National Center for Toxicological Research (NCTR)

Science Advisory Board (SAB)

November 1, 2016

Crowne Plaza
201 S. Shackleford Road
Little Rock, AR 72211

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

Agenda Item: Welcome and Overview

DR. PHILBERT: Good morning and welcome. It's good to see many old friends and some new friends. Before we get underway, let's go around the table and introduce ourselves. We will start with the Science Board.

(Introductions around the table)

DR. PHILBERT: You have before you the Agenda which we will follow. Since it is in the Federal Register we will follow it unerringly. We, at present, have no public comment so we may recoup some time which will allow us time for conversation.

For the members of the Board, I am delighted to welcome all of the staff members from FDA, and we would like to have an open, fertile discussion. In the interest of process we will first allow the Board to get into the reports and discuss the reports, but then we will be actively seeking input from you, and your perspectives on how the reports and how the work of the Center affects you would be greatly received, so please don't hold back. Just indicate that you would like to say something and I will call on you in rotation.

I would like to call one our member of staff.

Syed Ali was just elected as a fellow of the Royal Society of Chemistry of the United Kingdom, and I think we should congratulate him.

(Applause)

DR. PHILBERT: We are a FACA committee so I will hand it over to Donna who will lead us through the process.

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

DR. MENDRICK: Good morning. I am Donna Mendrick, the Designated Federal Official, DFO, and I would like to welcome everyone to the NCTR Science Advisory Board meeting. We appreciate the time and diligent work of our Board members in preparing for this meeting and for the forthcoming deliberations. I, and the Board, wish to thank the FDA Regulatory Centers and NIHS for their participation in this meeting and my NCTR colleagues for all their efforts in preparing for this meeting.

Now I need to say a word about my role. As the DFO for this meeting I serve as the 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 SAB. Also in my

role as DFO for the Board, a critical responsibility is to work with appropriate Agency officials to ensure that all appropriate ethics regulations are satisfied, and I have done that. 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, among the other 10 forms or so, unfortunately, that you are required to fill out.

Regarding the meeting operations, we have a full agenda yet strive to ensure adequate time for the presentations, public comment, and the Board's thorough deliberations. This is a special note for all presenters, Board members and other participants. Please speak into your microphone and identify yourself as this meeting is being recorded and a transcript will be posted to our website.

You will notice that, pursuant to FACA, we will have a public comment period today from 1:15 to 2:15 offering the public to provide comments about the topics being considered by the Board. 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 -- and I have

gotten no pre-registrations -- please notify me if you are interested in making a comment.

I would like to add that during presentations and discussion, if Board members require greater clarification on an issue requiring participation from attendees in the audience, they may request such information during the meeting through the Chair or myself.

In accordance with FACA, minutes of this meeting will be prepared as will a transcript, both of which will be presented to the website, so please remember this is a public meeting. In closing, I wish to thank the Board for your participation in today's meeting.

Now some housekeeping notes. For coffee, there is a Starbucks down in the lobby. The bathrooms are right outside here. At lunch time, for those who pre-ordered lunch, Kim will walk us down to the area where we will get our lunches. Again, please sign in if you haven't signed in because we have to under the law.

DR. PHILBERT: Thank you very much. I suppose for purposes of FACA I need to ask SAB, are there any new conflicts to declare? No, okay. Thank you. Then I'll hand it over to Bill.

Agenda Item: State of the Center

DR. SLIKKER: First let me say that it's really wonderful to have this opportunity once again, which is an annual event for us, to bring together our full Science Advisory Board group as well as our representatives from the various centers of FDA. It's an opportunity for us not to only discuss what is currently going on in NCTR but also look toward the future. That's why I am very pleased to have this opportunity to invite your comments along the way, as our esteemed Chair just mentioned, and to really look forward for FDA and find out what is the best thing we can be doing together to get us there. That's what this is all about, is to get your input, so I really appreciate each of you being here to do that.

We are going to do it in a two-part way. We will have the full SAB today and tomorrow, and then following that we will have the in-depth review, of course, of the Division of Systems Biology, and many of you will stay for that activity as well and new individuals will come who will look in-depth at that particular division. We do it this way because it saves time and it saves transit cost, and we think it's more efficient.

The other thing we're doing is that we're meeting here this time. We are not at the NCTR; we are right here in Little Rock. The reason for that is that we thought we would try this new model because it seemed like we would save transit time, which is about 45 minutes to an hour each way, and we can concentrate more of that time on questions and answers and interactions around this table. So we'll see how this works and we would really like your feedback on this new model.

For those that haven't seen NCTR -- many of you around the table have on multiple occasions -- we are going to offer the opportunity to visit and tour NCTR tomorrow afternoon. So, for those who didn't stay and want to see NCTR, we will have a full tour tomorrow afternoon and you'll get a chance to meet a lot of the staff, see the facility interact and that sort of thing. We'll hopefully cover both sides of this coin by using this approach.

However, it is an experimental model approach. We haven't done this before. So we do enjoy your feedback on this so we can see if we can improve the kinds of interactions that we make happen here at this NCTR Science Advisory Board meeting.

In any case, I'll go ahead and get started with

a little overview. I have to apologize in advance to some of you who have seen this many times before. I like to give the basic information about NCTR and its history for those who are new to the Board, so the rest of you will please understand.

We really feel that NCTR is a very unique resource within the FDA. It was established in 1971, so we're almost 45-plus years old now. The idea is really to kind of examine those kinds of issues where you need an integrated approach, you need input and collaboration with those from the rest of FDA, from industry and from academic facilities, and to really look at those kinds of risk assessment needs that are pointed toward public health.

The vision, of course, is to be a global leader in this particular area and to provide not only the fundamental research that's useful for FDA decision-making, but also to look toward training opportunities and, of course, innovative scientific approaches to develop new technologies to hopefully make the FDA decision-making more rapid and less expensive and more critical to understanding the importance to human public health and personal health. And, of course, the mission is to do the same and to do this in such a way that we

really try to improve the general public health of the U.S.

Also, this has many global implications as well because much of our work is done in a global context. Just like FDA now has offices in many different countries around the world, our outreach is definitely global in nature. A lot of our collaborators are from around the world. A lot of the training that we do is done with investigators from many parts of the world. At any one time, we may have 70 or 80 postdoctoral fellows and I would say at least two-thirds of those were probably born outside the U.S. and came to the U.S. to train. Many of them stay but many of them also go back to their home country, the idea being that the training that they received at the NCTR and FDA is really valuable to raising the standards and the safety net around the world. So, a lot of global outreach as well as outreach within the local community, the state of Arkansas and the United States as well.

This is the organizational structure of the NCTR. Many of you recognize the idea that we have this layer of very important administrative functions. It’s not a big percentage of the NCTR staff because most of our staff are researchers, but these are important

functions that occur here including the health and safety ideas and the idea of finance, et cetera, and the general management.

But the important business part of NCTR is the research. Down here you see the research divisions, the microbiology, genetic, molecular tox, biochemical tox, neurotoxicology and the Division of Bioinformatics and Biostatistics. And, of course, coming back to the Division of Systems Biology, that is the division that will be evaluated on Thursday and Friday of this week.

Each year we usually look in-depth with a subcommittee of this group to evaluate a division. We will have some discussions about this, but it could be that we do another division next year or perhaps we also look at some of our special functions such as nanotechnology, imaging, bioanalytics and other kinds of approaches that -- imaging, for example -- that are really coming to the front. We will discuss that with the Science Board members and make a determination as to what will be on the docket for next year.

These are the divisions that get the work done. Many of you have sat through reviews of these recently. Of course, the most recent one is the Division of Bioinformatics and Biostatistics, and you will hear an

in-depth response from the SAB members as well as from the staff of the Division of Bioinformatics and Biostatistics later on this morning.

What is the size and scope of NCTR as far as the number of FTEs? As you can see here, if you put together the research scientists, staff fellows and visiting scientists it's over 150 positions, and support scientists, about 45 or so. These include individuals that have a Bachelor of Science or perhaps a master's degree and are not principal investigators but do a lot of fundamental efforts here at NCTR.

We do have administrative staff which is a relatively small percentage of the total but very important to the smooth running of the operation. As many of you know who are within the FDA, over the years it seems like the reporting of information is ever more frequent and rapid and on a shorter and shorter string. In order to meet all those obligations that Headquarters and even above that, Health and Human Services, throw at us we do have to have some administrative support to allow us to respond quickly to those information requests which happen very frequently now.

Then, of course, we do have the FDA fellows, and these are a special group that have not only didactic

training across the FDA -- and each one of the centers has a few of these; we have a total of four. We usually allow two or three to come in each year so this is our number at this point in time.

This is a really important group of trainees. This includes our ORISE postdoctoral fellows which don't have to have a green card or U.S. citizenship to train at the NCTR -- a very important group -- as well as graduate students, summer students, et cetera, which adds up to a little over 100. This group is very important to us because not only does it allow us to train -- and usually this happens in two or three-year segments of time -- then they rotate and move on to other positions. We have been fortunate to place these individuals, many of them within the other centers of FDA but also in various kinds of medical schools or graduate training programs and various kinds of academic settings of course, as well as in industry, et cetera.

And onsite contractors are a very important part of NCTR and have been from the very beginning. Part of the reason is that we have to maintain this entire physical plant of the National Center for Toxicological Research. It is owned and operated by the FDA. It represents about 25 percent of the floor space that is

outside of White Oak. It is 500 acres that belongs to FDA, and the 30 buildings and the million square feet of floor space all belong to FDA.

So, to maintain that we have to have a physical plant workforce, and those are contractors as well as the diet prep and the animal husbandry contract and the pathology contract. All these are individuals that oftentimes the actual people who do the daily work have been at the NCTR for many, many years, but as you know, every three to five years we have to have a new contract, so the management may change but a lot of times the people remain the same. These are important functions and we do this through a contractual mechanism in order to have that available for the maintenance of this large totally FDA-owned facility. So you add all those up and you get a little over 650 individuals who are currently at the NCTR.

And this goes along with our partners at the Jefferson Labs, which is the ORA, and this group has over 100, 110 or so, that function there in the Arkansas Regional Lab of the ORA right there on the campus, so we call the entire campus the Jefferson Labs. We are really proud to be working with them in so many different areas but certainly in the nanotechnology area where we have

confined our resources to develop that area for use for identification of nanomaterials in foods and to study them in drugs and devices.

Let me move on to the NCTR research goals. Of course, these may modulate a bit each and every year, but the ones I really want to focus on at this particular time are these advances in scientific approaches and tools required to support personal and public health. This is an area in which many of the centers invest, but we find that this is an important area in which we can collaborate and invest here at NCTR.

Of course, this is a high priority in the FDA's Advancing Regulatory Science Plan as well where we want to stimulate and evaluate emerging technology as well as develop new tools to support precision or personalized medicine. These are areas in which many of the centers are working but we feel like this is certainly a growth area and expansion area for us as well. For those who get a chance to stay for the second two days in the Systems Biology Division, a lot of the work going on there certainly supports this area of precision and personalized medicine as well as emerging technologies. But you will hear a lot about that today from the various presentations and tomorrow from the various divisions of

NCTR. A lot of this is done in tight conjunction with the other centers, and those who are representatives here realize there are many collaborative projects that we do with the other centers to approach this important goal.

And, of course, the other thing is that we generate a lot of fundamental data for FDA decision-making, so a lot of the work that goes into the collaborative studies between the FDA and NIEHS, National Institute of Environmental Health Sciences, through the National Toxicology Program. That work, of course, is proposed and followed closely and collaborated on with the other centers. That work, such as the large Bisphenol A study, the work that had been completed on a variety of agents including B-1 and acrylamide, and now the emphasis is moving toward arsenic, a large number of studies there -- those are done to generate fundamental data for FDA decision-making along with much more in the area, let’s say, of the anesthetic agents being used in children, and a whole host of other agents that we’re working on together.

The other sort of second main goal is really to enhance this collaboration with the other centers. To this end, we had the opportunity to have a visit from the current Commissioner of FDA, Rob Califf. It was really

great to have him down for a couple days. Also, at the same time, we had representatives from several other centers to really talk about how we could improve this collaboration, and some really good points came out of that discussion.

But the idea is that we have established points of contact within each one of the other centers so that we have a way to communicate, and we know that that contact is going to allow us to understand how the centers are going to interact with us in certain kinds of opportunities, whether it be new kinds of goals in terms of new protocols or completing existing ones and delivering that data to the literature or to a point that it can be useful to FDA.

We also use this process where we solicit input from the other centers, first through a two-page concept which they get a chance to review and give us back comments. At that point in time, the other centers can say this is going to be useful but we have this idea to improve it. We also may or may not have someone who will work on it with you from our center. But the idea is that it can move forward. In some cases, it comes back to saying, well, no, this is already being done, or, this is not important to us at this point in time; therefore,

the project gets put on the shelf and is not completed.

But if they are approved and they move forward to a protocol, this is usually a five to ten-page document, sometimes up to 15 pages, that really spells out the in-depth work that's going to be completed. Then you layer on top of that the animal care and use or human use and many other forms that have been done, safety, et cetera, and you end up with a fairly thick document that we call a protocol. At any one time we may have 100 to 200 of these protocols active at the NCTR and usually the lifetime of each one is around three years, although it can be extended another year or so under special circumstances.

But this review process also is done in conjunction with the other centers and we get feedback on how to make that protocol even better. So this interaction with the other centers is key to us and key to what work actually gets done at NCTR in support of the other centers of FDA.

Also, the idea of building strategic partnerships -- I'll get into that a little bit later on in this presentation -- and talking about the virtual centers of excellence which is something that's going forward, as you know, in the oncology area, but we think

there is certainly room for this kind of virtual center activity in the area of developmental talks, modeling and perinatal medicine, so we're working on that with many of the centers currently and would like to also have you weigh in on what you think about this opportunity.

Finally, promote global interactions -- I mentioned this a bit earlier -- especially the regulatory sciences. Here, we have a number of opportunities to do this. Some of them are led by NCTR, especially those areas in the global coalition and global summits that have been happening every year since 2011 that really expand the issues and underline data behind the regulatory sciences. We'll get into that a little bit more in a moment as well.

One of the areas I want to talk about as far as accomplishments is really to build these scientific partnerships especially within the FDA. One that we are especially proud of that we have been working on with the Center for Tobacco Products since its beginning five or six years ago is this area of tobacco research capacity. We really feel like we're an important part of this whole process for the Center for Tobacco Products and that we do, in conjunction with their scientists, a lot of the wet lab work that's important to back up their particular

movement forward in the area of tobacco evaluation.

We have studies that are going forward now in addiction, both in the rodent model and in the non-human primate. Inhalation toxicology -- I know someone will be talking about that while we're here during these next four days because there has been some really good data generated on inhalation of various kinds of tobacco products and how they are eliminated and metabolized and how they affect animals.

Then biomarkers, of course, identifying those new ways in which you can compare and contrast various components of tobacco. It's very important for this new center to be able to evaluate and make sure that any new product that comes on the market is no worse than those that are already out there, so, having biomarkers to compare any harmful effects is really key. We help develop those.

Also, the idea of using bioinformatics and being more predictive with toxicology. There has been a lot of work going on between our Division of Bioinformatics and Biostatistics and the Center for Tobacco Products in developing knowledge bases and various kinds of text mining capability so that we can be more predictive with existing data as well as using new

data more effectively.

Finally, toxicology and adverse health consequences -- Again, a number of models have been developed through work with this. Some of them are brand new using cell culture and/or stem cells, using models that have a combination of cells and even flow-through capability, so, moving in the direction of alternative kinds of models that may be useful for evaluating tobacco products in the future.

Those are just some examples of the work that has been going on with the Center for Tobacco Products and we are very proud to be working with them to move this area forward.

Other areas include work that we have been doing, for example, with the anesthetic agents that are used in children. We had the good fortune of this being reviewed by the FDA Science Board about two years ago. This area is moving forward and in fact there is some draft information about how to use this in terms of moving forward the regulatory aspects. But the important thing is that this work has been done together mainly with the Center for Drugs and the researchers there, and at NCTR we are working quite closely together to develop this database.

I would say that we've moved a long way from first understanding that there may be an issue with exposure to anesthetics for long durations during certain periods of sensitivity in the developing animal and its effect on not only the brain cell death that could occur due to this but also the behavioral deficits that may be associated with this exposure to, again, longer-term exposure -- we're talking about over five or six hours of exposure -- to all these different anesthetic agents you see here and in combination in some cases to produce this effect.

It also has a very specific developmental window in the non-human primate; it's somewhere after mid-gestation up to at least five or six days of age, but at day 35 it seems to go away, so it's a very limited window. Unfortunately, in the human situation, which we think this is all associated with areas of synaptogenesis, it's longer in the human. It could be somewhere between pregnancy up to maybe a year or two of life. You're talking about the potential for millions of children to be affected annually with exposure to these kinds of agents, so we think this is an important issue for both CDER and for NCTR to continue to work on.

We also have signed a Memorandum of

Understanding with CDER which continues the work on monographs for sunscreen ingredients and other non-prescription drugs. Dan Acosta, who is our Deputy Director for Research, has been the main link to the colleagues in CDER. It has been a really good relationship running now on a year and a half, almost two years, and it really has been a fruitful interaction between the scientists here and the researchers there at CDER to move these areas forward.

And an area I just want to highlight is the work that we're doing with the genomics area and the use of bioinformatics to really move forward knowledge bases in this area. Rita Tong and colleagues within the Division of Bioinformatics and Biostatistics have developed this Research2Review program via knowledge uptake. This has been really useful not only to the reviewers within CDER but we think it can apply to reviewers within other centers as well. We're looking for that opportunity, but also the idea of interaction with these various systems you see listed here that allows one to be more effective in getting the right data in front of the decision-maker, in front of the reviewer at CDER, so they can move more rapidly and more efficiently to reach quality decisions.

And, of course, also the idea of using text mining to search large numbers of documents, and this is certainly true within the area of Center for Tobacco Products, but CDER and some of the other centers also can use this approach as well.

These are just some examples of building these partnerships within the FDA. In fact, we can sort of qualify and quantify this by looking at the number of projects that are running in NCTR, and it's somewhere over 50 percent in conjunction with the other centers. As you can see, these numbers may change day to day and certainly month to month because we do have new projects coming on and other projects winding down. But the idea is that most of the work that is done in NCTR is done with projects where we have signed protocols from members of the staff from the other centers.

And, of course, there's federal work that is done independent of this, but it mainly is in methods development, first steps in projects, trying to get the approaches built to a point where they can be useful to us and to the other centers as far as being reliable tools that we use in research protocols. So it's a stepwise process that we're engaged in here.

Let me just finish up on these accomplishments

by talking about the built scientific partnerships. We have this long-ranging interaction over 24 years now with the NIEHS and National Toxicology Program, an interagency agreement with FDA and NCTR being the leaders of this for the Agency. You can see the whole list of compounds that have been evaluated or are under evaluation currently. Triclosan has been quite interesting to CDER, of course, making regulatory decisions there. And the work in nanosilver between CDRH, CFSAN and NCTR has been moving that area forward.

Of course, dietary supplements come into play with CFSAN, as well as the work in modeling exposure to arsenic. This is an important area of research now, as you can imagine, and in conjunction with CFSAN we're moving this area out, first with modeling and understanding the effects during development but then also later with electronic studies to understand what happens with arsenic exposure -- and this is inorganic arsenic -- from preconception through pregnancy through development into adulthood, and what kind of residual issues may be there.

And finally, just talking about these new technologies and understanding more about how the microbiome is going to influence toxicological outcome;

pharmacokinetic and modeling exposures; also, 3D cell culture and stem cells -- All these are new approaches that are being evaluated very thoroughly in conjunction with other centers to understand how they may be useful to the safety assessment of the future and how they may be predictive of toxicological and efficacy outcomes.

Let me finish this up by looking at some of the areas that we have been advancing along with our regulatory colleagues within FDA. Certainly, safety assessment procedures is an area that has long been supported by our bioinformatics and biostatistics group. Many new kinds of approaches have been evaluated there.

Biomarkers is something each one of our research divisions worked on. You'll see a lot about that today and tomorrow.

Bioimaging has been a growth area for the last 10 years or so. We now have the capacity to look at not only young primates but we now are getting a larger bore MRI where we can look at adult primates. We also have micro-PET both for primates as well as for rodents. We will soon have four machines that are quite state-of-the-art that can do imaging along with CT, and this micro-PET and/or MRI plus CT -- very powerful tools that can be used to assess preclinical and nonclinical issues. And

it is, of course, of direct relevance to humans because those techniques are also available for the human for extrapolation.

We have done a lot more work in the last five, six, seven years with 3D models and stem cells. You'll hear more about that today as well. The microbiome -- Our Division of Microbiology has been studying the microbiome for many, many years. It was nice that someone coined this term, and certainly Steve Foley can tell you more about this later, the microbiome, because it has really been brought to the front. But the kind of work that the division has been doing for many years has supported this important interaction between the microbiome -- that is the rest of the genome that lives with us constantly -- and how it interacts not only with drugs but with many of the chemicals that we find in the environment.

Precision and personalized medicine -- you'll hear a lot about that in the next four days. It's an important area of expansion -- how to develop these new kinds of biomarkers and new tools to push this area forward.

Nanotechnology has been a growth area between ORA, NCTR and also the National Toxicology Program. We

have probably one of the best facilities to look at nano materials in the entire world right here at NCTR, and it's because we've done it in a group way with ORA and many of the other centers and NCTR working together. This area is certainly something that's important. Now that we have around 20 new applications each year coming in to some of the centers regarding compounds that contain nano materials it's more important than ever to be able to evaluate these, characterize them and look for their effects in biological systems.

Inhalation toxicology is something we developed much more recently, just in the last two or three years, with the Center for Tobacco Products. We're very pleased to have this capability now and to have staff here and also at CTP to evaluate this inhalation toxicology data dealing with tobacco products.

Modeling is a big area of expansion over the last five or six years at NCTR. We have always had good modeling but now it has been taken to a higher level that is not only PK but it's also linked to pharmacodynamics, or PD. This combination is a very powerful predictive modeling tool and you'll be hearing more about it this morning.

Bioinformatics I already told you has been a

growth area for us but very important, along with biostatistics.

And then regulatory science training -- I alluded to that, that we have tens of students on campus that are training in this area. Not only do they understand and can use what exposure they can get from the FDA, but also we have a training program at the Medical Sciences School of Public Health where they can get accreditation or a certificate in regulatory science, and many of our students have taken advantage of that, and staff, over the last year or so.

Let me finish up by talking about the enhanced regulatory science, the outreach globally. This is really important to us because we formed the Global Coalition for Regulatory Science Research in 2013. We now have the European Union, and that includes over 25 countries, along with nine other countries that are involved in this particular process, so we're very pleased to have that group running at full steam. We have been able to host a variety of meetings, some most recently with the European Food Safety Authority. They helped us co-host the 2015 Global Summit on Regulatory Science, and that was very effective in looking at regulatory science as it applies to food as well as to

drugs. There we had over 25 countries attend.

We have also been working on the more recent one which occurred in nanotechnology, and this was fostered by the Nanotechnology Working Group and also the Nanotechnology Task Force within the FDA. This was one of the bases of our Sixth Annual Global Summit on Regulatory Science and looked at nanotechnology standards and applications and was held right there at NIH at the national auditorium with over 150 attendees from 19 different countries.

So, what we're doing is globally looking at some of these important issues to FDA, trying to drive that forward not only by training but by exposure and collaborative studies and all these things are moving forward. White papers are coming forth from these meetings that sort of set the stage for how to best think about important areas like nanotechnology and regulation of foods, et cetera.

So this was really the research behind that. We are not talking about setting policy or setting regulatory standards. What we're talking about is what research needs to be done to support those efforts, and that's where we focused this attention in the Global Coalition for Regulatory Science Research.

And just thinking about things that we have done -- in summary, the idea that we built these partnerships with the rest of FDA is very critical to us. We have been optimizing these kinds of facilities by revising and realigning our organization right here in NCTR to be more collaborative, and also the expansion of the Global Coalition membership. And we are looking for new members now and we are very close to inclusion of a group from China as well as a second group from Japan, and also looking toward South America as the opportunity to have our next Global Coalition meeting which is scheduled right now to be in Brazil next fall.

By moving this Global Coalition and Global Summit around the world we think we will bring in more participants and really show the other countries that we're sincere in our efforts to move this together in a group way with many countries being involved in addition to the FDA and the U.S.

Let me turn to a couple issues I want to put on the table. Succession planning is very critical to the NCTR. You can imagine that NCTR, like many other groups that were focused on toxicology, occurred during the 1970s and early 1980s. When you think the EPA was created, the NCTR was created -- many of these areas have

focused on these kinds of activities. But that also means that many of the people who joined early on are also perhaps starting to look toward retirement, so we're really doing a lot of fine-tuning and making sure that we have succession planning in good control. We are looking toward our research divisions and our offices of management to either have deputy directors and/or branch chiefs, so it increases the opportunity for training as well as increases the load-sharing of the many different management things that occur.

As you know, HR issues have done nothing but increase in terms of the amount of paperwork that has to be done as well as the amount of reporting that has to be done and financial reporting that has to be done. All of that makes a bigger load for management within the various divisions of NCTR, so we're looking toward this deputy director or branch chief structure to help improve that situation as well as provide training for these individuals should there be an opportunity for them to move into even a greater leadership role.

We have transitions occurring in each of these other divisions. I won't go into any detail but just give you the idea that we know there are changes that are occurring that will reflect realignment in some cases or

at least new opportunities for either deputy directors or branch chiefs to move into the system. It's just important to keep this in front of us, that it's something that we all need to be thinking about, how to improve and make the best alignment of our resources to be most effective for the future.

I will close with just two new proposals. I will not talk much about the analytical and imaging quantification group. This is at its very early stage of proposal, but the idea is that there are so many activities going into, let's say, imaging, into better electron microscopy, into various kinds of quantitative bioinformatics approaches and modeling approaches that we really need a group that's focused on how to not only generate this data but then to manage it, to save it, to analyze it and to use it moving forward.

So we think that we may be forming a group -- We're not thinking about anything structural here; it's sort of a virtual group, but the idea is, in collaboration with other centers of FDA and also university systems, to kind of build a group that will be able to advise us and move this area forward. We think it's so critical for the future how to handle that massive amount of big data that's coming in from so many

sources, including genomics, and to use it more effectively and more rapidly than we do currently. So we think this is a growth area for us.

The other one I want to spend a couple minutes on before I close is this virtual center on perinatal medicine/developmental/toxicology/modeling. It seems to me that there is so much activity now going on across all the centers in this area, and yet, oftentimes, different components of FDA are not necessarily speaking to each other about it. You may have the opportunity within CDER, for example, to use a drug for the first time in children, so what dose do you use? We think we can help with the modeling procedures to make that kind of decision.

In the area of food safety, there are so many agents that are exposed in our food and our diet. After all, children, on a milligram per kilogram basis, have more food intake than adults do, so you have even a greater opportunity for exposure there to any sort of contaminants that may be in foods. So we think this area is key.

Just to give you sort of an idea about that, if you look at the National Toxicology Program activities over the last five years, you can see that almost every

one of them included exposure during development. In many cases, it was preconception through pregnancy through development on up to adulthood. But the idea is that development is included in so many of these fundamental assessments -- BPA, arsenic, et cetera.

So the deal is that we think bringing people together in this area could not only make FDA more efficient but also we could help each other, because a lot of the tools that you would use and a lot of the modeling that you would apply cuts across all those different kinds of applications in the various centers.

So we have reached out now not only at the very top to many of the center directors but also to the staff that are working in this area at the various centers, and we have also broached this idea to the Commissioner and it really seems like something that is starting to catch on. Jeff Fisher and I are leading the effort here at NCTR but we want others to join from all the other centers and really sort of put our heads together for how we can improve this area, work more efficiently together than we can separately, and share concepts, new approaches and analysis techniques.

So, why is it beneficial to have a virtual center focused on this perinatal period? I already

mentioned this, but maternal-fetal pairs represent a unique regulatory responsibility. You can imagine that the number of drugs that are approved by FDA that are available to pregnant women is very limited. Compared to what's available for the non-pregnant adult it's extremely limited, so there is certainly work that could be done there.

The idea of preterm and term birth neonates and infants representing a vulnerable population is basically under-studied. Again, if you go to the NICU and look for those drugs that are approved by FDA that are being used there, it's only about one or two out of ten. It's really a situation where the academic physicians and other physicians are taking it on their own to use these drugs during the perinatal period, and FDA could do more work there to help them make those better decisions.

Also the idea that it really provides an opportunity to address unmet FDA needs across the centers by creating these expert teams that work together, share their approaches and share their data outcomes.

Why now? Well, it's a period of time in which we know much more about this area than ever before, and the issues that I just outlined are certainly in front of us. Also the idea that we can work in these

multidisciplinary teams better now than ever before. We can communicate better; we have better computer systems to allow us to do that, and the *in silico* approaches and extrapolation methods are ever better. So I think it's time to move in this area and to use these new tools to help us move this area forward.

And, of course, it would be a coordinated approach across the various centers, prioritizing those certain areas that it would be most effective to look at first so that we can move together in a concerted way. And also to really start looking at the skill mix that we need. We have had a lot of success recently in cell culture systems, other alternative models, mathematical modeling. Of course, there are traditional laboratory animal studies; we are very strong in that area, and many of our studies now, as I mentioned, are being done in developing animals. So, how we can pull all that together so we can share it across the centers we think is a critical piece of this.

I want to give you just a couple ideas of what we are all doing together. This is not new. The idea here is to bring people together in a virtual center to be more efficient and more effective in our interactions, but there have been many interactions going on for a

number of years and I will briefly cover some of those.

We have pediatric toxicology between CFSAN and NCTR. You can see a couple areas in which many publications actually have come forth with investigators from other centers including bisphenol A, of course, and that sort of thing.

The pharmacokinetic models have been developed and I know that some of these have been studied and actually used by folks sitting around this table, but the idea is what are we doing with methylphenidate and the idea that now there are concerns about the similarity of those new generic agents out there and traditional sources of methylphenidate, and are you getting the same drug to the target site. Well, these models can help you determine that so we can make really good decisions.

Not only that, but there's a lot of work going on with renal function and a lot of work has been done across the entire FDA through other organizations such as ILSI-HESI working groups on nonclinical models for neonatal pediatric drug development. Multiple centers are involved in this strongly led by CDER, but we've been involved with this every step of the way. So this just gives you the example that there are many activities already ongoing that we could build this virtual center

on.

And there's not only work within FDA that we think is so strong that we recommend it at this point in time, but also the interactions with other agencies where we can get additional support and additional outreach. Of course, for years we have been working with NIEHS and the National Toxicology Program and we've already talked about this with bisphenol A and arsenic, for example, that was supported by NTP.

And then, of course, the NICHD and the Best Pharmaceuticals for Children Act -- we've been working with them. As a matter of fact, the methylphenidate work that we have been talking about began with an interaction with NICHD and NCTR.

And then, of course, EPA and the National Center for Computational Toxicology, we are very good colleagues with folks there, and we have already collaborative projects ongoing that we could expand upon and bring in their expertise as well.

I just want to close with some questions for discussion. I always like to put out some questions that the Board and members of the FDA staff can help with. Some of these revolve around this particular proposal. Can animal models be better utilized for preclinical

decision-making? What tools would help? This is a very difficult area to study in humans. It's very unlikely that you're going to get IRB approval to expose pregnant humans or infants to certain new or experimental agents. A lot of times, this work does fall to using animal models and we think we can improve those animal models so they will be even more effective in those first stages of assessment.

And what alternative models need further evaluation? There are many out there. You're talking about the microphysiological systems, you're talking about some of the genomic and omic approaches, or you're talking about imaging tools -- how can we move those to the next level where they're going to be effective for use?

And then, can *in silico* approaches help? We already have good evidence that they can but they have to be developed and applied appropriately, so we need to look at that opportunity.

And is there a need for additional *in vitro* to *in vivo* extrapolation? We think that as these new alternative approaches come to be further developed that the need to extrapolate the *in vitro* approach to *in vivo* is going to be even more important, so pharmacokinetics

and modeling can certainly help there.

With that, I would like to close and thank you for the opportunity to present these ideas to you and to ask these questions that you might want to consider, but also just for being here and being involved in the review that we have annually of the NCTR and the cooperation with all the centers of FDA. Thank you very much.

(Applause)

DR. PHILBERT: Bill, could you remind us how your budget model works? The reason I'm asking is you listed a number of new programs. You have a number of legacy programs and a number of collaborations, and I'm trying to work out in my mind how it is you shape shift into a forward-looking organization while maintaining or sun-setting legacy programs.

DR. SLIKKER: That's a good question. We have a process -- I talked about the concepts and the protocol development before they're approved to work on. Each one of those protocols has usually a three-year life; sometimes two but usually a three-year life. As they begin to hit that second year and move toward the third year, then we expect those to be written up, the collaborations, to say yes, this is a good bit of data that needs to be put out for public consumption, and have

those particular projects then be published, available to FDA and then that project winds down. That means that we always have the opportunity for new projects to come into the fold because we're sun-setting those that already exist.

The other area is that we give a lot of opportunity for the division directors to make use of the funding they have available to get these projects done. The division director has the responsibility of making sure that the most important projects are done that already are approved, and those decisions have to be made at the division level, and sometimes there's input from Dan and I as the Deputy Director for Research and myself, to help them with that process. A lot of it has to do with getting the people who are at the front of their field to make the decisions on which projects are going to make the most emphasis and, therefore, get the most amount of funding.

Those two approaches I think are in play. The natural progression and finishing up of some projects and beginning of new ones and then the idea that the prioritization within each division is based on where the most important cutting edge research needs to be done.

Does that help answer your question?

DR. PHILBERT: It does partially. It is unlikely that FDA is going to see massive increases in appropriations in the foreseeable future, but you outlined some really big, audacious programs. Bioinformatics alone will sink your budget. So how do you view the balance between critical mass and significant contribution and good enough?

DR. SLIKKER: The thing is the bioinformatics program has actually been in existence at NCTR for many, many years. Even though the Division of Bioinformatics and Biostatistics has only been here for about three years, the bioinformatics portion of that certainly was very active for another 20 years before that. The biometry or biostatistics part used to be a stand-alone division that was one of the premier, first divisions in NCTR.

We have modulated that so we could have them work more closely together, and there has been funding going there through the approved protocols. But we're not talking about a change in the actual monies going to that group; it has always been going there. Before it was a division a lot of the individuals were in more of a contract mode. Eventually that contract was closed down and those monies then were used to support the division.

You're right. The NCTR's budget, if you look at it, is relatively flat. There hasn't been a great deal of increase over the last five years or so, if any, but we have been very effective in using the resources that we have. It's a matter of just moving those monies from existing protocols that are shutting down to new protocols and emphasizing these areas. A lot of this is work that is ongoing.

We also, as you know, besides the allocated funds from Congress -- which has been, as I said, very stable and not growing -- we also have funding coming in from non-allocated sources, which is really nice to have those other revenue streams, and some of that is from the National Toxicology Program and NIEHS. That is a program there, as I mentioned, for some 20-plus years. And really, the last six years, support from the Center for Tobacco Products has been really key to keeping that area and to growing that area out.

There are also CRADAs and IAGs and that sort of thing that we may have with EPA and National Institute of Drug Abuse or even DEA more recently. So there are those opportunities. We do try to get about one-third of our resources from non-allocated locations and then about two-thirds from allocated. Yes, it is a tight budget

situation. It hasn't been growing, as you probably are well aware. But we have been very effective with what we've been using.

And these collaborations -- we're not talking about spending new money, for example, on this virtual center. That's one reason it's virtual. We're not talking about bricks and mortar; we're not talking about moving people. We're talking the ones that are already there who work in the area just to work more closely together and be more effective because they're sharing and developing activities together. So it really doesn't take new monies to do that.

It would be nice, of course, to have additional funds but not necessary to build a virtual center where you're really talking about trying to increase efficiency and be working together on projects with existing funding streams that already exist.

DR. PHILBERT: Thank you. Any comments or questions from the Board?

PARTICIPANT: (Inaudible)

DR. SLIKKER: That was over 50 percent and it varies. It has been as high as 60 percent or even 65 percent depending on the day and the time of the year and which year you're in. These are ones that are in

collaboration to the point where there are PIs from the other centers present, plus there has been a signature from an administrator above that to make sure that they really want and can be involved. So that has been very useful to us, to have those kinds of collaborations.

But you can imagine there is also work going on -- Let's say just recently there was an interest in developing a bioassay or a new analytical technique to look at one of the ingredients of some of the projects that we're doing in conjunction with some of the regulatory centers. That particular analytical approach had to be developed independently before the main project could be done.

So you've got to do those kinds of things and you usually do them on a very short-term basis, so they do represent a smaller percentage of the total effort going forward. But they are essential to either making sure that new technology will work and is useful, or to make sure that a new analytical technique is developed so that then the larger project can be done in conjunction with the other centers.

DR. STICE: I just wanted to get Bill a little more information on how you set priorities. I heard the word arsenic several times in your presentation. Maybe

you could briefly walk us through how -- it sounds like it's a bubble-up type of scenario where it's at the scientific level. How did that become a high priority area?

DR. SLIKKER: Good question, Steve. Arsenic or like agents are those that have been getting more and more attention. New datasets coming out suggest that with inorganic arsenic, perhaps the developing animal is more sensitive than the adult. Those kinds of new data spurred a lot of interest in understanding more about how to deal with arsenic.

Unfortunately, as you know, arsenic is an important element but it's also one that is found around the world and it gets into the water supply and into too many different products, and, therefore, we have to understand how to deal with that. It's not something that we necessarily created; it has been there from the very beginning and we have to learn how to deal with that.

The idea is that these new studies suggesting that there may be more sensitive indicators in the adult and more sensitive indicators in the frank neurotoxicity, that there may be more subtle forms of neurotoxicity that occur during development or they may be, of course,

potential for cancer. One of the models, as you know, has to do with stem cells being involved with that during development.

So this new data that has come forth in the last 10 years has spurred the regulators to take another look at this. Of course, EPA is heavily involved as well as FDA, and the idea is that that sort of brings that topic forward. So, in collaboration with CFSAN, which is one of the main groups that has that concern within FDA, we developed a series of protocols that the National Toxicology Program is also supporting, and the idea is to do the pharmacokinetics first looking at developmental exposure in mice, rats and nonhuman primates and compare that to the human where possible so we can model this to really understand what the exposure is to inorganic arsenic, and then move that forward into your more traditional bioassay looking at exposure throughout preconception, pregnancy, development through adulthood to see what sort of effects are actually occurring at what dose level.

If you look at the existing data out there, the doses are not very filled in. A good dose response does not exist. So, all these things need to be done in a very comprehensive way using good laboratory practices

and appropriate kinds of systems, and using a lot of modeling of the data once it comes forward to make sure that we have really accurate data for FDA to make decisions. Does that help, Steve?

DR. STICE: Yes. I was mainly interested in the process. So data comes forward and from there the scientific people are interested in an area, and then there are larger projects.

DR. SLIKKER: Yes. And it's a very intense process because you have to make sure that the folks who have the regulatory authority are interested in seeing those new data and want to have it to fill in data gaps. So we try to fill knowledge gaps that are there.

You have the interaction with the National Toxicology Program, which is a whole other array of scientists from NIEHS and from other agencies that lend a hand in making sure that the study is designed to its maximum. And then, of course, you have the researchers at NCTR in conjunction with the CFSAN researchers really devising those protocols and making sure that the protocol that we use is going to answer as many questions as possible and do it in the most efficient way. All those things come together. We're talking about months of work by many to make sure that priority comes forward.

So that's just one example, but it happens again and again and again at so many different levels. And it happens with much smaller projects that are focused on, let's say, a new kind of alternate process where you may use stem cells from the airway of either humans or animals to evaluate transfer of various kinds of tobacco products perhaps across from the lung to the blood. It's a whole variety of projects, but all of these are done in close concert between the review centers, scientists there, with NCTR scientists moving these projects forward. Some of them are one, two or three-year projects; some of them we know are going to be multi-year projects. For example, the arsenic one will actually be a very important project that will set standards for around the world.

What we find, of course, is that there are many countries who either don't choose to generate these kinds of data or do not have the resources to do such, so when these data come out we get requests from WHO, from EFSA, from many different countries, Japan, et cetera, and they want to see these data. Of course, we make them available to them so they can make the best regulatory decisions, because these studies are oftentimes just not possible to be done without the kind of resources and

functional capability that places like NCTR have to offer.

DR. REISS: Thanks, Bill. I have a question about translation, I guess. You talked very passionately about collaboration sort of within like minds -- bioinformatics, across countries, different institutes and so on and so forth.

But a lot of what you do is develop new knowledge that has to have an impact in the real world so it has to be translated. We'll just use the example of biomarkers, let's say renal injury biomarkers. How do you guys -- other than working with the reviewing divisions at the Agency and so on and so forth, the institutes at the Agency -- help to facilitate that translation or qualification of those biomarkers? Do you have direct links, for example, with the CERCI program or any other mechanisms to be able to do that?

DR. SLIKKER: This is an important question, how you actually reach the opportunity to have a qualified biomarker. As you know, by definition, especially the CDER program, it's a very defined roadmap.

A lot of our work is on more the development and/or initial evaluation of biomarkers. Not all of them are going to reach the pinnacle of being able to go

through the biomarker qualification process. But we have been involved in some of those activities. As you know, oftentimes that really is a multicenter, multiagency kind of approach and takes a number of years to do that well.

What we try to do is look at those opportunities where we think a new biomarker will come forward, get it to a certain stage and then look for partners to take it to the next level. This means that not only do you have your partners who are right there in the other centers, but you oftentimes develop partners. Some of these partnerships are with HESI; there could be outside groups that help coordinate these, and we are active in a lot of the HESI projects serving as co-chairs and that kind of thing. Or it could be a collaborative work with another agency either within the U.S. or sometimes abroad. We have done things with EFSA and other groups.

You're right; it's a very multi-step kind of operation but you need some folks that are working at the ground floor trying to get that biomarker idea forward. Then, to take it to the next level you may need a good study within FDA, and then, finally, to take it to qualification as you well know takes multiple groups working together.

DR. PHILBERT: -- a lot of client-vendor relationships and somewhat stochastic bumping into people who are interested in what you are doing or have an interest in your capabilities. How much of this is guided *a priori*? Say there's a need for a biomarker of liver or kidney injury, what have you. They come to you and you take the project so far, and then there's a sort of orderly hand-off.

DR. SLIKKER: Yes. It's a really important question because, right here, we have leadership from Donna Mendrick, who is the Chair of our Emerging Technologies Working Group within the Office of Science of FDA, and it's that kind of leadership where you pull together representatives not only from FDA but you get input from the other agencies both here within the United States and abroad to try to look at those emerging technologies that they feel are coming down the pike that could influence how we do business within FDA. So we have that kind of forward looking and talking about five and 10 years out type opportunities.

Then, more immediately, we have sort of the groundswell approach. For example, being able to understand, by working with CDER on some of their needs for having data in front of the reviewer -- so, working

hand in hand with the reviewers and the scientists within CDER that have responsibility of putting that data in front of the reviewers -- we have been able to develop this R2R approach which you will hear more about later today. The idea is that that allows us to have direct opportunity to influence the research and the products that are available to the reviewer so it makes the reviewers faster. It comes from an intense interest of FDA to move that area forward.

So, all these things have to relate to the importance to FDA but some of them can be done within centers and between the NCTR and other centers, and some of them are taken to an even higher level where you're meeting with other agencies or maybe even folks from around the world to make them happen. We use the appropriate approach based on what the actual opportunity is.

DR. CENTENO: Jose Centeno from CDRH. Thank you for the overview. I really appreciated very much your last slide about the questions for discussion. I think that kind of brings together a lot of the things that the interactions with the different centers can do with NCTR. These are very kind of visionary questions and very tough to address, but I believe that there are

resources that can be used to look at some of these questions.

One of the resources I would encourage you and the staff to look at, at least from our center's perspective, is looking, for example, at the regulatory science priorities that we have in the center that address some of the issues that you are looking at here with these questions. Tomorrow I am going to briefly touch on that.

I would also think there's quite a bit of a link here with, for example, the new directions in which we are doing the integration between, for example, toxicology and reviewing the review process. One of the recent publications we had was the biocompatibility guidance, and within that guidance that we published there is a new program looking at the risk assessment approach and looking at the issue dealing with *in vitro* testing, materials characterization and computational toxicology and how we can actually integrate all those areas to address the issue of biocompatibility of the biology of the interaction of materials with the human body.

I think this is one of the areas that will very likely address some of the concerns you have in these

questions here if we can work together on the implementation of that type of collaboration.

DR. SLIKKER: I agree fully. CDRH has been a good partner to us in many, many ways over many years. One recent one, of course, is the interest in metals, how they're distributed. You've got the pharmacokinetics of metals, the distribution of metals within the interaction of those metals with all kinds of endogenous systems including the immune system. We have already been working together a little on that interesting question. We could do more in that area.

That just gives you an example that when the regulatory centers bring problems forward and say this is something that we're concerned about, we're listening, and we want to work with you in trying to solve those problems because that's what we're all about.

DR. PHILBERT: We are way over our time. Are there any other burning questions or comments? If not, thank you very much.

(Applause)

DR. PHILBERT: We will move on to the subcommittee report on the Division of Bioinformatics and Biostatistics, Katrina and Pam.

Agenda Item: Subcommittee Review, Division of Bioinformatics and Biostatistics

DR. WATERS: What Pam and I talked about, we're just going to briefly walk you through some of the high points of the report that we wrote.

Last year, we convened a meeting reviewing the Division of Bioinformatics and Biostatistics. Pam and I were included in that in addition to Dr. Ken Ramos, John Quackenbush, Ying Lu and Cindy Afshari, who is also on the FDA Science Advisory Board.

The Division of Bioinformatics and Biostatistics is divided into three branches. There's the Bioinformatics Branch, the Biostatistics Branch and the Scientific Computing Branch. The review was performed in such a way as to focus on five different theme areas, so that's how we broke out our report for the review.

The charge to the subcommittee was to review the quality of science conducted by the division, whether it was state-of-the-art, and to evaluate the impact of the activities on the mission of the FDA, if the division activities were critical to Agency mission, and if they were forward-looking and anticipatory of the needs, and to provide feedback to the division on how it could

position itself for the future.

The first theme area was the area of precision medicine. In this area, it was very clear that the division plays a major role in advancing the adoption of emerging technologies to the FDA review process, increasing the understanding of the technologies within the Agency's workforce and advancing the application of technologies for regulation.

One of the major activities under this theme is the microarray QC process that Weida has really spearheaded for a number of years. Currently on the table is their MAQC and SEQC4 initiative that they're working on. This project really focuses on understanding quality metrics and sources of variation in these data types to be used for regulation, and their MAQC model that they use really enables a community consensus approach for understanding fit-for-purpose use of next-generation sequencing and use for regulatory purposes.

One concern that the Advisory Board had was that, given the rapidly evolving nature of technologies and new data types that will be brought to bear for drug regulatory purposes, this could be kind of an infinite loop of doing a new QC process every time a new data type evolves. Our recommendation was that, through the course

of this project, they focus on developing relative rank metrics that could be used for data QC and evaluation more broadly so that every time a new data type comes to bear there isn't another QC process that starts over again. But, clearly, it was evident that the activities of that group were highly relevant to FDA and drug regulation.

The predictive toxicology area is another major research area for the division, and the group has been developing and applying a broad range of methods including toxicogenomics, systems toxicology and computational toxicology methods for the FDA. Specifically, some of the resources they have created, like their liver toxicity knowledge base, have been very important in advancing and understanding the field and developing predictors for different kinds of drug-induced liver injury.

One of the second projects they presented within the review was a study of microRNAs as potential biomarkers for hepatotoxicity. One of the findings of the review panel was particularly focused on the microarray project, that it appeared to really be kind of under-powered and focused a lot on correlations as opposed to using both correlation and sequence-binding as

predictors of whether or not microRNAs would be regulators of response.

In that regard, we thought there was some improvement that could be made, but overall, the knowledge bases, even the new one with the endocrine disrupter knowledge base, are important contributions to FDA mission and, working more closely with some of the regulatory centers, could be even more high impact to the mission of the FDA.

Theme 3 was biostatistical approaches and applications. Within that area there's a lot of focus on both data analysis support as well as support of the National Toxicology Program programs at NCTR -- a very strong group of people that work within the Biostatistics Branch.

Throughout the presentations and discussions, one of the things that came out was sort of a concern about how the scientists within that theme area are promoted within the NCTR. There was some concern that their promotion is linked to high-quality publications as an output as opposed to metrics of success directly impacting FDA mission. For example, the biostatisticians that contribute in a support mechanism don't necessarily have their own publications that they could use for a

promotion metric, so one of the recommendations of the committee was to come up with metrics of success for contribution to impact of the mission as opposed to just being promoted on the basis of publications themselves.

A couple other suggestions that came out from that discussion was also, as Bill mentioned, the state-of-the-art imaging equipment and experimental data being generated by NCTR as an area of opportunity for which the folks within the division could have more contributions, and expanded collaborations between the Bioinformatics Branch and the Systems Biology Branch at NCTR. I imagine that committee will hear about that later this week.

The fourth theme area is the R2R framework, the Research2Review and Return program, which represented a really important collaboration between NCTR and the FDA centers, particularly CDER. That was a very impressive program to hear about and really to hear the contribution to product review and overall to the FDA, so much so that the committee felt like the program could really benefit from some of the Biocomputing Division spending some time on the White Oaks campus working with those folks to make sure that the project could be highly focused on product reviews. Having those staff do some rotations there would help them to really understand the use cases for

how the software is used and how they could better improve the software.

Again, within that discussion, it also came up about coming up with the metrics, working with CDER to understand how that framework is helping to advance and make more efficient the product review so that there could be tracking metrics of success for that program.

The fifth theme area was called Service and Support Function. Largely within that theme area is the Scientific Computing Branch led by Ted Bearden. The Scientific Computing Branch actually supports the entire IT infrastructure for NCTR, so it's not necessarily just supporting Bioinformatics and Biostatistics, but really important contributions there just in terms of modernization of the IT infrastructure, network bandwidth and what have you, as well as creating customized software solutions for NCTR. It's a very strong group and very impactful.

One of the other issues that we raised through the review was a little bit of a confusion about how support versus research is defined within the division in terms of the breakdown being stated as 40 percent research and 60 percent support. If you include the Scientific Computing Branch as entirely a support

function, it gives a little bit of a skew toward support as opposed to research. Although the division itself is very large, there appeared to be minimal support, only about 1-1/2 FTEs for data analysis support working collaboratively within NCTR, so that was an area we felt there was some room for improvement to provide actually more support for internal data analysis and support at NCTR.

Overall, I think it was a very good review. We were very impressed by the strong output and significance of the division for what they provide to NCTR and to the FDA. They have done a very good job of supporting the needs of the divisions and the product centers. And because biology and toxicology itself is increasingly data-driven, the value of this division overall to NCTR and the FDA is very important.

We listed a number of recommendations at the end of the report. Specifically to summarize our findings, one of the major ones was just a question and kind of a concern about mission creep for some of the research programs, not that they’re not necessarily valuable for FDA but that they didn’t necessarily present the research in terms of how directly it would impact either product review or the regulatory mission of the

FDA, although they are utilizing a lot of the new data types that would be used for the Agency. So there was just a recommendation to evaluate those programs to make sure that they are focused on the impact to FDA mission directly.

Some recommendations in there are about some of the programs that could maybe provide more depth to the FDA as opposed to broadly applying the same types of approaches over and over again, one example being the endocrine disrupter knowledge base and liver toxicity knowledge base, both of which are very valuable, but creating new knowledge bases in different areas is maybe less impactful than creating more depth within those knowledge bases to provide more impact to the Agency.

Again, a couple things I have already mentioned -- coming up with success metrics for the impact of some of their tools and, like with the R2R program, the value to the Agency mission so that the professional reward systems could appropriately accommodate for those things for the staff within the division that support those activities.

Finally, just hitting again on something that Bill already talked about -- success planning within the division. It’s a very strong division with a lot of

strong scientific leadership, but thinking about the future and where there are some opportunities to grow some of the leadership within the division and come up with some succession planning for what the future leaders of the division might be would be important.

DR. PHILBERT: Thank you very much. That was very thorough. Are there any questions or comments from the Board?

That was a very well written and clear report and the recommendations are quite explicit. It is our duty as a board to decide whether or not we accept this report of the subcommittee. If there are no questions, is there a motion to accept? And a second?

(A motion to accept was made and seconded)

DR. PHILBERT: Any additional discussion? If not, all in favor, please say aye.

(Chorus of ayes.)

DR. PHILBERT: Any opposed?

(No response)

DR. PHILBERT: One absent, no abstentions. Thank you very much.

We will reconvene at 10:00 o'clock.

(Break)

DR. PHILBERT: Next on our Agenda is the

response to the review by Dr. Joshua Xu.

Agenda Item: Response to Review

DR. XU: Good morning. I am Joshua Xu. I am giving this response on behalf of our Division Director, Dr. Weida Tong. I'm sorry he cannot be here. He is out on a long-planned family vacation. Good for him.

His overview of our division -- thanks, Dr. Katrina, for giving the overview. We have three branches in the Bioinformatics Branch with 17 FTEs focused on research, and the Biostatistics Branch has 9 FTEs split half and half for research and services, and then the Scientific Computing Branch with 17 FTEs. As Dr. Waters mentioned, this is part of the center investment and they are doing database development and software development for the whole NCTR, and also the IT infrastructure expansion to support the whole NCTR.

In the immediate office we have two administrators and one senior advisor, and on a usual basis we have about 10 post-doc fellows, and right now we have just one graduate student. We usually have two or three. The division activities are split about 40 to 50 percent research and 40 to 60 percent in services, and I will have more on that later on.

Our mission is to conduct research in

bioinformatics and biostatistics and also to provide services and support to NCTR and FDA scientists in bioinformatics, biostatistics and scientific computing. We really focus on FDA relevance. We strive to have direct impact to the review process, and we do that through strengthening our linkage with the product centers and the continuing evolvement of capacity to meet current and future FDA needs.

How do we do that? We developed the R2R framework, meaning Research to Review and Retain, and research to review meaning support the review process, and once we got the review, feedback the requests and we will also conduct research and data liberation. We do that through partnership between us and the Office of Computational Science at CDER, and really that partnership is the most important factor to our success.

Some of the projects were presented during last year's review. The first one is data analysis host system that is in collaboration with Office of Translational Science at CDER. That system tracks progression from IND to NDAs or BRAs and the approval of NDAs and BRAs. It began when they asked us to help us with upgrading the technology to make the system more efficient and easier to use, and then we provided a means

for text mining and analytical documents to further enhance the system.

And then FDA Labels -- that is the result of our research in liver toxicity and then we developed an application, and now it is a web-based database for FDA approved drug labels. We have been working with CDER and Office of New Drugs, particularly the label development team and CDER. That is really a collaboration. Within NCTR we have Office of Scientific Coordination and Division of Systems Biology working with us, so that it's a very good project and very successful.

We also have a collaboration with ORA to develop an intelligent recognition system for food contamination pests. They get to process the food and it's very challenging to detect them.

DR. PHILBERT: (Inaudible) -- flora or --

DR. XU: Like flies --

DR. PHILBERT: Insects.

DR. XU: Yes. And it could be -- eventually there are some other types like animal hair and rodents that are interesting to detect as well. And even some kind of metal glass as well, but that would be future in the line working with them.

PARTICIPANT: So, any biological contaminant?

DR. XU: Well, not this part. That would be -- probably sometimes they carry toxin would be a different category.

At the time of review, we group our activities into five themes. The first one is precision medicine, and that is focused on assessing enabling technology for precision medicine. We also present the application of the technology is the repurpose of drugs for rare disease.

Theme 2, predictive toxicology focused on using various bioinformatics and computational models and to predict drug safety.

Theme 3, biostatistical approaches and applications. In that, we present safety assessment and big data methodologies. With big data here we are referring to those multi-dimensional complex data -- for example, genomics data, but not limited to genomics data.

And Theme 4, R2R framework. In the last slide I mentioned it, and really here we strive to impact the review process and how to translate our research, our activity, and to help with the review, to do their job.

Theme 5, services and support functions -- That includes a lot of biostatistics support and support in the service in the Scientific Computing Branch and other

support for the data analysis. I will have more on that later on.

The subcommittee report -- Dr. Waters gave a very good overview. Mainly the structure is it began with an overview of the subcommittee review process and then a shorter review of our division. Then, the report gives specific comments for each theme and followed the order from one to five, and at the end there are overall subcommittee conclusions and suggestions. I call them overall comments.

In our response we followed the same order and we responded to the specific comments and then the overall comments. We took an approach like a point-to-point response.

I really want to take this opportunity to thank the subcommittee, the chair, and the members for their thorough and thoughtful review of our division and, of course, some very good, positive comments that we were very glad to receive, and there are also many very constructive comments that will help our division in future growth. We really appreciate that. We also want to thank the FDA representative that participated in the review.

In this presentation of our response we will go

to the overall comments first and then follow with the specific comments. I hope I can keep you alert but if not, the overall comments will at least give you the big picture.

Overall comment number one, the bioinformatics research and surveys, and throughout my presentation I gave the page number that referred to our response document and the comments I shared without quotation marks.

This one -- and this is following that the Division has done great work in research -- says the Division has also contributed significantly to the mission of the Agency by creating resources such ArrayTrack, EDKB and LTKB, and then also the representative from the FDA product center provided strong support for the division service and support activities, so we are very glad to see those favorable comments. Thank you for your recognition.

This comment is about the boundary between research and service. You heard that from Dr. Waters' presentation. This comment says, while the work of the division is overall commendable, its dual role -- meaning the research and the services -- might be the source of some of its weakness. It is not clear in some instances

where the boundary was drawn, and the balance between the primary research and the service to support the mission was a recurring issue.

In addressing that, I would like to start with clarification of certain terms -- conventional service and data analysis support and primary research. Of course, it's easy to say primary research is -- FDA has instituted a protocol for doing the research. And for the conventional service, it is very much legacy support mainly in the Scientific Computing Branch and the Biostatistics Branch, and both have well-engrained working mechanism for many years. But data analysis support is to support the emerging data and the large complex data and involves research in methods development. I will have more on that.

During the subcommittee review, both conventional service and data analysis support were described as support and may have confused the boundary between support and research.

As you are all aware, many new technologies are continually being adapted at NCTR and FDA; they generate large and complex data and they demand enormous bioinformatics and biostatistics support. An analysis of those data, managing the analysis and transferring all

this can be difficult, particularly analysis, and often for the method research and development. In order to provide such support we have to work closely with domain-specific experts to learn about the data, to learn what the question is they're asking and what kind of analysis they would like to do. With that, it really blurred the boundary between research and support. So, how do we do that moving forward? We put a proposal forward to enhance the division's data analysis support. We have been discussing this extensively with NCTR leadership; we want to give that kind of support an identity to enhance it.

Moving to the last overall comment, I think it is related to what Dr. Waters mentioned about making sure that research aligns well with the mission and doing internal review. I am glad to say that we have been diligent with that, and NCTR over the years has developed a thorough and rigorous vetting process to ensure NCTR projects align well with the Agency mission. This process has several components like internal division review and to get endorsement from other FDA centers, and often we have co-investigators from other FDA centers to be part of the proposal. An important part of each research proposal is to state the relevance and

anticipated impact to the FDA regulatory mission.

With that said, it doesn't mean that we took the comments lightly. Really, we continue to be diligent to have internal review and make sure that our project aligns with the FDA mission and has big impact.

This comment is about the subcommittee has some impression that there was a disconnect between projects, between branches within NCTR, and between NCTR divisions and other product centers. Also it was mentioned about the missing opportunities such as applying our activities to imaging data.

We do apologize for not conveying the message clearly during the review, and sometimes when we present the research, our presentation and preparation of the written materials are more focused on the division-initiated research projects. Indeed, our division is very collaborative and that comes naturally because we are inherently multidisciplinary and we certainly generate our own data. We are working to analyze the data.

We have many projects that we are very collaborative such as FDALabel that I mentioned, and there is a collaborative effort with several divisions at NCTR and also with OND and Office of Translational

Science at CDER. And the R2R project is very collaborative and requires us to work with the product centers to support that review. We also have the SAQC2 project, as Dr. Waters mentioned, and we followed the traditional MAQC and work with the community. And then CTP projects.

I am happy to report that NCTR has formed a bioimaging data analysis planning group -- not a working group. That group will survey what we have, what challenges we are facing and what are the approaches we may take, as Dr. Slikker mentioned, to build a virtual call to tackle the challenge and pull the resources together. All divisions have a prominent presence in that planning group. I am very excited to see the development in that direction, personally, because I was trained in imaging analysis.

We have many projects across the centers, and the one I showed here is because cardiotoxicity is very important these days and a lot of people pay attention, so we are getting into this area as well.

And beyond the walls of NCTR and FDA we also engage in many regional activities, and we are a long time partner with Mid-South Computational Biology and Bioinformatics Society -- I think 14, 15 years. Right

now, we have one staff who is the president for 2016 and then we have another staff serving as a board member.

Also, in 2014, we helped to formulate the concept and facilitate establishment and development of the Arkansas Bioinformatics Consortium. That is comprised of the major Arkansas universities plus NCTR.

This comment is about the R2R program. It has great potential to improve and enhance the Agency mission; however, it will not result in many publications. Thus, the subcommittee recommended that we define and collect metrics for assessing the impact. It also recommended that the R2R program be integrated into all division activities. We really appreciate the recognition of the importance of the R2R program and we agree with the subcommittee in defining the metrics and collecting data and assessing the impact, and I will have more on that later on in the theme-specific comments. We will make the R2R program a focal point of all our division activities to guide our growth, and maybe after several years we will achieve the goal and we can really motivate a division of R2R -- relax and refresh.

This comment is about a professional reward system is needed within the division that extends beyond publication. Dr. Waters mentioned this. Indeed, we

agree with the emphasis. A properly designed and implemented reward and career advancement protocol is very important for the support professionals and for the research scientists as well. That will help us to retain high caliber staff, and we will work with the center leadership toward this goal.

Now I'll move on to theme-specific comments. Precision medicine -- This general comment is about emphasizing the future plan of our research, emphasizing the issue of toxicological significance. During the presentation we presented a lot about SEQC2 which assesses the reliable use of genomic technology in regulatory decision-making, and I am glad to say that toxicological significance has been an essential element in all MAQC projects. In MAQC1 we have a project to assess carcinogenicity in the liver and kidney using rat models. In MAQC2, the development of validating classification models, we have three 50-assessment datasets. In SEQC1, we also have a toxicogenomics project to compare the MicroArray data versus RNAseq. So we will continue to make that an important element of our project in SEQC2.

For rare diseases, we aim to repurpose marketed drugs for the treatment of rare diseases, and we have

developed a new concept and just published using oncology drugs to treat rare disease. Of course, with that, safety is important, so we are developing methodologies to understand how the dosage should be modified to mitigate the tox effect.

This comment is about SEQC2. The subcommittee recommends sharpening of focus and closer alignment to the Agency's mission to facilitate continued maturation of the division. We have been working closely with many reviewers and scientists from FDA centers to make sure that our efforts support their regulatory application. We have three specific aims of the project: develop quality metrics for reproducible NGS results particularly in whole genomic sequencing and targeted gene sequencing; to benchmark bioinformatics methods for development of standard data analysis protocols; and also to assess the joint effects of key parameters.

Our initial emphasis was evaluating the challenge outlined by FDA discussion papers and other internal documents. We want to make sure that such alignment will further sharpen our focus on closer alignment. Indeed, such alignment has been maintained throughout the MAQC project series. Actually, the first MAQC results were incorporated in the draft guidance for

the industry for pharmacogenomics data submissions. Currently, this draft guidance has been revised and incorporated in the parent guidance, and our results from SEQC about RNAseq will be incorporated in the guidance as well. We are working with the committee.

This comment is about SEQC2. It says caution should be exercised in making investments to define best practices and standards in a fast-evolving field. The team should not lose sight of the fact that the ultimate goal is to systematically evaluate quality metrics and standard practices, and also recommended development of relative ranks and amounts of variation.

We are glad that the subcommittee recognized the fast-evolving nature of the emerging technologies and the challenges in evaluating such technologies. We do think that understanding the source of variation and the impact has been emphasized through the MAQC project and they are important to evaluating emerging technologies. And we will evaluate the source of variation such as sample pre-processing, gene capture panels, library preparation and sequencing instruments. We think that studying these parameters with various biological samples to get a comprehensive and diverse dataset will help us to provide insights into what practice may enhance or

negatively affect the validity and reliability. We will focus on quality metrics and standard practice, and then also develop relative ranks of those variations.

Moving to Theme 2, predictive toxicology, this comment is about LTKB and more specifically RO2. This comment lauded our effort in this and also suggested we expand our work and leverage what we learned in this effort to other drugs' administrative routes, to study other compounds that cause liver damage or to incorporate other data and information in LTKB. Actually, we have been doing that and I am glad to report some progress in that direction. This year, we are publishing a paper called DILI Risk that incorporates formation of reactive metabolites also in LTKB to predict the severity of DILI risk.

The second comment says it is not clear that the division has worked closely with other FDA centers to assess using the R02 as part of the regulatory process. We have tried to present this clearly; sorry if we were not making it clear in the review. Actually, we have co-chaired the Liver Toxicity Interest Group at FDA which has over 30 reviewers, most of them from CDER. Through this interest group we have been communicating our results with the FDA reviewers, and R02 has been used to

evaluate liver toxicity in 16 submissions. Those submissions cover across IND Phase I, Phase II, Phase III and NDA submissions.

As a result, we have co-authored a paper with a CDER reviewer on the success of the R02 rule in predicting hepatotoxicity potential of a specific class of drugs and direct-acting antivirals for treatment of chronic HCV. We also developed a bioinformatics tool to assist the reviewer in applying R02 rules and LTKB in their regulatory review.

This comment is about our MicroRNA project. At the time of the review I think we were at the early stages of the project, and the presentation talked a lot about the data analysis pipeline. This comment says the analysis allowed the group to identify a candidate best practice data analysis pipeline, but the small sample size and overall experiment design was not sufficient to find robust candidates. I would like to take the opportunity to talk a little more about the project.

Actually, the primary objective of the project is to understand whether expression profiles of microRNA in the rat liver tissue can be used as mechanistic biomarkers of human DILI. We think that those mechanistic biomarkers will provide better predictive

value, and we chose NGS to profile microRNA expression because they provide the utility to discover novel microRNAs, but, as you know, the standard data analysis pipeline is not ready and is not available so we have to evaluate them as well. Then, for our specific study design it involves some multiple doses, multiple time points and there are some specific requirements we have to take care.

So, with that project, we have four phases. The first phase is to determine which bioinformatics pipeline would best meet our needs. Once we identify that we will move on to generate more data and to discover microRNA biomarkers for liver carcinogenicity and, in the third phase, still generate more data to discover microRNA biomarkers for drug-induced liver injuries. Finally, in phase four we will have the validation dataset to confirm the biomarkers. The whole project will generate a pretty comprehensive dataset with more than 500 samples.

I am happy to provide you a progress update. We have produced two manuscripts, one on microRNA biomarkers for liver carcinogenicity. This one has been submitted. And another on microRNA biomarkers for drug-induced liver injury, and this one is currently under

internal review.

This comment is about the EDKB project. It represents an important contribution to the overall mission, and the information gathered is essential for the development of predictive models of response to endocrine disrupters, and additional work to use this resource to develop robust, predictive quantitative models has potential to be of broad interest and use across FDA and beyond.

I am happy to see the subcommittee's recognition of our contribution in that area. Indeed, we have been working diligently to develop models over the years. We have published a diverse set of models with 1,000 citations such as 3DQC model and then chemometric classification model, ensemble models and docking models and we will take the comments of the subcommittee to heart and will continue to work hard to develop more models to make the EDKB database more of use to the community.

As part of the effort we are anticipating a large predictive toxicology collaboration organized by the EPA. This overall comment about the predictive toxicology program it says has made important contributions, the most significant of which are the

knowledge bases that have been assembled. The division should consider working more closely with the regulatory centers within the Agency to explore how they could be used.

As I mentioned earlier, we are diligently working in that direction, and we have, for example, the LTKB knowledge base, and we initiated the interest group to communicate our results and work with the review. We are using the interest group and the R2R framework as a vehicle to facilitate the translation of LTKB for use in the review process. We are developing tools for making it easy to use for the reviewers.

We are also working with CTP to develop a tobacco constitution knowledge base, and this one contains chemical structure and available biological effects for over 9,000 chemicals.

Moving to Theme 3, biostatistical approach and application, this overall comment about the branch lists that there are four research areas and then says, these are important research areas and critical to the mission of the FDA. The group has expertise and significant achievement in all these areas. We are happy to see this positive comment, thank you. We do strive to continue our work that is critical to the mission of FDA.

During the review, we asked the subcommittee about guidance and how the biostatistics group should get into the big data research. The subcommittee replied with very detailed encouragement and guidance and expressed certain areas that can help their research into this area.

Since then, the transition of our research priority to big data analysis is underway, and we have developed three proposals and they are submitted to the FDA Office of the Chief Scientist. If we get funding from FDA Office of the Chief Scientist, this is out of our regular budget, so it's good.

We have also taken the suggestion to incorporate EHR. We are working with hospital systems to have the electronic health record data be combined with the FDA adverse event database. We are collaborating with CDER, CVM and CDIH in an effort to use big data to promote regulatory science.

It is good to talk about this question. The subcommittee recommended strategic planning with the branch to identify research directions and priorities. We talked about four research areas and then we talked about big data and really that the field of biostatistics can proceed in step with advances in biotechnology.

Nowadays, biotechnology such as imaging and genomics generate multidimensional, complex data. And also even just the traditional data streams like text mining and text become huge, so traditional statistics tests and procedures are more for risk factor identification. Now, since statistical models and testing procedures are developed to identify biomarkers for higher-dimensional and macro experiments, we want to take this and make some strategic planning and focus on the following two areas: statistical and machine learning for high-dimensional big data, and then, statistical methods for precision medicine, and particularly one area is clinical trials using those genomic biomarkers.

Of course, I mentioned strategic planning and also I mentioned about the vetting process, and we need to work with the center to review the new projects so that they go along with the center priorities. We are also actively seeking guidance from FDA product center colleagues to make sure that our projects meet the needs of the Agency.

Moving to Theme 4, this is my favorite theme, R2R framework and activity. This comment lists a lot of strengths of the project and says it is a major project with a goal that is wholly integrated with supporting the

FDA initiative of evolving FDA's regulatory science. And in a short amount of time, significant progress has been made. It is also highly integrative and collaborative with other product centers. The project will develop the tools. It is reiterative and recursive, and we are making sure the tools will be impactful and useful and user friendly. We really appreciate the detailed, insightful and positive comments about the R2R program.

Then the subcommittee recommended that we collect the right metrics for tracking the important output. I am glad that the subcommittee recognized the importance of the R2R program and that the Commissioner seems to agree with you. This year, we just won the highly-prized Commissioner's special citation for innovative, cross-center bioinformatics projects benefiting the regulatory business processes. One of the projects, the FDALabel which I mentioned earlier, also won a Scientific Achievement Award from the Office of the Chief Scientist for outstanding inter-center scientific collaboration.

We are actively developing metrics for tracking the impact. Mainly, we want to see to what extent the tool has been used, to what extent it impacts the review process, such as when we log in the users how long they

use and which area or which page of the tool they are using more. And we also collect user cases, specific cases of how the tool has been used and then feedback to further improve the tool.

And, of course, we will definitely work on further input from the Board and from the audience on how we can improve our collection of the right metrics.

Moving to Theme 5, services and support. Earlier I mentioned this service in scientific computing is more for the whole center, and this comment is talking about the emerging area of precision medicine and integrating the need for privacy and HIPAA compliance into the NCTR computing plan is essential. Given that it has taken multiple years for strategic planning and capital investment, really the subcommittee encouraged us to work with strategic planning so that we could have investment early on and have a better outcome.

We are working with the FDA Office of Information Management and Technology in pursuing increased bandwidth and access to cloud platforms such as AWS hosted by Amazon and Sale Force. We have been working with them addressing many security concerns, and we are getting close. I'm glad we are getting close. I hope that we will get access soon.

Of course, with strategic planning, one of the things we often encounter is persistent funding constraints. I'm hoping that the Board can help us with that concern and help us to get more support in that area. Our staff is also representing the center on multiple working groups and subcommittees to ensure that the needs of NCTR are considered when assessing cloud access and network infrastructure improvement and other IT infrastructure improvements.

This comment is about the Scientific Computing Branch. Earlier I mentioned that the branch developed a database and software to support the whole center. The subcommittee recommended that the value of customized software should be balanced with the amount of effort that is spent supporting legacy applications, and some of them may be under-utilized.

We agree with the subcommittee and we are actually not just agreeing with you but we agree with HHS and FDA, and they have adopted a commercial or off-the-shelf first approach meaning that when you have a need, go off the shelf and see if there is anything that meets your needs in order to eliminate redundant, outdated and under-utilized software. NCTR has been and will continue to follow their lead and we are working diligently. We

have performed inventory and will continue to do so, and to see the applications and databases hosted on NCTR servers and to evaluate whether solutions are available elsewhere in FDA. Open source products or off-the-shelf can help us to reduce our legacy customized software and better utilize our resource.

Of course, I have to caution here that “buy” versus “make” has and will continue to prove to be challenging. Sometimes our experience is that -- Actually, we have some experience using the off-shelf and it actually requires much more effort to maintain. In terms of our legacy applications, many of them are linked intricately through the shared data structure and the database, so simultaneous changes may be required to ensure they are working together. That is a challenge that we are facing now.

With that, I close my presentation and thank you for your attention.

(Applause)

For the question time, I am glad that my colleagues are here, specifically, Dr. Jim Cheng, the Statistics Branch Chief. He and I will together answer questions.

DR. PHILBERT: I will allow Drs. Waters and

Lein to respond to the response.

DR. WATERS: I just have one comment to make just to clarify with some of our feedback. When we talked about metrics for success and metrics of impact, we didn't question the value of the research projects to the FDA mission itself; it is more along the lines of how those projects were presented in terms of their own impact to the scientific community as well as impact through manuscripts that are published.

I think what the subcommittee was really trying to express was the fact that your stakeholder is the FDA and the centers as opposed to the scientific community, and that your metrics of success should be focused on how they impact FDA mission, not necessarily how many publications you achieve with that work.

So, as you think about some of the responses you have here in terms of the impact through microRNA work to manuscripts, manuscripts are not the impact that we were looking for in the subcommittee; it was impacts to the Agency itself. Like with the R2R framework, your impact is not usage; it is how you impact the product review process in terms of speeding it up, making it more efficient and that sort of thing.

DR. LEIN: I would just echo what Dr. Waters

just said. The other bit of maybe confusion about the intent of the Scientific Advisory Board was with respect to -- We didn't question your collaborative interactions. You guys are great at collaborating with the other center products and that was not a concern at all for us.

What was maybe something we want you to pay a little more attention to was whether the projects that you are doing within your division really are hooked up to other ongoing projects within the other centers. There seemed to be a disconnect in that. Not that you weren't collaborating, because you are, but just some of the projects we didn't see and it wasn't clear that you could explain to us what the connection was with ongoing priorities in the other centers.

DR. XU: Thank you for the clarification. We will work hard to improve the connection.

DR. PHILBERT: I have a more higher-level question for you and maybe for Bill. Where do you see the balance between building your own bioinformatics infrastructure and actually asking the scientific questions? There are lots and lots of very heavily resourced institutions across this nation and the world that have all kinds of computational and software expertise, but they are looking for questions. You have

questions and appear to be building infrastructure.

Where should that balance lie given that you have limited resources?

DR. XU: That's a great question. I'm glad that when you raised the question you said this question is for us and Bill.

DR. SLIKKER: Let me just make a couple of comments about that. I appreciate your question. NCTR and FDA in general, have to have a basic fundamental ability to handle large datasets, so we need pipelines that allow us to communicate between the centers and even within the centers. They need to be adequate so we can ship some of the larger databases around and analyze them.

We also need the capability to be able to analyze our data within our own FDA because, obviously, that data cannot be put on the street for analysis; it's very much protected data, and we appreciate that industry trusts us with that obligation to keep that protected. So, there is a certain amount of work that has to be done within FDA and, therefore, within NCTR to manage the data that we have. We are not going to over-build, obviously. That would be impossible because the budget just isn't there to do it, but there is a certain amount.

And then, of course, you have to layer on top of that the protection which is so important. We have to protect our data in many different ways, more so than university systems do and most other kinds of data-handling operations.

We do have certain obligations to be able to handle certain amounts of data internally and to protect that data, so that has to be there, but we're not talking about building exceptionally large systems beyond that. And we are able to collaborate with others and have done that many, many times. We've collaborated with university systems, for example, right now to help us in this arena dealing with the image analysis process. We have new employees who just have been recruited to UMS, for example, that are very high level on human imaging capability. So we're collaborating with them; they're investing in 10 or 20 new people plus massive amounts of infrastructure. We can collaborate with them and other universities to garner some of that higher-level support, but we do need a certain quality protection and support within the Agency to deal with the databases that we are deemed to protect.

Does that help a little bit?

DR. PHILBERT: Yes. So, within the alphabet

soup of the government there are a number of three-letter agencies that have enormous data-mining capacity. Why not -- If you don't want to go outside the walls of the government, is it possible to work with them?

DR. WILSON: One of our major challenges, as you may or may not realize, is that we are a few generations behind with regard to our network and bandwidth. As an example, we did a pilot project with NCTR last year to move some next gen sequencing data from NCTR up to the Center for Biologics and it took three months to move the data and two weeks to do the analytics. So that is the problem.

And we are working closely with our Office of Information Technology to make them aware that we need to invest in that infrastructure to get up to 21st Century standards but we are not there today.

DR. PHILBERT: And even if you get to 21st Century standards --

DR. WILSON: We will be behind again.

DR. PHILBERT: Yes. It's like buying a VCR. You buy it and it's out of date.

DR. XU: May I add a couple comments on that? In terms of bioinformatics research and the support, not always are we just trying to do something new. We have

taken things that have been developed in the community and added that to our infrastructure to help support research.

Sometimes we evaluate the tools that are there. For example, we started the evaluation with CLC Genomics Workbench, and we adopted and are promoting its use at NCTR. And later on, the whole FDA had a side license and coordinated the effort.

Another example is you may have heard there is a so-called Galaxy project that has built a platform for biomedical big data including imaging data and genomics data, and we just borrowed that and implemented our own high-performance computing cluster to support our data analysis of next-generation sequencing.

So, indeed, we try to make the best investment of our time and resources -- limited at times.

DR. YEAGER: I just want to note that you're talking about building a computational program and in the sense of programmatic that it can address different types of computational modeling, predictive toxicology, next gen sequencing. And in that, you have to evaluate what internal resources you need to make the program function, but what external resources are available to make it operate in a manner that is efficient inside the

government. So it's always striking a balance of internal and external resources.

But, in addition, NCTR has to have some added flexibility, and that added flexibility is each center within the FDA has a fit-for-purpose for their computational modeling, and Caroline did address the broader agency-wide. But within each center there are specifics needed, so NCTR may need to have flexibility to address those changes in each type of computational model that's being addressed.

I think, in the end, balancing internal and external resources and also considering that the model and the interest fit for the purpose that it's being designed.

DR. PHILBERT: Coming from southeast Michigan as I do, I am mindful that GM cratered under the weight of 20-plus platforms, all fit for purpose, and unsustainable. And the Japanese had half a dozen platforms and out-competed.

Again, I hear the need for tailoring and so on, but it just seems to be inherently inefficient to be building one-offs, apparently. Carol.

DR. LINDEN: -- at the Agency level are to continue to maintain some nimble flexibility in the R&D

space, but as projects mature and harden and become operationalized, for example, for supporting regulatory review, then we go into a more centralized support system for those high-performance, competing resources. So we're working again internally to build up that expertise and capability to be able to support that so that we have a hybrid model.

Then, of course, the other issue is that as technology changes and cloud computing could potentially in the future become a better platform for us to use for big data, there are other opportunities for how we address all these needs. So we are really in the process of trying to figure out the most efficient way. We realize that right now we have been doing this sort of organic, single resource development and we're trying to become more strategic about how we approach this as an agency. Those are conversations that are maturing into organizational structures for governance and that kind of thing.

DR. PHILBERT: Since we don't issue a formal report from this Board, please take this as support for an agency-wide effort but encouragement to go beyond what you can do as an agency to what can be done federal-wide and with the appropriate protections and so on, actually

building a public-private partnership. Again, by the time you set up the system it's out of date, and security issues -- I have said enough. Ted.

DR. REISS: Very nice presentation. I do have a question for you and maybe there is no answer to it. I'm not sure. It's a variation of the question that Steve had asked before, and it gets back to what you were talking about with research and support. They are a little bit different but in many ways they sort of overlap.

My question is how do you guys figure out what you are going to work on? How are ideas generated? Where do they come from, and how do you decide what's important to work on?

DR. XU: I think that our guiding focus is to maximize impact on FDA mission. So, in terms of precision medicine we are assessing the emerging technologies because various FDA centers have received NGS data and they are evaluating the application of such data. Predictive toxicology really is to the heart of NCTR, and drug safety is to the heart of FDA, so we utilize our expertise in cheminformatics and bioinformatics and to build models and predict drug safety.

And then, of course, biostatistics on approaches and applications, we emphasize how new biostatistical methods can be developed to address the important issues that FDA is interested in.

So, really we took two things. One is the FDA mission and impact, and then another one I didn't mention is the collaboration request and to support other NCTR divisions and FDA centers.

DR. CHEN: Jim Chen, Division of Bioinformatics and Biostatistics. The way NCTR has the protocol, every PI needs to have a protocol in order to get center director funding. The way the process works is first you have to get division director approval. The division director usually has a good concept about what is important to FDA and to NCTR. Then we write a concept paper and it goes to Dr. Dana Costa and review the concept paper. Then we send the concept paper to FDA product center to say whether the concept is potentially useful and also whether any of the centers wants to be a co-PI of this project.

After the concept paper is approved, if approved, then the PI will write a protocol and it goes through the same process. Usually, (indiscernible) wants each protocol to have a product center co-PI to ensure it

has some impact for this particular research. So that is the typical NCTR protocol and process.

Since our project, usually, everybody needs resources, so we submit the protocol and submit a grant proposal. FDA has at least three or four chief scientist funding in Office of Science, Office of Minority, and Chief Scientist -- the other one I forgot. In Biostatistics, usually we don't do experimental research, so what we need is kind of a post-doc so we submit a protocol together – chief scientist funding. That's the way we set our priorities. So the way we set it up is kind of based on the resources and also approved by the center director.

DR. REISS: I was also getting at the idea of how reactive are you guys to requests that come to you and then you try to help solve the problems versus, since you're doing a lot of what others have termed support, so you're working across the FDA in a number of different ways. You're exposed to a lot of issues and a lot of potential problems. How much of the stuff you're doing is trying to identify problems and then helping to solve them rather than just being given the problems that you're trying to solve? Do you see the distinction I am making? Proactive versus reactive.

DR. XU: That's a very good question. I think I should defer this question to my director.

DR. SLIKKER: You saw the numbers up there of protocols that are ongoing and over 50 percent we have actually a collaborator and a sign-on from one of the other centers, so there's a lot of that collaborative research going on.

But you have to imagine that our staff, just as you acknowledged that Syed Ali has been admitted to the British Chemical Society, we have leaders. We actually have -- If you look across the publication record you will see that nine out of the ten most published authors are within the FDA or at NCTR, and eight out of the ten most quoted are cited authors at the NCTR. We have 13 out of the 35 or so people who are internal to FDA in the senior biomedical research service. So we have leaders here. Many of them are active in other activities such as leadership in societies and leadership in thought processes across all kinds of different committees.

So, we do garner something from that kind of leadership to help see into the future and understand where investments need to be made. So there is some of that going on in addition.

But I must say that it still comes back to is

this going to be relevant to the product line centers. It could be a great idea, but if it doesn't affect FDA and how it's going to move forward then it really isn't important to NCTR, so it has to have both of those qualities associated with it.

DR. PHILBERT: He was rather modest in not mentioning the former president of the Society of Toxicology, to my left. Greg, I would like to formally welcome you to the Science Board. Would you mind introducing yourself?

DR. LANZA: I'm Greg Lanza, Washington University Medical School. I'm a cardiologist but I work broadly across a lot of internal medicine issues, primarily in biomedical and imaging and in targeted drug delivery, particularly nano-based.

I have one question. In the end, there is clearly a lot of activity here and that was a great overview for me of how broad it is. But the key thing, as I see it, is the result, so if I can use your microRNA example as one, you will be banging against the database that people give you data from and come up with some ideas about what these things mean. But these technologies are in their infancy. We know far less. The pile of what we don't know is far greater than the

pile we do know.

So I'm wondering, once you go into a project like that, are you already planning to independently validate what you see so that you can quickly get it into recommendations that might be important moving down the line rather than years down the line? Whenever you go up against a database, your conclusions can be biased by that database. In your case, even what was provided by the different sponsors will create a bias because you may have lots of holes, a dirty database, if you will.

How are you going to validate anything you come up with so that it will be independently validated and then something that can actually be not only implemented but transmitted to the other sponsors who can then pick it up as part of their regimen for submissions?

DR. XU: First, I want to ask for a little clarification. You're saying to translate to other sponsors -- what do you mean by that?

DR. LANZA: Let's say I'm doing some drugs and I'm working and the drugs have some effect in the liver, and you're doing microRNA work and you're trying to figure out which ones might be markers of, say, toxicity, hepatotoxicity, biliary toxicity, whatever it is. So you go up against data that you have that's coming from

diffuse sources and then try to generate a metafile of information that you can then make what if, what if, or a rough correlation, if that's what you're doing, in conjunction with some sponsor or other part of the Agency interested in this.

So the question is you find two or three or you don't, but that's in this database. And I wondered are there plans that you would even either internally or externally validate independently those conclusions so that it could get into either something that's useful for regulatory use or get off the list? When we do things like this on a database we usually get our ideas, we set our models and stuff, and we don't generally try to predict because that's usually poor. We try to then go backwards and take another database or do actually hard experiments to test that hypothesis to see how robust it is. And if it is robust we implement it.

I was going through the mining, the idea part, but I didn't hear much about how you then go from what you might find to something that you might make a regulatory rule or guideline, or actually go the other way where people are using it to support themselves that something is not there when you think it really doesn't mean anything; it's just noise.

DR. XU: My understanding of your question is talking about two things. One is how do you deal with the knowledge and the database and the understanding you already have in the community, and then some of them that may not be reliable, how do you deal with that --

DR. LANZA: How do you validate what you get out of the database?

DR. XU: Yes, that is one question. And then, two is how you translate your results to impact the review process. About the second question and the big picture, DILI is very difficult to detect. Most of the drugs if they have DILI they will have passed the animal experiment; there is no problem. So our hypothesis is we want to see whether the genomic biomarkers, particularly microRNA, would provide utility to predict DILI potential risk in humans.

The dataset that we're generating, the samples we got are from the Japan toxicology study where they profiled 131 drugs. Some of them are structurally similar but you can compare them like drug pairs but one is clean and one has DILI. So we provided them with rat and a very comprehensive design with micro-dose and low, medium, high, and then multiple durations like four days, seven days, 14, 28 days, and then they collected blood

sample and liver sample and did the histology and all this. They even have an experiment with rat hepatocyte and human hepatocyte and generated homologous microarray gene expression data.

This is the one we worked with them, and we were able to convince them to give us their liver tissue samples so that we can profile the microRNA to generate data for microRNA expression, because the microRNA regulates gene expression, and then integrate those microarray data with the microRNA next-generation data. And through our comprehensive analysis we're hoping to identify some biomarkers, microRNA biomarkers, that can predict DILI risk in humans.

The way that we develop and validate this biomarker is that we generate samples from some chemicals, pairs, and build the model and then find the biomarker and then, using the other independent biological experiment samples to validate those markers. That is the approach we're taking. Of course, we are always limited by the knowledge such as how the microRNA regulated gene expression and how the dose -- very intricate lab work there so we are limited by those, and we will try to do our best Maybe if some of the biomarkers are found maybe we can contact with other

people to generate further data or conduct mechanistic study to validate that.

I gave it a shot. I hope it is reasonable to you. I will relay that question to my division director, Dr. Tong, and we will think about it and hopefully we can have a better answer we can communicate later.

DR. PHILBERT: Other questions or comments? Any thoughts from our friends across the other centers?

Hearing none, thank you very much for a thorough and detailed response.

(Applause)

DR. PHILBERT: I am going to ask that Dr. Manjanatha present after lunch because as yet we don't have any requests for public comment. We will need to come back as a Board promptly at 1:15 just in case there is somebody who hasn't formally requested time. Then I'm going to ask Fred to be right on time so that we have time.

Agenda Item: NCTR Division Directors: Overview of Research Activities

Agenda Item: Division of Biochemical Toxicology

DR. BELAND: I am Frank Beland, Division Director for Biochemical Toxicology. We were asked to use sort of the standard format so this sort of follows

what Bill did, and you'll see this over and over again.

This is just a general overview of the division. For those of you who have been here in the past, it really hasn't changed very dramatically. We have around 60 people. This gives the distribution. The way the division is organized, we're organized into teams. The teams are led generally by a principal investigator, a PhD. We have about 12 or 14 teams. We don't work in isolation; we tend to work together.

This division really collaborates with other divisions within NCTR. They're listed on the top of this slide. We also have strong collaborations, as I think I'll be able to convince you, with other product centers. We get a great deal of funding from the National Toxicology Program. We receive funds from the National Cancer Institute. We have collaborations with the EPA, CDC and a lot of different universities.

As far as global leadership and outreach, we're involved with IARC, with the World Health Organization, the European Food Safety Authority and with the OECD, so we're heavily involved in a lot of activities.

The mission is stated at the top. I would like to talk about the goals. What we tend to do, not exclusively but a great percentage of the division, is we

characterize toxicity and carcinogen risks associated with chemicals, especially those of interest to the FDA. The way we go about doing this is we conduct bioassays.

In addition to doing bioassays we do mechanistic studies. These mechanistic studies, we do a lot of pharmacokinetic measurements. We do these in experimental animals; we do mice, rats, non-human primates. When we can obtain human samples we will also look at human samples to see whether or not what we're seeing in experimental animals corresponds to what goes on in humans.

We measure mutations, we measure DNA adducts, we do epigenetic measurements. We try and interpret the bioassay results. More recently we have increased our ability to do computational modeling. We have done it for a number of years but we've increased the emphasis and we currently have five individuals who are doing modeling. The way it works is we have the bioassay data and we have the mechanistic data and all of this feeds into the computational modeling, and we hopefully can present a total package to the FDA product centers that are interested in what we're doing.

This slide lists our portfolio of what we've been doing in the last two years. The ones on top are

more mature; the ones down at the bottom are things that we're just getting ready to start, so the protocols are being developed.

I have also listed the product center that has either requested the work or has an interest in the work. Everything listed on this slide with the exception of the last one, the nattokinase/lumbrokinase, has been funded to a greater or less extent by the National Toxicology Program.

The way I would like to do this presentation is I would like to present two accomplishments from the last year that I hope you will find of interest, and then I would like to go on to three new things that we're going to start but have not yet started but the protocols are being developed. This is the time, if you're going to give us advice, it would be nice to have it now to see if we need to modify what we're proposing to do.

The first thing -- Bill had this on his slide earlier this morning -- is I'd like to talk about the work we have done with aloe vera. A lot of people don't realize that you can buy a lot of aloe products in the marketplace that are taken orally. You can go to Walmart and buy it by the gallon. Some years ago there was a concern and it was nominated for evaluation to the

National Toxicology Program.

Aloe vera is a dietary supplement and it's regulated by the Center for Food Safety and Applied Nutrition, CFSAN. So, in conjunction with CFSAN, we did a two-year chronic bioassay. At the time, no one thought it would do anything. We did it in drinking water because this is how people normally take it. You think of topical applications and cosmetic products, but the concern here was oral administration.

These are data that I have presented previously. It shows that oral consumption of aloe vera is a very good colon carcinogen. This is in rats. It turns out that mice tend to be resistant, but in rats it was a very good colon carcinogen. The question is why. I want to show you what we discovered in the last year.

Aloe vera contains a compound called aloin which is shown in the top left hand side -- it's Aloin A and Aloin B, depending upon the stereochemistry. Aloin is resistant to hydrolysis by acid in the stomach but gut bacterial will hydrolyze it to aloe-emodin-9-anthrone. This occurs to a larger extent in rats than in mice, and actually humans are quite good at doing this.

The aloe-emodin-9-anthrone goes to aloe-emodin, and this is a genotoxic compound. What we hypothesized

is that the reason you're getting colon cancer in the rat given the whole aloe vera product is because of the aloin going into aloe-emodin. So, with funding from the NTP, we did a study, a 90-day study with aloin. These are the data here.

Across the top in the blue I'm showing what the concentration of the aloin is. The yellow bar is the equivalent of how much aloe vera extract you would get. What I'm showing here is goblet cell hyperplasia. We don't get cancer in a 90-day study, but in the 90-day study with the aloe leaf preparation we got exactly the same pathology. So the point I would like to make here is I think we have fairly good evidence that the compound in the aloe vera plant that may be responsible for the colon cancer is this aloin.

This is Mary Boudreau's study, but we would like to go on and do a two-year study. The idea would be that this would get CFSAN -- they could regulate this product based upon the aloin content.

The second thing I'd like to talk about is furan. Furan is used in industry quite a bit. It's a component of tobacco smoke, so from that perspective it could be regulated by the CTP. Our interest in it is it's found as a contaminant in many foods. CFSAN needed

to have dose response data to set regulatory levels, so we did a bioassay.

I have shown this before. This shows the induction of cholangiofibrosis in rats administered furan. The study we only did in male rats because there had been a previous bioassay and the idea behind this was to generate dose response data. The dose response is really bizarre. I have never worked with a compound that -- At .09 there was basically nothing and then you went to nearly 100 percent incidence of cholangiofibrosis.

As far as the regulatory impact, this bioassay -- we have an NCTR report on this. It has been furnished to the German government and it has been furnished to the European Food Safety Authority; it's been furnished to the Canadian government. Those people have come back and asked additional questions. We furnish them with any data that they need. It's being used by the FDA but it's also being used worldwide to help set regulatory levels in other countries.

The question is, first of all, what is the mechanism behind this. When we entered this study we thought that furan was metabolized to this oxirane driven by cytochrome P450 2E1, and then it rapidly rearranges into this cis-butane-1,4-dial shown down on the bottom

left hand side, and we thought it could react with DNA. In fact, Lisa Peterson at University of Minnesota has characterized DNA adducts from this.

We have looked very carefully and we can't -- as Bill Slikker indicated, we really have good equipment here and we have looked very carefully by tandem mass spectrometry, we've developed methods, we have all the standards, we have heavy label standard, and we simply can't find the DNA adducts that have been characterized.

But what we have found in the last year is kind of an interesting observation. This shows that furan goes to this cis-butene-1,4 dial, and that in turn reacts with glutathione. And you normally think of glutathione as detoxification, but in this instance, this product with the glutathione bound reacts with protein. So we've characterized this very carefully.

We had this idea that if it's not a genotoxic carcinogen maybe it's acting by some epigenetic mechanism and we have evidence that there are some epigenetic changes, so what we did is we looked at the histones. And indeed, this type of an adduct is found with one lysine in histone H2A. This is in rats. It's not 107; it's 108 in humans. Again, this product is very unique; it must have something to do with the structure of

histones. But I thought it was very curious because you normally consider glutathione to be detoxifying and here we have an active metabolite with glutathione bound to it.

Peter Fu has done the same thing with pyrrolizidine alkaloids. Showing that a glutathione metabolite is actually electrophilic.

So, new initiatives, I want to talk about three of them. The first one -- and this is a project being led by Goncalo Gamboa Da Costa, and this has to do with brominated vegetable oil. For those of you who are not familiar with brominated vegetable oil, this is the thing that makes Mountain Dew look like a citrus product. It makes it look like orange juice. It has been used for years and it was generally recognized as safe until the 1970s when there was some cardiotoxicity associated with it in rat studies.

So then CFSAN, who regulates this, said 15 parts per million, all right. But then there were some additional studies and I've listed them at the bottom of the slide, and there were problems with heart, liver, spleen. There was accumulation of brominated fatty acids in tissue, and I'm not sure how good that is. Anyway, CFSAN needed dose response data to help set a regulatory

limit, and they asked us to do this. The studies are being funded by the National Toxicology Program, and this is the design that we have. It is undergoing review and we will present this next week at White Oak to the review committee that involves the NTP.

We have had extensive discussions on what dose range we can use. The lowest level shown on this slide is estimated to be about tenfold higher than the 90th percentile of human consumption. It's basically the lowest we can go to and still do analytical measures. What we will be doing is a standard sort of toxicology study and these are the endpoints we will be measuring. Also, we will measure the brominated triglycerides in liver, heart and inguinal fat.

The other thing we're going to do is we'll have a bioaccumulation arm and we'll take the highest dose and the lowest dose, we'll measure triglycerides in the liver, heart and inguinal fat out to 90 days. Then we'll take them off the compound and see how rapidly, if at all, these brominated fatty acids are induced.

This is what we're proposing to do; it has not started. If anybody would like to see the protocols we would be happy to furnish that. I realize I'm presenting a lot of data at the moment, but if you want the

protocols we'll be happy to furnish them. We would be happy to consider anything that you would like to suggest.

Bill Slikker mentioned arsenic this morning and I would like to talk about that. If you are not familiar with arsenic -- and I'm talking about inorganic arsenic here -- the average concentration is two parts per billion in water. It can go up really high. In parts of the western United States it could be up to one part per million. The guidelines of both World Health Organization and EPA are 10 ppb in drinking water.

I'm showing the estimated human daily consumption here, and the important thing to note here is that children under the age of one get about five times the level that adults get. This is because they tend to eat rice-based products and rice is where you find most inorganic arsenic.

The reason we're concerned about arsenic is, basically, as a result of this bioassay that was published by Mike Waalkes two years ago. This is a whole life exposure. By whole life exposure, the dams and the sires were given inorganic arsenic before breeding, during breeding, and during pregnancy the mothers continued on. When the animals were weaned, they kept

on. It was a drinking water exposure.

On the top I show the lung adenomas; in the middle, the lung carcinomas in the middle, and then combined on the bottom. I should point out that the lowest dose is only fivefold higher than the EPA and WHO guidelines, so that's a concern. Where I have asterisks I'm showing a significant increase compared to the control. This study showed a significant increase at a very low level of arsenic.

The problem with this is it's obviously not -- it's a non-monotonic dose response. There are other issues here. I maintain that they should get liver cancer, they should get adrenal tumors. This study has caused a great deal of concern for the FDA, the EPA and the USDA.

I'm going to just talk about the bioassay but we have a lot of pharmacokinetic studies. These are being led by Dan Doerge where we're looking at various life stages and we're looking at mice, rats, nonhuman primates, all of the data that will be generated from the pharmacokinetic data. We're also doing a lot of epigenetic measurements, and all of those data will be fed into a modeling effort that is going to be led by Jeff Fisher.

What we want to do for the bioassay is we're going to do this whole life exposure, so we're going to have the sires and dams before breeding, during breeding. The dams we expose during pregnancy. And this is drinking water exposure.

We are going to differ a little bit in that when the pups are born we will directly gavage them. We're quite good at doing that. The idea behind this is that then we will know exactly what the pups are getting rather than depending upon lactational exposure. And then they're going to go from weaning to two years, so that's the repetition of the bioassay.

What we also want to do is have a perinatal exposure only, and I think this is really critical because if you look at the data, if you start an exposure to arsenic as an adult in animals they will not get cancer. It's this perinatal exposure that's critical. So, what we want to see is -- we know that perinatal exposure only will cause cancer. The question is does lifetime exposure increase the cancer incidence. What we want to do is compare the perinatal-only exposure to the lifetime exposure. The doses on the lifetime exposure go down to the 50 ppb up to 16 ppm. For the perinatal-only exposure we're just going to use the highest doses

because I think this is where cancer is. I believe this should be a monotonic response. I believe that these are the doses that will cause cancer, so we should be able to compare the slopes to see whether or not you get the same tumorigenic response.

The first two projects I was talking about, both will be funded by the NTP. If you're asking about funding sources and how we set priorities, this is it.

This last one I consider to be -- When Bill was talking about the projects that are NCTR initiated that don't necessarily involve other product centers, this is one of those. It deals with acrylamide. The interest of FDA is acrylamide in food, so we did a two-year bioassay. When we started the bioassay, we said, okay, acrylamide is going to glycidamide, and glycidamide is going to react with DNA, and indeed that is true. We characterized the DNA adducts and we did a lot of pharmacokinetic measurements, and all of this has been published.

And we got a lot of cancer. We got cancer in male rats and female rats. We got it from acrylamide and glycidamide. This shows what happens in the mice. There was harderian gland, lung cancer, skin cancer, forestomach -- it's a very good carcinogen. There's no

question about that. And acrylamide and glycidamide behave identically.

Taking the tumor data, you can do risk estimations. What I'm showing here are some food carcinogens. There's PhIP, which is a heterocyclic amine; there's benzopyrene, there's aflatoxin of course. I'm doing benchmark dose modeling here and I'm showing what level it takes to get a 10 percent incidence in rodents. It could be mice -- That's the most sensitive rodent that I'm showing here. PhIP is 710; benzopyrene is 290. You can see aflatoxin in rat is a roaring carcinogen. Acrylamide is very good; it's sort of like benzoapyrene.

Then I have the mean dietary exposure and you can see that we are exposed to a lot of acrylamide. Very little aflatoxin. And in the last I'm talking about marginal exposure, and the bigger the number the safer the exposure, so anything over 10,000 is generally considered to be safe. People for years have worked with dietary exposure to benzopyrene and PhIP. Well, I would suggest that it's really not much of an issue. I do believe that acrylamide is an issue. We are exposed to a lot of it.

So I presented these data and then an

epidemiologist gets up behind me and says we've looked and we can't find that acrylamide causes -- acrylamide doesn't appear to cause cancer in humans. So I don't understand this disconnect. Well, I do understand the disconnect; it's because everybody here is exposed to acrylamide. The high exposure and the low exposure only differs by a factor of three. If you asked somebody what do you eat, you know, it's dietary questionnaire data. If you measure internal dose in dietary questionnaire data it looks like a shotgun. It's not very good.

So, we want to look at this, and the way we want to address this is I want to look at mutational signatures. What we want to do is we'll take the tumor data -- we have formalin-fixed tissue, and here I'm showing the tumors that we would look at, and we want to sequence the tumor data by whole exome next-generation sequencing. And we're talking about looking at the mutational profile across the entire exome, and we want to compare mice to rats, we want to compare males to females and we want to look at compared tissue and just see what the mutational profile looks like. Then, having these data, we then want to compare it to human databases where they have done the same thing for mutational signatures.

Again, this protocol is under review somewhere. It has been submitted and it's being reviewed. We have the skills to do this. I don't know how it will turn out, but we believe we can do this. We have the equipment to do it. If it does work, then we have other bioassays that we have conducted previously that we can then apply to the formalin-fixed tissue that we have. I think it would create a very nice database. We'll just have to see what happens.

Just to give you an idea of the power of this method, I'm showing you a paper that was published in Cancer Research in 2014. This is to look at Chinese, and the question that was being addressed is, okay, you have people who smoke and get lung cancer and then you have people who get lung cancer who have never smoked but may have been exposed to second-hand smoke. Can you look at the mutational profile to see do the people who smoke have the same mutational profile that people have who do not smoke and develop lung cancer?

What is shown along the bottom is, the first two are transition mutations, g to a, and a to g mutations. The next four are transversion mutations. You can see this hierarchical clustering analysis. The people on the top, there were 16 nonsmokers, three ex-

smokers and one smoker, and then on the bottom there were the never smokers, and you can see that there's a clear separation.

I think this is a very powerful technique. We're working in close collaboration with people at IARC who are currently conducting this type of analysis. They are willing to do it for us but, as a scientist, I like to see if I can do this. We have these bioassays that we conducted previously, the formalin-fixed tissue exists, and I'm just curious what we will find.

That's it. I'm happy to answer any questions.

(Applause)

DR. PILLAI: The mutational significance -- the acrylamide question I thought was resolved about 10 years ago in the food industry because of all these potato chip manufacturers being concerned because acrylamide formation was there.

The other bigger question I want to ask is that mutational significance is only genetic potential. Why aren't you going down the proteomic and metabolomics levels, because that's really where the action is with these small molecules?

DR. BELAND: I believe that these things cause mutations, and I'm curious. First of all, people don't

necessarily agree with this interpretation that acrylamide is a mutagenic carcinogen; there are people who think it's by other mechanisms. So I will be able to establish that. I will be able to compare across tissues within a species and between males and females and so forth.

I can then go to the human database and see are there mutational signatures that correspond to what I'm observing in rodents that would be indicative -- Can I look at the database for colon carcinogens and see does this pattern mimic what I'm observing in an experimental animal? That would provide evidence that acrylamide could be responsible for colon cancer in humans. I think I'm interested at a different level than what you're suggesting.

DR. PILLAI: The comment I made is sometimes genetic signature scar on the DNA doesn't necessarily translate down to actual phenotype. There are a lot of mutations that you can, by next-gen sequencing, you can detect a lot of sequencing --

DR. BELAND: Oh, yes. This compound will cause the same type of mutations in two genes that are responsible for tumors but also throughout the genome. I'm just looking at the pattern of mutations. I'm not

ascribing them to a particular event.

DR. PHILBERT: Let me be impertinent. Why? What do you hope to learn from -- other than there are difference?

DR. BELAND: In the end I want to see -- you know, they have these mutational signatures for particular types of human cancers. Those data are published. I want to see in my experimental animals, given acrylamide, does the mutational pattern that I observe -- first of all, is it the same across tissues, which I don't know. But does this mutational signature that I get from acrylamide in experimental animals correspond to any of the mutational signatures that have been observed for particular tumors in humans?

DR. PHILBERT: To Suresh's point, do you have sufficient statistical power to pick up your Type 1 and Type 2 errors?

DR. BELAND: I'm working with a statistician about that and she is going to keep me honest.

But this has been applied in other instances where they have taken the animal data, have done this analysis for a particular type of exposure and correlated it with the database of human mutational signatures. That has been done. I can't give you the precise

citation but I'll be happy to give you the protocol where this --

DR. PILLAI: My only comment is I think the tools that are available now to understand toxicology have moved to much higher resolution than just looking at DNA damage or DNA mutations. You can drill down to a lot finer detail to understand biomarkers that may be related to acrylamide exposure and then cancer and things like that. That's my only comment.

I'm not saying that this method is not the right method, but it's probably a comment more than anything else.

PARTICIPANT: One follow-up question on what you're thinking about for this. Given that exposure to acrylamide is particularly high in smokers would there be a benefit, if you're going to look for comparisons in mutational signatures or some other technology, to focus on tumors in smokers only? You have about an order of magnitude higher intake, or exposure.

DR. BELAND: I realize that. I would like to focus on the first part to see what happens in experimental animals. We're not going to generate the human database; that exists, and whether or not it has been separated as far as smokers and non-smokers, we

would have to look at it at that time. Those data, we will rely on the literature to do that.

DR. PHILBERT: There is so much else in tobacco smoke, how are you possibly going to pull out the contribution of acrylamide?

DR. BELAND: I didn't say I was going to -- I am going to do the experimental animals first and see what that looks like. Then we can start doing comparisons. It all depends upon whether or not the data exist, where they have separated smokers from non. I would think they would have, but that is down the road. I have to do the experimental animal portion first.

DR. PHILBERT: But epidemiologically, you would have to pull out everyone who has eaten bread or potato chips or a pastry --

DR. BELAND: No. You can't. We have done the thing, take out this, take out that. The only way you're ever not going to be exposed to acrylamide is to quit eating. When I have an epidemiologist tell me that acrylamide is not involved in the etiology of human cancer, I believe that's because everybody is exposed. I don't think they -- between a high and a low -- you know, a consumer is only a factor of three.

I don't think the epidemiologist has the

resolution to tell me whether or not this is involved in human cancer. I was hoping that by looking at these mutational signatures I might gain evidence to show colon cancer has a mutational signature similar to what animals exposed to acrylamide do.

DR. PHILBERT: And one last technical issue -- are you going to make sure there is no acrylamide in the chow?

DR. BELAND: There is acrylamide. We know the levels of acrylamide. These animals have been dead for years. I'm talking about formalin-fixed tissue. We have the data; we know the tumor incidence; we know the levels to which they were exposed. The diet we used was the lowest acrylamide diet we could find, but we analyzed lots of diets. No. I'm talking about we're not treating animals here. All the tissue exists. We're talking about taking blocks that are sitting at NCTR and going on from that.

DR. STICE: Steve Stice, University of Georgia.

I'm interested in the BBO study, and all of them are really interesting. You mentioned the two-year study with dogs and pigs showing that there wasn't an effect, at least in that study. So does it mean that you really should be doing this in multiple species, co-

morbidity and age of animal as well?

DR. BELAND: The way this study has been constructed is we had to listen very carefully to what data CFSAN needs to make a regulatory decision. At the present time, they feel that the design we have given them with the rats will be sufficient. Now, once the study is done they may come back and say mini-pig or dog or whatever. But for the present time, they feel these data will be sufficient to help them.

And they are under pressure -- Pepsi and Coca-Cola have said they're going to take this out of Mountain Dew and so forth, so there's public pressure upon CFSAN to come up with some decision regarding this.

DR. STICE: So it's enough information for them to make a decision.

DR. BELAND: Would you agree with that, Goncalo?

DR. GAMBOA DA COSTA: There are essentially two components to this study; one is the dose response, the other one is to ascertain bioaccumulation of what is, by all means, a food ingredient. There is no doubt that given enough brominated vegetable oil you are going to have cardiotoxicity, so the question lies at this stage what is the threshold of exposure that triggers

cardiotoxicity, for example.

There are problems with previous studies when it comes to their design. In some studies the controls were not adequate or did not include males and females, so what we are trying to do here is do a clean study to ascertain the dose response.

Much more interesting I find is the bioaccumulation study where we are going to ascertain to what extent you see bioaccumulation of brominated fat, and we are going to be doing that in a much more detailed fashion than was done before whereby people were just essentially reporting brominated fat. We are actually going to look at which particular triglycerides are synthesized *de novo* by the organism and detailed levels of tetrabromostearic acid in the target organ. So I think this will provide a much richer dataset, and then there are more controlled circumstances than what was previously reported in the literature.

DR. PHILBERT: I noticed no electrophysiology. Is there a reason for that?

DR. GAMBOA DA COSTA: Regarding the heart? Well, I'm actually not entirely sure if anyone has actually looked into the heart function. The deleterious effects that were observed in the heart were essentially

accumulation of fat as a general observation in the organ, so you actually observe hypertrophy of the organ. Also, deposition of lipidic droplets, which I believe are brominated fats in the tissue.

But I don't think anything like that has been done, and we certainly have not been requested by CFSAN to look into that, but it's a reasonable observation.

DR. PHILBERT: (Inaudible)

DR. GAMBOA DA COSTA: Probably not. The most sensitive endpoint that we'll have will certainly be a bioaccumulation of brominated fat by mass spectrometry. What we are really hoping is that we're going to be able to link that lowest level of exposure, which is only tenfold about the 90th percentile of human exposure, to bioaccumulation of a food ingredient.

DR. LANZA: In addition to -- Mass spec would be great, but MR, cardiac MR even at high fields for these mice would be great because you can assess the fat through T2 and T1 estimations of the relaxivity or mapping. So I would definitely suggest that you consider that on the live animals or even the hearts, post. That may even give you some idea about the distribution because you're going to do a grind and find versus it may be selective, like atrial versus ventricular or RV versus

LV.

DR. GAMBOA DA COSTA: We are actually doing a detailed histopathology of the heart so we're going to get to even a lower level to get resolution than what is achievable by MRI.

The one concern I have about integrating the MRI as an endpoint in this study is that you necessarily need to anesthetize the animals to put them there, and I don't like -- I mean, either you have a separate arm where you can do that, or I don't like to put some of these animals under anesthesia. Either you put them all or you don't put them at all. If we put them all, it's a very substantial amount of animals to analyze under magnetic resonance imaging.

But I do like your suggestion that that would allow us to observe the evolution *in vivo* throughout the study rather than just have a snapshot at the end of the exposure. So I think there is value there. But it would have to be a separate arm and not done on these animals, I suppose.

DR. LANZA: Yes. One of the things we find in general is that you can do, as you mentioned, serial imaging and you can use fluorine gas on these animals and you minimize any anesthetic effects. But the other thing

about it is that we always have this sampling error when we take histology, and especially if it's not uniformly that you get pockets in distribution.

So, one of the things the MR might be able to do, if you do the mapping, is to actually localize where you take your samples versus others, and then put the picture together in a more 3D way in terms of what you have versus coming to a conclusion. We see this all the time in biopsy. I always think that MR is helpful in at least getting the big picture, because you won't see a function effect.

The other thing you could do, but you could do it with echo maybe, is strain analysis dynamically using spectral track on these animals because that may show -- and it's being done now by some labs, but that may show changes in the matrix in the twist that are related to the distribution of lipids that are going to later manifest into function. This is what we're doing now just clinically for cancer patients and others.

There are a couple different non-invasive modalities that might help you dynamically see how you got to where you are. The trouble with the echo is that it's not high resolution; it's kind of a global measure. The MR would be higher resolution. And then the

microscopy would be the highest resolution.

DR. GAMBOA DA COSTA: Just out of curiosity, isn't it a problem the fact that you have a beating cart for the timeframe where you acquire your MRI?

DR. LANZA: No, because you gate it, is essentially what you're doing. You're going to gate it and you're going to time-average it. That's not the problem. Same even with the echo. The framing rate is in 300, but you time average it for the strain.

DR. GAMBOA DA COSTA: Thank you.

Just to put things into perspective, I think the intention of the product center at this stage is to have the dataset that they deem, more so than investigate in detail the mechanism of toxicity -- is to reach a level of evidence that allows them to regulate the product. But I like the idea of following the bioaccumulation with time. That is certainly a really good idea.

DR. PHILBERT: Any other questions or comments?

PARTICIPANT: (Inaudible)

DR. BELAND: By resources I meant people. This is a group being led by Jeff Fisher, and we have two senior staff fellows and two postdoctoral fellows. Jeff is -- remember when Bill Slikker talked about the virtual

center. He is looking at perinatal exposure and perinatal development in the modeling.

We're starting to do modeling on Tamiflu, and we have done the experimental where we have given it to pregnant Rhesus monkeys, so we have all the data. Now this is being put into a model and being done by Annie Lumen.

We're looking at doing modeling of thyroid exposed to perchlorate and other thyroid-active compounds either alone or in combination. This is being done by both Jeff Fisher and Annie Lumen. Are you interested in the specific programs? I am not knowledgeable enough to comment on that.

PARTICIPANT: (Inaudible)

DR. BELAND: And, of course, Jeff Fisher and Dr. Yang have done extensive modeling on bisphenol A where they have taken across species including humans, and these data have been published. I think Bill has a listing of those publications on his slide.

Dr. Yang, who is one of the senior staff fellows -- they're staff fellows because of citizenship and we intend to keep them and continue to have this program. She has been interested in looking at extended release formulations and modeling of drugs and comparing

that to immediate-release drugs. She has a concept paper that deals with that. I think that is being reviewed right now by someone at Center for Drugs. They've talked about at the concept stage, concept papers being reviewed by someone at Center for Drugs. And assuming it comes back favorably then she will develop a full protocol on that.

DR. YEAGER: Phil Yeager, CTP.

DR. BELAND: Oh, just one more thing. Jeff Fisher is also heavily involved with the Center for Tobacco Products doing the modeling on nicotine and other components that we've done -- experimental data are being generated. Sorry, I forgot CTP.

DR. YEAGER: That's all right. I think you'll remember us now.

Back to the acrylamide, you talk about acrylamide in food and acrylamide in smoke, but acrylamide in food is orally ingested and smoke typically by inhalation. And you also list a number of target organ effects, multiple targets. Have you given any thought to trying to look at a route-specific, tissue-specific mode of action that might give you a better idea of a question you're looking at, because it seems like a pretty broad swath in the manner it's set up.

DR. BELAND: I have not considered doing inhalation. The exposures we've looked at, we've looked at drinking water and also mixed with food, and it gets distributed very widely very quickly. If you were to do it by inhalation you may get a bit more in the lung but I still think it would be a very systemic distribution. I don't know for certain.

DR. PHILBERT: Questions or comments? Susan?

DR. FELTER: I have a question. Can you tell us a little bit more about the proposed doses for the arsenic bioassay? Knowing in the whole life bioassay you saw increasing tumors at the point of 5 ppm, and now in the perinatal-only exposure the thinking is to look at only the higher doses so you can see the relationship of the dose response at these higher doses, how will you translate that to lower doses that are more relevant to human exposure?

DR. BELAND: First of all, I don't believe the responses that were reported. I think there's something not right with that stuff. That's my belief. I'm sure Mike Waalkes would disagree with me but that's my interpretation. And the reason is because if you do benchmark dose modeling, actually there are two whole life studies. The previous study only used the higher

doses. And there have also been studies where they have done only perinatal exposure. If you take the total sum of the data they should also have had liver tumors; they should also have had adrenal tumors and perhaps uterine tumors. So, in addition to a very odd dose response there were tumors that were missing.

These doses, I have the .05 because that's what was reported in the previous study. I should also point out that the diet will give you twice this. We've looked at lots of diets, and the animals exposed to the .05 will get an equivalent amount of inorganic arsenic from the diet, the cleanest diet we can find. We can't use an AIN diet; we have to use a natural ingredient diet.

The 16, the 5 and the 1.6 -- the 16 and the five, based upon all the other literature data, I should see cancer here. This will be a non-balanced design so the lowest doses will have 100 animals per treatment route of each sex.

So, doing benchmark dose modeling on all the previous studies, I think 16 and 5, probably not 1.6 if this response is monotonic. If it's not monotonic then we're going to learn that by doing this study.

The reason I'm restricting to perinatal only is because I think that's where you're going to get cancer,

so I want to compare the slopes of the lines when I have cancer. It doesn't do any good for me to compare slopes of lines if I don't have cancer. So that's why those three doses were selected for the perinatal-only exposure.

DR. PHILBERT: Won't the higher doses of arsenic interfere with sperm viability? Will those males be healthy enough to produce viable sperm?

DR. BELAND: Yes. These doses were used in a previous bioassay just like I described -- It's Tokar, et al, 2010 -- and it was administered in the same way. They didn't comment on reduced fecundity. But it's a valid point; I'll go back and look just to make certain. But the way the exposure was done is that both males and females were exposed before breeding.

DR. HONG: My name is Huixiao; I'm a senior scientist at DBD. My question is about your acrylamide studies. You mentioned that you're using tissue treated with FFTs, right?

DR. BELAND: Formalin-fixed tissue, yes.

DR. HONG: My concern is formaldehyde or formalin is causing the mutations. So, if your sample has been treated with the formaldehyde, how do you distinguish this mutation signature from formaldehyde

from the acrylamide?

DR. BELAND: We have control animals. We have tumors from control animals and we can make that comparison. People have been able to do this type of sequencing from formalin-fixed tissues to develop -- this is what we're talking about and all the literature that I reviewed was using formalin-fixed tissue.

DR. HONG: If you don't know what's the mutation signature from acrylamide, what happens to the same signature as from formalin? What if the mutation signature is the same for both treatments, both chemical agents? That will be confusing which source it comes from.

DR. BELAND: I don't have the answer for you until I do it, but presumably, what I'm hoping is -- well, we have tumor tissues from animals that were exposed to acrylamide and we have tumor tissues from control animals, and I would maintain going into this that those mutational signatures will be different. If not, then your point is valid I guess.

DR. PHILBERT: Thank you very much.

(Applause)

We are going to have an extra 15 minutes for lunch. I'll ask the Board to be back here promptly in

one hour at 1:15.

(Lunch recess)

A F T E R N O O N S E S S I O N

Agenda Item: Public Session

DR. PHILBERT: Last call for public comment. If not, then we will close the public comment period. Then we can just wait for people to drift back in and we will get going again soon.

Agenda Item: DGMT Division Directors: Overview of Research Activities

Agenda Item: Division of Genetic and Molecular Toxicology

DR. PHILBERT: Our next presentation is by Dr. Manjanatha, Division of Genetic and Molecular Toxicology. Thank you.

DR. MANJANATHA: Good afternoon. I am Mugimane Manjanatha from the Division of Genetic and Molecular Toxicology. I'm not sure which one is better, speak right before the lunch or right after the lunch, but I hope my talk will not be soporific. I'll try to make sure that at least I won't doze off. Bob Heflich is the Division Director, and unfortunately he is not able to make it, so I am going to present the research activities in the Division of Genetic and Molecular Toxicology. Before I start, let me make a statement that views that are

presented in my talk this afternoon are mine and not endorsed by US FDA or other agency.

All right. As Fred said, we all were given sort of a template slide so this is our first slide. It talks about the Division staff. I guess DGMT -- which stands for Division of Genetic and Molecular Toxicology; from now on, which I will refer to DGMT, that way I can save a couple of minutes -- is probably one of the smallest divisions because we are 36 people all together, but 36 fine people. And out of 36 people, 27 of them are fulltime employees as shown here, and there are eight ORISE postdocs as indicated here, and two externally supported staff members, and then one FDA Commissioner. So all together, there are 36. As far as 27 fulltime employees or FTEs, these are the ways they have been recruited. There are eight research scientists, then eight staff fellows -- two of them are externally supported -- and nine support scientists, and two administrative. Actually, one of the administrative persons works both as administrative 50 percent and maybe research 50 percent.

In the bottom, I indicated staff changes from 2015 to 2016. Actually, this is not, it shouldn’t be plus one. One of the staff fellows was converted to a

permanent employee and then we recruited three additional staff fellows and three postdocs. The reason that we were able to recruit three staff fellows is because many of our senior scientists over the last couple of years -- maybe three, four years -- have been retiring, so we eventually decided to fill those positions.

As far as personnel issues are concerned, we have one Commissioner fellow converted to staff fellow, that happened just this year; one ORISE postdoc externally funded for the year on staff fellow appointment, that's based on CDER funding source; and one Division Director appointment in process, that's more than two staff fellows in process of being converted to permanent employees.

Next three, four slides, I'll talk about outreach with the local outreach and then FDA-wide and then global.

DGMT scientists, in collaboration with the University of Arkansas Medical Sciences, are developing a human reticulocyte PIGA assay -- I'm going to talk about this briefly the next few slides to come -- for use in monitoring gene mutation in cancer patients receiving platinum-based antineoplastic therapy. So this is with

UA, that's a medical school, and many of DGMT folks are adjunct faculty at this facility.

The second is, as part of a Memorandum of Understanding between the State of Arkansas and the FDA, Division scientists performed research on the genotoxicity of the nanomaterial graphene, in collaboration with the University of Arkansas at Little Rock. So this is another university located in Little Rock.

As far as outreach FDA product centers are concerned, our number one priority is to respond to agency needs for chemical-specific data, and then we have several collaborative projects with CFSAN, CDER, CDRH and CTP, and we have done quite a number of studies on the Gene-Tox of nanoparticles. Just to name a few, silver nanoparticles, graphene, titanium dioxide and so on and so forth. Botanicals, that's with CFSAN, aloe vera, we did genotoxicity evaluation of that, and lately, we are working on black cohosh extract. As far as drug impurities with the CDER, ethyl methanesulfonate contamination or impurity, using transgenic mutation models, we have done that work in collaboration with CDER. And of course, with the CTP, tobacco products such as NNK and cigarette smoke extract and other stuff.

In most of these cases, we either used standard Gene-Tox assay or adapted and performed mechanistic studies.

As far as our global outreach, DGMT scientists led an international workshop on genotoxicity testing, popularly known as IWGT, a team of industry and academic and regulatory scientists, to develop a consensus report on the state of *in vivo* PIGA assays development. I will also talk about this in the next few slides to come. And then leading Health and Environmental Sciences Institute or ILSI/HESI team to validate and develop OECD test guidelines for the assay spanning 2015 to 2022. Also, we developed and validated regulatory tests or assays, most OMA, HPRD, transgenic mutation assays and PIGA. And then DGMT scientists are members of OECD development workgroups and worked on many of the tasks, including nanomaterial testing and lately, revision of existing OECD test guidelines, which led effort on revising *in vitro* HPRD gene mutation guideline, and this test guideline is classified as TG476.

What is our mission? DGMT mission is improve public health by providing the agency with the expertise and tools necessary for comprehensive assessment of genetic risk, and by strengthening approaches to

integrate knowledge of genetic risk into regulatory decision-making, so where all our research goes. Our number one priority is to respond to agency needs -- this is FDA Product Centers -- for chemical-specific data, some of the examples I've already given, like nanomaterials and tobacco products. And then maintain DGMT's tradition of leadership in regulatory assay development and validation, and some of these are mostly in form as HPRD, transgenic mutation model and PIGA. The ones that are highlighted are [09-8.47] -- I'm going to talk about that in the next few slides. And then establish new paradigms for regulatory decision-making that migrate -- sorry, that integrate -- measures of genetic risk with biomarkers of toxicity.

The next slides, or several of them, show our research strategies. Of course, we want to engage or we have been engaging FDA Product Centers, National Institute of Environmental Health Science, National Toxicology Program, and ILSI/HESI and other national and international organizations to set research priorities. Many of the DGMT scientists are members on the committees of many of these organizations, so they are involved. And develop better biological model for assessing human risk, such as 3D *in vitro* model, just an example. And develop

more comprehensive approaches for monitoring genetic radiation, which can be accomplished through development of allele-specific competitive block or PCR, next-gen sequencing and other techniques or assays that we have developed. And then develop better ways of evaluating data to determine human risk, sort of a dose response or benchmark dose as point of departure doses and things of that nature.

Okay, the top three accomplishments are listed here. We have a lot of top accomplishments to share with you but in the interests of time, these are the ones that I selected as our top accomplishments, and I'm going to go over each of them and also spend maybe a couple of slides on each of them to expand what's going on. Number one, received approval from OECD to develop and validate an OECD test guideline for the rodent PIGA gene mutation assay for regulatory genotoxicity safety assessment. Second accomplishment is conducted an Office of Women's Health-funded project comparing the oncomutation profile of breast cancers in Caucasian and African-American women. And the last one is developed a new transgenic hairless albino mouse model for potential reduction of animals used for NTP photocarcinogenicity study.

So the next couple of slides, I'll talk about these accomplishments. Let's start with PIGA assay. PIGA assay is an endogenous mutation assay. It's extremely versatile because this assay can be done across the species from rodent to monkeys to humans, and all you need is a drop of blood. And PIGA stands for phosphatidylinositol glycan class A gene -- I know it's a mouthful but it's an extremely interesting and important assay -- and the gene product is required in the first step of GPI anchor synthesis. Interestingly, GPI anchors attach several surface proteins for the mammalian cells such as red blood cells, and these proteins, for example, listed here, the CD59 and CD24. And interestingly, of all the genes required to form GPI anchors, only PIGA is located on the X chromosome. As all of you know, one of the copies of X chromosome is inactivated, so this means all you need is just one hit that can produce a cell surface or a mutant phenotype. And also, this sort of provides the opportunity, this phenotype can be accessed with flow cytometry, as shown in the next slide.

On the left-hand side -- this is basically, this slide is basically a cartoon presentation of what I described in the previous slide. On the left-hand, the wild type PIGA mutant -- sorry, wild type PIGA gene --

the cell is a red blood cell, as shown here; and the right is the mutant phenotype. So, as I indicated earlier, the wild type PIGA produces the GPI anchor, as shown here, and to which a surface protein is attached -- CD59. And interestingly, we can develop an antibody, which is fluorescently labelled, to this CD59 or other surface protein. That way, it can be accessed by flow cytometry and it can be sorted out and differentiated between mutant and the wild type. So as far as the mutant, there is no PIGA gene, functional PIGA gene, so there is no GPI anchor, so there is no surface protein. And interestingly, using flow, we could scan millions of cells for a PIGA mutant in a fraction of minutes.

I included this, this slide shows about how to sequence some of these mutants, because it’s extremely important to show that PIGA mutant phenotypes have PIGA mutation in them, not only for the OECD acceptance but also for a validation of any assay that uses a reporter gene -- in this case, of course, the endogenous gene. So basically, scientists in the DGMT came up with a neater method to analyze PIGA mutants, because the next-gen sequencing is not very accurate and in fact, it’s error-prone, and depth of coverage is also insufficient when analyzing many different mutants. So they use this

technique called MARDI -- mutation analysis by random DNA identifiers -- and basically, I don't want to get into too much of technical detail here. Oh, by the way, this is T-cells from rats exposed to a potent mutagen, DMBA. So you attach a tag, it's called random DNA identifiers, as shown here in different color box -- yellow, red and blue -- and then you repeatedly sequence the cDNA fragments. And interestingly, if this is a true mutation, it will show up in, as shown here as stars, in all the copies that you have sequenced. This way, they have identified hundreds of mutants and, interestingly, they all showed a very relevant DMBA-induced factor which is consistently produced as A2T mutation, which is the hallmark of DMBA damage.

Okay. This is a sort of arduous journey, what's shown here, to get to OECD acceptance. I'm not going to go on each of these, but suffice it to show that a few interesting sort of milestone achievements. The one that I want to emphasize is the 2008 first publication co-developed with NCTR. Actually, NCTR has an excellent program called ISEP -- International Scientists Exchange Program. So with that program, we were able to invite scientists from Japan, who came in in early 2008 and worked on this PIGA assay and co-developed, published a

paper working with DGMT scientists. That sort of laid the foundation for a PIGA assay and then of course, since then, we have journeyed a long way through.

And I guess this may be important as well. 2014, M7 guidance compliance for impurity qualification, PIGA assay was selected. That's a CDER-relevant issue.

And then 2016-17 -- actually, you could add 2015 as well -- as shown on previous slide, research at NCTR and demonstrating PIGA mutations are responsible for the assay phenotype was an important contribution to get this PIGA assay to OECD acceptance.

And 2018, as shown here, a detailed review paper and validation report are likely to be approved, and Bob and other co-authors are working on writing this detailed review paper, and actually there's a typo here.

2022 actually, we are expecting OECD test guidance acceptance.

Accomplishment number two: the scientists in the DGMT have characterized using ACB-PCR. Actually this stands for allele-specific competitive blocker PCR. And then they use this assay, which is a sensitive assay, to detect the rare mutation they actually develop in-house and they characterize, using this assay, ultra-low frequency of cancer driver mutation present in normal

tissues. So that was a surprise. So even normal tissues do have, but a low frequency, of these cancer driver mutations. And this frequency changed depending on the type of tissue screened and the age of the individuals and their gender. And they also showed that breast and colon, lung and thyroid cancers encompass subclonal population of PIK3CA and KRAS mutations, which may be the drivers of therapeutic resistance and the relapse of cancers in many instances. These are the two important publications by these authors.

The third accomplishment is the development of a transgenic hairless albino mouse. I know this is a busy slide. I'm not going to go over the breeding scheme of how to develop this strain, but suffice it to say, our interest was to transfer two reporter genes from gpt delta mouse as shown here, and transfer it to SKH-1 mouse, which is NTP Darling mouse, for conducting the photocarcinogenicity, as these mice are hairless and albino.

So why did we do this? The main purpose of doing that is we wanted to investigate if short-term mutagenicity by incorporating these reporter genes into SKH-1 mice, will it meet the requirement of predictivity of a long-term carcinogenicity that is done using SKH-1

mice. This is sort of an approach to address three Rs, although this won't be the replacement of animals but would address reduce and refine animals used in the carcinogenicity assay.

Over several backcrosses and a couple of years' labor, we did finally obtain transgenic hairless albino mice, as shown here at the bottom. And we wanted to test if these mice respond to UV exposure, so this slide shows that basically we exposed these transgenic hairless albino mice to two doses of UV. These are the same doses used for carcinogenicity, photocarcinogenicity, but the animals are exposed for longer time, like 40 weeks. And in our case, since it's a mutational model, we exposed them for just three days to give a cumulative dose of 20 low dose and 40 high dose mJ/cm^3, and as shown here, the mutant frequency on the y-axis per million copies and on the x-axis through doses of UV and unexposed control, and this is the mutant frequency at the GPT site, which is the reported gene. Interestingly, ten- to fifteen fold increase in the mutant frequency compared to the background. So this suggested that this model works fairly well.

As I said earlier that it's important to show that in these mutants, the mutation is involved in the

target gene, not only for validation purpose, also for publication it's important that these mutants are induced by the test agent. So I don't want to spend a lot of time on these.

These pie charts show, on the left-hand side is a control unexposed spectra and then on the right-hand is UV exposure, with the different types of mutations with the different color indicated here. But I want to draw your attention to one major type of mutation, which is G2A or C2T, and which is also a background predominant type of mutation which is 66 percent in control mutants. And interestingly, with the UV exposure, 84 percent, because UV is known to induce predominantly C2T or CC2TT because 6,4-photoproducts and pyrimidine dimers are known to target these bases. So we found 84 percent of these spectra included mutation at C2T. What is interesting is among the background mutants, 90 percent of these mutations occurred at the CpG side -- it's a methylated cytosine side -- that's where the most mutations are targeted; whereas with the UVB, there is a shift in this. Only 46 occurred at the CpG.

Also context of these mutations are extremely important. Interestingly, 92 percent of the C2T mutation occurred at the dipyrimidine side in the UVB-exposed

animals, whereas in control, it was only 67 percent. So this suggested to us that this model is useful and it responds to UV, it has UV signature and mutations. So Mary Boudreau in biochem tox has taken the leadership to test or validate this further, and hopefully NTP will fund that project.

The next few slides I'm going to discuss the future areas of emphasis. As I indicated earlier, chemical-specific data is extremely important for us to provide to the FDA Product Centers, and then we want to use conventional Gene-Tox such as NGS or next-gen sequencing to study some of the new emerging areas such as CRISPR. CRISPR and Cas9, I'm sure many of you heard that this technology is used from making monkeys to development of even plant. I heard in Sweden, a cabbage plant was established using this technique. It's a gene-editing technique, and it's extremely versatile. But for our purpose, we are not interested in technology as such. Our interest it in a collateral effect or an offsite effect, the upstream of where editing is done or downstream if there are any adverse effects of that. So we are studying that.

And then our second project is sort of autophagy. I'm not going to spend, well, a lot of time on

this. Autophagy has been in the news lately, but it’s a sort of recycling of cells, especially when organelles are degraded, the nutrients recycled. This is a physiological process followed by an organism. Especially in the stressful situation, autophagy goes up, but lately it has been implicated in many diseases including Alzheimer’s and cancer. But our interest is one of the autophagy genes, Beclin-1 I think, we want to use as a reporter for a mutation assay. And then we want to develop new biological and analytical approaches, for example error-corrected next-gen sequencing, which I talked about it, and digital display PCR. This is actually a new quantitative method. Again, here, the technologies are based on water/oil emulsion droplet technology where you fractionate your sample, thousands of droplets, in fact 20,000 droplets, so literally you will have one template per droplet and then you amplify your gene of interest or your mutation of interest. It uses the same TaqMan technology and the idea is the samples are fractionated and you'll get big picture, and also the sensitivity is slightly higher. That’s what DGMT scientists want, to compare the sensitivity of these assays with already-established allele-specific compared to block PCR, NGS and others.

And then human *in vitro* organotypic cultures, we do have one already established at DGMT funded by CTP. It's called ALI system. It's airway liquid interface bronchial epithelial *in vitro* system. It's been responding quite well and they are testing tobacco products using this. And they also are already establishing microphysiological systems, microfluidic systems; these are all ideas. It's like, you know, organs on chips.

And then lastly, develop new approaches to using Gene-Tox data, which is going to lead to -- this is Bob's favorite slide so I want to include it here -- instead of a one-size-fits-all standard Gene-Tox battery and using Gene-Tox data in a yes or no manner to identify carcinogens, we are asking why don't we consider mutation as a true toxicological endpoint, rather as a reporter of carcinogenesis, and then consider both somatic, cell and germ cell mutations as key events, as sort of apical endpoints? I know there are thousands of inherited diseases where you have germ line mutations, but mutations in somatic tissue not be manifested, but it may change the homeostasis and maybe we can develop approaches in that regard.

Then consider a mutation in an integrated fashion with other toxicological endpoints perhaps in the context of adverse outcome pathways where ILSI and HESI and other organizations are already discussing in this direction -- I mean going in this direction -- and many of our members are part of this committee and so we are working in that regard as well.

And then lastly, consider a mutation and the shape of the dose response curve in a quantitative manner to evaluate risk, for example benchmark those point of departure and things like that.

Well, I guess that was the last slide and this is about requesting your feedback, what emerging sciences or technologies can you advise me or us to pursue, and what future directions do you recommend for this division that would impact the FDA? Thank you for your attention.

DR. PHILBERT: Questions or comments from the Board?

DR. MANJANATHA: Right. I wish Bob was here but I'll try to answer as much as possible to my best capacity.

DR. PHILBERT: Suresh?

DR. PILLAI: A question. (off-mic)… roughly still looking at these mutations or are you looking at transcriptomic responses as well?

DR. MANJANATHA: Well, which mutations are you talking…?

DR. PILLAI: Like when you're talking of the DNA level mutations, are you referring to just the…?

DR. MANJANATHA: No, that one was a reporter gene. So it’s a transgene.

DR. PILLAI: Okay, okay.

DR. PHILBERT: Steve?

DR. STICE: Steve Stice. Was interested in your future areas of emphasis on the CRISPR. I guess I don’t quite fully understand how you're going to look at CRISPRs and off-target events, and is it related to a certain cell type or a certain treatment or what's the emphasis?

DR. MANJANATHA: I think, if I remember correctly, the concept was just submitted. PD-1 gene, I’m not very familiar with that, but supposedly the immunity-related, so I think it’s they want first to look at mammalian cells, maybe TK6 to start with. These immunological or immunity directed against tumor would be prevented by this PD-1 gene, so they are trying to knock

this out so that the immune response can suppress the tumor formation or tumor advancement. So that model is being used, but then nobody has looked at the collateral effect of this, so there are a couple of scientists that the DGMT, they look at that and also there was another one with HIV-related infection in mammalian cells. It's mostly *in vitro* system to start with. I guess this person that we recruited lately, recently, is a next-gen sequence expert so they can come up with a well-corrected method to sort of analyze the whole genome and look for some effect associated with that. So basically looking at the off-site or off-target effect associated with that.

DR. STICE: Just a follow-up real quickly. I think that's interesting. One area that really is getting hot and is hot is the whole CAR-T immunotherapy side, and there are groups at UPenn that are starting to do CRISPR to do CAR-T. So if you think about applications in the future --

DR. MANJANATHA: Sure.

DR. STICE: The whole CAR-T area, immunotherapy is going to be important.

DR. MANJANATHA: Yes, and I was also told when FDA Commissioner came to visit us, it's also one of his priority areas he is interested, although right now, I

don’t think there is any submission of CRISPR-related at the clinical studies, but eventually they may start to come in. And it’s good to know some of these studies we conduct, at least we are sure of absence of any collateral effects associated with this gene editing or genome editing.

DR. PHILBERT: Carolyn and then Pam.

DR. WILSON: (off-mic) … human gene therapy that would involve CRISPR-Cas, I just wanted to make a few clarifying comments. So I think what you were talking about PD-1 is in fact the CAR T-cell UPenn CRISPR-Cas protocol that’s been reviewed by the RAC, and I just wanted to say that as a center that’s regulating the technology, that of course we do require that the sponsor perform these types of studies to look for off-target effects. I do want to just clarify that those studies are either, have either been done or will be done before anything would proceed in humans.

And then secondly, Center for Biologics has also recently recruited a new PI to work in this area, and so I would ask that NCTR engage CBER and coordinate that aspect of the research because it is important for you to be aware of what we’re doing and vice versa.

DR. MANJANATHA: Oh, definitely.

DR. LEIN: Pam Lein, UC Davis. So a couple of thoughts that occurred in terms of emerging sciences and technologies, where are you guys involved in the epigenetic field or not? We heard that microRNA was being looked at in another division within NCTR. Is that something that your groups is engaged in? Then also, what about DNA repair, which seems to be becoming more and more prevalent in terms of neurodegenerative diseases?

DR. MANJANATHA: Yes, one of our investigators is working on microRNA as a biomarker for carcinogenicity but as I can answer a little bit more elaborate on the epigenetic studies, actually I recruited a Commissioner fellow, and I think he is in the audience, exclusively to work in this area, but not epigenetic field per se. But one of the ideas was to modify Comet assay, which is a single cell gel electrophoresis assay which detects DNA damage, but there is an enzyme, restriction enzyme, McrBC, which identifies or detects methylated C and cuts it. So we want to incorporate this into the Comet assay as sort of epi Comet. So he was [010-6.24] successfully able to establish this assay. The only thing is it detects very successfully the alteration in methylation as such, but it’s a global methylation, so now we want to expand it to a gene-specific methylation. So in a way,

some small studies in that area is going on. But we are not big into epigenetic like Fred Beland's group, Igor's group is heavily involved. We do collaborate with them but this technology, we have used it to modify one of the Gene-Tox assays.

DR. LEIN: Thank you.

DR. REISS: Hi, this is Ted Reiss from Celgene. So I was wondering if you could give us a little bit more flavor of the next to the last bullet on the future direction strategy slide. You're talking about integrating mutations and other pathways, or other toxicologic endpoints. So can you give us a vision of sort of what that would look like and then how would that be operationalized?

DR. MANJANATHA: Well, I think we have been working, you know, [create 010-7.38] with the EPA, it's a sort of a mode of action type of studies where we want to know if mutation is involved initially, in the initial step, for a carcinogenesis. But I was talking about in terms of the dose response curve, we can do a benchmark dose. We don't have to use mutation just as a report of carcinogenesis, but we can always say, well, if there is a threshold using drugs and then this low-dose response, we can evaluate that and we can say, well, at least up to

a certain dose, it is safe. There's no other adverse effect that we'd expect from it. That's something that we have done with impurities, EMS contamination, to show that up to 30 mg/kg was no response or an adverse effect associated with that. So it may be safe up to this level. So those are qualitative and quantitative information you can get by doing a dose response curve, using the mutational model, so you don't have necessarily use these models to answer yes or no as far as the reporter gene to predict the carcinogenicity.

I guess there are a couple of studies ongoing but majority have been with a CRADA and we helped publish some of those already. I don't know whether I answered your questions or not.

DR. REISS: Yes. Ted Reiss again. That was very helpful. What about the bullet above that which talks about the integration of various different endpoints including the mutation rate with other toxicologic endpoints? What were you considering there?

DR. MANJANATHA: Well, these days, we are moving away from using mutation alone as a report for carcinogenesis. So like a Tox21 paradigm, NIEHS and NTP or after that -- although that's *in vitro*. So we want to know exactly what are the toxicological endpoints that

come up, so which may include mutation as well. So then we can associate that with the toxicological endpoints rather than mutation alone. So there are a lot of studies or ideas in that as well. Kerry Dearfield actually heads one of the committees that's interested in the adverse outcome pathways. He is at the USDA. So we want to know whether we can use mutation to understand whether it is a part of these pathways as well. So that information, as well as other toxicological endpoints, we can integrate all that and come up with better risk assessment rather than just using mutation as a report of carcinogenicity.

DR. PHILBERT: So if I could follow up on that, how much of your work gets at the question of necessary and/or sufficient for any given endpoint? So AOP gets you part of the way there but how well are your data blended with other -omic platforms and more sort of data-rich analysis?

DR. MANJANATHA: Yes, we do work on gene expression profiles and also microRNA. I guess [Towers 010-11.43] group is involved in that, in putting all that information together in addition to mutation. But as far as the adverse outcome pathways, we just started to do this at the individual level. Actually, we are looking at the high throughput measuring different endpoints

including Comet assay and micronucleus assay in addition to other toxicological endpoints. So probably, we just started, then maybe hopefully we'll continue more in that direction. I can't think of any study that we set up where we are looking at adverse outcome pathways as such in the Division, but that's the direction that we want to go as well.

DR. PHILBERT: And do you have a guiding analytical framework, again so that you know when you have Type I and Type II ARAs? Because you can get lots and lots of false positives, but you can also get false negatives.

DR. MANJANATHA: Right.

DR. PHILBERT: Because it's not, it's rarely -- these endpoints rarely have single gene endpoints.

DR. MANJANATHA: Right.

DR. PHILBERT: You have gene-gene interaction, protein-gene, protein-protein and other molecules, large, medium and small.

DR. MANJANATHA: Right. We have a world-renowned bioinformatic and biostatistics division at NCTR. So every protocol when you write up, we work with one of the statisticians and they're always there to help us out, and I'm sure we can seek their advice and approach this

that way because as a statistician, they are more well-versed to understand some of this and advise in the right direction. So that's what we want to do.

DR. PHILBERT: Yes, Suresh?

DR. PILLAI: You mentioned twice -- that's Suresh Pillai -- you mentioned twice error-corrected next-generation sequencing. Why are you -- I mean, is there a problem with the current next-gen sequencing with errors?

DR. MANJANATHA: Yes. See, when you have thousands of mutants, as I've shown in that slide, if I can go back, so you are not sure, for example, DMBA-induced mutations will be real mutations or not because when you amplify, you can pick up all sorts of stuff, mutations associated with that, but that's probably not what we are looking for. We are looking for specific DMBA-induced mutation. So they use a technique like MARDI. So those are the techniques that are used lately to get the sensitivity of the assay, of the next-gen sequencing, to be improved and then detect those kinds of mutations. So I'm told when you sequence the whole genome, you will have all sorts of mutations. Many of them may not be real mutations associated with amplification and stuff like that. The error rate is

fairly high with the NGS. I mean, it's still a long ways to go to get the sensitivity where you could detect one mutation with the million background, wild type. So in order to know whether this is a real mutation or not, so they developed that sensitive technique, so by which they were able to show that those mutations in all the copies that they sequenced, they all showed up in the same location, same type of mutation. So that proved that DMBA led to mutation. So there is quite, always, some error correction associated with the NGS.

DR. PILLAI: If I could get a comment from Carolyn about it because FDA uses next-gen sequencing now for bacterial fingerprinting to [sort-identify 010-15.58] the whole genome sequencing, and they have, I thought that they had dealt with these error issues.

DR. WILSON: So that's really more of a CDRH question because they're the ones who are regulating diagnostics for using -- using NGS for diagnostics. But I can just comment in general that it is an issue that, depending on the platform that you use to do the sequencing, that there will be a certain error rate.

DR. PILLAI: Error rate, right.

DR. WILSON: We've also, in house, we've developed algorithms to sort of factor in that background

rate depending on the platform. We're looking, in our case, at mutational effects of, for example, vaccine strains after passaging in cell culture and things like that, so like a product quality. But it is a known issue with next-gen sequencing but really CDRH should answer from the point of view of diagnostics.

DR. PILLAI: CDRH, okay.

DR. MANJANATHA: And also, I want to draw your attention, this was a mammalian cell, T-lymphocyte, versus bacteria. You're talking about ten or maybe a hundredfold increased number of bases that you had to sequence. So the more bases you have, the more errors associated with that. So that's one of the reasons with the NGS being error-prone.

DR. PHILBERT: Please identify yourself.

DR. XU: Joshua Xu from DBB. I just want to add a comment to that. Actually, that is part of our SEQC2 study as well, and they're evaluating deep-target sequencing to detect rare mutations. Rare is not in the population rare; it's that in the sample, because of tumor heterogeneity and really, some of them just have very low percentage, like 1 percent, 0.1 percent, and actually that is a very important application is liquid biopsy and Dr. Don Johann from the University of Arkansas

for Medical Science is going to give an invited talk on liquid biopsy and he'll have a couple of slides to further explain this, the challenge and its important application.

DR. PILLAI: Thank you.

DR. PHILBERT: So what does that mean biologically to have a rare mutation in a heterogeneous sample? Will you get into that, Don?

DR. JOHANN: Yes.

DR. PHILBERT: Okay.

DR. PHILBERT: If it might impose that you sort of contrast out with the rare mutation population level and what that means therapeutically. Thanks. Any other questions or comments? Thank you very much.

Agenda Item: Liquid Biopsy to Further the Development of Lung Cancer-Based Precision Medicine

DR. PHILBERT: And now I'll hand it over to Bill to introduce our speaker.

DR. SLIKKER: Right. From time to time, we've had the opportunity to have speakers from outside the NCTR give presentations here at our SAB. We've had David Ashley from CTP as Director of their Office of Science and Research give a description of CTP activities early on after the creation of that center. Just last year, we

had a presentation by Rob Califf, who at the time was the director of the group that did the medical products and tobacco products management. Of course, since then he's ascended to the leadership of FDA as the Commissioner. So we won't put too much pressure on Don Johann in his role here as giving an opportunity for us to get some insight into activities that are going on in collaboration between NCTR and the University of Arkansas Medical Sciences campus.

Actually, this grew out of an activity that the Arkansas Bioinformatics Consortium started several years ago, and this was to pull together bioinformatics people from around the State of Arkansas in conjunction with the Arkansas Research Alliance, to sort of enjoy and appreciate the input from the five major research universities in the State of Arkansas and NCTR working together through an orchestrated approach.

And out of that, we challenged them about a year and a half ago to come up with a proposal that would address an important issue that incorporated not only bioinformatic approaches but also understanding the state of the art, in this case, of cancer assessment and treatment. And so they put together a really nice proposal and through the FDA's Broad Area Announcement,

it was selected competitively and funded as a contract. So we're going to hear some information that arises from this collaboration, the contract mechanism being in place for the universities in Arkansas, which UAMS is leading this charge but other universities are involved as well, and in working with the FDA NCTR in moving this area forward.

So with that background, I'd like to introduce Don Johann, who will give this presentation. Thank you, Don.

DR. JOHANN: Well, thank you for that very kind introduction, Dr. Slikker, and I'd like to thank the NCTR and the Scientific Advisory Board for asking me to come and speak today.

And so just as my background, I'm a medical oncologist trained at the NCI. I was at the NCI for ten years, and now I've been at UAMS for approximately four years, and what makes me a little different as a physician is I became a physician as a second career. So I actually worked in engineering for seven years for the Sperry Corporation, that rapidly became Unisys, on a variety of avionics projects, and attended medical school -- attended graduate school and I did a project, a computer science graduate degree, got interested in

medicine and wound up going to med school at Case Western.

So with that background, I'd now like to explain to you our very exciting project. It involves liquid biopsies, and when you think of a liquid biopsy, it can refer to a number of things, either a cfDNA, which means circulating free DNA; CTCs, circulating tumor cells; or exosomes. So all three fall under the rubric of liquid biopsies. For our particular study, we're just looking at the cfDNA, and this is a very big application of liquid biopsies, should have very interesting applications in cancer screening and the monitoring of patients as they undergo therapy, especially it's now showing up now in clinical trials to demonstrate clinical utility, and it's really expected to be a central piece in the future of precision medicine.

And I would just say for a very, very long time, it's been kind of the holy grail of medical oncologists, the ability to, just from a routine blood draw, get an idea of what, number one, is there a malignancy; number two, is my therapy actually being effective?

So now, there are applications of next-generation sequencing to study this molecule very

closely, and we are very engaged with SEQC2 and the Deep Sequencing Project because this is an application of deep sequencing.

So, we'll just continue to define some terms in the beginning here. So the precision medicine problem statement really, over 70 years, for cancer systemic therapy has really relied on drugs that are just marginally a little bit more toxic to the tumors than they are to normal tissue. That's why we get these horrible toxicities, people's hair fall out, they have tremendous bone marrow suppression, they get neutropenic, many die from infections. So one of the problems we've had is we haven't had molecular markers to predict benefit or understand therapeutic resistance. So the proposed solution now is to use NGS-based technologies to study tumors, to study other body fluids to get a better idea of basically how am I doing with this treatment. Is it really benefiting the patient?

So this slide here just kind of defines, further defines, what some of these terms are. So the terms, ccfDNA, you may have seen the literature, that's circulating cell-free DNA; and then cfDNA, so free DNA. These are really umbrella terms. Now for cancer, what's very important is ctDNA, circulating tumor DNA. So these

are shed products from the tumor that get put into the bloodstream that we can capture with a routine blood draw.

Now, as you see, this is a picture of a blood vessel and this is different tissues, some healthy tissue, some inflamed tissue but all tissues, when they turn over, put some of their genomic contents into the blood. So this is put in -- the processing can be either plasma or serum. Now, the Vogelstein lab has been studying this for close to fifteen years, so they've advocated really plasma versus serum. The half-life of this molecule is about two hours, so it rapidly disappears. The elimination is through the kidneys. The size of it is about 120-180 base pairs, so it's tiny fragments of DNA. And as I said, it's released from healthy tissue and diseased tissue, whether that tissue is inflamed or from a malignancy type process.

Now, the current applications of cell-free DNA are really in the non-invasive prenatal testing area, and why is that? Well, it's much safer than the prior methods, as amniocentesis or chorionic villus sampling. So as you see in the cartoon here, here is the fetus, the placenta adhering to the uterine wall, and we have small pieces of the placenta sloughs off and then the genomic

material goes into the mother’s blood, and that can be picked up. And these diagnostics are highly, highly effective for screening for the routine trisomies here. Now the other applications that are rapidly coming online are for solid organ rejection for transplants, for the chronic graft versus host disease, not acute, and in oncology, which is extremely exciting.

So the potential applications of this in oncology are the early detection and monitoring for various solid tumor malignancies. Now remember, a lot of these solid tumor malignancies will grow in a patient over a ten- to up to a thirty-year time period, and it’s usually only in the last few years that it becomes clinically evident. A patient comes in because of unrelenting back pain despite NSAIDs and maybe a little physical therapy. They have an x-ray and you see that there is a moth-eaten space or obvious blastic growth, and you have a biopsy and you work them up, and it’s prostate cancer with mets to the spine. So now you have a metastatic process and it’s much more difficult to treat, rather than if you could detect this very early when it was localized, when it was amenable to maybe just a surgical correction without maybe even adjuvant therapy.

So what we would like to do is to be able to use this approach for early detection and monitoring, as well as when a patient is being treated, to start to monitor them for resistance mutations. So this is very common now with lung cancer where we have the T790M mutation which, for patients who are EGFR-mutated, a lot of time happens. Sometimes you don't see this mutation early on, and it could be a clonal phenomenon where it's just at too small of a level or you are killing off one clone and that gives therapeutic pressure for other clones to start popping up. So we want to be able to do that, and actually there is now an FDA-approved test, the cobas test put forth by Roche Diagnostics, which does monitor that using a PCR technique for the T790M mutation. I'll show you that, a little bit more of that later.

Now this slide, the take-home message here is that the circulating tumor DNA in advanced malignancies, the detection varies and it's really a function of the type of tumor and the burden of disease the patient may have. So here on this part of the diagram, you see a lot of GI malignancies, bladder cancer, and 100 percent likelihood of detection under those circumstances; and all the way at the other end, you see gliomas where, for

reasons we do not yet understand, regardless of the burden of disease here, it’s just very hard to detect. So this is a very active area in translational medicine of trying to understand the molecule better, the circulating DNA, as well as the biology associated with that.

So here is an example of some disease monitoring for the detection of recurrence and resistance. So in the A graph here, here is an example of a patient that has a solid tumor malignancy. They have surgery and the tumor is completely excised. Over time, maybe there was micrometastasis, maybe there was a small met someplace else that we didn’t quite see. Maybe the margins weren’t quite as clean as we thought. But if we know, we can start to now monitor somebody for evidence of recurrence. This also kind of starts changing the equation of what we think about with adjuvant therapy. Can we now use more empirical results and monitoring to then modulate whether adjuvant therapy should be used or not, rather than just have it based on large clinical trials where it’s some sort of a categorical type assignment and you're looking at very aggregate statistics. Here, it’s individually based, you're sampling the patient’s blood over time, and you're looking for evidence of that malignancy coming back.

In the B graph here, this is an example of a patient getting systemic therapy and with systemic therapy, I want to make sure that I'm hitting the target, especially if this is some sort of a targeted type therapy, or even with chemotherapy when there may be some very sentinel mutations that we're watching. If the tumor is really being eradicated, then we should see a drop in that. Also, over time, again, you have the selective pressure from therapies and you may see the emergence of new and different clones. So here, the red and green would show you different clones, because the tumor does change over time, due to its own genomic instability as well as the selective pressure that different therapies induce.

So some of the recent -- from the literature, some of the recent ctDNA applications and clinical studies. As I mentioned that the FDA has newly approved EGFR tests, the cobas test by Roche Diagnostics. It requires 2 ml of plasma. It has a turnaround time of four hours and the level of detection goes down to about 25-100 copies of this target per ml.

Recently, by Geoff Oxnard up at Dana Farber, he showed in this *JAMA Oncology* article, rapid genotyping for non-small cell lung cancer for both KRAS and EGFR

mutations, again with a turnaround time of approximately three days, and this used a ddPCR technology which I'll be showing you a little bit more and which we are also using on our clinical trial which is a part of this very exciting project.

This *Cancer Discovery* publication showed serial ctDNAs can correlate with outcomes and sometimes even predict disease mutations. And just this morning, there was yet another study published by the UPenn group where they used ctDNA to monitor over 100 patients with different sort of lung malignancies and with very good efficacy of how some of the treatment was going.

So what about the technology to assess these mutations? Well, in broad strokes, there's two basic ones that we use. There is the digital droplet PCR and the next-generation sequencing test that can be panel-based or whole exosome sequencing. So the difference is with ddPCR, you need to know what you're looking for. So in this, for instance, with our trial, we will sequence the patient's tumors using multiple modalities -- DNA sequencing, RNA sequencing and also looking at the methylation -- and so we'll have a very good understanding of the solid tumor and then based on that,

could then use ddPCR to go down and find certain mutations in the blood.

Now the advantages of that, it has a much faster turnaround time -- we’re talking about hours to days, versus days to weeks with an NGS prep. The false discovery rate is very low, where the false discovery rate for NGS when you're looking for very low-frequency mutations is quite high, but we’re addressing that with some of the DNA tagging technologies which I’ll show you. The complexity of the bioinformatics for the ddPCR is minimal, where with the NGS approaches, you have these very complex pipelines that could easily have 12 to 20 steps depending on the modality. The expense for the ddPCR is relatively cheap, where NGS is of a moderate value.

Now the other exciting thing happening in the ddPCR area is panel testing is coming out. So now for instance, for the KRAS mutation at codon 12, you can screen for a variety of players in that area, so that panel. And so now I see as panel testings become more and more mature, one can envision a panel test of maybe 96 targets, where again, with very rapid commercially available instrumentation, you can start doing this for a minimal expense with a very quick turnaround time.

So here’s a slide regarding the accuracy of the NGS platforms in the top table here, so you can see they all have error frequencies that are relatively small but still quite significant when you're looking for the needle in the haystack. And when you're looking for ctDNA, especially with patients that may have very early stage tumors, you're looking for a needle in a haystack and because of that, these tagging techniques are now coming to the commercial market, and they improve the false discovery rate in a very significant manner. So as part of SEQC2, I’m working very closely with Joshua Xu on this, and so we already have access to some of the tagging techniques used by IDT and also used by Rubicon Genomics, and there is now, just the other day I got access to yet another one from Qiagen. So a lot of companies are getting on board because they see the necessity for this, especially in this very exciting area, to reduce the false positive rate in the liquid biopsies.

So we have a precision medicine advanced clinical trial. The disease focus is lung cancer. The novelty involved in this is we’re using a co-clinical trial which means for every patient’s tumor, we build a mouse model, a PDX. We’ll also purchase a GEM -- a

genetically engineered mouse -- if we see a very characteristic tumor present in that, and then the patient and the mouse get treated with the same type of cocktail and also are subjected to a liquid biopsy every 30 days. In the trial, we're using a blending approach of ddPCR and NGS-based approaches for the liquid biopsies, and that's really out of necessity. It helps us stretch the budget a little bit more and also helps us to join in on some of these panel-based approaches which are quite exciting. The project also involves a lot of very advanced bioinformatics and this is being undertaken by the five research universities in the state as well as working with very talented bioinformatics group at the NCTR.

So we chose lung cancer because lung cancer is a killer. It is the number one killer, as most people in this room know, for men and women. So 80 percent of the people who get a non-small cell lung cancer are smokers. Now, if we could find them early enough, we could give them a five-year survival of approximately 70 percent. However, there's no symptoms with early stage disease; there's no pain. We now have these low-dose CT scans which we can scan somebody for about $200, and in a study, the 2011 *New England Journal* study, of those

people scanned -- so these were patients who had more than a 30-pack-year smoking history, so they have significant risk for having lung cancer -- 24 percent of those had a nodule but when they were biopsied, only 4 percent were cancerous, so 96 percent did not have any cancer. And so you have to remember now, a patient, most patients with lung cancer, they're elderly, they have a lot of other comorbidities as well. They have bad lungs, COPD, most of them have heart disease. They've lived tough lives. So as an oncologist, you really have to think closely about biopsying them, especially for our trial where we're asking some patients to undergo a research biopsy.

Now, a lot of times with lung cancer, we can make the diagnosis just by cytology, so that's maybe going down the esophagus or going in with a bronchoscope, just getting a few cells and looking at those cells, you see a mass in the chest or in the mediastinum and then with those cells, you know you can make the biopsy -- you can make the diagnosis. However, you may not be able to genotype it from that. So with a liquid biopsy then, I can look at the blood and get a much better idea of what is their malignancy all about anyway, because lung cancer isn't really four or five diseases; it's hundreds of

diseases. And really, we should be sequencing all these people so that we can treat them much better, especially with some of these very advanced checkpoint inhibitors where there is about a 20 percent survival that's durable for these patients with even advanced lung cancer, but it's hard to figure out. We don't really have a great biomarker yet to figure out who benefits and who does not.

So, lung cancer is also a problem for the State of Arkansas, as many Southern and rural states is, and because of that, there was clinical relevance which dovetailed very nicely into one of the UAMS missions is to have an NCI-designated cancer center in the country here, and so when the external advisory board came in, they said, well you know, what would look great in your application is doing something scientifically interesting and nice regarding your lung cancer problem. So that was another reason why I decided to base the project on lung cancer.

So here is a cartoon of our clinical trial and as I mentioned, there is basically three legs of this stool. Number one is the liquid biopsy, which I have given you some background on. Number two is the co-clinical trial, which I'll explain a little bit more, and

number three is the necessity for very advanced bioinformatics.

So we have here a patient with lung cancer. We get a piece of their tumor. We can take the tumor out and have it grow in a PDX, patient-derived xenograft, so these mice have a modified immune system that allows the tumor to grow, and we would also sequence the patient's tumor by multiple modalities, DNA sequencing, RNA sequencing and methylation analysis through an integrative multi-omic study to really get a good idea of what's going on with that tumor because you know, when you look at the mutation in just the DNA, it doesn't mean that it's expressed in the RNA. And so if you are then going to subject somebody to an expensive targeted therapy, and let's say it's going to cost $10,000 a month for this magic pill and it's not expressed in the RNA, you're kind of wasting your money. And even with a 20% co-pay, that's a lot of money for people. So we can do this and just look at the data a little bit more thoroughly, and we are doing that in this project.

So once we grow out the mice, both either serially or parallel, the mice and the patients are both treated with the same drug cocktails. So in effect, we're using the mice as a test to see what may be beneficial to

the patients. So these co-clinical trials are an area that the NCI wants to move much more definitively in to get better models, and it’s much better than cells in a dish, and to get much more realistic models where we can use them to possibly prognosticate on what therapies may be best for a patient, have a little bit more evidence for that.

Now besides solid tissue analysis, every patient and their mouse models will get a liquid biopsy every month, and then all of that data gets analyzed by the consortium members. So, as I said, solid tumor with extensive molecular profiling, liquid biopsy with circulating tumor DNA analysis, looking at the evolution of the tumor over time as it undergoes treatment, so this is very exciting. And additionally, when I was first thinking of this and working closely with Dr. Wenming Xiao, who spoke earlier, we said from a regulatory science point of view, there’s going to be more and more applications coming in with circulating type DNA. It would be good to look at other groups as well. So we’re also looking at heavy smokers without lung cancer. We’re looking at patients with inflammatory disease, basically hep C and rheumatoid arthritis. We have access to a heart transplant rejection dataset that was done at Stanford,

and then with normal volunteers both before and after cardio level exercise, between 30 to 45 minutes. So all of those different cohorts are part of this clinical trial which is being run out of UAMS.

So, as part of this, I initially simulated the clinical trial using mice and lung cancer cell lines. So in this example here, I used an intratracheal, which is an orthotropic approach to inject approximately one million cells directly into the mouse's lung. So remarkably, you can do this to a mouse and they don't aspirate. They don't die from like a pneumonitis or something like that. So they are introduced directly into the blood, so then we can say that there is no venous or arterial introduction of this malignant material. So we used a classic cell line present at the ATCC, the H460 that has KRAS mutations, PIK3CA and a checkpoint mutation as well.

In this picture here, the mouse has been -- this is day 41 after intratracheal injection -- and the cell line has also been infected with luciferase so that allows us to do optical imaging. So you can see here, in mouse number 1, there is a good growth of the tumor and now one week later at day 48, you see even more progression of disease in mouse number 1 and just the

onset of disease in mouse number 2. Now as part of this experiment, every seven days, besides being imaged, the mice are also having a blood draw. So we can draw out 100 microliters and after we spin it down, we get about roughly 50 microliters of plasma and from that, we can then interrogate that with ddPCR because we know what mutations we're looking for based on the cell line.

So here is the result of the ddPCR where we're looking for the KRAS, the Q61H mutation, and basically we don't see it at all in the beginning but then at day 41, we just start to see that and day 48, we see these copies even more. So all the little blue dots represent a copy of the template for the Q61H piece of DNA. The rest of this slide just has controls on it.

DR. PILLAI: Twenty microliters, is that plasma?

DR. JOHANN: Fifty.

DR. PILLAI: Fifty?

DR. JOHANN: Yes. And we also repeated this for -- I then repeated this for human results. So this is a human who I knew had an EGFR mutation in them, the T790M. I got an extra blood draw on them and again did the ddPCR and then was readily able to find the evidence of this mutation with also good performance of the controls.

This is yet a different sort of optical imaging technique where the material would not require being infected by luciferase, but we can instead look at the metabolic activity using material called DG-750. So this is more akin to like a PET scan where you're looking at the metabolic activity, and tumor tissue is more metabolically active than tissue that is not malignant. And so in this case, this is an actual tumor from a patient. This is patient number 1, she had Stage IV adenocarcinoma. We got a research biopsy on her and was able to implant a very tiny piece of tumor tissue in the subrenal capsule area and then watched it over time. So here’s day 14, day 21, day 28 and you can see from the optical imaging that the tumor is indeed progressing.

So a lot of this has to do with, the liquid biopsy has to do with being able to monitor a nonlinear process. So more complex systems have nonlinear processes and when a patient has a malignancy, it’s not a linear process. So sometimes, we have an opportunity, a limited window of time to really catch something early and to do something much more definitive to really change a clinical outcome. So I believe that the liquid biopsy allows us to do that because it’s a routine blood draw. It doesn’t have the risks associated with it that you

would with regular cancer type biopsies, and it really allows, it really changes the practice of oncology both for disease monitoring and for the whole concept of adjuvant therapy.

So with this study comes a tremendous amount of data and a tremendous amount of complexity with handling this, and the TCGA project has been a very big success. But what's been found of that is that really only a few institutions can really benefit from that because not everyone has the infrastructure to really take advantage of that and the know-how how to get in there.

So we're doing NGS at UAMS and with this project, since UAMS did not have a big infrastructure for data storage for these very advanced servers, I basically proposed, well, let's rent this from Google. So we'll use the Google Cloud. So the Google Cloud is HIPAA-compliant and UAMS entered into a business associates' agreement with Google and now we get instant infrastructure, and also the people behind it to handle this infrastructure. Because again, just buying all this equipment and you have this problem that's going on in the life sciences where the IT's ability to keep up with the data generation that's coming out of these advanced high-throughput techniques is just not working. So I didn't

want to be at one of these administrative meetings where you have to explain, well, we spent millions and millions of dollars and now you're telling me our hardware infrastructure can’t handle the latest sequencer, which is now a fraction of the price of the one we bought two or three years ago. So our business model here is, UAMS, is healthcare and research, not computing or data storage. So it just makes more sense. And the NCI realizes this and so what's being forth now by Lou Staudt and Warren Kibbe is really the Cancer Genomics Commons where you're bringing the data to the investigator, it’s up there and using cloud-based techniques, you can get in there and apply your pipeline.

We've also established a working relationship with the Institute for Systems Biology on this project so that this institution is one of the three NCI Cancer Genomics Cloud providers along with Broad Institute and Seven Genomic and -- the company up in Cambridge, Seven Bridges. And this allows us to talk to them and also get access to maybe some of their more advanced software early on that they're being funded by the NCI to provide to the community.

So here is a cartoon of, from a computational standpoint, what things look like. So the sequencing is

done at UAMS, both for the patients and their mouse models. That data is then uploaded into the cloud and then the different team members can sign into the cloud, get access to the data, run it through either the best practice pipelines that we follow or experimental pipelines, and be able to do that all in the cloud and then keep that in a cloud repository. So we're allowing easy access to the data, we're allowing innovation for the bioinformatics as well as comparing it to some of the best practice pipelines as well.

And this is just a quick cartoon about some of the levels of bioinformatics that the ISB is bringing to the table through their funded Cancer Genomics Cloud initiative.

So precision medicine really envisions a knowledge network of disease, and this is a graphic from a study that was put forth by the National Academy of Sciences back in 2011, which really calls for a new taxonomy based on molecular medicine to study disease now that a lot of the technologies are ripe enough, and that cancer should really proceed first. So what this involves is not just the molecular data but integrating that into the EHR and all of the other data that we know about a patient so that we can really have a much richer type

description, and also a much better understanding of some of these cases that we now call exceptional responders where somebody had an advanced malignancy, they were sequenced, somebody saw an interesting mutation, they tried a drug on it and the patient had a remarkable response. And we see about, we read about these all the time, and they're prevalent in lung cancer certainly. We don’t fully understand them but with these individual cases, as we start looking for these rarer type mutations and getting more and more of a knowledge base based on that, we’ll get more and more accurate on how to apply that and bring these better type therapies to patients much quicker.

So the oncology precision medicine is really a puzzle. It requires multidisciplinary group efforts, and we have that with this project. We have molecular profiling and the handling of big data. We have a very advanced clinical trial using animal models, the complex bioinformatics with the consortium members, molecular assay developments, high-performance computing.

So in summary, we really anticipate liquid biopsies as a cornerstone of precision medicine. As I mentioned, it’s been the holy grail of oncology, especially for disease monitoring. Screening is a much

more difficult problem because you need tremendous sensitivity and specificity. It's a much harder problem but certainly right now for disease monitoring and looking for resistance mutations, or trying to genotype a patient from the blood when it's just not practical to get solid tissue, those things are ready to move forward.

The NGS-based liquid biopsies are an application of deep sequencing and because of that, we're working very closely with Joshua Xu for the SEQC2 project because of the high false positive rate, so we can remedy this with a DNA tagging approach and these approaches are coming now commercially.

The ddPCR, as I showed you, is much quicker. It's less expensive but it's not discovery-based. We want to do no harm. This is a very new and exciting technology, but a bad test is just as dangerous as a bad drug, and that's a quote from Dan Hayes, who is the ASCO President.

The liquid biopsy is very efficacious, especially when tissue is impractical. It also avoids the spatial heterogeneity issues, especially when a patient has a primary tumor with multiple metastatic sites. We know from the *New England* paper a few years ago that the molecular profiling from all these different sites can be

quite different. So you can think of the blood as a reservoir where all the pieces of the tumor are contributing to that, and we can get a much more complete description of it because of that. It also allows for more frequent monitoring to assess efficacy of therapy and early signs of resistance.

As I mentioned, the cobas assay is now approved for the T790M from the FDA and also starts to change the concept of what we think about regarding adjuvant therapy. You can also, in the future, see this as being a synergistic assay, especially combining this with imaging. So we try to screen people who have high risk. Somebody may have a nodule. The other thing that’s going on in medical imaging is every year, the techniques become more and more sensitive, but now you have the specificity problem. So teaming that in with a blood-based diagnostic could help to remedy that to a degree.

We’re using the Google Cloud for data sharing and collaboration. This involves the five research universities plus the NCTR. It’s HIPAA-compliant; we have a BAA in place. It gives us tremendous scalability and it’s also very financially attractive. It’s the taxi cab model; you only pay for what you use. If you're not using

it, you don’t get a bill. So with us, we see very episodic usages with that as we’re…

And I showed you a very advanced clinical trial that incorporates the liquid biopsy, a co-clinical trial approach and then also the necessity for the very advanced bioinformatics.

And this is certainly not all of my work by any ways, and the AR-BIC is really the brainchild of Weida Tong, and a lot of its success -- I would say most of its success -- is really due to Weida, and it’s been a real pleasure for me to be able to work with the computational scientists here, Wenming Xiao, Joshua Xu and Dr. Hong as well as the other investigators at the other research universities, and there is also a wide array of people at UAMS involved with writing the clinical trial, getting it through the IRB, getting the samples, all the patient care work. So there’s many hands involved in this. And I’d be very happy to take any questions. Thank you.

DR. PHILBERT: Questions for Dr. Johann? The floor is open. Yes.

DR. LANZA: Is this working? Okay. That was great.

DR. PHILBERT: Who are you, Greg?

DR. LANZA: Greg Lanza at WashU. The two questions I have, they are very short. In terms of the overarching, not just lung cancer or anything, but any pathology that you would know the genetic mutation for and that it's a progressive pathology like let me just give you one that's off the top of my head: hypertrophic cardiomyopathy. You know this. It's going to be progressive over time. Would you be able to use then ddPCR in that case? If you have a characteristic mutation, known mutation?

DR. JOHANN: Yes, so I was involved in some -- a number of years ago while still at Bethesda with a KRAS-based mouse model for OCUM.

DR. LANZA: Okay. The other question I had was you had the nonlinear curve.

DR. JOHANN: Yes.

DR. LANZA: And as I go through this, I'm trying to figure out how to quantify the signal and how to normalize it for individual variation, whether covariate or whatever you would do. What would your recommendation be to try to normalize the signal so you could look at relative rates of progression in individual animals so that you can then compare the heterogeneity of the population and the differential response to treatment?

DR. JOHANN: So the quick answer is more frequent monitoring. I just showed that to show that the window of opportunity, sometimes you can be fooled by when you start -- when the curve all of a sudden takes off like an exponential process. You could be fooled there, and it's only by more frequent monitoring that you would be able to catch it where you could do something definitively to change it. You would have to catch it very early.

DR. LANZA: No, I get that point. So the question I have though is I assume it is nonlinear.

DR. JOHANN: Yes.

DR. LANZA: Is there a way to normalize the data or you just take the raw number, whatever you compute. Like in PCR, how many cycles is something?

DR. JOHANN: Another way, so that you can look for it in an animal serially and get that curve, a progression of disease. So we're looking at our mice serially over time, as I showed you there. I just showed you a few snapshots of basically where the action is. I guess that's something really, that's something that I have kind of under study along with just trying to understand a little bit better the actual mechanisms

involved in ctDNA being put out into the blood. I mean, yes. So it’s more fundamental stuff.

DR. PAULE: Merle Paule, NCTR.

DR. JOHANN: Yes.

DR. PAULE: I see things are cleared renally, so are they detectable in urine?

DR. JOHANN: Yes. They should be, sure.

DR. PAULE: So why not just take urine samples instead of blood samples?

DR. JOHANN: I think that the urine, like most nephrologists know, the urine varies a lot. You can kind of dial a patient’s urine a little bit sometimes, like usually the first in the morning urine is, you know -- I’m not a nephrologist but I just remember that they always wanted the first in the morning urine. The blood, I don’t know, I guess we would have to maybe go to a clinical trial and see what's better. Some, certainly you would think bladder cancer should be great for that. It is cleared -- I know that people looking at all sorts of body fluids using ddPCR to find small pieces of DNA with somatic mutations in it, but honestly, I don’t know enough of why isn’t urine being used.

DR. PAULE: Because it seems like it might be easier for your animal models and so forth to get samples.

DR. JOHANN: Well, it’s hard to get a Foley in a mouse.

DR. PAULE: But you can collect urine from the mouse.

DR. JOHANN: Yes.

DR. PHILBERT: Yes, put him in a metabolic chamber.

DR. JOHANN: I just haven’t done that.

DR. PHILBERT: Suresh.

DR. PILLAI: Suresh Pillai. I have a question. In terms of the circulating free DNA, is the size always specific to so many bases long or either base pairs, or is there variability in that? Because when I look at it, because you’ve done a lot of work on virus transport in the subsurface or in distribution lines, when you sample anything, it’s never going to be -- it’s going to be sort of a very stochastic type process because it binds to edges, etc. So this S-curve that you're seeing, is that a function of its transport behavior in the vessels rather than actually disease manifestation?

DR. JOHANN: What we're finding is that the size of it, just like the literature says, seems to be, for the most part, between 120 to 180, and it's really the smear that we see by the fragment analyzer or something like that in that area that gives us a lot of confidence that moving forward with the assay is going to find something. So that's what empirically we're seeing, and I know other investigators, like the people at Hopkins who I've spoken with at conferences, what they are seeing as well. So we rely on these smears, where we look at the fragments of these molecules from the blood and it gives us the relative abundance of the different sizes of the molecule in the specimen.

DR. PHILBERT: Are there any other questions? If not, then it just remains for me to thank you very much for a very interesting and engaging presentation.

Agenda Item: NCTR Division Directors: Overview of Research Activities

Agenda Item: Division of Microbiology

DR. PHILBERT: Okay, ladies and gentlemen, it's my job to interrupt useful and interesting conversations. There we go. All right, Carl, thank you. Oh, sorry, Steve.

DR. FOLEY: All right. No, that's all right.

DR. PHILBERT: Brain damage.

DR. FOLEY: It says Carl up there. So good afternoon, everybody, and thank you for the opportunity to come and discuss and give an update on the Division of Microbiology. I am Steve Foley and I will be giving the talk today for Dr. Cerniglia, who is travelling.

So the Division of Microbiology, our mission is to serve a multipurpose function with specialized expertise to perform fundamental and applied research in microbiology in areas of FDA’s responsibility in toxicology and regulatory science. So I hope that as we go through the presentation this afternoon that you'll see that we are striving to meet this mission. We've got a diverse group of scientists working in areas from virology to bacteriology to mycology as well. Our vision then is to be a valued resource for advancing regulatory science research in microbiology for FDA.

So in order for us to meet our mission and our vision, to be successful, we are working in a number of areas to help to contribute to the FDA’s guidelines and regulations, and so we’re working to try to do this better. Some of the things that we’re doing is trying to understand the regulatory process in order to identify issues that are important to the FDA and our regulatory

centers, and then integrate our resource then into the programs within kind of the FDA infrastructure by coordinating and working with these other centers, and then also to contribute to NCTR’s and the wider FDA mission that Dr. Slikker talked about earlier today.

To be successful, we want to also enhance our FDA research and our interactions, and to do that, one of the things that we’re doing is trying to assess the needs of the FDA serving on different working groups or reaching out to center colleagues, working through Donna Mendrick’s office in there to try to assess the needs and then to conduct research then that’s critical to FDA’s regulatory mission. And through this, we've been trying to expand our collaborative relationships and then trying to build upon what we’ve done to foster future interactions as well too -- the old having your ear to the rail, if you will, to see what's coming and where there is a need.

Within our program, we are also trying to strengthen our research program management by focusing our research on priority areas for the FDA, maybe moving away from some of those that have less relevance in there and focusing on those where there is more regulatory impact. We’re looking at and we've been establishing

benchmarks for scientific excellence and making sure that our investigators have active protocols, they're making good progress on those protocols, presenting data at conferences and publishing either in impactful journals or providing technical reports to the centers in there, and communicating that in an effective way, using plain language, is one way.

One of the things too that we're constantly looking at is our facilities and trying to make sure we're utilizing those maximally and then looking at areas where we can improve with equipment or other sorts of things to help address the needs.

Our division staff, there's approximately 40 members that are in the division currently. That includes 28 fulltime employees, including 19 that are research scientists or staff fellows, or those that we would consider principal investigators for projects. We've got four support scientists that are helping with research. We've got four on the administrative side that are helping with purchasing and HR and those types of things to make sure our division runs smoothly -- very vital contributors to the division. We've got one FDA Commissioner's fellow and then ten ORISE either postdocs

or graduate students, and two visiting scientists right now from a couple of different countries.

Our outreach, our investigators and the people in the division have been very active in trying to establish those collaborations. Right now, we've got collaborations with each of the different FDA centers, and we'll look at a lot of these projects in the next few minutes, and there we've got either ongoing or planned work with each of the different research divisions as well. To some of our more recent successes that we'll talk about, we've gotten funding from the National Toxicology Program to carry out microbiome analyses. We've been working with other federal partners, USDA and CDC as well as on the state level, the Arkansas Health Department. We've got several collaborations with different universities within the state, with UAMS, the University of Arkansas at Little Rock up in Fayetteville at the university, I think some up at Jonesboro as well, and then with universities across the nation too, and internationally where we can leverage resources and help to address things that are important to public health. On our global and national outreach, we've got Dr. Cerniglia for example serving on WHO committees on food additives and pesticide residue, people in the division that are on

science advisory boards, several on journal editorial boards. We've got investigators who have served on USDA annals for example, looking at ARS research priorities and research programs with EPA and NOAA, the Microbiome Interagency Working Group. We've got our visiting scientist program where we have had some international scientists who have come in and have worked in the division. They've learned stuff and we've gained very good quality research from the work that they've been doing. And a number of our investigators are serving on FDA-wide committees in areas like antimicrobial resistance or the Institution of Biosafety Committee, different working groups within the Agency on Microbiome with the Office of Foods and Veterinary Medicine. So these, especially these FDA committees and working groups, have helped us to understand some of the needs of the agency and are helping shape some of our research.

So our research areas currently, they fall into areas related to the microbiome, especially looking at the interactions between the microbiome and things like antimicrobials or food contaminants, food additives or supplements, and other FDA-regulated products. Another area is in the development of methods to detect and characterize foodborne pathogens and other pathogens.

We've got work again in virology with norovirus, so foodborne viruses and bacteria like salmonella, *E. coli* and other pathogens, listeria as well. On the antimicrobial, we've got work going on antimicrobial resistance and virulence mechanisms. A lot of that work is being done with the Center for Veterinary Medicine and looking at kind of that interplay of antimicrobial resistance and virulence in these organisms. And then research groups that are working to try to improve risk assessments of priority of pollutants. These include the polycyclic aromatic hydrocarbons and different drugs that may be in the environment or other products. And utilizing kind of systems biology approaches, taking multi-omics approaches to answer or try to understand these priority pollutants and their degradation and things of that nature.

And then a fifth area is conducting research that is sponsored by other parts of the agency, addressing needs in women’s health, with tobacco products and nanotechnology.

When asked to look at the top accomplishments for the division, this was a challenging thing and when Dr. Cerniglia and I were talking about that, we came up with these three.

And one is getting the funding from the National Toxicology Program and approval to begin conducting host microbiome assessments to evaluate the impact of different compounds on the gastrointestinal microbiome as well as immune response. And then helping to begin to set up standardized approaches for microbiome analysis and data analysis associated with that for the NCTR -- excuse me, for the NTP program -- to help in risk assessments.

The second area is the development of methods to characterize *Burkholderia cepacia* complex in pharmaceutical products. There has been a number of recent outbreaks associated with contaminated drug products with this organism or related organisms, a big problem with immunocompromised individuals, causing illnesses and as we'll see in a couple of slides, those organisms are a very fastidious bug.

And then a third area is in the determination of microbial populations within smokeless tobacco products and then beginning to assess the impact that these organisms may have on the formation of carcinogenic tobacco-specific nitrosamines.

So on the NTP-funded studies, NTP has been conducting studies for a long period of time, as others

have talked about, assessing critical knowledge gaps in toxicology testing risk assessments, and these have included acute or chronic exposures to different test compounds and then trying to extrapolate these experimental models into human exposure risk assessment.

And one of the areas that hasn't been included in this has been the microbiome, either the toxic effects in changing the microbiome or how the microbiome may impact the different compounds that came in. Dr. Beland had mentioned earlier about some of the compounds that had been biotransformed by the microbiome. So beginning to work in this area is something that is important.

And so these studies looking at a number of compounds that are being looked at by either other divisions here at NCTR or at NIEHS -- triclosan, arsenic, silver nanoparticles and aloe vera -- and seeing, again, how they impact the GI microbiome as well as the immune response. And then also very important for the NTP going forward is to kind of set up some standardized approaches on how to handle the microbiome data both from the sample collection portion and then the methodologies for the analyses. And then once you get that sequencing data, that's kind of the first step, and then the analyses of that data is very important in trying to develop some

standardized approaches to do that, and then where to store that data and extract it, I think the previous speaker talking about how they're using the Google Cloud because of the ability to store those huge datasets highlights the need to look at ways to handle these.

On the *Burkholderia* work, again there's been several outbreaks, some fairly recently, that have been associated with the contamination of these pharmaceutical products, and these *Burkholderia* are kind of fastidious. They're not really easy to grow in the methods that have been published by USP don’t work very well, and so our investigators, Dr. Ahn and other, have been working to look at new methods for culturing as well as developing some molecular methods to detect these organisms in pharmaceutical products.

One other challenge with these organisms is that they tend to be able to grow in disinfectants at relatively high concentrations, things like benzalkonium chloride and chlorhexidine, and so some of this work is providing data on the susceptibility towards these antiseptics and then showing that there is key importance to making sure that the concentrations are correct in these pharmaceutical products to keep these bacteria at bay.

On our smokeless tobacco work, that’s some work that’s being done in our laboratory, we've been using culture-based and next-generation sequence-based methods to look at the microbial populations of both bacteria and fungi that are present in smokeless tobacco. In some of our work that was recently published, we found that the bacterial species identified in a number of the products may serve as opportunistic pathogens. But also concerning was that a number of these species were able to reuse nitrates and nitrites, which may provide precursors along the pathway to form these tobacco-specific nitrosamines as reactions to the nitrosation of nicotine to form these carcinogens. And so there's been some studies that have shown as product has been stored that the carcinogens go up in those products, and so we’re trying to see if the microorganisms contribute to that.

So in addition to these accomplishments, we've got a number of additional projects that are going on that are at various stages of completion and again, wanted to highlight that we’re working with quite a few of the different centers within the agency. Some of those with the Center for Veterinary Medicine involve looking at residue levels of antimicrobials that consumers may be exposed to from residues on meats or other things. And

then looking do these residue levels of antimicrobials impact the microbiome, or do they lead to resistance development in the intestinal tract.

Another project that we're working on is looking at plasmids where these extrachromosomal DNA molecules that are present in salmonella and related bacteria, and looking at their contribution to antimicrobial resistance and virulence. We're working more heavily on the virulence piece here, the Center for Veterinary Medicine colleagues on the resistance side, and trying to assess how readily these plasmids that contain resistance and/or virulence genes transfer from one bacterium to another.

So we have several projects that have been in the nanotechnology area, looking at nanoparticles or nanodrugs and seeing how they impact intestinal microbiota or impact the immune function. A recent project that CFSAN has asked us to work on is looking at microbial contamination of tattoo inks. There's been a number of outbreaks associated with individuals who have gotten tattoos and getting infections. One of the key ones are the mycobacteria.

Some projects related to *Clostridium difficile*. *C. diff* infections have been a real challenge for people

who have been on antimicrobial therapy and causing significant morbidity and mortality, and we've got a couple of investigators who are beginning to look at the fecal transplant type areas, and then a group along with colleagues at CDRH looking at the development of some molecular methods to serve as a reference method for tests that come in for evaluation by CDRH.

And the seventh project is a project that had been funded by OWH looking at the development of an *in vitro* vaginal tract model to look at the probiotic potential of lactobacillus strains towards toxic shock syndrome, toxin 1-producing strains of *Staph aureus*.

And so we'll look at each of these briefly and kind of give a highlight of where they're at.

So Dr. Khare and Dr. Cerniglia in our division, they've been working on this project looking at or trying to evaluate whether the ingestion of residue levels or low levels of different antimicrobials can lead to -- are trying to determine is there a shift in the microbiota populations within the GI tract. Do we see the selection of antimicrobial-resistant bacteria? Do the bacteria in the GI tract degrade or inactivate the drugs that are taken in? Dr. Cerniglia and people in the division had done earlier studies where they looked at

fluoroquinolones, an important class of drugs, in trying to set up -- that help to kind of feed in some international guidelines and Center for Veterinary Medicine guidelines trying to evaluate the safety of the residues of veterinary drugs in human foods. And the data that they generated then helped to establish this approach for generating a microbiological acceptable daily intake.

Even with this work, there were some data gaps that were shown and so they, along with the Center for Veterinary Medicine, have been trying to establish or have begun some studies looking at tetracycline and are planning to look at erythromycin as well too to see what their impact is on the GI microbiota.

On our plasmid work, we've been utilizing DNA sequencing, whole genome sequencing or plasmid sequencing, and then *in vitro* assessments to characterize virulence in these antimicrobial-resistant strains of salmonella that we have or we've acquired that center from CVM. The plasmids that are in a number of these strains allow for the transfer of antimicrobial resistance and/or virulence associated genes.

What we saw -- and this kind of builds on some of the work that was done in Dr. Pillai's lab a number of

years ago -- that exposure to certain antimicrobials can impact the plasmid transfer dynamics, that we saw as you increased concentration of antibiotics in certain strains and certain antibiotics, that you saw an increase in the rate of transfer of these plasmids from the resistant strain to the susceptible strain, and they're transferring their resistance. And when we looked at the plasmids that were those that encoded antimicrobial resistance transferring, but those that also encoded for virulence or increased pathogenicity were transferring too. So you may have a single transfer event where you're getting organisms that are both more drug-resistant but also more pathogenic, and which is a concern. It's these types of data that we've been working with the Office of New Animal Drugs Evaluation at CVM to help them understand and evaluate the risk of antimicrobial use.

We've had, on the nanoparticle stuff, research projects that we've been working on. These have involved looking at the impact of different types of nanoparticles on the GI microbiota, trying to see are there shifts in the bacterial populations in there. There has been, we've done both *in vitro* and *vivo* studies, and then some of this work, in addition to changing the microbiota, have been looking at the impacts on the permeability of the

intestinal barrier, so how readily do those nanoparticles get outside of the intestine into other areas of the body. Work done by Dr. Saeed Khan looked at bioaccumulation of these nanoparticles and macrophages in the spleen and tried to see whether there was an impact on or an impairment to clear listeria infection. And *in vitro* systems, it appeared that was impairment, in culture, but when they went to the rat models, there didn’t seem to be that impairment that they saw in culture.

Dr. [013-23.25], who is in the back there, has also done work with nanodrugs, looking at the impact on absorption through the intestinal epithelial barrier. And then this work too has then looked at the immune response as well by looking at pro-inflammatory and anti-inflammatory genes.

Our tattooing work, approximately a quarter of 18- to 50-year-olds in the US have at least one tattoo now. These pathogenic mycobacteria have been reported causing illnesses following tattooing, and a number of studies both at CFSAN and CDC and others have found that these infections, a key pathogen has been *Mycobacterium chelonae* and related species.

And so what CFSAN has asked us to do is to look at different tattoo inks. NCTR had done some toxicology testing on tattoo inks some years back, and so those tattoo inks have been stored and then looking at okay, are these organisms present or were they present in these tattoo inks, and then looking at a number of other tattoo inks that are on the commercial market to look for microbial contamination.

These organisms are also somewhat pesky to grow and so our group then is also working on and working with some folks at ORA to look at improved methods for detecting these organisms in tattoo ink.

The lactobacillus work that Dr. Mark Hart's group is doing, they've developed and tested, or developed, the in vitro vaginal tract model to help assess potential of naturally occurring lactobacillus strains, to inhibit toxic shock syndrome toxin-1 producing strains -- that's a mouthful -- of *Staph aureus*. And to do that, they've developed a defined media that supports both the growth of the lactobacillus as well as the clinical strains of *Staph aureus*.

And more recently, they have developed a strain in the lab where you've got lactobacillus that are able

to express and produce lysostaphin, which has been shown to inhibit *Staph aureus* growth.

And some of their other work in this area has been looking at the ability of *Staph aureus* to grow and produce toxins in different types of tampons that are on the market, and there they are supposed to not be able to support the growth of these organisms but some of the work that they are finding in the lab seems to show that they are able to, and they're working on trying to look at that some more.

On the *C. difficile* work, we were asked by CDRH to look at developing a molecular-based reference method that can be used by sponsors who are coming to CDRH with molecular methods that they want to have verified for use in detecting *C. diff*. The gold standard right now is a toxigenic culture method which is a laborious method; it takes quite a bit of time and from what I understand is kind of a pain to do, and so the sponsors have come and said, well, is there a molecular method that we can compare to. And so we're working with CDRH to help develop this composite molecular method, then we'll look at how well that performs versus the toxigenic culture method that's the accepted reference method, as well as some previously FDA-cleared methods, and then to see if

this composite method that's being developed can serve as that reference method.

In addition, a lot of the sponsors are using frozen samples. They'll collect fecal samples and then they'll freeze those back. So there is a concern that freezing may impact the viability, so another part of this study is to look at does freezing impact the viability of these, either the vegetative cells or the spores in clinical specimens, that may impact testing results.

On the fecal transplant work, we've got one study that was recently approved and then another one that's working its way through the development system. And this probably, instead of immune response following fecal transplant, it probably should be immune response associated with fecal transplantation in there. The work that Dr. Wagner is doing is trying to understand how the commensal intestinal bacteria modulate the immune response in response to the *C. diff* infections as well as when the *Clostridium difficile* is there with representative members of the microbiome and in culture and also rodent modules.

And the hope of this work then is that it will provide insight for the regulation of FMTs as well as

potentially provide alternative therapies than the fecal microbiota transplant, which is able to be utilized on enforcement discretion -- is that the right term for it? Enforcement discretion in there.

And then Dr. Bruce Erickson is working on a project with collaborators CBER to look at potential for pathogens in fecal transplant, developing methods to better detect pathogens in there or if there's *C. diff* in the specimen that's associated with fecal transplantation, some of the issues surrounding that. That's in the development stage.

And anyway, the goal of this is to try to better understand the factors surrounding fecal transplantation and making sure that it's as safe as possible, and again just helping to provide some data on that issue with fecal transplantation.

So our future directions for the division, we want to look at increasing our capacity and resources to conduct research on the microbiome, and it's been an exploding area and there's likely to be a lot of microbiome-associated kind of work coming towards the FDA and if we can help with that, we want to continue to help in those areas. We want to advance our scientific approaches to look at how different chemical or microbial

contaminants impact the microbiome. Continue to work on improving the risk assessments of human and veterinary drugs and priority pollutants through the integration of different systems biology approaches in our research. And utilizing some of the newer tools that are coming out to refine this research. We want to continue to work with the Center for Tobacco Products to help them with research initiatives and provide data that can help with the regulation of tobacco projects work. Continue to develop projects in the nanotechnology area in collaboration with the Nanocore as well as the FDA Regulatory Centers. Build upon some of our previously funded work with OWH to help address knowledge gaps and then address some of the new initiatives that OWH puts forward. And then we want to identify opportunities to leverage work that we're doing with other partners within the FDA as well as other federal, state and international regulatory and public health agencies as well as academia and industry, where it provides value.

So overall, the division is diverse in its expertise. We've got a well-trained group of microbiologists in different areas that we can assemble to meet the microbiological needs of the FDA. We are continuing to try to reach out to our stakeholders and

develop research projects that help to address the needs of FDA.

And one of the things that we've been doing over the last few years is to look at where the biggest needs are within the FDA centers. There are some centers where there may be plenty of microbiologists where they don't need a lot of help from us, where there are others where there's probably more pressing needs, and so we want to continue to look at those areas where there's a need and move to that. I think we have the flexibility to do that.

So as far as feedback, when we were talking about this earlier in discussions before the meeting, was as a division, are we addressing the needs of the FDA centers. Are there, or what emerging sciences and technologies can you advise our division to pursue. For example, on the microbiome, is there a need for more work in the microbiome area as it relates to regulatory science? Also, how can we do a better job engaging the centers to learn about their needs, and what future areas do you recommend for the division?

DR. PHILBERT: Thank you. Are there any questions or comments? We've got five minutes. Suresh and then Katrina. Or Katrina then Suresh.

DR. WATERS: So this is Katrina Waters. I have a question about your NTP collaboration on the microbiomes with the chemicals.

DR. FOLEY: Yes.

DR. WATERS: So is that focused mostly on looking at changes in the microbiome itself or is there a pharmacokinetic component where you would look at change in the metabolism of the drugs?

DR. FOLEY: Right now, as I understand, that's primarily looking at changes in the microbiome. So what we're doing is building upon studies that are already ongoing within -- and then just building that microbiome assessment component on top of those to see are there, yes, are there changes in the microbiome in populations that are associated with those that may develop, or where you may see toxicity or carcinogenicity or not. Sangeeta, is that -- you're probably better able to address that, since they are the ones who are working in that area.

DR. YAVAS: They are also doing microbiomes changes as well as the metabolism of the xenobiotic compounds, as well as the compounds they are using. We have a collaborative project to try to see the pharmacokinetics, pharmacodynamics and as well as metabolism, and we are doing both.

DR. PHILBERT: And your name please?

DR. YAVAS: Gokhan. I am Gokhan from NCTR, from the Division of Microbiology.

DR. PILLAI: Suresh Pillai. I have a question. I noticed that CFSAN is not here but very recently, norovirus has been, you can now culture norovirus in the laboratory and I know you have a good virology group here, as well as *Toxoplasma gondii*, which are two major pathogens of concern to the US population. Is there any work anticipated in those two areas, that norovirus and toxoplasma?

DR. FOLEY: Yes, we are doing some work in norovirus, and in some of that, we're looking at -- one of the projects is looking at the survival of norovirus in the environment, and then other is looking at kind of coinfection of norovirus and other foodborne pathogens. Because I was surprised when I looked at the literature at the -- it's about 20 percent of norovirus infections have another, where you can also culture out another bacterial foodborne pathogen, salmonella or other things. And so one of the work that the norovirus group -- and my lab has collaborated on this a little bit -- is to look at that coinfection as well too, to see what is the impact of having -- why do you see such a high,

relatively high percentage of cases where you see that coinfection. So those were the two areas.

DR. PILLAI: What about *Toxoplasma gondii*?

DR. FOLEY: We are not working in that at the moment.

DR. PHILBERT: Is there a kind of term of art than fecal transplant?

DR. FOLEY: Re-poopulate? I don't know. That's a coined term by…

DR. PHILBERT: I know.

DR. FOLEY: By a group in Canada, and I think that's what they call it, re-poopulate, so.

DR. PHILBERT: So who regulates microbes in things like active cultures of yogurt? Is it just CFSAN or is it…?

DR. WILSON: It depends on -- it's Carolyn Wilson with CBER. It depends on whether it's just nutritional, like if it's in yogurt and there's no health claims then it would be CFSAN, but if there is a health claim then Center for Biologics regulates it.

DR. PHILBERT: All right, thank you very much.

DR. FOLEY: Thank you.

DR. PHILBERT: And our next presentation is by Dr. Merle Paule, Division of Neurotox.

Agenda Item: Division of Neurotoxicology

DR. PAULE: Well, good afternoon, everyone. Thank you for the opportunity to come and speak to you today a little bit about what's going on in the Division of Neurotoxicology.

Our division staff consists of 39 full time employees as PIs -- or excuse me, 39 full time government employees, 20 of which are PIs or PhDs, 16 support scientists, 2 administrative. We currently have one FDA Commissioner’s fellow and seven postdoc graduate students and so forth, so our total is 46 FTEs.

Like other divisions, our mission is to develop and validate quantitative markers that can identify biological pathways associated with the expression of neurotoxicity, employing fundamental research efforts in several focal areas designed to broadly examine the involvement of a variety of systems, are used in this particular effort. We tend to focus on the N-methyl-D-aspartic acid and gamma-aminobutyric acid receptor complexes as mediators of adult and developmental neurotoxicity, primarily because of their involvement with the toxicity associated with the general anesthetics and the cytotoxins. These systems are incredibly

important in synaptogenesis, all sorts of development aspects of neuronal outgrowth and so forth.

We also do a lot of work in the monoamine neurotransmitter systems because they are heavily involved in drugs of abuse, and also in affective and movement disorders such as Parkinson's disease. We tend to believe that the mitochondria are really key for just about all the toxicity we observe, so they sort of serve as quite a common pathway in a lot of the toxicities, and not just neurotoxicity. So we look at a lot of mitochondrial dysfunction and oxidative stress.

And then lastly, we are starting to look at beta-amyloid [analysis in nucleic 2.03] navigation in the expression of neurotoxicity in animal models of Alzheimer's disease and Parkinson's disease.

Get this right. So the approaches that we use are varied. We use cell culture, in terms of primary cell types, organotypic and neural stem cells. We use both rodent and human neural stem cells. We're developing nonhuman primate sources as we speak. We tend to look at the health and development and differentiation of these cells and culture. We primarily use those for mechanistic studies and then something that I'm going to be talking a little bit more about, we're starting to develop organ-

on-a-chip with respect to blood-brain barrier models and how it might be useful to inform what goes on during traumatic brain injury.

Our whole animal work involves zebrafish, rodents and nonhuman primates. Zebrafish are wonderful creatures, particularly with respect to development aspects, because they’re see-through. They are transgenic zebrafish that have glow-in-the-dark neurons, glow-in-the-dark other systems so it’s very nice and very easy to quantitate the effects of treatment on these animals as they develop. We then move to rodents in terms of a lot of survey studies, before we move on to nonhuman primates. A lot of our work involves looking at the morphological aspects of these creatures both using light and confocal microscopy, and of course when we start looking at neuropathological endpoints, we use light fluorescence and confocal microscopy, PET/CT approaches, MRI and so forth.

We also spend a fair amount of time looking at functional aspects of organisms. So we've done some nerve conduction velocity studies. We do a lot with behavioral, whether it’s functional assessment both in trained and untrained animals. Untrained animals, we use observational or ethological observations, typically

referred to as non-operant behavior. And then trained or operant behavior allows us to train animals to specifically show or exhibit certain particular aspects of brain function such as cognition and executive function.

We have a fairly substantial effort in humans in that we are trying to utilize the same instruments that we utilize in our laboratory animals in terms of functional assessments in human situations, primarily in children. So we have what we call an NCTR Operant Test Battery. It’s a series of games that our animals and children play to, again, demonstrate specific functions that I’ll mention a little bit later. Currently, we’re doing validation studies, how does children’s performance compare to nonhuman primate performance.

We’re looking at population characteristics in performance of this particular battery. That is, do kids with ADHD, anxiety and depression perform differently than kids that don’t have those disorders -- and the answer, as you might guess, is yes. We've looked at drug effects on brain function in children, in particularly kids that have attention deficit disorder, on and off methylphenidate, demonstrating that methylphenidate in fact normalizes behavior in children with ADHD.

And we have a series of clinical collaborations, one of which is ongoing at the Mayo Clinic, in which we're looking at children that have been exposed or anesthetized with general anesthetics during what we think is a critical period of susceptibility before the age of one. They are using the NCTR Operant Test Battery in that population as well, and then we have another lab at, actually, in Mexico City that's operated by Mount Sinai, in which we're looking at a cohort with kids that have been exposed to relatively high doses of lead exposure.

In terms of outreach, we have a lot of collaborations within and without the center. In NCTR, we are collaborating with Systems Biology in a very exciting study looking at matrix-assisted laser desorption/ionization mass spectrometry brain imaging. Now, I think you're going to be hearing a lot more about this because it is very cool stuff. You're doing, basically, imaging brain slices with this technique such that you can look at specific neural transmitters, take the same slice of the brain, look at dopamine, look at serotonin, look at other neural transmitters or neurochemicals in a very sophisticated and a quantitative

fashion. So this I think is a big deal coming down the pipe.

We collaborate with our bioinformatics and biostatistics group. We have an ongoing study sponsored by HESI in which we're trying to look at the holy grail of the biomarker of neurotoxicity by giving a prototypic neurotoxin -- in this case, trimethyltin -- and then farming out all the tissue to a variety of consortium members to look to see if there is in fact a fingerprint of neurotoxicity. So these studies are ongoing and Syed Imam has been coordinating that effort on behalf of the division. But, it generates a lot of data and we need to use our bioinformatics people to help us sort through that and make some sense of it.

Biochemical toxicology, we coordinate with them on studies of arsenic. You've heard Fred talked about those earlier. In our particular case, we're going to be looking more at functional assessments in the animals that have been exposed to arsenic.

In terms of other FDA Regulatory Centers, we have a longstanding collaboration with CDER with respect to our pediatric anesthetic studies, probably fifteen years or so now we've been working on those studies in collaboration with that group. With that same group,

we've also been looking at MRI imaging and biomarkers of neurotoxicity. That’s a project I’m going to be talking more about later.

And then most recently, we've been asked by them to look at the issue of gadolinium retention using MRI. So gadolinium contrast agents are used frequently, and it turns out that these things, depending upon whether they're linear or nonlinear in terms of chelation, tend to accumulate in brain. So nobody thinks that’s a good thing but nobody really knows how bad it is or if it’s bad at all. So we’re starting to do some studies and looking at time course, duration, location and also eventually some functional correlations with deposition of these different contrast agents.

And then a number of us in the division sit on the neurotoxicity assessment subcommittee at CDER.

And of course, we have a growing and very productive effort in collaboration with the Center for Tobacco Products in our behavioral pharmacology efforts in which we have squirrel monkeys performing a self-administration study on tobacco-relevant products, primarily nicotine at this point, and we’re in the process of this next coming year setting up a parallel laboratory in the rodent model.

In terms of additional outreach with other government agencies, I mentioned that we have an ILSI/HESI multi-institute agency consortium looking at fluidic biomarkers of neurotoxicity. Here again, we're giving a prototypic neurotoxicant. We're taking CSF, blood, urine as well as tissue, and trying to do a shotgun approach to see if we can come up with a fingerprint of different entities. So we're doing proteomics, lipidomics, microRNA and metabolomic endpoints to see if there's going to be something of interest.

We currently also have an interagency agreement with DEA to conduct studies at the University of Arkansas for Medical Sciences in studies designed to take emerging street drugs to determine what their abuse liability is. So that's kind of a new approach for us but kind of parallels the kind of work we've been doing with the Center for Tobacco Products.

In collaboration with the University of Arkansas at Fayetteville, we are moving in the area of microphysiological systems development. Again, this is the blood-brain barrier on a chip that I'll talk more about. And we also have presence on the Coalition Against Major Diseases. Again, Syed Imam is our representative.

It’s a several entity-wide consortium looking at issues related to Alzheimer’s and Parkinson’s disease, coming up with biomarkers of treatment effect and so forth.

In terms of global outreach, we are participating with the OECD adverse outcome pathway identification in coming up with markers that we can identify as relevant to development on neurotoxicity, and then we are also working with ILSI/HESI on their developmental and reproductive toxicology team to come up with white papers and approaches on neonatal pediatrics.

So today, I just want to sort of throw out three recent accomplishments. We have a lot so we can’t talk about all of them, but I did want to talk about several I think that are of interest. One I've mentioned already is the development of the BBB-on-a-chip to model traumatic brain injury. Also want to mention our progress on qualification of an MRI T2 images as biomarkers of neurotoxicity, and then close with an update on sevoflurane general anesthesia-induced cognitive deficits in our nonhuman primate model.

So this particular effort, the development of the BBB-on-a-chip, is led by Syed Ali in collaboration with the University of Arkansas at Fayetteville, and in those efforts, they isolate primary brain

microendothelial cells, they collect these cells from the brain matter of whatever animal model you want to use. They've done rat, cow and nonhuman primate. After a mechanical and enzymatic digestions and several centrifuges, they seed these on collagen/fibronectin coated tissue. And what you can see here is those cells plated after isolation at NCTR versus the commercially available products from Abbott.

So the hypothesis here is that high-speed and biaxial stretch mimics the damage induced by traumatic brain injury in primary cultures and commercially available brain endothelial cells, and they can be used to study TBI *in vitro.*

So this is just a cartoon of how it's prepped. The cells are put on a high-speed stretcher and stretched to various degrees. And as you can see here, as a function of percent stretch, cells tend to get dead. So in this particular case, if you stretch them to 10 percent, you start to see significant increases in cell death.

DR. PHILBERT: Isn't that the elastic limit?

DR. PAULE: And another metric of cell health is lactic dehydrogenase released into the culture system and

you can see then a nice dose-related increase. As stretch increases, you get increases in LDH release.

The next project that I want to talk about is the progress on qualification of the MRI T2 images as biomarkers of neurotoxicity and beyond. Again, this is a collaboration with CDER. In this particular set of studies, we used mature Sprague-Dawley rats. We took ten known neurotoxicants *in vivo* in imaging at 7 tesla using our MRI, and developing T2 maps, and then followed up with neuropathology on the animals that were sacrificed by sending them to neurosciences associates for analysis. Eighty slices per brain; they analyzed them with silver cupric stain. And this is just a list of the compounds that we tested. Due to the shortness of time, we're just going to talk about kainic acid and hexachlorophene today, but you can see there were a variety of well-known bad actors.

And what this slide shows are three different brain slices. So there are slices through different areas of the brain in control animals, in kainic acid-treated animals and in time after treatment from 0, 1, 2 to 48 hours. And I think that you can see that within two hours, we're starting to see signal in the kainic acid-

treated animals where it’s not in the control animals, and this happens in each particular brain slice.

Taking the same animal -- so this was the animal image before kainic acid, two hours after kainic acid when we’re starting to see some signal, MRI signal -- sacrificing that animal and then doing the cupric silver stain, then you can see that this particular area does in fact show challenged architecture in these same animals. So this is sort of a qualification that the MRI signal is associated with frank neurotoxicity.

There are different ways to analyze these data. We can average baseline, take data from a number of animals, the average baseline information for untreated animals and compare that to treated animals and come up with a statistical difference to show, in fact, where the brain, these particular effects are significant.

I’m having a tough time here. Okay. The other compound that I wanted to talk to you about today is hexachlorophene. In this particular case, rather than a single treatment, we gave it for five days, and you can see from baseline here that the lesion or the signal increases out to a maximum of six days. We then stop treatment and in this particular case, the lesions or the

signal resolves. So there doesn’t appear to be any residual toxicity with this particular compound.

Using hexachlorophene then as the example, in a different way to look at things using diffusion tensor imaging, you can get nice signals of control animals after day six when the lesion is maximal from different areas of the brain here -- corpus callosum, the commissures, fimbria, external, internal capsule -- and that resolves after twenty days.

So we think that using the MRI will give us signals that certainly give us information as to where one should look if you expect to see any neurotoxicity.

The next example I want to talk about is studies that have been going on for a long period of time. They initially started with ketamine exposures that demonstrated not only neurotoxicity in terms of cell death in the nonhuman primate but also functional deficits in those same animals when tested even several years later.

So the studies here involve sevoflurane-induced general anesthesia during development followed by CT imaging of the neural effects and then assessment of the brain function in these animals using the NCTR Operant Test Battery. Again, these are all studies done in

collaboration with CDER. In these studies, we used postnatal day five or six nonhuman primates, one-week-old animals exposed to 2.5% sevoflurane, a clinically relevant concentration, and in these studies the exposures were for eight hours. Previous studies with ketamine used exposures for 24 hours, so this is a significantly shorter period of time compared to what we've done previously with ketamine. This particular exposure does in fact cause significant neural damage, cell death and glial cell activation.

So what we are showing here are some PET scans. Wait a minute. Yes, okay. So in terms of looking at neural inflammation using our PET approach, we used a compound called FEPPA, and FEPPA is a marker of activated glia. So there's a particular receptor on mitochondrial membranes that is expressed during glial activation. FEPPA tags that receptor and we can image that when we use an F18-marked FEPPA compound. So we’re going to use the PET/CT imaging post-anesthesia to describe the time course associated with this exposure.

These slides here show an animal prior -- an animal that has not been exposed to anesthesia -- versus an animal that has been exposed to anesthesia, and you can see in the cortex here that the signal is much

greater than in an animal that has not been exposed. This is a more complete sort of comparison here in which we've overlapped CT images with PET images to co-register them together to get a better indication for precisely where the PET signal is coming from.

And these are the data after 1 day, 7 days and 21 days after exposure. So what we're showing here is the scan, the PET scan is two hours long. The open circles here represent animals that have been exposed to sevoflurane for eight hours the day before. One day after exposure, you can see that the FEPPA signal is significantly increased for most of the duration of the scan.

Control uptake is down here, and I need to mention here that in addition, we gave these animals a compound called acetyl-L-carnitine -- remember I said that everything goes through the mitochondria. Acetyl-L-carnitine stabilizes mitochondrial cell membranes. It's involved in free fatty acid transportation in the mitochondria. It's also an antioxidant, and we've shown in previous studies, it's a very good agent to protect the adverse effects associated with developmental exposures to general anesthetics. In this particular study, we use them in collaboration with the sevoflurane,

and you can see that acetyl-L-carnitine virtually prevents this signal of neuroinflammation when the animals are exposed to sevoflurane.

The other interesting thing is that one week after exposure, we're still seeing signals of neuroinflammation, and you could argue that even three weeks after exposure, there's an elevated signal indicating continuing neuroinflammation even though this is not statistically significant. These are only three or four animals per group here so we just don't have the power to show what's going on.

Okay. So we've taken these same animals, put them back with their moms, let them grow up until they're about six or seven months of age, weaned them from the animals and then trained them to perform a series of cognitive function tests in the NCTR Operant Test Battery. So we have tasks that assess learning, motivation, color and position discrimination, and short-term memory. And I'm not going to have time to talk about all of those today, but those are the tests that are contained in the battery.

This is the intelligence panel that we utilize to give those tests to either our kids or our monkeys. Kids work for nickels. Monkeys work for banana-flavored

food pellets but the panels and the game are the same. Kids are very nice to work with, simply show them the videotape instruction set and tell them this is how you play the game; not so easy with monkeys. You've got to spend a lot of time training them.

And in fact, this is basically training data for monkeys. The top panel here represents 66 weeks' worth of training in animals that were exposed to ketamine, in the red, or not exposed to ketamine in black. And you can see that generally, at least initially, both groups continued to improve in performance of those learning tasks. So this axis here is the percent of a learning task they finished in a certain amount of time. And you can see that after a while, the control animals outperform the ketamine-exposed animals, and I can tell you that as these data have gone out for literally years beyond here, the ketamine animals never catch up with the controls and in fact, after this time, actually diverge a little bit more than this. So they actually get worse.

The interesting point about this figure is that these -- and remember that the ketamine exposure was 24 hours, okay. The sevoflurane exposure was only eight hours and look at the data that we see here. There is no

indication that the sevoflurane animals ever start to acquire performance of these tasks. This is like chemically induced mental retardation in only eight-hour exposures, so very interesting.

So to sort of summarize, a single episode of eight hours of sevoflurane-induced general anesthesia during a sensitive period of brain growth spurt can cause subsequent cognitive deficits in these animals. These effects appear to be permanent and worsen with age. These effects were seen in behaviors that reflect aspects of brain function related to IQ, and where I didn’t show the data but in kids who performed the same tasks, the performance of this task is significantly correlated with IQ. And although I didn’t show the data today, there is preliminary data that suggests that we could protect against this behavioral deficit with acetyl-L-carnitine in much the same way we protected against the neuroinflammatory response.

You might remember from previous presentations that I made that the ketamine data actually were published with the rollout of this public-private partnership between the FDA and the International Anesthesia Research Society called the SmartTots organization, and this organization exists to try to

generate funds to do more research in this area because it’s very difficult. Certainly, pharmaceutical companies are not interested in pursuing this. So, go to SmartTots, dig out your wallet and give some money.

Future directions. Those of you that have been on the panel for a while know that Fluoro-Jade is a compound that has been developed by Larry Schmued in our division and used to really become the industry standard in terms of identifying dead and dying neurons. But all that work was really done in fixed tissue. So recently, we've been looking at whether that’s also applicable to unfixed tissue, which would be nice; and then even more recently, to see if we can use that in living tissue culture. And the preliminary data tend to suggest that it might be possible to actually use Fluoro-Jade in living cell cultures and identify those cells that are sick or dying.

I previously mentioned this MALDI-MS imaging of brain slices. I am totally excited about that. I think that’s going to be just wonderful.

And then we’re also adding a larger-bore MRI to our toolbox. So right now, we have a very powerful 7 tesla MRI that has a small bore so we can’t image animals any bigger than small rhesus monkeys. With our larger-

bore 4.7 tesla instrument, we'll be able to image even adult animals. This is going to be valuable I think for translational purposes because we have preliminary data now for children exposed to general anesthesia before the age of one showing that there are MRI changes associated with level of exposure. So if we can replicate that and validate that, or demonstrate that in the animal model, I think that would be very powerful. We can only do that if we have a larger bore.

And then future directions, again, Larry Schmued is developing rare earth metal chelates. This is very interesting stuff too. He's come up with a compound called Euro-Glo, which is fluorescent, high-intensity emission, has resistance to fading, is compatible with multiple labelling protocols, and stains myelin and amyloid plaques. Now the cool thing about this stuff is that you could also stick it into animals and image it with your MRI. So because it's got this paramagnetic quality to it -- and believe me, I don't understand the physics -- it should be possible to get images with the MRI using these tracers as well.

And just to give you an example of what it looks like, the top two panels are tissues stained with Euro-Glo. This is low magnification and higher

magnification, in which you've stained these amyloid plaques. And for comparison here, this is his other stain, Black Gold, which stains just myelin. And you can see the myelin all around the plaque but doesn't actually penetrate the plaque. Another compound that he has developed is called the Amylo-Glo, which just stains the amyloid plaque but not the myelin. This is the Fluoro-Jade that I mentioned earlier, which actually does stain the plaques as well. And then periodic acid-Schiff reagent stains both the myelin and the amyloid plaques. So this is a very interesting thing, so this is very preliminary data but I think it's going to be pretty cool if this is actually useful in MRI as well.

So to close then, I would ask that we've toyed around for a while with doing electrophysiological endpoints. I came from an EEG background. I love EEG, but it's very difficult to interpret. We have used EEG to determine the level of anesthesia in our infant animals so that when we treat with acetyl-L-carnitine, we know that we're not changing level of anesthesia so it doesn't impact the endpoints that we're looking at. So we do use EEG currently and we have used nerve conduction velocity in a somewhat limited fashion, and of course nerve conduction velocities are just one form of evoked

potentials. But I've been getting some input from colleagues who think it might be advantageous to incorporate microelectrode arrays, especially in our cell culture work. So we have limited resources. Do we want to go into this area as well or do we think it's going to be not as great a value added as just continuing with what we're doing? And of course, are there emerging sciences and technologies that you suggest we pursue? Now, I've already mentioned the MALDI-MS brain stuff. I think that's one we definitely want to go for, but are there others out there that we haven't mentioned and we might not be aware of? We'd certainly like to hear what you have to say about that.

So with that, I think I'll just close with a listing of all the wonderful people that I have a chance to work for.

DR. PHILBERT: Carolyn.

DR. WILSON: So great, Merle. I have tons of questions but I'll limit it to a couple.

DR. PAULE: All right.

DR. WILSON: So first of all, with the acetyl-L-carnitine studies, was that administered prior to the sevoflurane or after?

DR. PAULE: So it was administered one hour prior to and then four hours into, so they get two doses. And really, they were pretty high doses. They were 100 mg/kg injected IP twice. That seems to be, 50 to 100 seems to be around the dose that's needed to cause this protection. Now, I must say that we have some preliminary data that suggests that acetyl-L-carnitine at those doses by itself is not without effect.

DR. WILSON: Not surprising.

DR. PAULE: Yes. But when you combine it with the anesthetic, it completely blocks the anesthetic effect.

DR. WILSON: Right.

DR. PAULE: And so we need to know a lot more about the pharmacokinetics and all that sort of stuff to maximize best practices with that stuff.

DR. WILSON: Right, and then in terms of emerging technologies, I actually had MEA written in big letters in my notes before you got there.

DR. PAULE: Good.

DR. WILSON: So I think that really, if you're going to continue with the OECD and development neurotoxin, really getting into cell culture, that's probably a tool that really would be useful to add to

your arsenal, and it's relatively straightforward. There's sort of a big investment upfront to get the equipment but once you've made that, it's really not, it's cost-effective. It's much more cost-effective than doing electrophys. And people who don't do electrophys can still do MEA.

And I would also say that the other technology that might build upon your *in vivo* imaging would be calcium imaging. There is some really cool stuff out there now which is not single cell but it's more population-based calcium imaging, like the FLIPR, which allows you to screen a bunch of chemical simultaneously, and we have found that that actually corresponds really nicely with the EEG data. So you can get some EEG data-like information from cultures.

DR. PAULE: That's very valuable, and in fact, we have done single cell calcium imaging to look at the NMDA receptor mechanism in culture, but getting to a population would be better. Yes, thank you.

DR. PHILBERT: Steve.

DR. STICE: Steve Stice at University of Georgia. I would second what Pam said about the MEA. We work with MEAs and we're not electrophysiologists, we're cell culturists, and it works very nicely. The only thing

is I think it works better with primary cells or mouse embryonic stem cells. The human cells are much more difficult…

DR. PAULE: Yes, they seem to be all the way around, yes.

DR. STICE: The human stem cells, to get. It takes a longer time to culture them out. That’s a big expense and a headache. But if you're looking at rodents, either primary or stem cells, that’s really good.

I had a question about the BBB. I guess I’m not quite in tune with what you're trying to do with TBI with that. Are you trying to disrupt the BBB, and how are you going to measure those types of things, I guess?

DR. PAULE: So Syed Ali is here and he can answer that as well, but I think the point is that it’s a model of the blood-brain barrier in that these endothelial cells are the ones that are being cultured. And of course with traumatic brain injury, that’s a problem. So I think to understand what happens when you torque these cells and try to figure out perhaps mechanistically what's going on, you then can be in a position to perhaps ameliorate what damage is going on. Syed, do you have anything to add?

DR. ALI: Yes, because of the time, Merle didn't include -- we did a lot of protein expression in those blood-brain barrier cells. There’s a lot of protein associated with this which affects the permeability of those blood-brain barriers. So when we are doing this stretching or these things, we are looking at what it affects, not only the protein but also the morphology of the cells and whether they are dying, what percent they are dying, how they are dying. So we are looking at the whole array of biomarkers during the insult to those blood-brain barrier cells.

DR. STICE: Okay, so that sounds like you're doing it on a cellular basis, not on a whole structure tissue type…

DR. ALI: Yes, we are culturing those endothelial cells when they are viable, they are having networks, then we put those -- we are actually growing on these [3DM 4.59] chips, [3DMA] chips and put on a stretcher.

DR. STICE: Okay, I understand now. And I guess there are human-sourced endothelial cells and stem cells or neurons and so forth.

DR. ALI: That’s the next goal actually. We are trying to put the whole network and we are looking at not

only endothelial cells but also neurons, astrocytes, pericytes next to each other, and then we will put on the same [3DM] chips to look at…

DR. PHILBERT: So what I was going to say, is the 10 percent greater than the modulus of the elasticity for the cell membranes or are you just --

DR. ALI: Oh yes -- I'm sorry, what did you say?

DR. PHILBERT: Ten percent would exceed the modulus of the elasticity for the cell membranes.

DR. ALI: Yes, it's stretching but back to the normal. So really, we're stretching to the cells and bringing back, because this is stretch -- yes.

DR. PHILBERT: But if you exceed the modulus of the elasticity…

DR. ALI: Oh yes, yes. Yes, it will come back. It will come back and it has a different -- we can do up to 15, 20 or 50 percent stretch.

DR. PAULE: Well, some cells come back but 10 percent dies.

DR. ALI: Some cells die during this process actually.

DR. PHILBERT: Yes, so I think, so somebody that I think you need to talk to is Michael Sheets out of NYU who has done some really brilliant work on extracellular

matrix interactions, because I think you are sort of moving into the nonphysiological with 10 percent.

DR. ALI: All right.

DR. PHILBERT: And so you can get really great results but they may not mean anything.

DR. ALI: All right, definitely.

DR. PHILBERT: And then, sorry, have you finished, Steve? Yes, so I'm only mindful of this because the University of Michigan football team is good again, but there are two things that collide there with football players: one, TBI of course, and this idea of spinocerebellar disorders and neurodegenerative disorders; but also they take a lot of opiates to manage pain, and then there's the sort of wider public health issue of prescription opiate abuse. And you have focused a lot of your work over the last almost a decade now on pediatric exposures but there is this really important transition out of adolescence into young adulthood where there is a lot of entraining of neural networks and so on. Is there any plan to move into that area of endeavor?

DR. PAULE: Yes, I think that adolescence is probably just as important as early development, for the reasons that you state. We're also actually doing some adult studies with anesthetic agents because there is

this postoperative cognitive disorder that’s associated in adults after exposure to general anesthesia. So we’re already starting to look at adults, but I think clearly we need to also start looking at the adolescent period because it’s really kind of a no-brainer, forgive the pun, but that’s what's going on. And why wouldn’t you expect something to go wrong when you muck around with development of neurons during adolescence? We all know teenagers are crazy. That’s because all this weird stuff is going on.

DR. PAULE: Katrina is nodding wildly. And then the last point I would make is there are huge advances in signal processing now and neurorobotics, where people are using EEG for intentional movement of robotic prostheses, and there are a number of institutes, including the Neuroscience Institute at UCSF. They're working with veterans in the VA system out there and are deconvolving all sorts of really interesting patterns, and people who speak maths understand the signals much better than we who speak English.

DR. PAULE: Yes.

DR. REISS: Ted Reiss from Celgene. So I have a slightly different question. Excuse me. So for many of the reviewing divisions in CDER, suicide/suicidality, the

neurotoxicities there are quite important. You mentioned depression on one of your earlier slides but you didn't talk about it much during your presentation. So is there any work you guys are doing in that area, because it seems to be a more general neurotoxicity issue for the reviewing divisions?

DR. PAULE: Right. It's a very important area of course, and the only work that we have done in that area is to look at children that have been diagnosed with major depression. I mean, I didn't even know that children got major depression but they do. And when you assess them using our cognitive assessment tool, they do not perform normally. Their short-term memory function is very abnormal. So we've just used the instrument to see if it's sensitive to that particular patient population, and it is. We haven't done anything beyond that. But it is a very important issue and of course that's one of the reasons we look at the model aminergic systems in terms of neurotoxicity after exposure to methamphetamine and things like that, but not specifically targeting suicidality.

DR. PHILBERT: I think most kids are going through depression now that they've gotten off their sugar high.

DR. LEIN: Just one last comment. The MALDI-TOF imaging, which is beautiful…

DR. PAULE: It is.

DR. LEIN: Are you guys looking at anything other than neurotransmitters or just looking at neurotransmitters?

DR. PAULE: So we're looking at neurochemicals, not necessarily all of which are neurotransmitters. So we will do whatever the team -- and of course this is Rick Beger's team in the Division of Systems Biology -- so whatever they're able to do, we will milk them for. I'm not sure exactly what their capabilities are going to be, but we have seen, I would say, probably eight to ten different neurochemicals that they've been able to image using the same slice.

DR. LEIN: Because I know that we're optimizing it to look at xenobiotics, so that might be something else to think about expanding into if they've got that capability.

DR. PAULE: You mean administered compounds?

DR. LEIN: Yes.

DR. PAULE: Yes.

DR. LEIN: And then secondly, if you're not already doing it -- you might be -- but you might want to

consider looking at the ratio of the excitatory to inhibitory synapses if you can do that with that technique, because that’s really been pretty informative for the Mind Institute on neurodevelopmental disorders.

DR. PAULE: Yes, I don’t know what the capabilities are. I mean, this is just so new. I was just amazed by it. So we haven’t really tested the capabilities yet, but it has a lot of potential like that.

DR. PHILBERT: Lots of excitement. Any comments or questions from our friends at FDA? Jose?

DR. CENTENO: Not a question but it’s a comment. In CDRH, we have been seeing an increasing number of submissions dealing with neuromodulation devices, mostly dealing with cochlear implants and the platinum-based implants. There is a re-emerging interest on the neurotoxicology of platinum. So this might be an area of research interactions.

DR. PAULE: Yes, all right, thank you.

DR. PHILBERT: If there are no more questions or comments, thank you very much.

We're in the home stretch; I'm going to invite everyone to stand for 30 seconds, while we change over to Bill.

[BREAK]

Agenda Item: Division of Systems Biology

DR. PHILBERT: Ladies and gentlemen, please take your seats. And our final presentation this afternoon is from Dr. William Mattes, Division of Systems Biology.

DR. MATTES: Bottom of the eighth, no hits, I'm the closer. So, and I'm standing between you, the end of the game and the end of the eighth afternoon and perhaps a good stiff drink, so I'll try to make this as painless as possible.

Okay, so here are the specifics. In terms of the division staff, we've got 23 FTEs in terms of research scientists, 11 support scientists, three admin staff. Right now we don't have any Commissioner fellows. We've got seven ORISE postdocs, no grad students really. Total of 49 people. And in fact, as one of my people pointed out, well, these numbers aren't quite right because some of them are -- actually a few of them are -- open positions.

But let me talk a little bit about outreach. So we have collaborations, and I went through all of our protocols and we've got folks from all the other divisions working on some of our protocols. We also, in terms of having protocols and projects with other

centers, we have projects with CDER, CDRH, CBER and CFSAN. We have interactions with NTP, NIH and the VA, and a number of universities. I've noted a few there, Medical College of Wisconsin, University of Pittsburgh, Ohio State University. Didn't list them all but anyway, we've got some things going out there in terms of external interactions.

I'd like to call attention to a few collaborations of note. We have a tyrosine kinase inhibitor systems toxicology project that really has pulled in a couple of folks from CDER working with us, providing input into our protocols.

We have a group at CDER who is very interested in a mouse obesity model we're trying to develop, looking at immune cell effects in that model.

We have recently gotten involved in aptamer technology and that was, I would say, driven by the fact that I found a colleague in CDRH who had prior experience with it and a great deal of expertise in it, and I felt that that really qualified it as a project to jump into.

And we have an ongoing project with CFSAN for listeria detection and quantitation.

Now, here's the old mission statement. To address problems of food, drug and medical product safety

using systems biology approaches and innovative technology. But why systems biology? Why would we throw that choice word out there?

Well, the way I look at it is it really provides you tools and approaches that will bridge, importantly, your nonclinical models with your clinical settings and focusing on both your adverse events in these different settings and individual responses. I coined the words “translational toxicology and precision safety assessment” and mostly because you’ve got to have buzzwords to move ahead in this world.

In terms of system -- and I’d like to say what it means in terms of systems thinking -- is that you have these models and then you have these clinical situations, and the idea, I’d say, I would posit in terms of this discussion, that systems thinking focuses on how these systems -- and I’m obviously focusing on a pathway here, the AMPK pathway -- but you can actually expand this, if you will, to the concept of physiology in a broader sense, not just a molecular level or a cellular level. But it’s using this systems thinking to connect your model systems with your clinical settings and vice versa, because in fact, you can use this as a way of asking and qualifying whether your model systems are behaving the

way you would like them to in terms of predicting or informing your clinical data.

The tools are, well, the traditional -omics kinds of things: transcriptomics, proteomics and metabolomics. But these become the way that you can gain that systems information.

In terms of goals, I would say that what we're looking for are translational biomarkers, either prognostic or predictive, and right now we're focusing on hepatotoxicity and cardiotoxicity. We're very much interested in the mechanistic bases for species tissues, sex and subpopulation specificity in drug toxicity or adverse reactions. Also, we'd like to develop *in vitro* models for better evaluation of various toxicities, repro, developmental and clinical toxicity. And at the same time, we've been working on *in silico* models for looking at relevant toxicities and, as an addendum, robust technologies for pathogen detection in outbreak characterization.

Strategies -- working with drug classes where you have known toxicities. These, you would call them really kind of tool compounds, anthracyclines, acetaminophen, tyrosine kinase inhibitors. Some of these are oldies but goodies, or oldies but baddies, but

because of the information around them, they form a good knowledge base from which to work off. We want to characterize systems biology effects with these various -omic tools, and at a variety of levels; and integrate data with the systems biology informatics, accounting for species, tissue, sex, subpopulation differences; and finally, incorporate the innovative *in vitro* computational and instrumental technology.

I would venture to say within the division, we have a certain number of themes. The first theme, and you can see how this develops, is translational safety biomarkers and mechanisms. I've already referred to that. Alternative models, already referred to that. Technology to assess food safety, computational modelling and cross-species prediction. In fact, those of you on the subcommittee that will be here on Tuesday and Thursday will see that I've used those themes to organize our presentations, or organize our work for the purposes of evaluation and review.

Of course all of this is done with an eye toward how we're going to apply this in use and evaluation of FDA-regulated products.

And in terms of tools, our model systems are *in vitro*. We've got primary cell cultures, cell lines and

induced pluripotent stem cells. We have rodent models and specialized mouse models. And we, in fact, are blessed with being able to invoke some clinical studies or access to clinical studies where we have blood, urine microRNA, protein and metabolite profiling -- in clinical studies.

So let me get into a few accomplishments that I feel are worthy of note. We have some data, preliminary data I would say, on translational biomarkers of liver injury. We have some really, I would say, exciting accomplishments in terms of a flow cytometry-based approach called RAPID-B for detection of listeria. We have demonstrated mitochondrial injury in cardiomyocytes after tyrosine kinase inhibitor treatment. We have identified some protein changes in mouse plasma very early after doxorubicin treatment that could be useful for identifying the cardiomyopathy doxorubicin induces. And we also have a unique molecular modelling approach called 3D-QSDAR, and we have models that have shown that the toxicophore for phospholipidosis is actually very similar to that for hERG binding, suggesting that the target molecules for those two endpoints have some similarity.

So let me jump into this translational biomarker, namely what Rick Beger’s group has found using

metabolomics is that palmitoylcarnitine shows up very early in overdoses -- or I should say toxic treatments -- of acetaminophen, APAP, in both rodent models and in human samples where there is an overdose.

And what you can see -- and I think actually what I do is I click on that, yes -- that the palmitoylcarnitine peak appears before the ALT peak, which is in red. So the palmitoylcarnitine peak appears early before the ALT peak. And this is, in this case, these are subjects who have been treated with acetylcysteine, which is the antidote, later than perhaps they should have been treated but anyway, that makes their particular situation closer to that of the animal model. But it demonstrates the power of using a metabolomic approach to look across species for biomarkers.

Let me turn to this flow cytometry method for detecting pathogens, which we call a RAPID-B. And in this case, what I'm showing is the specificity of this particular probe to RAPID-B -- I'm sorry, to listeria -- versus other bacterial species. And you can see the multiple counts showing up for listeria, various listeria species, but not for the non-listeria bacterial species. The important piece of this, and the reason it's called

RAPID-B, is the assay time is eight hours or less and the throughput 24-48 samples at a time. This can be compared to the bacterial, the standard plate methods, which are 24-48 to even longer time periods.

Also, I pointed to our investigation into tyrosine kinase inhibitors. In this case, we're examining cardiotoxicity and in fact, this is what I would call delayed cardiotoxicity. It is well-known that cardiotoxicity may be viewed as arrhythmic effects or proarrhythmic effects. In this case, we're looking at effects after seven days of treatment so it is not an acute effect. And what you can see is if you compare two particular tyrosine kinase inhibitors, gefitinib and vandetanib, where one is considered to be safe in terms of the cardiac level and the other has a black box warning, you can see that after seven days of treatment, you see even at the Cmax concentration, cardiomyocytes are showing a decrease in ATP. So it confirms the structural cytotoxic effects of this compound, which is consistent with the clinician reports. What we're looking at is this as a cell culture model for exploring various cardiotoxic effects of tyrosine kinase inhibitors.

And I mentioned identifying protein markers, early protein markers, of doxorubicin toxicity. What's

exciting in this particular slide -- and I did not include a descriptive slide -- is we're using a technology that's out of a company called SomaLogic, which they have a technology called aptamers, which is basically using a synthetic DNA molecule to mimic, basically, an antibody. You may think of it as a DNA version of an antibody. And it offers some unique advantages, particularly in terms of reproducibility. They have an approach which allows us to look at 1300 human proteins at a time in plasma or serum and in this case, we're looking at mice and you're seeing these proteins, these six proteins showing up as early as two weeks after dosing, and at a very low dose of doxorubicin. So there's real promise in this technology for picking up some early, early biomarkers of doxorubicin effects.

Also, I referred to a unique molecular modelling approach that makes use not of three-dimensional structure in terms of points in space, but it makes use of the NMR spectrum of a particular compound. When you think of it, NMR captures not only the distance between molecules but really their quantum mechanical space, and that's what is kind of unique about this approach, putting together the NMR structure and the NMR

characteristics. And then the NMR characteristics of several molecules relating to their biological activity, you end up with this QSTAR, this spectral data activity relationship.

When that was done for two classes or two types of biological endpoints with a series of compounds, it was found that you could determine the toxicophore or the structure of the toxicophore for these two, and there was similarity. In this case, the compounds were monitored for their ability to bind to the hERG channel, which has been implicated in cardiac toxicity for a number of drugs. And also, in this case, the compounds were being assayed for their ability to induce phospholipidosis, a different type of toxicity. What was found is that the phospholipidosis structure was a subset of the hERG binding structure, namely, you can see these aromatic groups separated by a certain distance, adjacent to an amino group, again separated by a certain distance. So it offers some interesting mechanistic viewpoints as to the relationship between these two toxicology targets.

I want to talk real quickly about current projects we've got going. One is the evaluation of serum metabolic biomarkers to predict AKI severity -- acute kidney injury severity -- and this is in a clinical

study. Also, a look at cell-free microRNA as a clinical biomarker of drug-induced liver injury, evaluation of an *in vitro* testis organ system as an alternative model for male reprotoxicology, and also a comprehensive examination of tyrosine kinase inhibitor toxicity.

Just a few details because I don’t want to delay us any more. In terms of the clinical AKI biomarkers, this is a collaboration with the University of Virginia Medical School, and we’re looking at plasma both with metabolomics but also using this aptamer technology that I showed you was so powerful in terms of the doxorubicin treatment.

We've also been involved in looking at microRNA biomarkers of drug-induced liver injury at two levels, but in particular we’re looking at urine microRNAs in patients from the acute liver failure study group, and the results are very suggestive for a set of microRNAs that would actually be prognostic for patient survival.

Finally, I want to refer to a project that we have ongoing at different levels, a comprehensive examination of tyrosine kinase inhibitors. We’re doing data mining of mouse, rat and human kinome sequences for species, sex and organ differences in targets. We are doing *in vitro* comparisons of hepatotoxicity in primary

hepatocytes as well as iPSC-derived cardiomyocytes, and we're trying to develop an *in vivo* systems biology study of sunitinib. In a mouse model of cardiomyopathy that was actually developed for doxorubicin, so we're kind of taking what we know about doxorubicin and its induction of cardiomyopathy and comparing that with sunitinib and its well-known documentation of cardiomyopathy.

In terms of the TKI, the whole reason we're looking at this is because you see its interaction with so many multiple targets and pathways.

In terms of future directions, we are considering stem cell models for both hepatocytes and cardiomyocytes, and this has brought us into some collaboration with outside laboratories, both at the Medical College in Wisconsin and at Stanford, and the idea is to be able to have the potential for really looking at inter-individual variability.

We're also running studies to investigate adaptation in drug-induced liver injury, and the reason that, I think, is important is it's easy to talk about toxicity but as you know, many of us have taken Tylenol in the past and still are listening to me speak, unfortunately. But the reality is somehow, the body really can adapt to certain insults, and that's kind of

important in a critical evaluation of drug safety. At what point do you consider something simply toxic, or is there going to be a progression to adaptation? So we're exploring *in vivo* and *in vitro* studies to look at models for adaptation to therapeutic doses of acetaminophen.

So this is a point at which I do kind of turn to you and hope you're still with me, in terms of some feedback. I've considered the area of TKI toxicity as a systems biology problem but is this really relevant to FDA regulation? What other aspects might I consider, and what toxicities might be relevant? I think that's my last slide. No, it isn't.

Clinical collaborations, how important are these? I have considered this nonclinical-to-clinical connection important for biomarkers and mechanistic work. Is that correct? And finally, what other directions might be considered? And how might interactions between systems biology and other FDA centers be enhanced, and the age-old question whether there are emergent sciences and technologies could you advise me to pursue? And what other future directions might impact the FDA? And with that, I think I go back to thanking you for your attention and hoping you'd give me some ideas.

DR. PHILBERT: Questions for Bill. Pamela and then Katrina.

DR. LEIN: Pamela at UC Davis. So this goes a little bit back to the neurotox, the presentation we just heard, but you might want to consider using MEAs if you are going to persist with the cardiotoxicity. The microelectrode arrays. They have proven --

DR. MATTES: Oh, we are.

DR. LEIN: Okay, so you are.

DR. MATTES: Yes, we have already -- I didn't show that data but we have that. Yes. Yes.

DR. LEIN: Okay, okay. So Merle, talk to him.

DR. PHILBERT: Katrina.

DR. WATERS: Katrina Waters. So a question referring to your last feedback slide about the nonclinical and clinical sort of connection there, I think the clinical connections are really, really important, particularly for the systems approaches, because there are a lot of things that you see in a nonclinical model that just simply don't happen in humans. And there is so much regarding, like you said, adaptation. The human body is so resilient to the different perturbations that at least what we have been seeing with some of our clinical samples is they are

really very insightful about what we do see with our nonclinical models, and we can gain some understanding of predictors for survival, for example, that can come from the systems data that are really interesting and can link back mechanistically to the nonclinical models.

DR. PHILBERT: Ted.

DR. REISS: Ted Reiss from Celgene again. So I'll just make -- excuse me, my cold's catching up with me here -- I'll just emphasize the point that was made and just at a very high level. It was part and parcel of the question I asked Bill earlier this morning. I think it's absolutely critical for you to do that. It's not just should we do that but that's how to do it, because these ultimately are clinical questions that sort of need to be addressed and answered, and I would encourage you to think more and more about how you can develop those relationships, whether those are public-private partnerships with academia through the CIRCEs, other mechanisms through industry, so on and so forth, to help solve these problems because they're critical.

DR. MATTES: Just as an aside, one of the things that, you know -- and my background, if you didn't know, I led the Predictive Safety Testing Consortium in its first kidney biomarker qualification thing. And one of

the things that always bothered me was the difference between nonclinical study designs and clinical study designs. And one of the things I feel that would be huge would be to say here's my clinical study, here's how I've measured it. Let's make my nonclinical study identical. And part of that is, I think a key part, is to be able to pick up, be able to sample in a survival fashion, and I didn't go into that but that's of course one of the features you can do with metabolomics but also very sensitive assays.

DR. PHILBERT: Bill.

DR. SLIKKER: Yes, well you know, I think this whole idea for these translatable biomarkers that move across species is really key here, and also the implications for human health. I think it was one of our FDA colleagues that a few months ago was talking about DILI and was talking about the effects of acetaminophen and saying how important it was to the clinician to have this knowledge about biomarker indicators really early on, to make critical decisions about whether or not they use an agent to try to reverse the effects of acetaminophen or were looking down the stream for a situation where you may actually get liver replacement in order for survival.

These critical decisions have to be made in a timely fashion to protect human health, and yet the kind of tools that we've been using are not, as we sort of understand now, particularly up for this particular challenge, okay. There is a fine line as to whether or not a clinician says hey, we're going to add an agent to reverse this effect, or we're going to send you home, not knowing the next day whether your liver is going to be intact or not because the biomarkers aren't good enough.

DR. MATTES: Right.

DR. SLIKKER: So we really need to get here faster and we need to get here better, so we have these translatable biomarkers. It looks like some of the ones that you guys are uncovering certainly are earlier, and perhaps better, to help the decision-making process of the clinician during these critical times of care.

DR. PHILBERT: So something that needs to be taken into account is we do, in toxicology, experiments on inbred or narrowly outbred strains of mice, and humans are inherently heterogeneous and nine times out of ten, you send the patient home and they're fine, and it's the tenth time that gets you. And so actually building into the models some degree of heterogeneity and paying attention to the outliers.

DR. MATTES: There’s an interaction we recently had with Ivan Rusyn, who is in Texas, in terms of exploring the use of collaborative cross animals for that. And I think it’s very intriguing and a real opportunity. But you're absolutely right, you know.

One of the things that I feel is also critical when you talk about the sampling is in whichever study you do, sample before the effect if you can. Of course, many clinical studies you're not depending on how it’s set up. but in the animals, the classic toxicology is enter four and here’s the mean. But the reality is you may have ten animals with two responders. What was going on beforehand? What was their blood chemistry beforehand, if we can sample that? So I think there's ways to capture variability in a nonclinical setting.

DR. PHILBERT: So I think we’re beginning to all go in on a theme that I’ve heard all day, which is how might one position NCTR strategically to think about this transition from animal models into human populations, because we come up with all sorts of fanciful numbers based on extrapolations? But is there a way, in systems biology, of accounting for these distributions -- normal, skewed, whatever -- and can you actually model that to get to a better estimation of what's likely to happen in

a population? And is there then a way of validating, so in between, in high-throughput or moderate-throughput assays, can you actually model the variation in responses that you are likely to see for any given organ?

DR. MATTES: It's a very exciting concept, just a lot of work.

DR. PHILBERT: But that's how you might think of actually building a strategic advantage in servicing the centers. Ted, were you about to say something? Suresh.

DR. PILLAI: Just a question. Why is listeria pathogen detection this division rather than the microbiology division? Suresh Pillai.

DR. MATTES: I don't have a good answer.

DR. PHILBERT: Greg.

DR. LANZA: How do you differentiate from an early change that is going to just be transient and may be actually part of the response leading to recovery versus an early change that actually is indicative of something that's going to be progressive, whether it's of itself or the first of a cascaded set of changes?

DR. MATTES: Yes, I think that's a critical thing because it gets into this question of adaptation, and you know, in terms of that, doxorubicin, the doxorubicin study that I showed, what we did not have and

what we don't have in that study design is a treatment and then stopping, and then treatment and then stopping.

We do have a study that's being developed in terms of protocol where it's an attempt to treat mice with a modest dose of doxorubicin and then wait and see what delayed cardiomyopathy would be. The trick, I think, will be to get some interim blood samples to see, of those that do not progress, are there appropriate markers; and of those animals that do progress to cardiomyopathy, are there appropriate markers? So.

DR. LANZA: This particular case of the anthracycline is important. If I put my clinical hat on just for a minute, so I see quite a few cases -- and I'm sure you do also -- of young people prior to going on, for breast cancer or something like this, and then next thing you know, we're looking at their heart is destroyed. Now we use different imaging techniques on strain -- we talked about it earlier -- but I'm wondering if maybe in this ability to correlate, is that you can focus down on the early markers against what we're clinically trying to use, like in this particular case, strain imaging. So look at, we see a small change in strain but we really only can have gross changes that we feel comfortable with. But if it could be supported by

biochemical systems biology that this change that we're seeing averaged over the whole heart is, you know, it's a later manifestation of this thing that's part of the downstream, then we'd have more confidence of being more sensitive to say it's time to stop this treatment.

DR. MATTES: Or, I have to get back to the -- or use an intervention or a more aggressive…

DR. LANZA: Or try to recover it.

DR. MATTES: Yes, yes, yes. Yes. And theoretically, if you have the right biomarkers, they become not just clinical tools but they become tools for assessing treatments, intervention treatments.

DR. LANZA: Greg again. So for the mouse models or rat models that you might use for this, there are technologies that would add, I think it's in VIVO still has it, but to make a long story short, where you could do the strain imaging on the rats and look for the twists/untwists with MRI for instance, but…

DR. MATTES: We have that. We actually, we have that. We just got that technology in place and are using that as part of the studies.

DR. LANZA: Right, because I think that if you correlate it with something we're using…

DR. MATTES: Yes.

DR. LANZA: That we can't use well, if you go we understand what it needs but only when it's too late.

DR. MATTES: Yes, yes, exactly. Exactly. No, I didn't go into the study design but the design is sampling longitudinally over time, along with the imaging, I think it is called VIVO 1000. Yes, the imaging that we can use then to correlate with the more clinical measures.

DR. PHILBERT: Was there a question from the chairs? Yes.

DR. HARRILL: I'd like to comment.

DR. PHILBERT: Please come to a mic and identify yourself.

DR. HARRILL: Hello? Hi, Alison Harrill from the NTP. I just wanted to go back to your comment and your admonition to the NCTR to consider genetic diversity in their biomarker development, and just as a rescue to NCTR, there was a contract with my lab at UAMS that was started in 2014 to look at inter-individual variation in DILI responses in diversity-outbred mice. And so we have expanded those studies to look at pharmacogenetic biomarkers related to susceptibility and now that I'm at NIH, that work is ongoing in Igor Koturbash's lab, but it

is an active area of research that has involved several of these individuals in this room over time.

DR. PHILBERT: Thank you for pointing that out. I think the missed opportunity is in addition to identifying the pathogenic pathway, you actually have an opportunity to compare that against the adaptive or nonresponding.

DR. MATTES: Yes,

DR. PHILBERT: And we tend not to look at the negative outcome because traditionally, as toxicologists, we push the dose till you see something.

DR. HARRILL: It’s interesting because some of our pilot data seems to suggest some adaptation events, and that’s an active area that we’re going to explore in our second phase of the study.

DR. PHILBERT: Great. Ted, you had a…

DR. REISS: No, I was just going to -- this is Ted Reiss again -- I was just going to state the obvious, that sort of the vision for the future would be to be able to understand not just the biomarker discovery part of this but the pathway sufficiently so that our job would be easier, and your job would be easier, to anticipate toxicities in addition to efficacy. So that’s the obvious vision here but I just wanted to state it.

DR. PHILBERT: Yes, I think in many ways, biomarker -- the term “biomarker” does a disservice because we don’t understand process, and these biomarkers are no doubt conditional, right, so it’s not just -- it’s there, bad thing or good thing. It’s there and a whole bunch of other stuff, and getting that contextual framing we don’t do terribly well.

DR. MATTES: And in terms of biomarkers, one of the things that really is now, I think, we’re getting that level of sophistication, is understanding yes, they're part of a process. They are part of a kinetic longitudinal process. You can have a -- you know, when you say a biomarker, there is what might use the word “endpoint” that one can measure at any given stage in injury or disease and its progression and/or resolution. So you know, I think you can -- I agree with you. Biomarkers is almost a disservice because you almost want to say what is the process we’re looking at, the progression, and what do we use to monitor the different stages?

DR. PHILBERT: And are there identifiable points of no return? Well, this is a great way to end the day’s discussion, I think, that ties many things together. I want to thank everybody from NCTR who showed up today, of

course our colleagues from the rest of FDA and the SAB. We are going to move to closed session so we'll give everyone five minutes to stretch, re-oxygenate again and clear the room, and we'll reconvene in five.

(Whereupon, the open session adjourned.)