILLAI: In support that -

DR. BELAND: I do not know if the statement has

been completed yet.

DR. PILLAI: The second question is more philosophical. With more analytical platforms coming online, you are going to start seeing a lot of new chemicals being identified and lots of potential toxic molecules being detected because the analytical instrumentation is becoming so sophisticated.

Along with that is also the sophistication of the genomic and transcriptomic platforms and metabolomics platforms. You are going to start seeing gene expression and signatures in all sorts of responses. How would you take all of this data especially with your division's data sets and make sense at a macro level? Almost 20 years ago, somebody detected this in Europe with analytics and they found out and now we have spent all these years on furan.

DR. BELAND: I think you are talking about acrylamide that came out of the European -

DR. PILLAI: That is one example. I was just curious about how would NCTR look at all of these data sets at a macro level and say it is of concern or it is not.

DR. BELAND: The way I want to look at it is by looking at the mutational spectrum. I think we are in a position to do that with experimental animals because we

have blocks - furan was not carcinogenic. It caused a preneoplastic change. It caused cholangiofibrosis, not cholangiocarcinoma. But we have acrylamide. We have benzopyrene. We have lots of other studies. We have aloe. We can do the sequencing, isolating DNA from paraffin blocks. I would like to approach it from a mutational point of view.

As far as transcriptomics and so forth, I am involved with studies like that. To me, they are far more difficult and far more speculative to come to a concrete answer. But this is just my personal belief. I am not young. In the years that I have left, I would like to focus on this mutational signature because I personally think it is very powerful and has the potential of taking animal data that already exists and then seeing whether or not the same pattern exists in humans. I can do this in collaboration with people at IARC and so forth. All the pieces are in place. We just have to do the work.

DR. FELTER: Susan Felter. I have a follow-up question to that, but I want to start by saying thank you for the emphasis that you provided throughout your talk on how your exposures compare to what we know about human exposures. I think it is super helpful and really important to always keep that context.

I wanted to follow up on the work you are just talking about with the acrylamide and glycidamide and looking at mutational spectra. A couple of questions. One is they both are associated with numerous tumor types and rodent models. Are you going to look across different hard tumors and look at differences in mutational spectra and different tumor types? What about non-tumor tissue from the same animal? If you had a certain hard tumor you are looking at, you may or may not find similar mutations in non-tumor tissue. I have a follow up about the humans.

DR. BELAND: We will do as much as - everything you have suggested we have talked about. This is not cheap to do. We are going to be limited by funding. We will do as much as we can with the funds that we have and collaborate to the extent that we have to get things done. The idea was to compare male mice with female mice, compare lung tumors with Harderian gland tumors, compare mice to rats and so forth and then just see -

As far as looking at tumor and non-tumor, I am not sure - I think we have talked about it, but I am not sure that we have come to any conclusion as to what we will do. Again, we are just starting on this. It is expensive.

DR. LEIN: Was there a question over here from

any - go ahead, Steve.

DR. STICE: A quick question. You joked, Fred, about the arsenic project and can you find a way to get funding for it. But I guess it really begs the question - limited resources within the center or limited interest outside the center? Where are the major roadblocks?

DR. BELAND: CFSAN did not think it was going to provide them with information that they required, I guess. My thinking is that these data are not just used by the Food and Drug Administration. There is interest - no one has contacted me from EFSA saying we want the data. But I just know that all these bioassays - the furan data - I have given every bit, including all the appendices, all the pathology and statistical analyses. That has gone to EFSA. That has gone to the German Map Commission. That has gone to Health Canada.

When we do something - we are one of the few places still doing - obviously, you cannot do bioassays in Europe any longer. But the data that we provide is used extensively. I think the data that Dan has put together showing that there is at least in the preliminary pharmacokinetic studies, there is a nonlinear response. I think it points to the need to conduct a careful dose response study, which we had set up. As far as funding, it

was going to be funded by the National Toxicology Program. What we did not do is get sufficient buy in from CFSAN.

DR. LEIN: Any other comments, questions from around the table or from the general audience?

DR. WALKER: To address the question about the sequencing of tumors. The NTP Archives are currently supporting the Sanger Institute, a massive sequencing of both rodent and human tumor samples to get exactly the question that you added because they have a lot of money. There was a massive worldwide call for proposals that went through a whole series of things and they ultimately got it and it was like \$10 million or something like that. Fred is absolutely right. It is really expensive, but someone -

DR. DORSAM: Just a very minor question. Bob Dorsam. On the PEG project, is the final destination of that - Bob Dorsam, CDER. Is the final destination for the PEG project a publication?

DR. BELAND: No. I have lots of publications. CDER came to us and requested - of course, we will publish it when we can, but the final product is producing something that is going to be of regulatory use to -

DR. DORSAM: I only ask because I am - it does sound interesting to me and I would certainly look forward

to seeing that information.

DR. BELAND: Of course, we want to publish it. I have learned through the many years that this Center has evolved, we really need to get something that is agreeable and that the product center can use. And then if it is possible then we will publish it.

PARTICIPANT: Just a follow-up comment about -

DR. LEIN: Introduce yourself please.

DR. LANIYONU: I am from Office of New Drugs. The reason that (indiscernible) that funded this project as well as NCTR. In addition, there are different stakeholders that might actually benefit from having some of these issues published. Because invariably, if they are published, they become publicly available. And sometimes it might actually be easier for an independent body such as NCTR to publish these data so that the drug companies themselves might know (indiscernible) comment about (indiscernible) when these PEG products are used in cosmetics perhaps because of the low levels that they have been used over the years, it might actually give rise to immunological responses. We are experienced in this. Thank you.

DR. BELAND: Maybe I did not respond correctly. The question as I took it is was the primary reason for

doing this is to get into publication. That is not - the primary reason we are doing this is to fulfill a need of a product center. Any time we can publish, we do. The only time we have had any hang up on publishing in the present time is with Center for Tobacco Products. It is a very somewhat cumbersome process to get things published. Even that is breaking down a little bit and things are now coming out. The reason we are doing it is to address the product center's need.

DR. SLIKKER: Bill Slikker, NCTR. I wanted to reinforce this process. We have Nigel Walker here representing the National Toxicology Program. I think he agrees that the reason that we are celebrating 25 years of this understanding between two different agencies is because it works. And the reason it works is that we have the product centers of FDA interested in the process. They request certain information be generated. We work with them to develop a protocol. Many of their scientists are involved. Scientists from the National Toxicology Program, scientists from NCTR are working together to come up with a protocol. It is going to answer the question and to make that data available so everyone can use it.

What is interesting about this is that we have requests all the time, as Fred pointed out, just in the

last year. PMDA from Japan are friends at the EU and also from other areas, France, in particular, have been asking for data sets. We will make those available as soon as we are capable of doing such. There is a process for doing that, but all the data will be made available. I think that it celebrates the idea that this relationship works to really focus attention on important problems because there are regulatory consequences.

DR. LEIN: Are there any other comments from around the table? Thank you very much, Fred.

The last presentation before lunch will be Dr. Robert Heflich from the Division of Genetic and Molecular Toxicology.

Agenda Item: Division of Genetic and Molecular Toxicology

DR. HEFLICH: I just wanted to say something first about Fred's talk. It seems we have lots of time here. Mutational signatures. This is not a new idea. I guess it got its biggest notoriety in the early '90s with Vogelstein and Kinzler paper in Nature. Carcinogens leave fingerprints or something like that where they published some incredibly compelling data about liver tumor signatures in the P53 gene in Africans who were exposed aflatoxins.

That was followed up by another paper pretty soon after it where they showed UV signature mutations. These are very specific mutations. Basal cell carcinomas from people who were exposed to sunlight. That kind of lit a fuse to everyone at the time. As was pointed out here - with most types of human exposures, it is not obvious. There are only a certain number of mutations that actually are predominant in mutational spectra. If you notice that paper which came out a couple of years ago and sort of reignited the interest in this field, it only lists a couple of mutations.

You need a lot of data actually to be able to define a signature. The best signatures are not only the type of change, but where it occurs in a particular gene. We are talking about cancer driver mutations usually now. PF53 is a good one to look at because it is a tumor suppressor gene and it has a big target for mutagenesis. You can see signatures there. As I said, it is not always obvious.

We have made a few pitches to various people that we can from my division about using this and following up on this for human exposures that might have a real strong impact on mutational signatures, but so far we have not gotten any takers. I wish Fred luck with his.

This is going to be an update on the Division of Genetic and Molecular Toxicology. I am Bob Heflich. I have been director for the last four years. In the past year, we have a deputy director, Mugimane Manjantha. Stand up or raise your hand.

I was just going to flip through these slides. They are not really of interest other than bean counting, I suppose. But we are the smallest research division. I think that is true. There are 31 people right now including everybody. Here is the list of - we have about 11 or 12 principal investigators, most of which have PhDs - PIs on particular protocols that are ongoing. They are listed here along with their expertise.

Here is our outreach slide. This is where I want to start with our mission. Improve public health by providing the agency with the expertise and tools necessary for comprehensive assessment of genetic risk and secondly by strengthening approaches to integrate knowledge of genetic risk into regulatory decision making. I consider these to be different. I am not real strong on missions and strategies and goals. I think you will get the picture as we go through this.

As you know, genetic toxicology became important to FDA in the '70s probably, a little before my time, when

Bruce Ames came out with this observation that you could identify carcinogens by whether or not they were mutagenic. There were a number of assays that were developed at the time. The measure of mutagenicity and some sort of comprehensive way because mutations are induced in a lot of different ways. You need different systems to detect them generally.

The regulatory agencies including the FDA incorporated mutation assays as part of what they wanted to look at as far as preclinical data. I suspect that every one of the product centers at FDA have some kind of language about how they use mutation data.

Our goals might be a little different than the other divisions' goals because we have such a history. There is a lot of use of standardized genetic toxicology for making regulatory decisions.

The first goal is to respond to agency needs for chemical-specific data. That means generating assay data where it cannot be compelled to other reasons. We have done work on recent years of nanomaterials, some drug impurities and tobacco products. We give the FDA a certain amount of expertise in particular assays that they have to regulate on.

There are not a lot of people left in the FDA

who actually perform some of these genetox assays. It is sometimes useful to talk to a person who actually knows something about the assay. Given as an example here, the CDER PTCC, which is the Pharmacology/Toxicology Coordinating Committee, I believe. There is a Subcommittee on genetox that reviews INDs and determines whether or not they want to put this particular drug or whatever into clinical trials. Genetox is an important component of that. I have been on that committee for a while. We actually have a teleconference on Thursday where we are looking at a drug impurity that has in vivo Comet data. I and two ad hoc reviewers from the division have chimed in what they call a consult on this data that is helping out the reviewer.

The second bullet is to maintain DGMT's tradition of leadership in regulatory assay development and validation. I would say we have gone back at least 40 years that I know of. I think we started with the center although I am not totally clear on that. Over the years, we have developed a lot of assays or had a major role in developing assays that became standard regulatory assays. I have listed some here.

Some of our newer efforts are in the Pig-a gene mutation assay, which was first developed here at NCTR

about ten years ago. We have had an association with it over the years to the point where now we are trying to get an OECD test guideline. That is my job mainly in running that effort. I am not going to talk about Pig-a. I promised everyone I would not talk about Pig-a. Pamela knows I gave a seminar for her at some meeting last year on Pig-a. That is enough.

Some other things that we think have regulatory value that might become assays eventually are the Comet assay variant and the hairless Albino transgenic rodent that Fred talked about.

I think I had some conversation getting a cup of coffee a little while ago about how some of these things sound real good when you develop in the lab. There is another component to this of actually getting these things accepted and getting people to think that the data from these assays is useful for them. A lot of times that requires consensus building with a lot of these international groups that include the regulatory people and the industry people and academia. I listed a couple here: the OECD, the IWGT. The IWGT is meeting - that is the International Work Group for genotoxicity testing. It is meeting this week. And actually, I am pulling an all-nighter with one of Susan's colleagues tomorrow night to

review 3D cell culture, state of the art. It is applied to genetic toxicology testing.

Recently, in the last year, Nigel has been good enough to consider instead of our usual types of projects that are more chemical-specific type projects like what Fred mentioned, some things that are assay specific where some assays look promising. NTP thinks it would be good to put some effort into developing them a little more. One of those is airway tissue model that I will talk about a little later.

My intention was to bold the things I was going to talk about is establish new paradigms for regulatory decision making that integrate measures of genetic risk with biomarkers of toxicity. I think the division as a whole is kind of gravitating towards in vitro systems. We are gravitating to in vitro systems that are more relevant perhaps than the historically used systems of bacteria and cell lines.

Also, we would like to incorporate besides genetox other elements of endpoints of toxicity into these assays so we can put into context what we learned from the genetox assay by looking at other things. That is often very helpful for interpretation.

Here are my strategies. I have divided this up

into biological systems. Metagenetic analysis. Lastly and I am not going to talk about this. I did not realize I would have two hours to talk. I am shooting for an early lunch here. Develop better ways of evaluating data to determine human risk. This is something we have been doing in the last couple of years in the realm of our own version of computational modeling. There has been a push on it in the world of genetic toxicology to make genetox data a quasi-apical endpoint that you could do quantitative risk assessment with. You need a lot of factors before you can do that. But the methodology to actually quantify and describe the dose responses is something that is somewhat available in the literature. We have been adapting that to our assays. A lot of this has been through the HESI Quantitative Analysis Work Group, which is - I guess there is nobody here connected with any of those people. It is a worldwide effort.

I am going to go to the biology. I probably said this before, but I probably forgot that I said it so I will repeat it. Historically, genetox assessments have been conducted in bacterial mammalian cell lines, and inbred rodents, often transgenic rodents if we are measuring gene mutation. It would be good if we are going to make some sort of assessment rather than just - I

believe Laura said this that historically genotox has been yes, it is genotoxic, no, it is not genotoxic and that is as far as it has gone. To be able to better represent human functions so we can get an idea of how humans might respond to the test agent.

Currently and listed here in three bullets and I will read the first one last is high-content/medium-throughput screening approaches using metabolically competent human cell lines and primary cells. We have a couple of protocols in this line. This is something we are working with Kristine Witt from NTP on. She has a whole laundry list of problem chemicals that they have identified as problem chemicals as far as genotox predictions are concerned that they have identified in their high throughput screening. We want to see if we can find a combination of the biological platform and endpoint that might do a better job than what they have done so far. That is that idea. These are 2D cultures by and large.

The other thing that has been going on for quite some time is to use 3D tumor models. These are tumor cells from humans that are grown in culture. And what we do is we look at the tumor driver mutations in these cultures. They are capable of propagating in vitro for several

weeks.

The idea here is that we are going to treat these models with some kind of targeted cancer chemotherapeutic agent. Probably that is targeted to one kind of mutation, one kind of product of a gene mutation and see what happens.

You probably are familiar with the Vogelstein model. That is history. The current tumor - the way people look at tumors now is there are a gemisch of clones of having different cancer driver mutations in them. And over the progression of the tumor that mix can change. If you treat for one mutation like the predominant mutation as is often done, what happens is you get eventually a relapse. The thought is that the other mutations that are in that tumor can take over the tumor, take over the job of expanding that tumor and you go back to the problem. You got a tumor, but genetically it is different. We hope that we can look at this in vitro and track it in vitro. And maybe we can come up with combinations that can treat this tumor. Ideally, we could take a patient's tumor and find out the right treatment and then give it to the patient. Personalized medicine.

Here are some proposed projects. Actually, these are both in development. I just was working on a protocol

last week that included both of these. I am going to talk a lot about germ cells because it is a personal interest of mine and as Fred indicated, our time is running out here. If I do not take a stab at germ cells as far as regulatory - I probably feel like I failed as a division director.

What we would like to do is develop some in vitro alternative germ cell assays. Part of the problem with looking for germ cell mutation or any kind of an effect in germ cells is that it is very animal intensive. From a practical matter - there are in vivo germ cell assays. In fact, genetic toxicology got its start with germ cell mutation in the '50s with Alexander Hollaender. Practically, it is impossible to study.

FDA and all the other regulatory agencies take a short cut and they say if we determine that something is mutagenic in somatic cells, we regulate on germ cells automatically without ever doing it. They do not really ever consider any other kinds of mutations other than what is considered the cancer mutations. They regulate on cancer and not on any other kind of genetic effects that might result in disease.

I have a couple of projects here I would like to propose. If anyone on the SAB thinks this is a good idea,

please speak up. Getting someone in FDA to say this is a good idea is really a tall order as it turns out.

Another thing we can do, which is the last bullet, is adapt the Pig-a assay. I have not told you what the Pig-a assay is. It is an in vivo mutation assay - somatic cell assay. It is kind of a traditional kind of assay. But it uses erythrocytes. You can do a mutation assay with literally a drop of blood. Actually, we only need 10 microliters of blood. OECD likes it because it is very animal friendly even though it is an in vivo assay. It integrates very well into a lot of standard toxicology testing. Industry likes it too because it does not cost very much as a standalone assay. It gives you another endpoint that complements the micronucleus assay, which is something that is done in vivo as a standard test.

It turns out that sperm also are amenable to Pig-a assay. One of the characteristics is they are single cells and you can put them through a flow cytometer and you can measure cell-specific antigens, which is the basics for the Pig-a assay. We would like to develop that as a relatively quick germ cell mutation type assay that we can use in vivo or in vitro as an endpoint.

I am going to talk a little bit about the airway tissue model that Bill alluded to in his opening comments.

This is probably the best developed of the typical models that we have been working with. We started this out - I think it was 2010 or 2011 as a collaboration between NCTR because I thought it was a good idea and CTP who strangely enough agreed with me. We went into collaboration to develop this model. You were not around, Cathy. This is Patricia - she was a lot easier to convince.

This is a model that is in vitro that generates a tissue like in vitro - I always call it model, but that is an overused word that is very like the epithelial lining of the airway. In this case, we have used human cells for this almost exclusively. And what you do is you get a biopsy from a human lung. It is always an autopsy biopsy. And the large airway, the G3 level, is very amenable to cell culture. You can just cut that out and dissociate those cells and seed them onto - you let those grow a couple of days until they get nice and confluent and then expanding the middle thing here.

And then you take the media off the top of the cultures. This media I should say is special media. It has a gazillion things in it. You create an air liquid interface in these cultures. Media on the bottom. There is a porous membrane that the cells are sitting on. The nutrients are coming up from the bottom. We think of this

as the blood supply.

You get something that looks like the cartoon on the right. A bunch of - you can see that the cells become columnar and they have cilia. Some of these look like goblet cells, which they are. There are these little cells under here that are basal cells. These are progenitor cells. This thing actually forms a steady state. The basal cells divide once in a while. We place the (indiscernible)-- or whatever, into media cells, the ciliated cells and goblet cells. It forms a culture that will - if you feed it every other day, you can make it lasts, people claim, up to a year in cultures. You can do long-term studies with these things.

Here are some stains. One of the nice things is you can make sections and do histochemistry on these. Here is a p63 stain, which I am told is a marker for basal cells. You can see them a little darker here. This really does not give good contrast. The Ki67, which are dividing cells. The basal cells and the dividing cells tend to colocalize. The bottom two are stains for mucus or mucins. And one is acid and one is neutral, I believe. I can never remember which is which.

You can see that the cultures tend to elaborate mucus into the apical side of the culture where it

accumulates at the surface. We have to wash these things off every couple of days to keep them from drowning in mucus. In the throat of course there is a lot more activity going on. You can see the bluer - stain or goblet cells. You can count the goblet cells. There is a thing called goblet cell hyperplasia that you can measure as well as the concentration of mucins and the mucus and all kinds of -

Here are some of the endpoints that we have measured. I am not going to show any data because all this is sponsored work and Cathy will be happy with this. To really publish it or bring it out for public view, it has to go through an elaborate clearance process. I am going to avoid any pitfalls here. I am not going to show you any data on this particular project. Here are some of the endpoints we have looked at over the years. Mucus secretion is an obvious one. We have assays for the different mucins. Some of them are inducible, some of them not.

Cilia. You can kind of see the cilia. They tend to be lost when you make sections and fix them. But they really beat. You can measure the beating rate with imaging microscopy. A lot of times the most - the quickest and most sensitive response to a toxicant is this mucociliary

clearance. Either the elaboration that the mucins changes are the cilia beating frequency changes. Sometimes when you give a particulate, it beats faster. Of course, it wants to get rid of it and with a lot of the toxicants that affect particular proteins or internal elements, it suppresses the cilia beating. It is kind of interesting responses.

You can measure tissue permeability, which is done electrically. You can also do immunofluorescence for tight junction markers. In the culture itself, you can measure all the kind of biomarkers, molecular biomarkers you want for RNA and proteins and things like that. We generally have measured Phase I and Phase II metabolism genes.

As I said, one of the neat things that you can do is make stains of these sections. You can look at morphological changes. One of the things that happens within a week often of daily treatments with a toxicant is the whole structure of the culture changes. You get the squamous appearance. People argue about exactly what we should call it. It could be squamous pre-metaplasia.

Another neat thing you can do is you treat for a week and you see something happen. And then you can stop. And very often, they recover, including with the

morphological change. They will bounce back and look like they used to.

When a culture goes squamous, it is sort of tightens up. It is circles the wagons. And the permeability of the culture becomes much less. You can imagine it is a sort of a defensive kind of mechanism.

In the media, you can measure a lot of secreted markers like cytokines and chemokines and NF-kappa B. Extracellular matrix proteins are quite responsive to toxicants in the system. That is half of it.

Although we do this in the government, this is not an unknown technology in pharmaceutical industry and in academia. We may be the only people in the government doing it as it turns out. But there are a lot of neat exposure systems that you can use for various kinds of inhalation products. I am not going to talk about the cigarette smoke exposure because I will get into trouble.

But we have a system for aerosol exposures. If you can make a solution out of a toxicant and then you can nebulize it so you get a cloud produced, you can do that with this unit in the middle here. Here is a nebulizer. You shoot this into the nebulizer. You get literally a gray cloud in the chamber, which settles out. Here is where the cultures are sitting in these little pockets.

There are 12 here. Actually, one of these is a balance. It is a quartz crystal micro balance so it can measure in real time the deposition of any particular matter. You can do this with nano materials also. Although we have never done it with this particular setup. This would be a nontreated control here on the left. Below this base here is media and a heating unit. You can keep these cultures happy while they are being treated. That is for aerosols.

And we also just got a unit that can be used for gases and vapors, semi-volatile materials that will vaporized with a little heat. This system maintains the right temperature to keep the test article volatilized. It has a way of making dilution so you can test different doses. And this over here is the exposure system, but it is kind of much smaller. The actual exposure - about this big and it is a big Plexiglas box - prohibit exposing us, but even so, it sits in a -

Here is just a list of our projects that are ongoing. We have two major projects. One is, as I said, with CTP. It is evaluating the toxicity inflammation produced by cigarette smoke using these cultures. Another one is a new one with NTP, developing an in vitro system.

Over the last six or seven years, I really think we have become good at this. This is not easy. Getting

these things to work, these cultures to work is not that easy to do even though it is pretty simple. We do all this in-house. We make all these - we get the biopsy material from various sources, universities. But we make all the cultures ourselves. Getting the cells to differentiate takes about 28 days. And then once you got them, we start treating them about six weeks. As I said, they will go for three months usually. They will change a little bit in morphology, but not a whole lot. They kind of age in place.

One of the things we would like to do and we have done it a little bit in the past is apply computational fluid dynamic modeling to scale in vivo to in vitro exposures. Exposing by inhalation is a complicated thing. As you think about a spot in the lung that we are modeling in these cultures, what gets to that lung from an inhaled substance is not so easy to predict.

There is a whole field of toxicology that deals with this as it turns out. But there are not a lot of extrapolations made between these kinds of systems and in vivo systems or vice versa as I have it here because we had some - in many cases, we have in vivo data that has been done with pure compounds. We can find out what doses of those pure compounds we want to use. That is the

reverse - kind of thing.

We actually have the software to do this. It cost about \$50,000. Luckily, we did not pay for it. But we do not have anybody to run it right now. That is something we would like to do in the future.

As I said, what we have here are human models because they are the best developed and obviously we are interested in humans. But there are rat and rodent models that can be readily made using the same things. A lot of the quantitative in vivo data is rodent data from inhalation studies. If you are going to find out how well your models are predicting responses in vivo, it might be a good idea to have a rat version of this model.

It also would be good to see whether or not the rat model is responding the same as the human model. Someone talked about comparative work. That would be something that we would like to do, having a rat and a human side by side.

From a genetic toxicology standpoint and very little of this has dealt with genetic toxicology. We have done the common assay on these things. I would like to make a transgenic rat ALI culture. Similar to what Manju has developed with the transgenic hairless Albino rat that has a reporter that you can recover in a bacterial

transgene that you can pop out and measure mutations in. Those things exist in rats in vivo, but no one has made an in vitro model. It seems obvious that you could do it. If you can make an in vitro rat model, you can make an in vitro transgenic rat model just as easily.

Of course, you can use NGS sequencing technologies. I will talk about that in just a minute. We can even do Pig-a if we got lucky on these. This is the simplest kind of airway model that you can envision. It is just the surface. It is cyto-contact responses. You can measure, as I showed you. You can measure a lot of kinds of endpoints using that. A lot of them are disease related type endpoints. But you can make this more complex by adding macrophages or fiberglass endothelial cells, kind of create whole multi-layered tissues. And people have done that. The problem with that is they are not very stable. You can imagine. It is like juggling ten balls instead of two balls to keep everything going.

If we want to quantitative kinds of endpoint assessment, we can do that with six or ten of these as far as measuring an endpoint. And a lot of these endpoints are non-destructive. We can do the endpoint determinations longitudinally. To me, at our stage of development, I think it is probably good to exploit what we have as far

as a model before we get into these higher powered things.

There is also MPS. Somebody is going to ask me. Why don't you use a microphysiological system? There are lots of reasons why. It is mostly related to dosimetry. You just cannot do the kinds of treatments in an MPS system as you can in this kind model, which has an open apical surface. Now there might be a lot of disadvantages. I am sure these people who are developing - in Harvard and elsewhere in the country are going to figure this out eventually. But right now, the technology is such that the state of the art as far as inhalation toxicology is with these things. I am holding onto that for the time being.

But I must admit. We had a teleconference a couple of days ago that Donna hooked up. This group in Germany has developed this microfluidic system with a downstream tissue from the lung. They used ALI culture as the lung component of it. You just pop the Transwell into this thing and then you start the microfluidics and you have a liver or a kidney or something else. They had as many as five, I think, in their system. That might be something that we should pursue as far as maybe a transitional MPS type system.

Two types of projects. The second is the monitoring genetic variation thing, which is another thing

that is near and dear to my heart that we have been working on for the last 25 years I would say. And with the advent of the next generation sequencing, a whole new list has opened to us that we should taking advantage of.

Regulatory genotox assessments rely on DNA damage such as you get in a Comet assay. Cytogenetic damage like chromosome breakage and annual ploidy and get that in a micronucleus assay. And we reporter gene mutation that you get in bacterial cells in the Ames test and mouse lymphoma assay or in the transgenic rodent assays. This is equivalent, as I like to say, as using a surrogate endpoint and surrogate systems for evaluating effects in humans. It is no wonder that genotox data is considered hazard ID data. It is kind of like a litmus test you put on your chemical and it tells you whether or not it genotoxic. That is far as it goes.

My long-term life goal is to change that and make genotox data pseudo-apical endpoint. Now we are not measuring a disease. Obviously, a genetic change is not something that is a disease phenotype. But if you can equate a genetic change with a disease phenotype, it is almost - it is as close as you can get to what people define as an apical endpoint.

For years, we have been using these kinds of

biochemical kind of mutation assessments to get at cancer-driver mutations. This started with Barbara Parsons about 25 years ago when she was a postdoc. She is now a GS15. It has worked for her. The major system that she developed was called ACB-PCR, which stands for allele-specific competitive blocker-PCR. It is a PCR technique that is capable of measuring one allele and 100,000 wildtype alleles. As it turns out, that is sufficient for many cancer-driving mutations because the backgrounds of some of these cancer-driving mutations are quite high in humans and in rodents. This is really hard work and it has taken Barbara 25 years to get to where she is. This is really hard work to do ACB-PCR for one mutation at a time. That is where it quantifies. Very good data, but very difficult to get.

People have come up with other kinds of protocols, some of which for diagnostic applications. This one with gd-PCR is something that we are doing in collaboration with CDRH actually.

Error corrected next generation sequencing, which if you use a couple of tricks that normally are not used in next generation sequence analysis, you can actually make NGS system much more sensitive to rare changes. NGS has the problem that if you just run it

straight, it is very error prone. You get a change that is just auto factual. One out of every 100 bases, it gives you a sequence on. This is just too error prone for our purposes where mutations generally occur in the ten to the sixth or lower range. But there are tricks around this that we have developed somewhat in-house and other people have been working on it.

We have used actually error-corrected NGS on a Pig-a project. One of the problems with proving that Pig-a is a gene mutation assay is we do it in erythrocytes. How do you prove that it is a really a mutation causing that phenotypic change in the erythrocyte, which does not have a nucleus? Well, you take one step back and you look at the progenitor cells. We fished out all the progenitor cells and the erythroid lineage and also all the granulocyte lineage and we tried to look for mutations in the nuclei that were left. A problem with these cells is they do not multiply very well at least in vitro in our hands.

What we did was we just took the single cells. If you place some tricks on them, you can actually see mutations in these cells because they have DNA. We can tell - because the granulocytes and the erythroid cells come off - I do not want to explain erythropoiesis, but

they branch off at different points. You can tell where that mutations was induced by comparing the mutations from the same animal in the granulocyte population and the erythroid population.

This is some work that (indiscernible) has done. He is in the back of the room. Incredible. It is astounding work considering what we knew about this five years ago or even two years ago. I think this is going to make the validation of this particular assay possible because we will be able to show that there is really a mutation causing this phenotype.

But to go on to the proposed and beginning projects, one of the obvious things you can do with NGS is look for germ cell mutations. You can do it in humans. You can do it in rodents easily. You need the parents and you need the offspring and you just do comparative genome comparison to look for new events. You do not need high fidelity methods. People are doing that. It is not unique to us.

But one of the problems with getting FDA's attention with the importance of germ cell mutation as far as an endpoint they should regulate on is because no one has absolutely shown that there is such a thing as a human germ cell mutagenic. In animals, yes. In humans, there has

not been sufficient proof yet. I think we are ready to make that proof given the resources to do it.

We have also tried duplex NGS for mutation quantification. This is a method that in theory will detect sequence changes down in one and ten to nine so one per genome. It is tough to do, but we are trying to work our way through it and maybe see some avenues that we can improve on.

The last two are projects I am going to show you in the last 15 minutes. This was cooked up by one of our staff fellows. He was very clever about this. High fidelity NGS and whole genome clone analysis. What I told you was that the limitation to looking at rare events with NGS is it is intrinsic error rate. That is a problem when if you take a single mutation like in the Ames test, we are talking about events that occur in one in ten to the eighth level. We are much below one in hundred. The noise is quite substantial in this system. But you can get around that using this little trick.

You start with a homogeneous cell population. It could be bacteria. It could be mammalian cells. It could be T lymphocytes from a treated animal. If it is T lymphocytes from a treated animal, they have already been treated. You can treat the bacteria with - this might be a

control. This might be treated. And then you plate everything out as single cells.

What we are going to do is let the organism, the cell do the work rather than trying to PCR this up and let next gen sequencing do the work. The cells are going to replicate. They will go through replications where they will produce a clone. You take those clones and you compare the clones, the mutations in the clones to what we started with, the genome of origin. Anything that happens within the first couple of replications you should be able to detect and it depends on where you put your screen. Unfortunately, I do not remember where Javier put his screen. You can see one or three replications on your damaged DNA. You can detect mutations from that because that is above the noise level of the system. And then you can tell the locations, the frequencies and the base pair changes of the somatic mutations for treatments A and B.

Here is some data. If I did not say it before, this is something that was cooked up by Javier Revollo who is our next gen sequencing expert, very clever guy. He treated some E. coli with ENU. That was a very good choice of chemical. I did not tell him to do that. I am sorry. With EMS, ethyl methanesulfonate. Ethyl methanesulfonate is an alkylating agent. It produces ethyl groups on DNA.

It is relatively long lived. It reacts with DNA over a period of hours.

What he did is he took three doses of EMS. He harvested cells. This is just three cultures essentially at one hour, two hours, and four hours. And then they went through this procedure of sequencing the entire genome. Instead of looking at one base like you do in an Ames test for mutations, we look at ten to the six bases. We are gaining real estate here. The error rate is not critical. We somewhat overcome the error rate. If we can see those mutations in the first couple of generations, we can confidently call them an EMS-induced mutation. This is the frequencies that he saw. Note that this is on a log scale. These are spectacular increases. In fact, they are greater than you see in the Ames test. It is related to the dose and it is related to the time of treatment. Perfect toxicology.

If you look over here, one of the things that comes out of this just automatically is the kinds of mutations that were induced. You can see that by and large, they are G to A transitions, which is the exact mutation that you expect from an ethylating agent producing 0.6 ethylguanine in a bacterial system. This was a mammalian cell system. A eukaryotic system - it would be

a different mutation. This is really a proof of principle, which one of the FDA reviewers asked for because he or she did not believe we could do it that this actually works.

We have also done with mammalian cells, in vitro, L5178Y cell, and a cell line derived - not a cell line, but a clone derived from rat T lymphocytes. The rat was exposing in vivo. We took lymphocytes. Actually, we did seven of each combination to get the error bars and looked at that and they all worked.

With the mammalian cells, we are talking about a much bigger genome. The chances are you will see a spontaneous mutation once in a while. There is a background. With the bacterial system apparently you do not see a background at least underneath these conditions.

What could we do with this? We are going to apply this. I do not think it is going to replace the Ames test if that is what you are going to tell me. But I think you can apply this to situations where you do not know where the mutation is going to occur. It is going to be a rare event. You do not know what it is or where it is going to occur.

One of the problems that we have been made aware in the last couple of years is this business about gene editing, using CRISPR and other kinds of technologies

where you correct or induce a mutation that is directed by a particular sequence or a protocol that occur in a particular place. You are going to use this product sometimes in humans. The risk here is what if it is not 100 percent correct in targeting that mutation. If you follow this in the literature, that has been going back and forth by different people. There is no real good way of knowing this. A standard way of determining whether or not CRISPR Cas9 has a fidelity close to what we would like or whether this particular target is error prone or something like that. Here is a way we could do that potentially. We are just going to take a homogeneous cell population. I sort of forget what the cells were going to be. I think part T cells. Actually, I can show you what they are. And human lymphocytes to the cells. Apparently, they are clonable.

We are going to target using some kind of CRISPR targeting. Apparently, these are genes that had been knocked out for clinical reasons and collect the cells that have the proper knock out and do the same thing. Expand the clone and then sequence them and see what we get.

To make this a good experiment, I guess we need a positive control. I am not really sure what we are going

to use for that unless we find something that is really error prone, but I guess we can just induce mutations in the cells and try that.

Genomic locations and frequencies, CRISPR-induced somatic mutations. Hopefully, we will be able to determine that. This has been done in collaboration with the PI in CBER. I believe there is a back woods project that CBER has proposed, looking at gene expression, using NGS in CRISPR-targeted clones. I am not sure what happened to that, but I am sure Javier will be able to tell me.

If you have any questions, please let me know. As I said, my big picture goal is to make genetic toxicology - advance the field of genetic toxicology. It is what it is now and it is very useful for regulatory. We can go on giving advice about whether a common assay is positive or negative for some test agent.

But I think what I would really like to do with our resources here at NCTR is to move the field forward. I think the forward field that I see is making genetic analysis or genetic toxicology into a pseudo-apical endpoint that we could use for quantitative risk assessment of disease endpoints. That would be a long-term goal. We need the right biological situation. We need the right analytical situation. But I think it is not too

crazy to think of that happening.

The other thing is I really think we are - FDA or the regulatory agencies are dropping the ball by not paying more attention to germ cell mutation and somatic cell mosaicism, which I have not mentioned here, which is another kind of mutation that really is not evaluated for a health risk by the regulatory agencies. If you look the guidance documents, they all acknowledge that this exists, but then they go on to talk about the Ames test. I would like to take a step back to the 1950s and treat these kinds of mutations more seriously because now we have the technologies that we can actually find these mutations in some sort of efficient way. We do not need a thousand mice at Oak Ridge National Laboratories to do these experiments. We can do them with a couple of mice or even - and using the technologies we have.

I will stop here. If anyone has anything to help or criticize -

(Applause)

DR. LEIN: Thank you, Bob. That was a really enthusiastic presentation. I really enjoyed it.

SAB, we have been asked some very specific questions here from Bob, which really do address this issue of in your scientific opinions and expertise. Is it

worthwhile to invest resources into germ cell models and also somatic cell mosaicism?

DR. FELTER: Susan Felter. Thanks Bob. Great talk. Help me understand. The focus on germ cell mutations. Is that because you anticipate a unique susceptibility to germ cells that somatic cells would not necessarily have. In other words, that you would see mutations in germ cells, but not somatic cells. I think some would say somatic cells are easier to work with. There could be a presumption that if it induces mutations and somatic cells that we would just assume that the same is true for germ cells as well. Is there an additional element that you think that they would be uniquely susceptible?

DR. HEFLICH: In certain cases, I believe that is the case. There are a few examples of that, which my adult brain cannot come up with of the top of my head. I think you can imagine that germ cells are very much different than liver cells or lung cells or anything else. They have a very different biology associated with them. They also have a different DNA metabolism associated with them. They replicate much differently. They go through mitotic cycles. They go through meiotic cycles. Different agents, this has been known for 40 or 50 years, will mutate at

different points. This is male germ cell gametogenesis. I do not think you can absolutely predict that something that is mutagenic in somatic cells is mutagenic in germ cells or vice versa.

Now EFSA, I believe, and perhaps ECHA, mix them all up, have recognized that and when they -- I think it is ECHA. It is the REACH program -- have recognized that. They are asking where there is exposure of germ cells because that is always a factor that you consider germ cell mutagenesis as an endpoint and whether or not it occurs. If there is no data on it, you might be compelled to generate the data. The chemical regulatory world has gotten very excited about this. They have been looking at the transgenic assay because that is the one mutation assay that can be done in vivo in germ cells that exist with an OECD test guideline. I think we can do the Pig-a assay in the same way we can do the transgenic assay and save people a lot of money. If you deal with multi-national chemical agencies that might be an advantage.

I also think that from a long-term perspective, if we regulate on risk and we look at somatic cell risk versus germ cell risk, things happen in germ cells as germ cell mutations that would never happen in somatic cells. The only reason we are interested in cancer is because

that is one of the few diseases that you can mutate as a somatic cell and have a phenotype in an adult or even in a young adult because it produces a phenotype where that cell can expand with friends and produce a tumor. But if it is a germ cell mutation, there are literally thousands of identified diseases that are associated with germ cell mutations. The human Mendelian database or whatever the heck it is has 5000 plus and counting different phenotypes that have been associated with a particular germ cell mutation. To me, it would be unbelievable to think that we could say treating this person with a potential mutagen might not affect that.

I just think that cigarette smoking for one thing - there is a lot of evidence, indirect evidence that suggest that it does affect germ cell mutations in a variety of non-cancer diseases in the offspring. I think we should really look at that.

And the ultimate use of this data is quantitative of course and germ cell quantitative dose responses may be very different than somatic cells in any particular tissue and in adult versus whatever.

I talked about somatic cell mosaicism. That feeds in very nicely with Bill Slikker. He probably does not know this. Bill Slikker's perinatal virtual center

because how somatic mosaicism causes a disease is if you mutate something early enough in development where a tissue expands from that mutated cell that has multiple copies of that mutation. You get a mutant phenotype that way. In fact, if you look on yourself, you probably have all kinds of moles and things. Those are somatic cell and mosaics that would probably induce during early development.

But there are a lot of other diseases also associated with that. It is becoming increasingly clear that this occurs because we can now sequence tissues from various parts of animals, diseased tissues, and normal-appearing tissues. We can see the differences in the mutational spectra in these tissues. It looks like this is a lot more common than we thought because of the relatively facile way we can sequence large genomes now.

I think these two things - if we want to look five or ten years down the road, I think people become aware that this is more and more important to government regulatory agencies looking for risk with a particular product. It just seems to me that - just because we have done things the same way for 50 years, it does not mean we have to think in that way for the next 10 or 20. If it is done anywhere in FDA, it is going to be done here. I am

putting up the flag. I am willing. If somebody says this is important, go for it. We have a couple of protocols in the queue that will likely to be shot down in the product centers because they just do not want to hear about it. It just creates more problems for them. If the SAB says, yes, this is important, I will feel like I have accomplished my mission.

DR. LEIN: Thank you, Bob. I have a quick question and then I am going to turn it over to the product center to see if there is any comment from you on this topic. Where does epigenetics fit into this scenario you just lined out for us?

DR. HEFLICH: In division of responsibilities at NCTR, we have generally left epigenetics to biochemical toxicology. Although we have two PIs who include epigenetics in their research portfolio. One who does micro RNAs as a biomarker in relationship to mutation of cancer. He seems to think that micro RNAs will tell you more than mutation will. I will not discuss that. Manju has developed an in vitro test for epigenetics that is quick and easy and can be included with DNA damage. You can get epigenetic and DNA damage in the same kind of little kit test. It has not gotten very far out of his lab, but it has potential.

DR. LEIN: If you do move forward with germ cells, this would be a prime opportunity to integrate the mutational landscape along with the epigenetic landscape.

DR. HEFLICH: I agree with you. There is a whole history of imprinting and methylation effects in germ cells and males versus females. There is a whole kind of universe of information that could be brought into and integrated into any kind of assessment like this.

DR. JAIN: This is Diwakar Jain, New York. You draw a big distinction between germ cells and somatic cells as far as mutagenic potential is concerned. By the same token, somatic cells. That is not a monolith. These are different somatic cells, hepatocytes, renal cells, fibroblasts or smooth muscle cells or any other. The susceptibility could be very different too. It is not that all somatic cells are going to respond exactly identically. And then there could be species variation as well. The issue is we are opening very complex issues of susceptibility to mutagenesis across different somatic germ cells or different species. Different studies may have been done using different cell cultures and they arrive at different conclusions, but they could all be due to the model they used.

DR. HEFLICH: Exactly. You will notice the second

bullet here - likely use different kinds of cells and see how they compare as far as these chemicals or these agents that we think may be toxic or we may think they are not toxic. Is this is a cell-specific effect? Is it a genotype effect? We do not know. This becomes important.

Most of these assays that we use now in genetic toxicology were developed in the '70s and '60s. They work well. People hopped on them, developed huge databases on them. They know their predictive capacity. That is not likely to change unless something comes along that says that we really should be evaluating things this way. We need this kind of data to make a final regulatory conclusion. We suspect that this is going to make a difference.

From a qualitative standpoint, if you are just talking about hazard ID - I do not the people from CDER very well. David Jacobson-Kram used to say, he only wanted to see an Ames test. That was enough for him. There is a lot to be said for that.

As far as hazard ID is concerned, you cannot beat a simple system that responds to a known mechanism. You can say that. And then you can build on a clastogenicity endpoint in a very simple system that you know is very sensitive to picking up clastogenicity. You

could use CHO cells or mouse lymphoma cells or assays. This is exactly how you get a battery of assays. From a hazard ID standpoint, this has a track record for identifying genotoxic carcinogens like 90 percent. I do not know what it is. It depends on how you do the math. We are going to have to come up with something that is an improvement on that.

I know from the industry standpoint, they do not want to lose lead compounds that might be genotoxic in vitro, but if they are put under more relevant circumstances. We have a compound called dihydroxyacetone. I am going to regret I brought this up. It is a tanning agent that gives people tans and reacts with proteins. It also reacts with DNA as it turns out. It is mutagenic in the Ames test. It is pretty mutagenic. It has been known since the late '70s that it has mutagenic in the Ames test. But the question is is that relevant for human exposure. It may not be because you are treating dead cells essentially when you are applying a spray-on tan. Dead cells do not mutate. That is the first thing I learned in genetic toxicology. You have to make some sort of rational assessment of the situation. That is a simple example.

But you can say the same thing about other kinds

of situations in humans that may impact the significance of a positive finding in vitro. Industry is probably well known. They spend a lot of their mental effort discounting positive in vitro responses by trying to develop a mechanism or a demonstration that this not relevant for in vivo. It is good that in vitro tests are so sensitive, but they only give you half the story sometimes.

DR. LEIN: Is there any input from any of the product center representatives regarding this issue of the germ cells and their relevant importance in the FDA mission? Thank you.

Any other questions or comments from anybody around the table?

DR. FELTER: Susan Felter. I will change gears and ask you just one question. You did not go into any details on the ongoing work on quantitative evaluation of genetox endpoints as if it is a quasi or pseudo apical endpoint. Is that effort focused on in vivo data only or are you also considering using it for in vitro?

One of the things that occurs to me is in cancer quantitative risk assessment, when you are working with a bioassay that was originally designed for the purpose of hazard ID and was not designed necessarily for dose response assessment, we might have done things a little

differently in the design of the bioassay if the intent from the beginning was to be used in dose response? I am wondering if you would see the same potential for genotox where if you want to use it for some kind of quantitative evaluation, does that suggest that there should be changes made to the protocols, which were designed for the purpose of hazard ID?

DR. HEFLICH: You are thinking really far ahead. As far as what data would be useful for quantitative risk assessment using gene mutation, my personal feeling is it has to be in vivo data. People right now are using reporter gene data to do it. I do not think reporter gene data necessarily is the best kind of data if we are going to say that there is a threshold response to a certain treatment and there is a safe margin of a dozen so based on this data.

I think my personal opinion is that the only way we can do it is to look at a panel of genes, perhaps the whole genome although I do not know whether or not that is practical. It is certainly not practical currently. And get an idea of mutational load in any sort of bias in a particular target or sequence that one might associate with a disease phenotype. I think you may be able to regulate on that as a basis of dose. But you have to have

the right biological system. That is why I think the biological end to this is very important also. I am not sure whether a rat or a mouse is the right system to use. It certainly is something. It has a lot of data behind it. Maybe I could be convinced that that is the right biological system that will give a quantitative assessment of risk. Obviously they used that quantitatively for tumor risk. I guess I would be on firm grounds to say using for mutational risk although we know - as someone said, there are all kinds of differences between rats and mice and humans. You take inhalation toxicology. That is a really different kinds of biological parameters involved in those kinds of responses. I am not sure I answered your question.

I think we have to get a couple more pieces to the pie before we can actually use data quantitatively. We have done some quantitative assessment for particular situations. If you want to know whether or not chemical X is more mutagenic than chemical Y or the smoke from cigarette X is more mutagenic than the smoke from cigarette Y, there are dose response modeling regimes that can be applied to that like the benchmark dose and potency quantitation that people have come up with recently. We have used them. But it gives you kind of a narrow sort of

answer that answers a specific question. You can differentiate between two doses on the basis of mass and assay mutations, for instance, two treatments. Does that have a human consequence? That is the \$64,000 question that I am not sure we can approach right now. But it is the one we should be trying to approach.

DR. LEIN: I believe there is one more question.

DR. STICE: Steve Stice. I just want to make one comment real quickly. I think this is really fascinating what you are trying to do. I think you couldn't have presented a more difficult aspect and the fact that you are working in a fast area of organ on a chip where thousands of labs are being funded in that area. Next generation sequencing is moving lightening quickly. Just recently a publication on CRISPRs where you do not necessarily will have any off target effects where it could be just modifying the RNA. With a group of 30 people, this is really difficult.

DR. HEFLICH: I agree. We are out-competed in many ways. These are hot areas. We have to rely on our uniqueness perhaps if we do have any uniqueness. I hold to that hope. I think this approach is relatively unique. Whether or not this will become a mute question in a couple of months or years about the CRISPR Cas9, I do not

know.

I can remember back with the Gelsinger problem with the integrative mutagenesis. I remember we discussed these in the genetox group. I cannot remember the official name for it. What can we do about this as far as safety evaluation so this does not happen again? No one had a good suggestion at that time. This was in the 1990s. I cannot remember. I think this would work for that in the same way it works for CRISPR Cas9. We can generate biological data or biochemical data that will put a number on this if a number exists.

A lot of this other stuff that I have seen in the past has been computational type analysis. I do not know. When you put a computer between me and the data, I wonder. You have to be able to track everything. Everything that goes in good comes out good also. This is something I can wrap my mind around a little easier.

DR. LEIN: Thank you. Are there any other comments, questions around the table?

DR. WILSON: Just for accuracy. The incident that you were just referring to was not Jesse Gelsinger. It was not in the '90s. It started in 2002 with retroviral vector based gene therapies and development of leukemia. That was in a very specific patient population of children who had

X-linked SCID. While there is still a lot of uncertainties around the exact mechanism, it is likely disease context specific in terms of why we saw that kind of insertional mutagenesis in that patient population. We have not seen it with some very similar retroviral vectors in a variety of other diseases.

We did institute, just for the record, a long-term follow-up policy to evaluate patients long-term for potential events that were related to insertional mutagenesis. I think it was a long, known mechanism that retroviruses can induce insertional mutagenesis. It was not a matter of having an assay to detect it. We knew that was a likelihood.

DR. LEIN: Thank you. Anything else? I think we are adjourned for lunch. We will reconvene at 1:15 for the public session. Donna, do you have any logistics about lunch?

DR. MENDRICK: The people who ordered lunch - they should be ready down in the café. We have to go down. We should go in groups and - come back. Apparently, there is an area to eat outside although if it is going to rain, I do not know. But, again, please make sure you get back here on time. Thanks.

(Luncheon recess.)

Agenda Item: Public Comment Session

DR. MENDRICK : This is the public comment section. I have not gotten any requests for public comment. Is there anyone in the room who wants to make a comment at this time?

(No response)

Okay, so we will go back to the regular meeting.

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

DR. LEIN: Okay, thank you. The next presentation will be by Dr. Carl Cerniglia, Division of Microbiology.

Agenda Item: Division of Microbiology

DR. CERNIGLIA: How does this work? Does someone put them on the screen? Okay, got it. Thank you. Good afternoon. Boy, I have to tell you a funny story. It just happened. I decided instead of eating lunch to go outside and take a walk. Everybody who knows me knows I

like to exercise. I went out, and I am walking. I am going toward the Clinton Center.

I am going down, and I see a familiar face. It was Dr. Raoul Cano who is a microbiologist from the University of California system who actually served on our SAB. He was driving through. He has family in Boston. He was just driving through. He was like 45 minutes in Little Rock, and he was headed to the Clinton Center. He was going to work his way back towards California. You talk about a coincidence and talk about lucky.

When I was driving down this morning, they said who is the luckiest man in the US today? I am trying to think, who could that be? I think I am now because I met Raoul. Anyway, they said Justin Verlander, the pitcher. He won the World Series and married Kate Upton within 10 days. Anyway, I feel I am pretty special and pretty lucky today to be here.

Now, I think you heard great talks from both Fred and Bob, biochemical talks and genetic talks. Now, we are going to switch gears and talk about microbiology. First of all, it is so exciting for me to speak to such a prestigious group of esteemed scientists as Dr. Slikker mentioned in his introduction. It is important to us to get scientific advice and recommendations from the board

and our FDA colleagues. It is very critical. Thank you for being here and taking the time from your schedule.

And also, I want to thank Donna for organizing this nice venue. I can look out here and see my walking path. I run every day, so it is kind of nice to see that, too, as well.

Anyway, I want you to kind of think about microbiology now. Being in the microbiome arena, I kind of want you to think of how I can relate the microbiome to some fundamental principles of this mission and vision. The mission of the division is to serve a multi-purpose function with specialized expertise to perform fundamental applied research in microbiology in areas of FDA responsibility and toxicology and regulatory science. So that is kind of the standard to submission we have had.

But I have to say in the spirit of the microbiome and when you think of the microbiome and the gut/brain access and these signaling pathways, and how the importance of the GI tract is to the brain in terms of producing neurotransmitters, signaling pathways and neuromodulators, and the fact that it can lead to different moods and anxiety, et cetera, and other behavioral responses, as well as some neurological disorders.

When I think about the Division of Microbiology and the fundamental mission and the vision, I have to say that this particular year, and about a year and a half since the last time we spoke, was a bit of a gut check. It really was. It was a true gut check for the major reason it was a challenge for myself and scientists. They have not really had really behavioral responses, but certainly they are very concerned about budgets and how we can kind of maintain our excellence from a lot of the advice that we received from the board over the years. How we can maintain this excellence and keep our leading role in these fields based on the fact, that was alluded to from Fred's presentation, that things are tighter.

It changed the way I direct and the way I manage because I had to think much more strategically now in kind of setting priorities on how we go. Again, this year was truly a gut check in terms of maintaining the mission and the vision to advance regulatory science within the agency. Because basically, what is it all about for any of us in this room is we want to maintain strong science, we want to make sure it is accurate.

Then we certainly, as FDA people, we want to make sure we are contributing to the FDA mission. They are fundamental values that I think about all the time.

These are the things that concern me. When things kind of get tight, we shift sometimes more from a research mode to a business model mode in our thinking, which certainly is different than we have in the past. And also, obviously the funding priorities can be skewed a bit towards more of the strategic areas.

I was very pleased in Dr. Slikker's strategic priorities that the microbiome was mentioned in the presentation. I have been division director since 1986. I have been here since 1980. It is kind of nice to see the microbiome actually being addressed at the director's office level for the importance of that.

In terms of division staff, I think there haven't been radical changes. I think it has basically been pretty consistent. From last year or the last couple of years, as was mentioned earlier, we have some adjustments in the ORISE and visiting scientists program. The numbers kind of fluctuate anywhere from 45 to 35 in that area. We now have about 37 FTEs. If you remember from Dr. Slikker's presentation, the total is 688 out here. So we are that percentage of that. You can do the math.

I think the other important point that Dr. Slikker mentioned that I would like to reflect here is

that succession planning, how critical it is to the institute and certainly to the Division of Microbiology. And this was one of the comments Dr. Pillai was on the Science Advisory Board when we reviewed.

And one of the comments, which I took extremely seriously, was the fact of succession planning. The old drop-dead theory, what happens if I get a heart attack on that running path? Who is going to be in charge type of thing? So actually, I took that to heart, so to speak. I had one of our staff members, Dr. Steve Foley. Steve, you can stand up. He is our new deputy director of the Division of Microbiology.

So during the period from the last SAB to now, Steve has been involved. I did all the coaching and mentoring, but basically he is pretty much of a self-starter. Certainly, everything that I handled and all these stretch assignments that I gave him and representing us in many different ways, he handled admirably. He is our deputy director for the Division of Microbiology. I am really pleased to say that.

The other piece, besides the staffing, which I want to comment on because I think it is important is the ORISE program or our visiting scientists program or our staff fellow program, and how important it is and critical

to us. I want to compliment certain Dr. Acosta because he has been taking that on to make sure that we can kind of maintain the program obviously as best we can. Obviously everything is based on funding priorities. But it is kind of nice to see that our upper level management, that we see the importance of these programs, as Dr. Slikker mentioned, as well.

The other aspect is infrastructure when you are talking about that. I think our division, we have our own footprint within a division. We have kind of maintained. I think we are well-equipped. Our facility is what it is. I think it is pretty state of the art to address that.

At NCTR, we have a space management committee, Rich Keach and I co-chair that. That is all NCTR. We kind of look at both lab and office space as requests come in at the NCTR level.

But even within the Division of Microbiology, in which Steve heads the committee, I have a space management committee within the Division of Microbiology, some of our principle investigators headed by Steve. So we looked to see how best we can use the space, especially now as priorities are changing and budgets are tighter and that type of thing. We have that in mind, as well.

Outreach, I think we do a very good job in terms

of our outreach program. I think it is pretty similar to what you heard from the other division directors so far this morning. I think we do have projects with all the FDA centers. In fact, what I did is, in preparation for this talk, one of the things that I did, I asked my staff to provide slides on what they are doing, certainly with a focus on approved protocols related to the FDA centers.

So then, I kind of went over those slides with Steve's help. We then kind of made them look good. I sent them to each of the FDA center representatives to give you some idea of kind of what we are doing because there will be no time in this presentation to cover all of the waterfront. I apologize for that in advance. But at the same time, you have the information. Certainly, there are websites and other things you can check. But I would be happy to talk specifically at the breaks or at dinner.

Also, the National Toxicology Program, which I will talk about later, has been very important to us. It has already been mentioned several times. Very active with other agencies, certainly at the universities that you heard. The Global Outreach, I just came back from two meetings in Geneva with the World Health Organization.

One was on what is called JMPR which is pesticide residues in foods. That was a two-week stint

kind of towards the end of September. And then, I just came back in October again for another two-week stint in a different committee on food additives, veterinary drug residues in foods.

We are very active at the international level, certainly in international harmonization with the VICH. My staff was involved in science advisory boards. Even Dr. Cano, to get back to my running on him, I am on the science advisory board for the California system. So I visited his university, as well as the University of California Santa Barbara and others along the California system. It is just one example of many boards that were on it.

Editorial boards, I want to highlight the Microbiome Interagency Working Group on the Federal Strategic Plan. The reason I want to highlight is because we had a representative from CBER, Dr. Paul Carson. He and I kind of represented the agency on the federal initiative. It was the Obama initiative on the strategic plan, which is we put together a nice document across all the federal sector. There are actually about 46 different components to that from various federal agencies and committees, et cetera.

We put together a strategic plan, so we will

have to see exactly how that goes. But anyway, Paul, working together, this is an example of how working together with another colleague within the FDA center. That was CBER.

The staff is very active in working groups. What I would like to mention, though, again it gets into the business side of it, is the seminar program. I always prided myself on having an outstanding external seminar program for the Division of Microbiology at NCTR, for example. We had three presidents of the American Society for Microbiology, Ron Atlas, Rita Colwell, Gail Cassell who came and gave talks at NCTR.

But now, due to budgets, it is just the way it works. I can either send my staff to go to meetings. We have limited budgets for travel now. I had to kind of put the external seminar program kind of on the shelf. It is a golden opportunity in a way because I even talked about it to my colleagues here like in neurotox with the brain/gut access. We could have nice joint seminars on the microbiome bringing in speakers. Certainly on the bioinformatics group, certainly all the computational types of things, having joint seminars in nano, biochemical tox, toxicology, systems biology.

I had this vision of having this really nice

seminar series where we could cut across and highlight that. Actually, the FDA does have like a microbiome seminar series now, part of the working group. But actually, before all of this, before even the microbiome got truly registered within the agency, the Division of Microbiology did have a microbiome seminar series, which Dr. Sangeeta chaired for us.

So sometimes, I guess these are the times we live in. You have to do what I call the PUSH principle, which basically it is Praying Until Something Happens. You do that a lot. You hope that you get some positive results and get some money in there, so some of these things can happen like a seminar program. We will just have to see what happens.

In terms of microbiology research areas, we kind of have five bulleted areas here. The first bullet is basically the microbiome host interactions. We are kind of looking to see the impact of a variety of agents, certainly those that are most important to the FDA in terms of the impact on the human microbiome.

The second bullet point, and I am going to go into this, I will give a couple of examples of that later. The other where we have been spending a lot of our time, and I will say again, this is where the science advisory

board, and when we had the meeting that Suresh was on, gave us advice to kind of diversify our research portfolio. So over the years, that is exactly what we have done.

We were more somewhat food centric a bit. We have kind of totally moved away from all of that. In fact, we have very few foods projects now. We have more of these other areas. People from FDA have that information.

So one of the areas is with developing methods to detect and characterize microbial contaminants in FDA-regulated products. I will give you some examples of that of how we are, I think, playing a major role within the FDA in that particular area. In both the foods arena, instead of isolating organisms that are potentially contaminants in food borne pathogens, our switch, because there is a lot of that already going on in the agency, certainly around the world, our focus more has been on antimicrobial resistance and virulence mechanisms. Are they pathogenic? Are they virulent? What are the antimicrobial profiles? So we are kind of honing in on that particular area.

The next bullet is conducting research to aid FDA in areas of women's health, tobacco and

nanotechnology. Now, that is all on one line. But in fact, it could be on there lines because we have separate research areas in nano, women's health and tobacco products. In fact, not too long ago, Dr. Anil Patri actually asked information on what we are doing in nano and the microbiology. So I had the principle investigators provide him with that information. There is actually quite a bit going on within the Division of Microbiology and the nanotechnology arena. But I won't be discussing that today for time purposes.

In tobacco products, we have two projects. I will be discussing one of those today. In women's health, we have two projects, which I won't be talking about. But we can always talk about those later.

The other aspect, and we have always had a history in the Division of Microbiology and Environmental Biotechnology in improving risk assessments of FDA regulated products, including integrated system biology approaches. And the example that I will give you that I think is a great example, and it is actually in the FDA strategic plan on regulatory sciences.

A highlighted project is the fact of how we combine both the environmental microbiome to the human microbiome in terms of risk assessment in the Gulf Oil

spill. It was very active in the blowout of the Deepwater Horizon oil spill. So we had one aspect on the environmental microbiome where we looked at biodegradation and bioremediation. How do the bugs degrade these particular compounds? So we did some really beautiful studies using all the latest approaches and everything.

And then on the other hand, the agency was also very interested in what is the impact of petro chemical residues in oysters and shrimp. We did a nice study of looking at the impact of that on the intestinal microbiome. So it is kind of neat because we talk a lot, we talk about political boundaries, we talk about walls. But when you are talking about bugs, there are no boundaries. In the environment, you got the bugs degrading the compounds.

Then you have got these petro chemical residues. It gets bio accumulated. And then we can absorb it, and what happens? So this was a great example where we played a major role. I could certainly send you any of these kinds of papers.

So the three top accomplishments, this is kind of the format, which is fine. I think you have got to hone down talks. We can talk forever on all our accomplishments, so it is fine just to talk about the

three main ones.

What I am going to do, instead of just talking about all of them all at once, I am just going to kind of go one-by-one, so this way, I save a little time. The first accomplishment is a project which we have with CFSAN is the detection of microbial contaminants in tattoo inks. Basically, the concept is think before you ink. That is the first thing you need to kind of keep in mind.

But then also, this is kind of the research issue, and this is from the Harris Poll figures, so whoever is into polls, probably some wiggle room there. But basically approximately 25 percent of those aged 18 to 50 years old have at least one tattoo. That is probably no surprise. They kind of went back, and it has gone up from maybe 2003, 15 percent to where we are, 25 percent or more.

But what happened a couple of years ago actually, there was an outbreak. It was in Rochester, New York. There were about 19 cases of people who had tattoos in the Rochester area. They ended up having rashes and abscesses and that type of thing on their skin.

So then the red flag goes up. The local health state health lab then did their due diligence and took the swab sample, sent it to the CDC. They did next generation

sequencing. Found out it was *Mycobacterium chelonae*. Then they did the next step, which is doing source tracking, using pulse field gel electrophoresis. And they found out that all of these 19 cases were linked to the same strains of *Mycobacterium chelonae*.

Then they go back and look at the ink. They found that the ink was contaminated with this particular organism, *Mycobacterium chelonae*. So this led the agency, and I am working with CFSAN on this particular project in the Office of Cosmetics and Colors. Dr. Katz is the director. But I am working directly with Nakissa Sadrieh and a commissioner fellow. We are working on doing a collaborative project surveying a wide variety of inks that they would like us to look at to see if they are contaminated. They are commercial tattoo inks and permanent makeups.

So basically, our techniques, there are very standardized techniques because this is also a multi-lab validation study. This is how we do business within the FDA. It is not only being done at NCTR. The same inks were being screened by a contract lab and also being screened with an ORA lab in California, the PRL lab.

These are all blind studies. They were all looking at these and seeing exactly what the results are.

We all have come up with the similar results. And basically, the techniques, and this is where it really is kind of fun because we are doing these methodologies, some Louis Pasteur technology, culture-based plating methods. But at the same times, we are also doing omics. In the culture of always using new terms, now it is called culture omics. It is a combination of these types of things.

So when we did this, and I need to give credit to the team, it is Dr. Seong-Jae Kim and Dr. Ohgew Kweon, in my particular group, and ORISE postdocs that are doing the majority of the work with our colleagues and guidance from CFSAN. Basically, we surveyed 84 unopened sealed tattoo inks and permanent makeups from 13 companies.

Now, if you actually do it on a company basis, 12 out of the 13 companies had products that were contaminated, so if you just look at it on a company basis. If you actually look at the percentage of the inks that were contaminated, about 49 percent of the inks were contaminated with bacteria and fungi, more bacteria than fungi. Seven inks had a combination of both bacteria and fungi, but most of them bacteria.

And then obviously, we had to identify because the concern is what are the organisms? They identified

these, and some of these are clinically relevant strains. So this held true with the other groups, as well.

Then we have a lot of conference calls and all this. Now we are doing another survey. We just had meetings on this last week. Again, we are being directed to fine tune and even look at even more inks. The obvious thing would be is if it continues, then you would have to do potentially some type of enforcement action.

This is a good example of how the NCTR is providing information to the agency. And also, it is really good training for my staff because this is truly, and I didn't really highlight it in the mission, but it is kind of regulatory science research. This is a perfect example because obviously, and I know the ORA labs, they know they do this every day. But on the research end, we had to learn about samples and all of this.

They all got training on how to handle these particular things, photographing all the bottles. There is a whole long list of things that they have to do even before they do the analysis to make sure everything is done properly. That has been great training, as well as getting them involved. That has been kind of interesting. It kind of leads, and the levels can be anywhere from 10 to the 1 to 10 to the 3. It is not usually, but some can

even be higher up into the thousands of cells per sample.

We have an expert in the room. Dr. Pillai is an expert on sterilization. On these ink bottles, they say sterile, so you wonder. And the second thing is, sometimes they even have a certificate, sterilization certificate. This can lead into discussion of exactly what type of processes do they have in place to sterilize these things.

Now, I don't want to confuse the fact that those who go to their local tattoo parlor, and they are getting inks there, that is a separate deal because the state and local authorities, they have their own rules and regulations in terms of the process, making sure the needles that are used are sterile and et cetera. But this is before all of that. This is the ink itself.

The next accomplishment that I want to talk about is our involvement with the National Toxicology Program. And basically, we just had a meeting a week or so ago, Dr. Khare, she represented the Division of Microbiology on that. I am going to give kind of the brief. I am going to give the 30,000 view of what we are doing. Then we can certainly get into details later on the data.

But the bottom line is what we are trying to do,

and we are trying to actually put the microbiome into toxicology risk assessment. That is the bigger principle. And the question comes up, you give talks. I am surrounded by toxicologists. Then you give talks. You always get the so what question. I mean, it is typical, especially if you are not grounded in microbiology. You get used to those questions. You know how to handle them. They are not difficult questions to answer. But at the same time, you get it, but you still have to prove. That is where we are going to try to do is, once we get this information, do we really need it in a toxicology risk? That is kind of how we are trying to think.

And so, I have to give a lot of credit, an enormous amount of credit is to Dr. Paul Howard because he was the one who made the connections with Dr. Nigel Walker in terms of thinking about getting the microbiome piece into this. We worked together on that. I gave a lecture actually at NIEHS. It was not on this topic, per se. It was on that environmental microbiome. This was during the oil spill deal. It was kind of that connection of the environmental bioremediation microbiome connection.

So anyway, Paul and I got together, with Dr. Khare's help, and said, Carl, if you had to design studies, what would you design? That is the starting

point, right? You have the problem. You have got the compound of interest and the rationale for that. But then how would you do it?

So this was a great opportunity just to kind of think, based on all the latest information bb you can remember the microbiome field, in 2003, there were maybe a couple hundred citations in the literature. 2007, when the NIH microbiome project hit, and then the international microbiome was right after that basically, they are pretty much together. To this day, now, there are about 27,000 references. The field has exploded.

So with that, with all the technology and everything, it was kind of cool that we could kind of work together to kind of design what I call the Cadillac type of risk assessment in terms of the microbiome. So basically, that is what you see here in the scheme. I thought that would be important for you to kind of see how we can link both the metabolism aspect because you want to know if we ingest the compound.

You have to think of, when you think of the microbiomes, you have to think of two things fundamentally. The first thing you have to think of is the gatekeeper. When you are ingesting something, if you are talking about oral exposures, you have got it in the

mouth. You got loads of bacteria in the mouth. It goes down, and there is a lot of architecture between the esophagus and the GI tract. But you get bugs in there.

So you get these kinds of interactions. When it gets in, does it affect compositional changes? Did they metabolize it? Did they bind? These are the kinds, so that is the watchman. The bacteria are the first. That is the gatekeeper part of it.

The second part is always the watchman. That the signaling pathways. Once it gets into the GI track, we know that connection between the vagus nerve and the brain and the potential metabolites that can produced. So you have that aspect, too. When we are talking about doing toxicology risk assessment in the microbiome, you have to keep those two concepts in hand. So basically, you have designed a really nice system. Obviously, we always look at the live bacterial community. Then we certainly do all the various omics technologies to determine what the impact is, not only on the composition, but also on the function of the bacteria and the host interactions.

And then the big piece is this whole system data integration. You have got to integrate all this information. So that is kind of a really nice model.

That is what we are doing in our studies. We are kind of using that approach.

So these are the kinds of test chemicals. I won't spend a lot of time because you can give a lecture on any one of these topics for a long time. But these are the kinds of compounds that we are working on with the NTP program. So you can kind of look for yourself.

Obviously, some of these, you are probably quite familiar with.

The efficacy testing, what we mean by that is on animal studies, they either use water, they use corn oil. No one truly knows when you are adding, what happens to the microbiome roof. Fundamental question, so we are kind of doing these efficacy testings, as well.

The other point that I should probably mention, and maybe I will get it on this next slide, yes, it is the first point. When we talk with the standardization of samples, when you looking at the microbiome literature, and you can be talking about obesity, you can be talking about IBD. You can talk about the various neurological disorders, behavioral disorders. And you look at the literature, especially if it is compositional changes. There is a lot of variability, an enormous amount of variability.

When you start looking at these papers, a lot of the reason for that variability is for this first point, you need to have standardized methods for sample collection. There is a lot of variance on how samples are handled. Do you do fresh is best? Do you put it in stabilization buster medium? Do you freeze it, which is typical? They are all going to give you different types of profiles.

It is very important to kind of standardize these kinds of things. So that is something we can do at NCTR to give some information. I think our overall goal is not only working on a specific compound and giving them all the data, but to have a process in place, so any laboratory around the world can do the same kinds of things.

I am not going to spend time since we just had the NTP meeting on all of that. I want to kind of keep us on track time wise. I want to make sure I stay within the time, so that people would have time for discussion.

Another key highlight accomplishment is this whole issue, and the general theme would be antimicrobial resistance. Certainly, it is a global health problem. The area we are working very closely with our CDM partners is this issue of antimicrobial residues in food, and what

impact they have on the testing the microbiome.

I have to say that the relationship between those working between our group and their group has been outstanding. At the lead, Kevin Greenlees gives us an enormous amount of input in terms of making sure we are following policies and all the language within our paper. Jeff Gilbert within that organization of Human Safety Risk Assessment and Sylvie and the whole team, we work very closely. We are communicating our results either with seminars or discussions on our research.

But basically, when we are talking about antibiotic residues in foods, and talking about exposure, there are three important points. One important point or microbiological endpoint that I like to say is what is the impact does the slow level residue have on the GI tract, so we measure that.

The second important point, which is getting into the resistance issue, what is the lowest concentration of the antimicrobial residue selecting or resistance. We know therapeutic levels do it. That is obvious. That is a no-brainer. But when you get to very low, even sub-MIC levels, does that select for resistance? We truly don't know that.

And also, do these bugs have the ability to

inactive or degrade or bind these things? We do all of that in our particular studies. We work very closely with our CVM counterpart in terms of the compound of interest. Previously, we did a nice series of studies on enrofloxacin. Now, we are working on tetracycline and erythromycin.

What was interesting, and this gets into when you are talking about resistance in human populations, especially when we are talking about the low levels, let me just give you one example. The first confounder when you are doing this type of work is the fact that if I took a fecal specimen from everyone in this room, there is going to be variability. That is one problem.

The question comes up how many do you sample to be statistically significant. Some people argue, well, you just do composites. To get around the variability, you just do composites of this study. Or you do you do individual samples? There are debates on that topic. There are pros and cons either way.

The second thing if I took those same samples, and I screen for tetracycline resistance, I would find about 80 to 90 percent of you are going to have low-level tetracycline resistance. This gets into the question of when you do get an exposure, what is that threshold of

increase that could be a potentially clinical impact. How do you measure it? How do you determine that? That is kind of where we are headed at. That is the kind of information we are trying to provide. This is another thing we can do very well to provide the agency this type of work on that particular subject. This is kind of cool.

Also with our CVM partners, because this is in vivo, we have in vivo tests. We have bioreactors set up that kind of mimic the GI tract, et cetera. But they are also doing an animal study in the Office of Research component of CVM on a pig study, so we can compare the in vivo work that is going on there with the in vitro work that is going on in CTR.

What we found, just to kind of give you a little snippet of the results in this particular case, is that we found that these levels, and not to get into the national regulatory authority kind of language of acceptable daily intakes and the levels and all of that.

But anyway, what we are finding is that the levels that are set by the regulatory authorities, both nationally and internationally, are really pretty good values. What we are finding is the impact of the intestinal microbiome is usually below that. That means we don't have a problem with it. The high dose, you have

an effect. The low dose, the ones that you would potentially see, and again, it is a potential exposure we don't seem to see have an effect, either on resistance or the compositional changes at the moment.

The other aspect that we are working on the same kind of study, which is also kind of really interesting because when you are talking about these microbiological endpoints, one I said is a selection for resistance. The other aspect is what we call this colonization barrier disruption. So basically, when you get an exposure of any toxicant, you can disrupt that microbiota. And then that can lead to all kinds of different dysbiotic effects.

In our particular group, Dr. Khare in her lab there, we have model systems set up with human intestinal cell line systems where we can measure barrier effects. It is really pretty neat work because we can look at permeability changes. You can look at resistance in the epithelial cells.

You can actually label the bacteria because the GI tract, just to back up slightly, not to give you lessons in microbiology, but we all have been affected one way or another with Montezuma's Revenge or something like that. You can get disrupted. So basically, it is a very protective barrier we have. You can do a lot of insults

of the GI tract, and still be normal, right?

If it is disrupted, we have the green fluorescent protein bacteria. We can look and do translocation assays, too. We have got some pretty neat methodology to get at this. To my knowledge on antibiotic residue issue, no one is doing these types of studies to truly understand the barrier effect.

And the other interesting aspect of this barrier effect that people don't typically look at because as I mentioned, because as I mentioned earlier, I could take a fecal sample from everybody in this room. But what I could not do is actually take samples, well, not easily, you can do it via colonoscopies or that type of thing, is actually look at the bacteria that is actually look at the bacteria that has actually adhered to the intestinal mucosa lining. They are very important. We can do studies looking at that, too. That is kind of the neat add-on that we have. Again, I want to compliment our collaborators on that particular project, which is the Center for Veterinary Medicine.

So additional projects, and this is one with CDER, and this another important area, is the microbial contamination of pharmaceutical products. The working we are working on with CDER is the organism is *Burkholderia*

cepacia complex. *Burkholderia cepacia*, it used to be termed before with taxonomic changes. It is quite common in soil and water.

This particular one, *cepacia* in Latin means onion. It was actually found on onion root and soils and everything. That is how it was isolated by Burkholder, and it is named after him.

What happened is that people who were immunocompromised and get exposed to a pharmaceutical product that is contaminated with this organism, it can be very lethal. It is very serious. There are some methods out there, but there aren't really good methods to resuscitate *Burkholderia*.

What the issue is, if you just look at this particular water here, and it is clear. Well, that is how the pharmaceutical product looks when it is contaminated with that organism. This organism can live in basically distilled water in low concentrations.

The key is how do you detect it? How do you resuscitate it? Why does it even survive in these things? A lot of times, in these pharmaceutical products, you are also going to have an antiseptic or disinfectant. Like chloride, you need something like that.

We have actually done all of those kinds of

studies and showed exactly how we validated the methods for CDER. We are providing a lot of information for them. I can go into the reasons why these organisms can survive. We did a lot of the omics, the proteomics, the genomics, totally sequenced these organisms and know exactly genes involved in resistance and survival under these conditions. We have papers published on that. So that is a good example.

Another example is with CTP, and Dr. Steve Foley and his lab are leading that particular project on actually looking at a wide variety of tobacco products for microbial contamination. So again, it is microbial contamination issue. They are looking at a wide range of tobacco products. They are also looking at the production of nitrosamines, as well.

So basically what they found on the variety of different storage conditions, obviously there are the microbial contaminants. They have been characterizing these. They have been giving that information to the CTP colleagues. This is another important area.

The other project we have with the Center for Tobacco Products is on actually the oral microbiome and how it is affected and smokeless tobacco products when they get into the oral cavity, how they affect the oral

microbiome. I didn't put that to save time. Obviously, I am not talking about that, but I could later. I think that is another part of risk assessment because the first site of exposure is actually the mouth. Most people don't even consider that as much. But you are going to have some residence time in the mouth.

So now the future directions of the program, and as I said previously, the only thing that truly matters to me is to instill in my staff to do good science and to be accurate at what you do and contribute to the agency. That is the overall theme. Certainly the strategies that we are probably going to try to continue is certainly in the microbiome arena, on these FDA-regulated products, certainly with NTP and other projects of interest to that.

We are always interested in doing these. We are using the most current technologies, point number two, to determine the impact of microbial contaminants in foods and regulated products. I can see us continuing. Both of those top bullets have been funded because money is tight. We have been fortunate to get money for these particular projects to keep these things going.

I think we are definitely going to continue the CVM work that we are doing. Certainly, that is all internal NCTR funding. It is nice that the director and

the Office of Research provide us those funds, so we can carry out those careful studies for the Center for Veterinary Medicine on the impact of low-level residue, antimicrobial residues in foods on the GI tract. So we hope to continue that.

We had two projects funded with CTP. Both of them have ended. I think it is very important to at least look at exposures in the oral microbiome. I think both projects have their merits in terms of the contaminants. But if I had a preference in strategies, I think that would be one that maybe we can talk about. When CTP did come down, and we had that kind of preliminary discussion, it was probably six or so months. They came down about six or so months ago or whenever. We will see how that leads in terms of priorities on that. I think nano is here. We want to kind of work closely with the facilities that they have here on our projects on that. We hope to continue that.

And then these other initiatives in Women's Health, we have been fortunate to get funding on both of our projects. We hope that we can continue in that direction. A lot of it is certainly trying to find leverage resources to do the work that we need to do.

I think the other important piece in the purpose

of this particular meeting is to improve communication. I think we have offices in place here and certainly in Washington that Donna leads. We have the Office of Research that Dan leads. Bill is always sending our projects everywhere to see if there is interest within the FDA centers.

We have a communications office, too. But still, I think we can even improve upon this communication amongst our FDA centers and colleagues, and maybe even better mechanism maybe can come out of meetings like this or in the future. I think that is something that would be helpful to all of us at NCTR, not only microbiology.

And then this gets into it again. A lot of it gets to be on budget in terms of we have always got to consider how we prioritize the research, especially under these particular tight times. So again, I think it has been an honor for me to have the opportunity to speak to you today. I am certainly interested in any type of feedback or question that you might have. Thanks again for your attention.

DR. LEIN: The floor is open for questions.

DR. PILLAI: Carl, thank you for the overview. I have a couple of questions. Is NCTR kind of the home for microbiome research within the FDA? Or is there CFSAN

and other agencies or divisions?

DR. CERNIGLIA: I think what is exciting now about what is exciting about the microbiome arena within the agency is, first of all, there is an FDA working group on the microbiome. That is representative from every FDA center. We have three representatives within the Division of Microbiology on that. I am involved in the upper one on the HHS level. But at the FDA level, we have three within our division, Dr. Khare, Wagner and Erickson. So each center has representatives. Dr. Paul Carlson from CDER leads that group. They have meetings now. Now, it is getting spread.

One of the exercises that Paul Carlson and I had in the beginning when we had to provide information to HHS is what is the agency doing. So we receive that information which we package. So we are not the only ones.

DR. PILLAI: I have another question. When you look at the antimicrobial resistance determinants, are you looking at an organism agnostic, or are you looking at it in the context of an identified organism?

DR. CERNIGLIA: What we are trying to do, and this is wonderful with all the new technology. So first of all, when you think of the microbiome, I think you have

always got to think of community. You can't think so much of individual organisms. Although when you are doing, as you know, when you are doing next generation sequencing, you can go all the way from the phylogenetic level to the genus level to the species level to the strain level. You can tease all that information out, so we can get that.

But still, holistically, you have got to think of a community structure. When you think of the microbiome, you think of whether it is on your skin or it is in your lungs or whatever, the anatomy actually is very different architecture depending on where you sample. The structure is different. That is point number one.

The second point, which I think is a critical one when you are talking about resistance, so we layer it with the bugs, so we get that. But then we are also looking, for example, with tetracycline as a good example. We actually looked at the ted genes. We looked, so actually there are 23 major groups of tetracycline-resistance genes. The catalog is four different areas, whether it is ribosomal protection.

There are different mechanisms. We looked at those. We keyed in on out of those 23, we found like four of those are actually representative of that population. I would almost assume that if I sample it, you would find

those four. We looked at it at that level, as well.

DR. PILLAI: I have just two more questions. One question is why are you looking at tet and erythromycin rather than some of the semi-synthetic antimicrobial that are in the market or getting developed.

DR. CERNIGLIA: They were requested. There was a reason for that. Early on, there was some litigation going on both on tetracycline and penicillin. The agency needed data on that. They are old drugs. That was one of the reasons why we got involved in those particular compounds to begin.

We did fluoroquinolones. Before that, they were very interested in fluoroquinolones. That was a big hot topic, if you remember, on the boiler chicken. So that was a big issue in which we provided the agency data.

I just came back from JECFA, as I mentioned. I did beta lactams. I did polylines and some quinolones, not quinolones. I looked at those classes of compounds. I am really up for some of these new emerging ones that we could look at. But again, we have to take the lead on what is important to the agency at this point.

I mean, early on, and this gets to some fundamental principles, do you want to?

DR.GRAHAM: This project actually started with

interest from CVM because these particular drugs are used in food animals. Antimicrobial resistance, a large portion of it is being experienced by humans is coming through the food chain. That is why we had asked NCTR to start looking at those particular drugs.

DR.CERNIGLIA: They are all there. That is a given. That is a given. These are coming from veterinary medicine. They are used in food-producing animals. I had that on my first slide. The issue really truly is not only exposure, it is cross-resistance. That is the key issue in the emerging public health. In other words, if you did get exposed to this particular drug, and then you took a drug of a similar class and it didn't work, that is the fundamental principle. Tetracycline is one that is being used.

And then as Dr. Graham mentioned, and he is absolutely correct, it is widely used. Tetracyclines are widely used.

DR. PILLAI: One last point. I think the work on the pharmaceuticals in the Burkholderia area is really interesting because with a lot of these recalls for medicine, pharmaceuticals and devices, et cetera. Your work with using omics technologies to integrate these organisms, you may be able to identify primary structures,

primary DNA or RNA sequence information that may be indicative of why a process failed. From FDA's perspective, recall is one point.

But then the second is if you are trying to remediate that situation, I mean, they may have done all the necessary step or whatever sterilization protocols. But sometimes, it passes through. To understand if there is a signature that is representative of why a particular product could help in optimizing some of these processes.

DR.CERNIGLIA: That is an excellent point. That would really be good. It is clear, you know, a lot of these when they are coming up is in the manufacturing process, contaminated water or something. It is not being tested. That is absolutely right. Thank you.

DR. LANZA: I just have two questions that are more clinical, but maybe you have been studying these. One of them has to do with *C. difficile*. The situation where not that you have been able to treat it quickly, but where it has been chronic. You have been a patient actually even seeing an ID specialist on vancomycin for chronically two or three long-term cycles, maybe even having fecal transplantation.

You are in a situation where now you have gotten over it, this whole thing. But now you are trying to

reconstitute a microbiome in the gut. They are asking you questions about they are afraid they are going to get it again and so forth. Have you studied this problem?

DR. CERNIGLIA: I am glad you mentioned. I didn't have time to talk on it. Certainly Carolyn and her group are expects in this area. But we have two projects with CBER on fecal microbial transplantation. It is an interesting area because in our aspect, one of the projects we are trying to do is just understand the mechanism of how it works. That is always a wild card in these things.

But as you pointed out so rightly, recurrent difficile is what you are speaking of FMT seems to have a success rate. So obviously, there is a lot going on in that particular field, as well as so you have a mix.

Those who don't know what FMT is, it is basically you have donors. These donors fecal samples are screened, so you want to make sure that there is no pathogens and that type of thing in there. Then they are either put in the capsule and fed orally, or they go in via colonoscopy or an enema. Then they get in because those that have *C. difficile* have very disrupted GI tracts. So you are trying to reestablish the microbiota. So that is the principle.

But the problem from the research standpoint, there are still some fundamental things that need to be understood. Certainly you don't want pathogens in your samples and that type of thing and how you store the samples and that. There is a lot involved on that. But it is certainly one are. The people in the probiotic field, I mean, they would like to have maybe more defined organisms versus this complex mixture.

One thing people never talk about, but I think you have to remember, in contrast to a chemical exposure, when you are taking a microbial exposure, that is in there. It can be there for life. You don't know. You do not know.

As a chemical, there is a dose. There is clearance, you can binding. There are some things in there that still need to be clarified. But we have two particular projects on that. I am sure that Caroline, you can add anything she would like to this.

DR.WILSON : Briefly mention that the point you raise is a critically important question. I think it is one that actually the scientific community doesn't know how to answer and exactly why fecal transplantation works and what are the critical consortia that are effective at reestablishing normal flora. What is a normal flora?

We are excited because in CDER, Paul Carson has been using a mouse system that is a functional knockout for mucosal-associated invariant T-cells. And mice that don't have these T-cells are completely resistant to C diff. This allows us to have a system that we can now titrate and figure out how to associate different consortia of fecal materials with this kind of resistance. It is an important question that he and a lot of people in the community are working on.

DR. CERNIGLIA: Yes. There is this whole concept like a super donor. Why is one donor better than another? I mean, they call them super donors. They work better than others. We don't understand that.

And then the other thing that comes up all the time is the record for C diff seems to be pretty good. But what about the other ones? It doesn't seem to be, like IVD, Crohn's disease and that. What is going on here? There is a lot.

DR. LANZA: If I could ask just one more question. The other one, drugs that are being used for diabetes that involve continuous excretion now, both in the urinary and the GI tract through these GP1 inhibitors. With the amount of sugar now being pumped into the tract, I am wondering does this have a chance for changing the

microbiome and being chronic effect? Or is it just diluted out so much that the amount of sugar doesn't make any difference? If it does make a difference, are we looking at problems that they may have downstream?

DR. CERNIGLIA: That is always an important excellent question. Thank you for that. When you are talking about dietary intake, and let's say the sugars in this case, first of all, as we eat, like whatever you had for lunch today, you are probably going to be changing some microbiota in there. The bugs, generation time, some bugs can be less than 10 or 15 minutes. You can doubling in the tract. You can get changes.

And then you guys remember, too, whatever you are eating, there is a microbiota component to that, as well. It is not sterile, the food that you are eating. You are going to have that. So you have that. But you are going to get some, what we call, fluctuation. People have done these kinds of studies where they have looked at diet. They just looked to see how the microbiome changes pretty quickly.

But at the same time, what they find in these studies is that there is a sense of stability there, too. There is usually a core of organisms that is pretty much intact. So even though you may be getting this dietary

change, unless it is a real radical change, like you really stay for a long time on like a low-fat diet or something, you are going to get some daily fluctuations in your microbiota.

But what is important I think is that what doesn't change is the function. Even though the population could change a little bit, the functions of these bugs, what they typically do, whether it is protecting an immune response, metabolism, production of fatty acids, barrier effects. These functions do not typically change. So even though you may get some differences in the composition of the bacteria, the function doesn't change.

DR. LANZA: The reason I asked the question, though, is because the secretion is on the other side, downstream side of the stomach. You are secreting tons of glucose, both in the urine, and I mean a lot, and in the gut. But now, you don't have a processing going through the stomach. It is chronic, 24 hours a day, every day for as long as we keep them on these meds.

DR. CERNIGLIA: That would be interesting to study. Is somebody looking at that, from the microbiome angle, do you know? That would be interesting to prove.

DR. LANZA: I have no idea.

DR. CERNIGLIA: That would be interesting to know. That would be interesting. Thank you.

DR. PILLAI: One comment, you are doing all of this work with antimicrobial resistance. It is also critically important, I think, to look at the antimicrobial resistance potential in these fecal transplantation and probiotic formulations. We just don't look at that. We just think of them because there are a lot of these resistance genes in the fecal transplant population. As I call it, fecal repopulation, is going to have --

DR. CERNIGLIA: That is a good point. That is part of our protocol. We are looking at that. We are looking at pathogens, viruses, antibiotic resistant markers, yes, that is part. It is important. I am glad you mentioned it.

DR. STICE: I wanted to get back to some of your feedback requested. You suggested you wanted to get it. Are you addressing the needs of the centers? How do you get information as to what their needs are, engaging with them?

I guess I am still a little fuzzy on how that process is currently conducted for somebody like you. I know that Bill is out there talking about your work all

the time. How does the process work for you to engage with currently, so we can help you think about better ways.

DR. CERNIGLIA: It comes at several levels. I am sure I will get help from others on this question because it is an important question that you have asked. The first level is a meeting kind of like what is going to happen tomorrow and Wednesday when we have the in-depth science advisory board meeting and we have our FDA center people there. We are presenting our work.

And then there is feedback, not only from the board, but there is also feedback from our FDA center colleagues that can kind of steer direction. That is one particular level, which is certainly an important level.

The second level, which in terms of feedback, and this was mentioned a bit in Dr. Slikker's opening comments is the fact that as we write projects, none of these projects any more, although I think I saw the number of like 42 percent of our projects at NCTR are FDA center-driven. I always thought they were higher than that. But anyway, that is okay.

But the blue sky kinds of questions that we all like to answer doesn't happen anymore, at least not in the Division of Microbiology. So they are pretty well

directed from an FDA need. That is point number two.

And then once we come up with that idea in this exchange amongst our colleagues in its layer, and these are the representatives. And then you have the research counterparts. And then you have the scientists under those organizations. There is this communication.

And then when some ideas kind of come, then we put it on paper in this concept format. Then once we do the concept, it goes to Dr. Acosta's office in the Office of Research. And then they look at it, to see indeed if it is even maybe worth moving forward. And then if it is, there are liaisons that the NCTR has that Dr. Acosta and Dr. Slikker have, in their office, the people who work in their office has. And then they send these concepts out to get feedback, whether it is a good idea, bad idea or would they support it or not support it. These types of questions come up.

And once that happens, again, it is a process. Then we write a protocol proposal. And then that proposal goes back out through almost a similar kind, to experts. It could be with internal or external experts to kind of craft it. That is one.

And the we also have these Office of Chief Scientists grants. That is another level. This comes

from the commissioner's office. Then the scientists have ideas. Each center has their own internal review process first for these. Then they take the best, going to kind of percolate back up towards the commissioner's office for evaluation. There are different levels of how we address the needs and maybe get feedback on what we are doing.

DR. STICE: I got that from Bill's presentation. I didn't know if there was some place where you all get together and have poster sessions? Or is there a way for you a survey, say 100 scientists.

DR. CERNIGLIA: Thank you for mentioning it. Well, yes, there is an FDA science forum. That is in the beltway area in White Oak. It gives people an opportunity to present their work at the FDA science forum.

There is an Office of Foods and Veterinary Medicine kind of science meeting. There is an opportunity there for our scientists to present things. Sometimes it gets more limiting now for our own attendance because of the budgets, travel. But they can still participate, send posters. There is Webex and that type of thing. So that is another aspect of how we can interchange. And then there are working groups within these organizations where there is communication, as well.

DR. STICE: From the feedback requested, it

sounds like there isn't enough, that you have information to engage.

DR. CERNIGLIA: Yes, the reason for feedback, like I mentioned before in communication, I think these mechanisms of pathways of projects and all those, creation, it could be a little bit smoother. That kind of feedback would be useful to get form the various centers and all that.

DR. GRAHAM: Steve, I want to follow up on that. I can only speak for CVM. But NCTR has been very cooperative and receptive to any comments that we have when we review a proposal that they submit to us. There are often back and forth modification of the proposal amongst our sciences, and then they move forward with it. Those few rare occurrences where we say, we just don't see a need for this, they drop it. They don't push it. So they are certainly meeting our needs.

They are very responsive back and forth in helping to develop the project in the first place. If we don't give it a, yes, it is fine, go ahead and proceed. More times than not, we have some suggestions on tweaking it. They actually listen to those tweaks and they modify their proposal accordingly.

DR.STICE: I guess to your point of view, there

really is enough information flowing back and forth that they are engaging enough with the centers to get the information.

DR. GRAHAM: That is correct. And one of the things that we had instituted maybe two years ago was that these proposals had to go through middle and upper management at both ends. It was no longer just scientist to scientist with the projects being a surprise to management.

So for instance, if a project starts out concept wise at NCTR, it comes to me through Dan. It doesn't come any other route. That way, I know when I receive it, NCTR management has already bought off on the potential concept, so that we are not wasting people's time.

DR. SLIKKER: I appreciate those comments. Just one more additional comment. The 42 percent, I think I didn't present it quite clearly enough, Carl, because you did a great job of talking about all the ways that we interact. But that 42 percent is either there is absolute funding from other centers coming into support it, or that there is a person who is actually actively working as a collaborator. It would be a co-PI and a co-author on papers.

All the concepts go through the route that John

just described. 42 percent was where we got this sort of active participation versus the idea that they are interested in an area, but don't have resources to invest at that time.

DR. CERNIGLIA: Yes, I was listening. Thank you.

DR. LANIYONU: I also want to say that we have had tremendous cooperation from NCTR that at least I was personally involved with very iterative process regard to carefully defined goals, that is what can work for us.

I agree with the comments made that sometimes you might identify a need that their interest is looking for. But there are truly many things that can impact that. I can speak from a division's perspective.

More often than not, there are such processes (indiscernible) division or a center is probably reactive. This is the support we want. We want the cost analysis. We want something to be done.

More often than not, that cannot be leading edge science. It is just we have a problem. It is unidentified, which NCTR does excellently well.

The other thing is that what can then be done that can prevent such in the future? This is my first time of coming to this meeting. What I am not too sure

why, what I have not heard about, is there a mechanism in place for the NCTR actually engaged in centers. And tell them that they may not know what they need today. They may be big ticketed that may actually involve eye level problem. The vision that they are seeing in five years' time, this would be (indiscernible). You may not think you need it now, but we should be prepared for it.

It may end up being big ticketed items. It may end up being items that (indiscernible) accounts. It may be you want to collaborate with NIH, for example. Maybe you cannot do this alone, but if you have identified, we can envision it and there is a need for it. Actually, those are the kind of projects that allows for leading cutting-edge science, as well as future and even the immediate applications for science. Because invariably, regulatory science is the follow up of science. You truly know, it has to walk. There can be cases about it. If you are not too sure, I mean, people might just simply say, go to the university and ask those questions. Those are not the type of questions that you are interested in. I wish you well.

DR. LEIN: Are there any other comments or questions around the table? Yes, John?

DR. GRAHAM: John Graham, CVM. Carl, I noticed

you mentioned a couple of times about developing new methodologies. You talked about it with rapid PCR detection for *Burkholderia*. You talked about it in tattoo ink.

I wanted to mention to you at least how CVM and CFSAN deal with these new methodologies, if you want those methodologies to make it out into ORA labs, they need to be validated in a multi-lab validation. We are taking a much more proactive look in preparing for those in advance, asking some questions up front. Like is the equipment necessary already out in the field? How difficult is this going to be? How much is it going to cost?

So my suggestion to you would be that when you write these proposals in the future to continue working on these methodologies, that you have transition to ORA in mind. And that you coordinate with the multi-lab validation Subcommittees that OFVM has. So that you are basically greasing the skids because there is a lot of work that goes into these. You don't want to go through all that work, only to find out that ORA is not interested or is not capable of running those things.

DR. CERNIGLIA: Thank you. That is a good point. We try to do that, but I think Steve is involved

in that process. We kind of run through. Dr. Foley keeps us on tap on that. But certainly, the tattoo ink actually is multi-lab validation study that I mentioned earlier.

But actually, the *Burkholderia* one is going through. It is recommended as going through the USB pharmacopeia 62 and 61. So we are already going through that process, as well. But I think in the future, for anything else, I agree, we are already doing that. But in the future, we will always consider that, as well. Thank you.

DR. LEIN: Okay, last call. Anybody have any other questions or comments for Carl? All right. Thank you. We are actually going to take a bio break right now. We will reconvene at 3:00. Thank you.

(Break)

DR. LEIN: I think if we can settle in, we will get started. Our next presentation is by Dr. Merle Paule, Division of Neurotoxicology.

Agenda Item: Division of Neurotoxicology

DR. PAULE: Well, good afternoon, everyone. It is a real pleasure to be able to give you an update on what we have been doing at the Division of Neurotoxicology. I want to thank Carl for setting up and reminding us all that the microbiome exists to support

the brain and its function. I also read something interesting the other day where it said that most adults share their microbiome with their dogs and not their children. I will let you just think about that for a minute.

So, the Division now has 53 persons working in the Division, 44 of whom are full-time government employees. 24 of those are PIs/PhDs. 17 are support scientists. 2 are administrative. And we have one Commissioner's Fellow. The rest are made up by ORISE Post Docs and visiting scientists. We have a sizable group doing a lot of work.

Like other divisions, the mission and the research themes of our division is to develop and validate biomarkers and identify biological pathways that are associated with the expression of neurotoxicity. To do this, we employ fundamental research efforts in several focal areas designed to broadly examine the involvement of several focal areas. One being the N-methyl-D-aspartate and gamma amino acid receptor complexes as a mediator of adult and developmental neurotoxicity. It turns out that these systems are incredibly important for the development on neurotoxicity of general anesthetics and excitotoxins,

in general. More recently, you probably have heard that ketamine is being used in pediatric populations to treat depression. This is a big area of concern for the agency.

We are also concerned about monoamine neurotransmitter systems as targets of neurotox because these areas are heavily involved in drug abuse - that is drug self-administration and affective and movement disorders like Parkinson's disease. We believe that mitochondrial dysfunction and oxidative stress really serve as mechanisms - as part of the final common pathways in the expression of all of these disorders and all the toxicity we are talking about. More recently, we have started to look at beta-amyloid and alpha-synuclein deposition in the expression of neurotoxicity. These are, of course, important for Alzheimer's Disease and Parkinson's Disease models.

So, to do these kinds of things, we employ a variety of approaches and systems, all the way from cells up to humans. In terms of cell culture activity, we use primary, organotypic and neural stem cells. Most recently, we have incorporated human neural stem cells along with our rodent models. We are now working on developing a protocol to develop nonhuman primate neuro

stem cells, so we will have a three-species comparison to see which ones are going to give us the best bang for our buck. For these particular efforts, we primarily look at health and differentiation of these cells. We use them primarily for mechanistic studies. We are currently developing organ-on-a-chip technology. I will say more about that a little bit later.

In terms of whole animals, we range from zebrafish to rodents to nonhuman primates. Efforts brought to bear in those studies typically involve morphological assessments using light and confocal microscopy. With respect to defining a neuropathology, we use light, fluorescent and confocal microscopy, PET, CT scans, and MRI.

We also conduct a variety of functional assessments in our animals because we believe that the final common pathway of neurotoxicity is a functional observational endpoint. We look at nerve conduction velocity. And, following the recommendations of this body last year, we have now established microelectrode array technology to look at cells in culture. We also do a lot of observational and behavioral assessment. In terms of observational behaviors, we look at what we call non-operant behaviors, which are not trained

behaviors. They are innate behaviors. We also look at trained behaviors or operant behaviors. Those situations allow us to tap into very specific aspects of cognitive function and executive function.

For our human work, we try to do a lot of translational work in that we utilize the same sort of executive function and cognitive function metrics in children and adults, even, that we utilize with our non-human primates, primarily, but also our rodent models. We conduct these studies using the NCTR Operant Test Battery, which is a behavioral battery that we developed at the Center. We use them to validate the endpoints so that we can demonstrate their reliability and importance, not only to humans, but then backwards to the animal models. In our children population, we have been using these behaviors to characterize populations of ADHD kids or kids with anxiety and depression. We have looked at, when possible, drug effects to demonstrate, for example, that kids with ADHD's behavior is normalized when you give them methylphenidate or other CNS stimulants.

And then recently, we have been establishing clinical collaborations. We have one that is just wrapping up at the Mayo Clinic, in which we have been

using our operant test battery to assess children who have been exposed to general anesthesia at a young age. We have an ongoing study with Mt. Sinai colleagues actually at a laboratory in Mexico City, where they are looking at the effects of lead exposure in children in Mexico City.

In terms of outreach, we outreach just like every other division. We collaborate with most other NCTR divisions. Systems biology, in particular, we are looking at MALDI-MS Brain Imaging that was mentioned earlier by Laura. I will talk more about that later. We collaborate with the people in bioinformatics and biostatistics, who have been very helpful with us in dealing with large datasets that we have obtained from a consortium study on biomarkers of neurotoxicity. That has been done in collaboration with HESI/ILSI. I will mention more about that later. Then Fred Beland mentioned the studies with arsenic. We are also working on those particular efforts, as well, with Sherry Ferguson.

In terms of other centers, we have worked for literally decades with CDER on the issue of pediatric anesthetics. More recently, we are now looking at magnetic resonance imaging and biomarkers of toxicity

and, in fact, are in the process of trying to qualify MRI endpoints as biomarkers of neurotoxicity. You may have heard recently about the concern of gadolinium deposition in brain. Gadolinium is a contrast agent that accumulates in brain that has been demonstrated in people. We are now demonstrating it in rodents. The question is does it do anything when it is there? That question has not been answered yet. We also serve on the Neurotox Assessment Committee with CDER.

For CTP, we are conducting behavioral pharmacology of tobacco products studies, primarily looking at self-administration of nicotine and related products.

In terms of outreach with other government agencies, we do - we just have completed about a three-year study with ILSI/HESI, which is a multi-institute agency and consortium, looking at studies looking for fluidic biomarkers of neurotoxicity. So, we administered a known neurotoxic in trimethyl-10. We sampled all of the body parts we could think of, including CSF, blood, and urine. Those analyses are undergoing and are about to be published. We have identified a very interesting cytokine that seems to be present in CSF, blood, and even in urine, which makes it nice because urine is easy

to get. Those concentrations tend to correlate with duration and severity of neurotoxicity. We are very hopeful that that is going to actually pan out and be useful. Of course, it is only for trimethyl-10. Maybe it is not going to be useful for other neurotoxicants, but we are going to check that out.

We have an interagency agreement with the DEA, where we are conducting studies at the University of Arkansas for Medical Sciences, characterizing the abuse potential of street drugs. The current focus, as you can imagine, is on opiates and opiate-like compounds, fentanyl and Fiorinal fentanyl. We are providing data to the DEA so they can make scheduling decisions and figure out whether these things should be decriminalized or not.

With University of Arkansas at Fayetteville, we are conducting a study to develop microphysiological systems. I will talk more about those later. Also, to utilize the MRI to improve synthetic heart valve creations. I will talk more about those later, as well.

We also have representation on the Coalition Against Major Diseases, which focuses on Alzheimer's and Parkinson's Disease.

In terms of global leadership outreach, we

participate at OECD Adverse Outcome Pathway Identification, primarily for developmental neurotoxicity. We have a seat on the European Cooperation in Science and Technology effort, looking at the - on the Nano4Neuro Committee. We also have representation on ILSI/HESI DART or Developmental and Reproductive Tox Committee, looking at neonatal pediatrics, where they are talking about developing models of retinopathy, prematurity, and so forth, other things that affect developing humans.

Today, I just want to briefly discuss three recent accomplishments, one of which is an expansion of our efforts to develop a blood brain barrier-on-a-chip model for using in modeling traumatic brain injury. I will bring you up to date on more progress on the qualification of the MRI T2 images of biomarkers of neurotoxicity. And then wrap up with a discussion of our studies on sevoflurane general anesthesia-induced cognitive deficits and ways we think we might actually be able to protect against that adverse effect.

So, with respect to the BBB-on-a-chip model, this is done primarily led by Syed Ali. They isolate primary Brain Microendothelial Cells. Basically, from any species you want, but they have done rat, cow, and

monkey. Through a series of digestions and separations, they then see these materials on collagen-coated tissues. As an outgrowth of that, we are developing microfluidic models of that system as organs-on-chips. So, the hypothesis here is at high speed and biaxial stretch mimics the damage induced by TBI in primary cultures using commercially available brain endothelial cells. This system can model TBI in vitro.

So, this is basically a photograph of the system. High-speed stretcher attached to basically a petri dish under which the chips - the cells are seated. The high-speed pull stretches them out. Here, you can see data from stretch ranging from zero to 15 percent. So, cell deaths. Cell viability decreases or cell death increases with increasing stretch. Dead cells are labeled on the left side. You probably can't see it too well in this light. Live cells are labeled on the right side.

It turns out that stretching those cells actually induces apoptosis, which is a mechanism of cell death. It may not be the only mechanism of cell death. There may be chronic cell death going on as well, but here is a marker of apoptosis, showing increasing stretch with increasing apoptosis. Now, these data were

generated from just stretching the cells. With the newer system, where you use medicinal air to put a burst down on the cells, you can actually get a two-dimensional stretch, biaxial stretch. It actually allows you to stretch the cells even more. Data here are shown for stretches up to 50 percent. You might think that is extreme, but in human TBI, it is not. Cells often are stretched beyond 50 percent. So, this is a nice model in which we are actually looking at a wide range of stretch. We will be able to utilize this in efforts to try to understand the mechanisms associated with the expression of toxicity and hopefully, mechanisms if not preventing it, at least treating it or ameliorating the magnitude of the effect.

Lastly, with this system, in collaboration with the University of Arkansas at Fayetteville, Syed is looking at the development of microfluidic system, where you can actually bathe these brain microendothelial cells with a sera-like fluid and look at the transfer of material across this in vitro blood brain barrier model. When you do that, the top series of slides just shows exposure time in minutes. Again, you might not be able to see it, given the light in here. But in monitoring the crossing of fluorescein dye across the blood brain

barrier, you can see that with time, there is increasing transfer of the material across the BBB. So, you can establish baselines and then look at your treatment to see if it is affecting the integrity of the BBB and its ability to prevent material from transferring across.

Now, moving on to our qualification of MRI T2 images as biomarkers of neurotoxicity and beyond. This was a collaboration, again, with CDER. We used Sprague-Dawley rats. We used 10 known neurotoxic compounds. We did T2 mapping using our 7 Tesla MRI. Collaborated with Neuroscience Associates to do follow-up neuropathology, which was, in this case, used as the gold standard of neurotoxicity. We looked at fluoro-jade C stain in hippocampus as our marker. Neuroscience Associates looked at 80 slices through the brain using silver cupric.

I think I mentioned last time and showed you the list of compounds we used. In fact, I know that last time we met I talked to you about kainic acid and hexachlorophene. Today, I want to show you the data that we have worked up for trimethyltin, pyrithiamine, and 3-nitropropionic acid.

So, here is a typical T2 MRI image. Showing TMT toxicity that has caused lateral ventricle

expansion. Not something you would necessarily think is a neurotoxic endpoint, but this expansion in ventricle size correlated very nicely with hippocampal cell damage. So, what you are seeing here is time after TMT exposure in days and increasing size of ventricles after TMT exposure. The small arrow points to the T2 signal that you see in the hippocampus. The large arrow is pointing to the size of the ventricle.

Moving onto the 3-nitropropionic acid administration example, it causes significant increase in T2 signal, as well. You can see - well, first of all, the top row in each of these panels represents baseline brain slices. Each slice is a different brain slice, different area. The top represents prior to treatment. The bottom is three days after treatment. You can see in slide six and eight huge increases in T2 signal. Whereas, you look at number 14 and you see an increase in apparent diffusion coefficient. So, both of these metrics are sensitive to TMT, but it turns out that the T2 signal was much more sensitive than the apparent diffusion coefficient, so that is what we have been focusing on more recently.

3-nitropropionic acid toxicity again. T2 MRI signals. This is just showing the correlation of the MRI

signal with traditional neural path. You can see, for example, in the top right, big holes in the hypothalamus that are associated with basically big holes in the brain slice. If you look at the individual cellular matrices, you can see the kind of toxicity associated with these T2 signals.

We then also confirmed that with a different compound, here, pyrithiamine-induced neurotoxicity, showing, again, that the T2 MRI signal corresponds nicely with histopathology.

So, we think that the MRI T2 signal is going to be a very nice biomarker to inform where one would look if you want to do traditional histoneuropath. For example, if you are a drug company and you want to get your drug approved, you can come in with three slices of the brain and say, see, there is nothing there. Well, you may have missed a whole lot. If you utilize the MRI and scan your animals and you can do this repeatedly over time in the same subject, you can use that information to then inform you about where you should be taking your traditional neuropath slides to look for neurotoxicity as proven by dead and dying cells.

That last example I wanted to mention was our studies on sevoflurane general anesthesia-induced

cognitive deficits in the nonhuman primate. We know that sevoflurane induces frank cell death as evidenced by increases in neuro apoptosis when you expose infants to this compound when they are young. In most cases, we are talking about postnatal day seven. I am going to show you some data today that we have looked at. We have utilized PET/CT imaging of neural effects. Then I am going to show you data from the NCTR Operant Test Battery, demonstrating long-term cognitive deficits in these animals.

So, the exposure regimen that we used was we took five or six-day rhesus monkeys. We exposed them to two and a half percent sevoflurane, a standard clinical concentration to do induced general anesthesia. Here are the exposures for eight hours. As I mentioned, sevoflurane did cause significant neuronal cell damage, and as I am going to hopefully show you, also, glial cell activation.

For these studies, we imaged these animals several days - several points after their eight-hour exposure on postnatal day five or six, using a marker of neural inflammation called FEPPA. FEPPA is a compound that binds to the transporter protein on mitochondria. This particular protein is expressed when glial are

activated. So, we believe that this is a marker of neuro inflammation. We, unfortunately, don't have a PET ligand that will label apoptotic cells in monkeys. We have one that works in rats, but we don't have one that crosses the monkey blood brain barrier. So, we have gone to FEPPA, which is kind of a surrogate marker, we think, of nerve cell damage.

Here is just a typical PET scan, left, pre-exposure, right, post-exposure. Combined with CT images on the top panel, with the PET images on the bottom panel, you can co-register them and get nice neuroanatomical locations for where these signals are arising.

The kind of data we can generate are shown here. So, these data were taken 1, 7, and 21 days after their eight-hour exposure on postnatal day five or six. The open circles represent the uptake of FEPPA after exposure to the sevoflurane. You can see one day after exposure, that signal is much higher than any other group. The other groups include a control that was not exposed and animals exposed to sevoflurane plus a compound acetyl-l-carnitine. We have been using acetyl-l-carnitine because it has been shown in other systems to be very protective of these neurotoxic events. It is

a good antioxidant. It stabilizes mitochondrial membrane. It is a mitochondrial protector. We talked about the fact that everything leads to the mitochondria. We think if we can protect the mitochondria, then we can decrease damage.

So, seven days after exposure - these data are all from the frontal cortex in these monkeys - you can still see an elevated signal. I would argue that even 21 days after exposure, the signal is elevated, but it is not statistically significant here because we had such a small group. There is only three or four animals in each one of these datasets.

So, we then took these same animals and ran them through our NCTR Operant Test Battery. So, we know that they have got this neuroinflammatory response going on. The question was do their brains still work the way they should after such exposures? For these studies, we used this particular operant test panel. You can see it is the same panel that we utilize with humans, in this case, children. Children are working for nickels that drop into the bucket. Monkeys are working for banana-flavored food pellets. You get the idea. The principle is the same.

What I am showing here are some comparative

data. Some six years ago now, we published that a 24 hour of exposure to ketamine-induced anesthesia, as proof of concept, was able to disrupt performance in the NCTR Operant Test Battery. The red line on the top panel represents data from animals exposed to ketamine over two years of training. So, this represents two years of training in this particular behavioral situation that I mentioned. I am not going to go through the details of it, but the point here is you can see they diverge from controls and stay diverged for a significant period of time. This is highly significant.

Down below - that is 24 hours of exposure to ketamine. Down below, the data are for 8 hours of exposure to sevoflurane. Significantly less duration exposure, but significantly greater adverse effect.

So, what we have here are our attempt to protect from this adverse effect of sevoflurane on the OTB performance. So, what I am showing here in red are the data you just saw before. Black are the data you just saw before. Now, we have given these animals acetyl-L-carnitine. You can see that when you give it the acetyl-L-carnitine plus sevo group, it is no different from control. So, not only did it protect against the neuroinflammatory response, it also

apparently protected quite nicely against the cognitive deficit that is shown here. Now, these data are only about a year's worth of data, but you can get the point. It was protective.

So, a single episode, eight-hour sevoflurane-induced anesthesia caused subsequent cognitive deficits. They appear to be permanent, actually, and they worsen with age. These effects are seen in behaviors thought to reflect very important aspects of brain function, those related to IQ, for example. Preliminary data suggest protection by acetyl-l-carnitine versus FEPPA labeling and the cognitive deficits.

The initial ketamine data were actually presented to launch a public-private partnership between the FDA and the International Anesthesia Research Society called Smart Tots. More recently, our data were used to support a warning label on I think 11 different general anesthetics that the agency is recommending physicians use caution for in infants under two years of age.

So, future directions. We want to further characterize the Parkinson's disease damage after a traumatic brain injury using the system that I mentioned to you earlier. We want to move along the MRI T2

biomarker qualification. We are collaborating, again, with others to look at matrix absorb laser - laser deionized - laser desorption/ionization mass spec. We are actually read to bring online our new larger bore MRI to look at general anesthesia studies using that imaging approach in our monkeys. We are going to be incorporating MEA assessments into neural stem cell studies and using hi-res MRI to quantify some heart valve stuff, which I will show you in a second.

So, the MALDI-MS imaging overview, Laura talked about that earlier. A spectrum is required at specific coordinates. You take frozen tissue, get a slice, apply the matrix, analyze the data, and then you can plot individual compounds on individual spots on slices and brain. This blows my mind. This is really amazing stuff. You can get a nice picture of where these compounds, where these specific neurotransmitters are in brain. Of course, you could do this before and after to see what is going on.

At very high resolution, this is the kind of information you can get, where you can specifically ask where and show where GABA is, glutamate, acetylcholine, norepinephrine, epinephrine, and so forth. At lower resolution, you can actually get higher sensitivity, so

you get a little bit of a different picture. So, depending upon the questions you are asking, you can change your resolution to get better or different distribution of your material.

With respect to using the high strength magnet, the 7 Tesla MRI, Serguei Liachenko has been collaborating with UA Fayetteville scientists to look very - in great detail at these porcine microvalves with the intention of creating a much better, much more accurate synthetic version for use in the clinic. This is very exciting stuff. You can see the kind of detail that is obtained using these ex vivo tissues to employ high strength MRI to get information that you really can't get any other way.

I told you we set up the multi-electrode array system. What I was showing here are data from that system. That system allows you to look at spike rate, spike counts, and spike bursts. So, those are the data you see across the bottom. We used control traces. You can see the electrical activity up here. The boxes show some more births are happening. The middle trace is after MPP administration, which is a dopaminergic neurotoxicant, and then a lower trace for nicotine. You can see that there is nice dose response data collected

from this particular system. So, this gives us a way to look at in cultures, the electrical activity of cells, and find out what is going on with them after exposure to agents of interest.

I think I also mentioned last time we are developing rare earth metal chelates. Rare earth metal chelates, the one in particular that we have gotten some data on is Euro-Glo. Its base is europium. These things are fluorescent. They have high intensity emissions. They are very resistant to fading. Compatible with multiple labeling protocols and have been used so far to look at - to label myelin and amyloid plaques. The key thing and the cute thing about these things is they are paramagnetic. The hope is that these will also be visible using MRI. So, you could have the same dye, basically, to look at, on your typical slice, on a slide, or using MRI. I think that the initial attempt to put it into a live animal was not successful in that the animal died. It could have been a dose issue. We are trying to work on that.

I will close with just showing you all of the 53 people that I mentioned earlier and also mention that we are in close collaboration with 3D imaging people at UAMS, who make all of the PET ligands for us, since we

don't have a cyclotron at NCTR.

And then close with just the feedback requested. So, there are things out there on the horizon that we don't know about. We think we are up to date, but who knows? There could be things out there we don't know about.

I haven't mentioned CLARITY, but CLARITY is a tissue clearing protocol that lets you take brains and other tissue and turn them invisible. It is absolutely amazing to me that this can be done. The question is can we utilize that technology to give us information that we are not currently getting from our other approaches? We think MRI is absolutely fantastic. PET, obviously, is good. PET doesn't have the resolution that something like CLARITY might have. You might be able to go in and label very specific fiber tracts, clear the brain of everything except those fiber tracts, and get some information maybe that we can't get anywhere else. I think, currently, now, this process takes weeks. So, it is not quick, but I think it has got potential. We would be interested in hearing your thoughts on that.

Then even more important, perhaps, given the big data that we are getting now - you know, that MALDI-MS stuff generates massive quantities of data. All of

the omics platforms generate massive quantities of data. The MRI files are huge. Can we - we can continue to collect all of this stuff, but what kind of percentage of our effort should we say - what kind of ratio should there be for us to be most effective in utilizing the data that we can generate? This is not a small issue. We need to figure out how we can grapple with this and mine the data that we are already collecting to the maximum and get the best bang for the buck.

I believe that is all I have. I finished within my seven minutes. All right.

DR. LEIN: You are one minute over, but we will give it to you. Thank you.

(Applause)

DR. LEIN: Thank you, Merle. That really was a great presentation. We have 15 minutes for questions.

DR. Aschner: Thank you. Very nice presentation.

I have a couple questions that are a little bit perhaps more specific. The first one relates to the second project. You are using T2. When I think about T2, I think about iron. I am wondering if you agree with me. If so, does changes in iron concentration in the tissue - is sort of a reference for the histopathology that you

think you should be doing next?

DR. PAULE: All I can tell you is that I know that iron is a problem. I can refer you to our MRI specialist Serguei Liachenko, who can address that issue.

DR. LIACHENKO: Hi. I am Serguei Liachenko. So, yes, T2 MRI is very sensitive to iron concentration. We have done a few experiments with that, investigating iron nano particles using this technique.

In this particular set of experiments, T2 is kind of reflective of the tissues microstructure, like water content and water composition, like intracellular/extracellular, temperature, and those kind of things.

Even without iron, this contrast changes tremendously. With neurotoxicity, it is giving us very high dynamic range. Certainly, iron is a part of the factors, which change this - we are looking at all of it. Actually, all of it, including iron content changes is kind of consistent with neurotoxicity.

DR. Aschner: I agree. You might have a Fenton reaction if you have - well, if iron increase. So, I agree. Thank you very much.

The other question relates to the specific -

the first project. You know, I like a lot the microfluidic system, but I am wondering whether when you are doing the experiments, is this just a media that you use for endothelial cells? Do you fortify the media with, for example, astrocyte condition media? That might have tremendous influence on the integrity of the barrier that you might be getting.

DR. PAULE: That is an excellent question. For that, I would refer you to our expert, Syed Ali.

DR. ALI: It is a good question. Actually, not only we are using epithelial cells. We are using neurons and plan to use the astrocytes, so then we can see the changes in the blood brain barrier - the physiological system. There are very standard techniques we have been using.

DR. LANZA: I have two questions. I wanted to follow-up on yours, Dr. Aschner. If you are looking at T2-weighted edema, essentially, can you detect mild TBI? The pictures you showed us looked fairly severe. What I am wondering is really the situation of mild and mild repetitive TBI.

DR. PAULE: So, the cases that I showed you are severe cases. We have not used this system to look at TBI yet. I can't answer that question. I don't know how

severe the TBI would have to be before we would be able to pick it up.

DR. LANZA: I think that is one of the key issues.

The other question is I saw the gadolinium in brain. Did you differentiate whether the gadolinium, say it is on DOTA, is chelated or is it free?

DR. PAULE: No, we didn't.

DR. LANZA: So, you just know that total gadolinium is there and then whatever effects you have seen to date, but not whether it is essentially chelated to something like DOTA, which is almost physiologically irreversible because it is a free metal like NSF.

DR. PAULE: As far as I know, all we have been able to do is to look at total gadolinium, not any of the speciation. That is going to be an important thing to find out.

DR. Aschner: I think that is going to be very important. As you know, it can be linear, non-linear. There are a lot of different species of gadolinium. They have completely different effects.

DR. PAULE: That is right.

DR. LANIYONU: I can shed some light on that because my division is actually trusted in gadolinium

retention in tissues following multiple (indiscernible) administrations of these products in humans. I appreciate the differences you are trying to make between linear and macrocyclic. It has been shown that linear is the worst offenders. My division actually took this issue to an advisory sometime in September, given that the European EMA has actually banned the use of linear or about to sometime. The public (indiscernible) is just working its way through the European pilot.

A question as to whether the deposited gadolinium is still bound, free, or complex with total materials, the jury is still out there because most of the analytical methods that are used to measure retained gadolinium could not actually differentiate between the species that are left behind. They are (indiscernible), which is still very challenging at this time to actually associate pathophysiological consequences of the retained gadolinium, but NCTR is actively working with my division, doing fantastic project on this issue that will bring clarity and answers to some of the questions that we are grappling with.

DR. PAULE: Yes. I didn't mention that we were working with the Division for Medical Imaging Products at CDER.

DR. LEIN: I had a question, as well - actually, feedback, based on what you asked for. In terms of CLARITY, I think, actually, is very useful and definitely worth investing time into this methodology. It is going to, I think, revolutionize in vivo imaging. We have actually been using it in zebrafish. It is actually incredible what you can get out of a zebrafish with CLARITY.

The other thing I would - I don't know how this fits into the FDA mission, but I think one of the big areas that is starting to become huge in neurotoxicology is actually outside the brain, but looking at innervation of peripheral tissues. This is where CLARITY has been really allowing remarkable advances. So, looking, for example, at immune organs and their innervation by the autonomic nervous system, looking at the lung, in terms of reaction to various inhaled stuff, in terms of the cholinergic and adrenergic innervation of the lungs. So, I think that is where you are going to see a lot of really interesting advances that could really inform safety issues and also toxicology.

DR. PAULE: Well, that is excellent. It is kind of what I thought, but we are just now developing that

protocol to look at a variety of different clarifying methods. Do you have a favorite?

DR. LEIN: We are also evaluating. We are working with CalTech, who actually was one of the inventors of the CLARITY method. Offline, I can talk to you and share some ideas on where we are going with it.

I would also say that you are absolutely dead nuts on with this whole in vivo imaging is awesome, but the resources you need for the data management are overwhelming. We are struggling with that exact same question in just a single lab, so I can't even imagine what you guys are facing, in terms of a center. We are roughly looking at about 60 percent of our resources on data management and 40 percent on data acquisition.

DR. PAULE: Yes. I can imagine.

DR. LEIN: It is a huge percentage of what we are doing.

DR. PAULE: Well, I think in the next couple of days, we are going to be talking about bioinformatics and imaging. Maybe some of this topic will come up in that. I know there has been a focused effort to try to look at data management and how best to go about doing that.

DR. LEIN: I am very jealous of you. I think

this is the only key to moving forward is to integrate efforts across your center and centers that have that type of expertise. It is just overwhelming, just in the data management. Let alone data analysis, just in managing the data and how you archive it so it is accessible later.

DR. MARGERRISON: Ed Margerrison, CDRH. Away from the data management and back to TBI, we actually have an animal model of very early TBI that we are using EKG and things to look at. I don't know if that link has been made with our group and your group. If it hasn't, then I think we need to make it.

DR. PAULE: Yes. Absolutely. We are going to have to do some validation and stuff. We are going to need the animal model to do that. So, yes, no question about it.

DR. LANIYONU: I would also like to add that the Division of Medical Imaging Products essentially also is very interested in the TBI models. It is a current problem that we are focusing our attention on.

DR. LEIN: We have time for one last question.

DR. STICE: I just second what Pam said about CLARITY. There are a number of commercial units - companies out there that are commercializing that

technology and it is getting faster. There are ways to speed that process up.

DR. PAULE: Good.

DR. STICE: I guess just a quick comment on the BBB is don't forget the pericytes, as well, because those are very important in that process. At least four cells are important in that process, as well.

Just a quick question then on the MEAs. I think I was one of the ones that suggested you look at MEAs. It is really interesting that you were able to specifically monitor dopaminergic cells. I would be interested to know how you were able to do that.

DR. PAULE: I would refer to, again, Syed Ali or Syed Imam, in the back of the room. He has been leading this work and has actually set it up and actually, was the principle at NCTR for coordinating the ILSI/HESI effort on the biomarker study.

DR. IMAM: Yes. What we have - these are progenitor cells that we differentiate into human dopaminergic neurons. They are very much similar to those that are actually in substantia nigra. What we did with these is that we can culture them for over three weeks and record after repeated dosings. In the data that we have here, it was just done for four hours. What

you see in this is that MPP plus, which is a stimulant for dopamine and nicotine, also, that is a stimulant for dopamine, it did make the change in different spike rates and counts. Currently, we are going to continue with these dopaminergic neurons. We also have the IPSC-derived dopaminergic neurons that are also being worked upon. Hopefully, in the future, we can try to do other kind of neuronal system.

DR. STICE: A word of caution. Do you have 100 percent dopaminergic cells? If you don't, then you don't know if you are specifically recording from dopaminergic cells. If you do, then you don't have the support cells like the astrocytes that might be very important. A way to tease out specific dopaminergic response is very tough.

DR. IMAM: So, the current cells that we have, they are actually pure dopaminergic - as far as we know, based on the TH expression of it. As well as we did develop a very unique set to identify them by looking at alpha-synuclein and parkin interaction, which is pretty unique at the confocal level. With that, we know that they are completely different from any other kind of neurons. We can probably figure that one out in terms of the dopaminergic neurons, itself, but yes, you are

right, if we add other cell systems like astrocytes, things will change in there.

DR. PAULE: I just wanted to share that with you because I encouraged them to get me a slide so that we could prove to you that we actually follow your advice.

DR. LEIN: I think we are done with this session. Thank you very much, Merle.

(Applause)

DR. LEIN: Our last presentation for today is by Dr. Weida Tong from the Division of Bioinformatics and Biostatistics.

Agenda Item: Division of Bioinformatics and Biostatistics

DR. TONG: My name is Weida Tong. I am the director for the Division of Bioinformatics and Biostatistics. I came to give a very quick overview of this division.

This division has four branches. We are a Bioinformatics Branch, Biostatistics, a R2R Branch - it is the new branch just established a couple of months ago. I am going to give you a little bit more detail about this branch later on - and the last one, it is a Scientific Computing Branch. Besides the branch, I

labeled them - there is research, support, and service. I am going to provide a little bit more clarity on what that means because our division was reviewed in 2015 and one of the comments from the site visitor teams indicated the boundary between the research and support and the service is quite blurred. Hopefully, we - I can add a little more clarity on that.

At this point, we have five postdocs and two graduate students. Our division actually is very multidisciplinary. Anything you can think of about computer science and IT, we very much have it in our division, particularly these core skillsets, such as like programmers and software engineers and system administrators, and so on and so forth. So, in terms of the research, we cover a very broad range of the bioinformatics, biostatistics, molecular modeling, computational chemistry. We even have a wet lab in our division.

So, the division serves three functions: service, support, and research. Now, before this division as established, we already have a Computer Center at NCTR, provide all kinds of different IT-related support. When this division was established in 2012, the Computer Center was split into two. More than

half has come to the Scientific Computing Branch. Another half belongs to the OIM. They still working side by side to manage this Computer Center. This consists of 135 servers, a petabyte of data storage, as well as manage a small and high-performance computing environment.

We also - before this division was established we had four statisticians designated to support the national toxicology programs. We consider these as a legacy activity. We also characterize them as a service.

Now, we are entering the data rich era, so we require a lot of the bioinformatics and the biostatistics support. This we consider as a support. This support functions mainly - resides in the R2R Branch - it is the new branch - and the Biostatistics Branch. Within NCTR, we engage two types of support: committed tasks and requested activities. I am going to explain to you what is the difference between these two. Beyond the NCTR, we are actively engaged with the other FDA centers to provide the tools and the bioinformatics capabilities to support the review process. In the next few slides, I am going to explain that part of the work, as well.

In terms of the research, what we do is not

much different from what you hear so far from other divisions. We focus on the PI-driven research activity in this division.

What I am going to do is I am not going to talk about a service because this is a part of the center-wide infrastructure and investment. What you have heard so far, that is it.

In terms of the support, I am going to talk a little bit more in the next few slides, just to give you an idea what kind of support we provide to the NCTR scientists, as well as to the FDA. I plan to devote most of my time to talk about the research.

Okay, so about support within NCTR. As I mentioned, we have two types of support. One is called committed tasks. Another is called requested activities. Now, you know just as much as I know. We are dealing with the big data. You already heard about bioimaging data at NCTR, next-generation sequencing, and so on and so forth. When we are dealing with these data, analyze these data, we need to have an infrastructure. This infrastructure does not belong to the individual PIs or belong to the divisions.

So, we consider this - it is a committed task. We take on this task to establish this environment so

that all of the PIs and the divisions can take advantage of it. For example, when we are dealing with the next-generation sequencing data, we implement the Galaxy Platform, so a PI can get in to analyze the data by themselves. We are also maintaining the high-performance computing environment at NCTR, particularly to scheduling the jobs to make sure most efficiently use the HPC and resources at NCTR.

We are also maintaining another tool called ArrayTrack. Now, ArrayTrack has been around for 15 years now. We developed this tool originally to support FDA volunteer genomics data submission programs. This tool is still actively used at NCTR to manage the microarray data and analyze the microarray data, as well as for the interpretations.

In order to facilitate the use of these capabilities at NCTR, we also provide the trainings and normally once a year.

Okay, so in terms of the requested activities, it is very clear. It was requested by the PIs or other divisions. Merle already mentioned that we work with the NeuroTox Division, but, of course, we work very much for every other division at NCTR. Most of the work we do is focused on the sequencing data analysis. Sometimes not

only just apply existing tools, we also develop or specialize algorithms to process these data. Sometimes we develop the database to manage these type of data. So, we also do the integrated data analysis and imaging data analysis and the biostatistical support.

Now, this is actually - it is a long-standing effort - actually, it was before our division was established. There is a tremendous grassroots relationship between our statisticians with other divisions. So, there is a lot of interaction. Sometimes the support was provided from our division, I am not even aware of it. So, I cannot give you everything. I just want to point it out this is very important component of our division, to provide statistical support to other divisions.

Beyond NCTR, we have a very active engagement with other centers, particularly CDER. We are very much involved in every component of the review process in CDER. We are trying to improve the regulatory submissions. Particularly, we want to provide the capability to enhance science-based review process and the management of knowledge derived from this process.

Let me just give you an example. For example, CDER has a lot of so-called template. Basically, it is a

Word document. It has several pages. There are a lot of fields. Reviewers need to fill out these fields. When they fill out these fields, they are using free text by reading a lot of the box to box document. Those are very valuable information. What we do, we don't change anything what they do. Instead, in the back end, we put the code behind these fields. So, every time they fill the information in, this information can bring something into the database. Later on, this information can be used to support the future review process.

Another project we are working on is called DASH. Dr. Slikker was mentioned in his presentation. DASH stands for Data Analysis Search Host. It is a database. We did not develop it. Actually, DASH was in CDER for a long, long time. It was used to track the approval of the NDA and the progression of the IND to the NDA. It was widely used in all the upper management teams, including Janet Woodcock was checking this database routinely. However, this database is very old on the Access Database. If you know about IT, Access is very, very bad. It is a big no-no. When several people head into the database, it becomes extremely slow.

So, they come to us. We upgrade the entire infrastructure to the Oracle Relational Database. On top

of that, we add web-based applications. Everything looks exactly the same as before, but entirely changed in the back end. Make it very efficient.

Because this improvement created a lot of opportunities. Other offices, who have a lot of the similar dataset, wanted to be incorporated into the DASH. Right now, we are working with CDER by including all of this information into the DASH. At least five groups are already in line to bring the data into the DASH programs.

The last one is the text mining. Clearly, every year, CDER was producing a tremendous amount of documents, such as like approval letters, patient narratives, meeting minutes, the PharmTox documents, so on and so forth. These documents just stayed there, either PDF or just a piece of paper. What we do, we apply and develop various text mining tools to extract the information from these legacy documents and then use this information to support the review and the process.

On top of that, we also - of course, as I said, a lot of the work we do right now is with CDER, but we also work with CBER, as well. One of the projects that is still active is to use the Next Generation Sequencing to assess the safety of the engineered

therapeutic biologics.

We also have several projects funded by CTP. Three of them were retired last year. We still have one still going using a molecular modeling tool to assess the addiction potential of tobacco constituents.

Because we have more and more interaction with the other centers, particularly we have more and more projects from other centers, several years back we decided to develop a framework to better manage these sort of interactions. So, the framework is called Research to Review and Return or R2R framework. This framework was coordinated by us and CDER Office of Computational Science.

We implemented four stages of the process. The stage one is exploration. Basically, we have a biannual face-to-face meeting and a quarterly videoconference to talk about various issues and challenges in the review process. Then we will rank order them to see which one we might be able to take on or to help out. So, this is stage one. Stage two is assessment. We quickly conduct a prototyping to determine whether to go or no go. If it is a go, then we move to stage three, which is execution. This time, we can develop a detailed outline and plan with the deliverables, timelines, and the

milestones.

Once the project is finished, we have three options. The first option is to proceed to production. That is going to involve an entirely different set of people. There is some sort of regulatory framework behind it. It also involves how to maintain the product and so on and so forth. Often, during this process, there is some new ideas generated from this project. Then we bring these new ideas and go back to stage two and put into the list. Of course, the option three is work finished.

So, currently, there is a number of the project under the governance of the R2R framework. They are across the various stages of the R2R. In order to better manage oversight of R2R - so, in this year, we established a new branch called R2R Branch. Now, the name makes sense.

Of course, we are not going to - this R2R Branch not just only focused on the R2R frameworks, but also encompasses other type of the supportive functions at NCTR. As I mentioned, most of the support functions this branch operates is dealing with the new data stream, such as data from the omics data and next generation sequencing, bioimaging, and so on and so

forth. Of course, with this new branch, we are much easier to prioritizing the project and coordinate the support functions. Most importantly, for the people working this branch - because these are support functions, so their responsibility becomes much more clear. The career development path is much easier to define.

With that, we have come to our division missions. Our division has two functions: service and support. We try to provide the bioinformatics and the biostatistics support to NCTR, as well as FDA. Most importantly, we are trying to strengthen our linkage to other FDA centers.

In terms of the research, again, it is not much different from the other divisions. We conduct integrative bioinformatics and biostatistics research to support FDA missions and improving the safety and efficacy of FDA-regulated products. So, in the rest of my presentation, I am going to talk about the research. I have finished the service; finished support. Now, I move on to the research.

Before I talk about the research, we do have a lot of the outreach and leadership activity. I am not going to go through all of that. I wanted to really

emphasize two activities we have that is mainly focused on to develop the bioinformatics competency within Arkansas regions. A few years back, we instrumentally to establish Arkansas Bioinformatics Consortium. We call it AR-BIC. This Arkansas Bioinformatics Consortium, right now, consists of the Arkansas major universities plus NCTR. We already participated in organizing three annual meetings. The fourth meeting is going to be the next April in Little Rock, again.

Another local society, called the Middle South Computational Biology and Bioinformatics Society or called MCBIOS, has been run over 14 years now. NCTR always important part of this society. Dr. Slikker, actually, in the early days as president of the society. Now, our division has one past-president with two board of directors.

Okay, now, talking about our division's research, as instructed by Donna. We can start with the three accomplishments.

Number one, MAQC, MicroArray Quality Control Consortium Project. We started this consortium effort way, way back in 2005. The objective of this consortium is to assess the technical performance and the clinical utility of the emerging technology, particularly

genomics technology. In 2006 and 2010, we complete two projects, both are focused on the microarray. Now, you can see where this consortium name comes from. It comes from microarray. Starting in 2010, we started a project focusing on next generation sequencing. So, this project consortium also called sequencing quality control. Sometimes people call MAQC Consortium and sometimes call it SEQC Consortium. It is the same thing.

In terms of the accomplishment, by 2014, we finished a third project after MAQC. One of the most, for me, important accomplishment from this project is by the time that we deposit the data to the GEO - this is the largest database in the world. Our dataset has occupied six percent of the old RNA sequencing data in the GEO. We feel very proud of it. We feel like we contributed to the research community by providing the high-quality data.

Accomplishment two is the Liver Toxicity Knowledge Base. Now, we have been working on this project for a long time. Our definition at the Knowledge Base is very straight forward. First, it is collect a lot of the data and then, develop a predictive model based on these data. We call it the Knowledge Base. The Liver Toxicity Knowledge Base and the reason we choose

the liver toxicity - it is very obvious. Hepatotoxicity has a tremendous impact to the drug development and clinical application, as well as the regulatory and applications. Over the years, we have collected a lot of the data. At this point, we already have a comprehensive dataset available for the public to use. We also developed various models.

I am not going to go into detail because we have so many different moving parts for the Liver Toxicity Knowledge Base. Years ago, Nature Medicine featured our project by describing what we do at NCTR and by addressing the important issues related to hepatotoxicity, not only just for drug development, but for the regulatory applications. Most importantly, I think what they did is they went out, interviewed the leaders in this field, and also talked to reviewers in FDA, to assess how this LTKB project are going to impact the research community and the review process. I think that their comments are very balanced. I really encourage you to read it. I am not going to go into detail here.

Accomplishment number three is about rare disease. As you know very well, there are more than 7,000 rare diseases that have been identified, probably

more than that and some of them we probably don't know. Only 500 of them have a therapeutic option. For the FDA, this is a pretty big issue. For the past 10 years, one-third of the drugs approved by FDA is for the treatment of the rare disease. Now, to my opinion on the rare disease, it is not necessarily difficult to treat. It is just not a lot of the people do it. Particularly pharmaceutical companies don't feel - have a tremendous incentive to study the rare disease.

What do we do? We feel there is a lot of the drugs already out there - if you Google it, go to the patient discussion forum, you will often find it said someone has the rare disease and they are using some drug, off-label use, and it relieves the symptoms of that particular disease. So, what we wanted to do is systematically look at all the marketed drugs and see which drugs can be used for the treatment of what kind of rare disease. Recently, we had a paper in the Pharmacological Sciences to detail our strategy how to reuse the oncological drugs for the treatment of the rare disease.

Okay, so, this slide will summarize some of the key projects in our division. Not meant to be a kitchen sink style. Just lists a few of them as a

talking point when I move on to the next few slides. I am going to talk a little bit more later on about the Sequencing Quality Control Phase 2 project, SEQC2. This is the fourth phase under the MAQC consortium. And in terms of the Liver Toxicity Knowledge Base, we have a project funded by the Office of Women's Health to develop hepatotoxicity database for herbal medicine and dietary supplements. We also work with the local VA to integrate electronic health records to understand the drug and host interaction, how they are related to hepatotoxicity. We continually push our models to the review process.

If I have time, I am going to talk a little bit about this project, as well. Also, we have another project that is a long-standing project, called the Endocrine Disruptor Knowledge Base. Very close to my heart because 1996, when I was hired to NCTR, was develop this knowledge base. Every knowledge base that came out from my group, there is some similarity of this one.

Of course, as division folks on the bioinformatics and biostatistics, we are working very hard to develop the big data methodology and analytics. We focus on data integration and text mining, as I

mentioned earlier on, the imaging analysis.

So, now, I am going to go to deep dive to talk a little bit more about SEQC2. As I mentioned earlier one and when we finished the third phase of this consortium effort, and that - SEQC1. That SEQC1 was focused on RNA sequencing. As a natural extension for the SEQC2, we decided to focus on the DNA sequencing. More specifically, focus on the whole genome sequencing and targeted gene sequencing.

This is a set of the milestone. The minute we finished the third project we are starting to discuss what are we going to do the next. Then we bring this idea back to the FDA. We approach every center in the FDA at this point. Every center has a representative on this project. We want to make sure what we do in here reflects the FDA's needs.

We have first kick-off meeting at NIH last year, September. (indiscernible) gave the feature speech in our workshop. We have the second workshop in the SAS Campus in April this year.

Throughout MAQC project, we always asking a very simple question. If you have the same samples, give them to different laboratories and they are using different machines to do it, and then you analyze

differently, are you going to get the same results? This is our core question throughout the entire MAQC consortium activities. For this project, it was the same. We looked at cross-lab reproducibility, cross-platform reproducibility, and cross-analysis reproducibility. We focused a little bit more on the data analysis. We tried to benchmark the various ways analyzed the next generation sequencing data. Hopefully, at the end of the day, we will be able to develop a standard analysis protocols.

We also wanted this project to be a little bit more clinically relevant. That is why we focused on the targeted gene sequencing and the whole genome sequencing.

Right now, we have seven working groups that were established in this consortium effort. Some are more active than others. The working group number one, it is very active. We have a little bit over 160 people from 70 organizations engaged in the working group for number one, focusing on somatic mutation. For the working group number two, it is equally active, equally number of the peoples and organizations, but a focus on the targeted gene sequencing. The working group number three is focused on germline variants. Working group

number four is focused on difficult genes. Now, I am not going to talk too much about difficult genes. Difficult gene means a very difficult, but really important to the FDA.

Okay, future direction. By now, you probably already realize our division is pretty diverse and serves three main functions - service, support, and research. In the service and the support area, we are continually focused on data rich analysis and data management. We are going to increase our data analysis support, such as imaging data and the next generation sequencing data. We are also going to continually invest in R2R programs.

In terms of the research, some of the activities here is the new initiatives. Some is existing activity. For example, we are going to integrate the LTKB models into the review process. We have a benefit to do that because we are co-chair of the Liver Toxicity Working Group at FDA. We also want to take what we learned from the LTKB by oozing into other endpoints. We are currently thinking about cardiovascular toxicity. We already are starting to have an initial dialogue and see whether the methodology we used in the LTKB can be used or equally applied to cardiovascular toxicity.

I will probably just finish here. Feedback. I have three feedback requests. First is how to efficiently utilize the growing size of the diverse datasets in the public domain to address the FDA issues? That is my first question. There is so many data out there right now. There is a journal called Scientific Data and only publish data without any hypothesis. If you say anything about a hypothesis, the paper gets rejected. Other interesting is about data - accurately describe the data so people coming in can look at the data and with a fresh eye, come up with a new hypothesis. This is a journal by Nature. Right now, it is only two years. Impact factor already very high. So, we have a journal club in my group every week to take one paper to see how we can do with this data. This is the first question.

Second question is there is a lot of emerging technology, such as organ-on-a-chip and so on and so forth. We would like to hear the SAB, whether our skillset can be useful in this area.

The last one probably - we debate a lot - is how we are going to reward the scientists who only provide the support function. NCTR is a research environment or community. A lot of promotions were

dependent on your publication and so on and so forth. If you ask a scientist to focus on the support, what type of career development path should we establish?

Thank you very much.

(Applause)

DR. LEIN: Thank you, Weida. Questions or comments from around the table.

DR. PILLAI: Nice overview of a presentation. I was just curious have you reached out to Google and Facebook about machine learning? Some of this dataset, unless you use their analytics, it is virtually impossible to make sense. Their data points are sometimes a lot more richer than a microarray or a next gen sequencing dataset.

DR. TONG: That is a wonderful question. No. We have not talked to Google and Amazon. I know they are very keen on deep learning and these sort of methodologies. We do apply the deep learning in the dataset we have. The datasets normally we are dealing with is not large as like these companies are dealing with. We found that the deep learning is sort of the technology we can take advantage of it, but the advantage is not that significant as we originally expected, particularly when we apply these methodology

in the Liver Toxicity Knowledge Base because this is the database we have the most data.

DR. LANIYONU: I do not have comments. I wanted to say thank you. You have described a lot of big ticket items that are extremely useful for regulatory work. One of the ones that you actually described that may simply be service to you - improvement of reviewers template is a big deal. Usually, within the - at least I can speak for Sudan OND, within Sudan OND, it is actually difficult to compare analysis from - say you are dealing with a class of drugs and you want to see what adverse events can be observed to be - it is extremely difficult. You write in your review - you will be less inclined to actually go in and do the search, despite the fact that they are sitting on tremendous amounts of medical data that I wish academicians, physicians could actually get their hands on some of the reasoning behind, which is not the case. So, it might be a very small item on your plate, but it is a big improvement in the way we do our business. I will just say that your group is involved with other colleagues at CDER, computational groups, say a big thank you.

DR. TONG: Thank you very much.

DR. LEIN: A quick question in response to your

third item for feedback. Who determines the criteria for advancement within NCTR? Who sets those criteria?

DR. TONG: We have a very clear mechanism defined. The mechanism was established some years ago. There is a very strong flavor in terms of the research. All these data analysis or support involved in the various research area and has been addressed to some degree. I just want to hear whether there is some other examples available.

DR. SLIKKER: I can just mention that we have a peer review system for our research scientists. Those individuals that are doing research in the bioinformatic area would fall under that same sort of approach.

I think one thing, though, that we do want input on is how can we provide fair support and give credit to those that deal with big data kinds of issues and large experiments that we are seeing more and more, where we are using multiple disciplines in one experimental kind of push? To do that, how do you reward individuals that may be amongst several, including 10 or 20 authors in some cases, because of the diversity of research being accomplished? I think this is a question for general discussion. I don't think we are alone in trying to find ways to reward individuals who are

involved in group research.

DR. LEIN: You are not alone. We do struggle with this in the academic sector, as well, because we have very different classes of individuals within our structure. It really is - the reason I asked who sets the criteria, if you set your own criteria, then you are the ones who will have to grapple with how do you define what is worthy of advancement and award. There are creative ways that are coming out of the academic world. They do have I think pretty direct translational applicability to what you guys are facing. I think it is a matter of maybe working with some of your partners or in academic to see how they are dealing with these very similar issues.

There are just ways to say, yes, we have to look at what is the contribution of each individual to a product, whatever that product may be, whether it is a report or it is a publication, and not just look at impact factors. Look at their contribution to that and figure out a schema that recognizes contribution to multi-person, big effort-type things.

You guys certainly have a mission, a very clear mission, to provide support to your various partners within FDA. You could probably assign some sort

of a weighting factor to that, in terms of its impact on the mission. We talked about R2R last year, I remember, which has been hugely important for the product centers. We heard from all of you that this has been a remarkably useful tool that never got published. Building that into your criteria that this is something that has been incredibly impactful and therefore, we weight it accordingly.

DR. TONG: Thank you very much. I bring these slides up for some of the activity we involve with other division. That is a credit with the co-authorship and all of that. More in the collaborative area. Some of the committed tasks like we go out to put the Galaxy Platform together and everybody can take advantage of it. How is that going to be - it is very difficult. If you publish a paper now, you have an impact factor, how many people published it, like quantitative measure. For those, a little bit more difficult. So, I thought I would just bring this up.

DR. LEIN: Any other comments or questions from around the table? Do we have anybody on the phone still? I think this closes the public session. Are there any last comments, Donna or Bill, you would like to make?

DR. SLIKKER: I just want to thank everyone for

a full day of activities, the presenters, individual on the Science Board asking really quality questions, individuals, likewise, from our centers that are asking good questions and providing good information. Tomorrow is going to be a very exciting day because we have the opportunity here, from each one of the centers. They can talk about not only their center activities, but those things that hopefully will impact and encourage interaction between us and their particular center. So, we are looking forward to that, as well as an opportunity to hear from FDA's Chief Scientist.

I will say that our commissioner unfortunately, has had to rearrange his schedule and we are no longer on that schedule. This is unfortunate. However, I will say that Scott Gottlieb, our new commissioner, came to NCTR within three months of him being selected and approved as commissioner, so we are very happy about that. Probably one of the most rapid opportunities to have a commissioner come and visit NCTR after they had been selected. That was really positive. We had a great interaction with him. I know he has a lot of other things on his plate right now. So, we have the opportunity tomorrow to have Captain Denise Hinton give us comments about the FDA and NCTR.

We are looking forward to those, as well as input from all of the other centers. We are looking forward to a good day tomorrow. Thank you very much for the interaction today.

DR. LEIN: Thank you, everybody. We will reconvene tomorrow morning.

(Whereupon, the meeting adjourned.)