Food and Drug Administration
Center for Biologics Evaluation and Research (CBER)
Cellular, Tissue, and Gene Therapies Advisory Committee (CTGTAC)

Teleconference Meeting No. 64

February 16, 2016

FDA White Oak Conference Center
Great Room (Salon C)
Silver Spring, Maryland

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PROCEEDINGS (1:00 p.m.)

Agenda Item: Welcome, Roll Call, Introduction of Members

DR. CRIPE: Why don't we begin. I am going to do a welcome and call to order. This is Tim Cripe, Chair of the Committee. I appreciate everyone participating in today's call.

Our first order of business is a roll call and introduction of the members that Janie is going to do.

DR. KIM: Thank you, everyone, for calling in. My name is Janie Kim; I'm the designated federal officer for today's meeting of the Cellular, Tissue and Gene Therapies Advisory Committee. Ms. Rosanna Harvey is the committee management specialist for CTGTAC. On behalf of FDA and the Center for Biologics Evaluation and Research, we would like to welcome everyone to the 64th CTGTAC meeting described in the Federal Register notice of January 21st, 2016.

Before proceeding to make administrative remarks and reading the Conflict of Interest Statement, I would like to take a roll call of the committee members who are on the phone for the official record.

DR. CRIPE: Here.

DR. KIM: Dr. Cripe, can we go down the list and see who else has phoned into the meeting. You can go ahead and read it.

DR. CRIPE: Dale Ando.

DR. ANDO: Here.

DR. CRIPE: David Bartlett.

(No response)

DR. CRIPE: Catherine Bollard.

(No response)

DR. CRIPE: William Bugbee.

(No response)

DR. CRIPE: Barry Byrne.

(No response)

DR. CRIPE: Leisha Emens.

DR. EMENS: Here.

DR. CRIPE: John Gearhart.

DR. KIM: Dr. Gearhart cannot make it on the call.

DR. CRIPE: Ole Isacson.

DR. ISACSON: Yes.

DR. CRIPE: Grace Pluhar.

(No response)

DR. CRIPE: Stephen Rose.

DR. ROSE: Here.

DR. CRIPE: Janet Wittes.

DR. WITTES: Here.

DR. CRIPE: Ann Zovein. Ann, I see you on the list, right.

DR. ZOVEIN: Sorry, I was on mute. I am here.

DR. CRIPE: Good. Anybody on the phone that I missed. I see a bunch of guests that are on the phone.

DR. EMENS: I am one of the guest. This is Leisha.

DR. CRIPE: I see your name as well.

Janie, do you want me to read the FDA participants or are you taking care of that?

DR. KIM: We will make the introductions around the table here in the conference room, starting to my right.

DR. WILSON: Carolyn Wilson, Associate Director for Research Center for Biologics.

DR. EPSTEIN: Suzanne Epstein, Associate Director for Research of the Office of Cellular, Tissue and Gene Therapies.

DR. PURI: Raj Puri; I'm the Director of the Division of Cellular and Gene Therapy in the Office of Cell, Tissue and Gene Therapies.

DR. BAUER: Steve Bauer, Chief of the Cell and Tissue Therapies Branch in OCTGT.

Agenda Item: Administrative Remarks/Conflict of Interest Statement

DR. KIM: And I am Janie Kim, the designated federal official for this meeting.

We will begin today's meeting with a session that is open to the public followed by an open public hearing

session, both of which are available via the live Webcast. It is important to make sure that no personal actions or any other confidential information is intentionally or inadvertently disclosed or discussed in the open session. If there are no comments from the public, the meeting will go to closed session, and that will not be Webcast.

For the closed session, the FDA staff being evaluated will leave the room and also the alternate industry representative, Dr. Dale Endo, will leave the telephone conference.

I have been given a note that Dr. Celia Witten may be on the phone. Is that correct?

DR. WITTEN: Celia Whitten, Office Director of the Office of Cell Tissue and Gene Therapies.

DR. KIM: To those on the phone, please remember to identify yourself before speaking and to mute your phones when you are not speaking to help minimize any background noise so that the transcriber can pick up everything that is being discussed.

We will also remind the committee that the information related to the personnel action for the FDA staff is confidential and must not be disclosed or discussed in the open session with others outside of the closed session.

I will now read the Conflict of Interest

Statement into the public record.

The Food and Drug Administration convenes today's meeting of the Cellular, Tissue and Gene Therapies Advisory Committee under the authority of the Federal Advisory Committee Act of 1972. With the exception of the industry representative, all participants of the committee are special government employees or regular federal employees from other agencies and are subject to the federal conflict of interest laws and regulations. The following information on the status of the advisory committee's compliance with the federal conflict of interest laws including, but not limited to, 18 USC Subsection 208 of the federal Food, Drug and Cosmetic Act is being provided to participants at the meeting and to the public.

FDA has determined that members of the advisory committee are in compliance with federal ethics and conflict of interest laws. Today's agenda includes an overview of the research programs in the Tumor, Vaccines and Biotechnology Branch and the Cellular and Tissue Therapies Branch, Division of Cellular and Gene Therapies Center for Biologics Evaluation and Research. This overview is a non-particular matter. Based on the agenda, it was determined that these overview presentations present no actual or appearance of conflict of interest.

Dr. Dale Endo is serving as the industry

representative acting on behalf of all regulated industry and is employed by Sangamo Biosciences. Industry representatives are not special government employees and do not vote.

The Conflict of Interest Statement will be available for review at the registration table. We would like to remind members and participants that if discussions involving any products or firms not on the agenda for which an FDA participant has a personal or imputed financial interest, the participant needs to exclude him or herself from such involvement and their exclusion will be noted for the record. Thank you.

DR. CRIPE: I would like to remind people on the phone to mute their phone if they are not speaking and unmute when they do speak because we are getting a lot of extraneous noises.

As Janie mentioned, we have two parts to this afternoon's meeting, the open FDA overview presentation followed by an open public hearing, and we will accept walk-ins at that time, and then a closed session.

To begin the FDA presentations I believe the first speaker is Dr. Carolyn Wilson, who is the Associate Director for Research for CBER.

Agenda Item: Overview – CBER Intramural Research Program, Carolyn Wilson, Ph.D., Associate Director for Research, CBER

DR. WILSON: This is Carolyn Wilson. I apologize for my voice. Something hit me over the weekend and I have a little bit of laryngitis but hopefully you can hear me okay.

I want to start by thanking the two Co-Chairs. This is sort of unusual that in this one session we're looking at reviews from two separate site visits, one for the Tumor Vaccines and Biotechnology Branch which was chaired by Dr. Emens and Cripe, so I want to thank them and the team they led for their insights and their report. And then we are also going to be discussing in closed session the report on the Cellular and Tissue Therapies Branch, and that was led by Dr. Isacson and Assan, and I thank them as well as their team for their report.

I'm going to try to quickly give you an overview about the Center for Biologics and our research program and how we use regulatory science. We have a strategic plan that we are actually in the process of working on to revise the strategic goals, but for now this is the strategic plan that we've been working under.

It had six major goals. I'm not going to read them now, but there are links to both the center's

strategic plan and the strategic plan for regulatory science and research that were both organized around the same six goals regarding national preparedness, improving global public health, enhancing the ability of science and technology to facilitate development of safe and effective products, safety of biological products -- and this is really talking about safety in the post-marketing component of the product life cycle -- advancing regulatory science and research, and managing for organizational excellence.

Because regulatory science is oftentimes a term that means different things to different people, I would like to just take a moment to at least give an understanding from CBER's perspective of what regulatory science means to us. We think of it as using science and regulation to advance product development. By that, what I mean is things begin with a public health issue that drives, in some cases, development of a novel product.

Sometimes those novel products -- and I would say particularly it is the case in Office of Cell Tissues and Gene Therapies, as you probably know from sitting on this advisory committee, that we don't always have all the science and knowledge and methodology in place to be able to address all the questions that we might ask from a regulatory framework. So this poses a challenge, and that's where regulatory science, through a combination of

discovery science and directed development of new tools, may help fill some of those gaps and perhaps may look at development of new animal models for assessment of new products, at developing better understanding of mechanism of action, for example, to inform development of potency assays. Sometimes reference materials need to be developed and so on.

As this new science emerges, it puts the agency in a better position to develop regulatory policy and make science-based decisions. As we get better policy out to our sponsors we then, in turn, get better data back from them, and it positions us in a better way to make benefit-risk decisions.

This, hopefully at the end of the day, results in the goal that I think we all share, which is a product that's licensed, that's both safe and effective, and has a positive impact on that public health issue that drove the cycle to begin with. And then, of course, after it's licensed, we still need to be vigilant in terms of post-market surveillance for potential adverse events or safety concerns.

CBER has what's called the researcher regulator or researcher reviewer model. This is where our research scientists do all the same activities as our full-time reviewers, meaning that they go out on inspections, they

review submissions to the agency, INDs, BLAs and so on. They participate in organizing and presenting at advisory committees, writing policy documents and so on.

And because these scientists both are active members of the external scientific community, going out to meetings and presenting and hearing about the novel science in the field while also seeing across the entire spectrum of products coming into the agency, it positions them in a really unique spot of being able to both look proactively at what hasn't yet come to our doors but is likely to in the future while also being able to look across an entire portfolio of a class of products and identify what kinds of scientific gaps might help move those products forward. This allows us to make sure our research is really being used in the most efficient way possible.

And, of course, we don't do all this work by ourselves. We actively collaborate. This is data from an FY2015 research reporting database just showing that we collaborate broadly across the United States, and globally, and with a variety of different sectors including academia, other government agencies, industry as well as nonprofit.

So, how do we manage the research portfolio? This is something that we're currently in the process of revising, but for the purpose of this review, since this was the way we were doing it for the past four years, I'm

going to explain to you what we have been doing.

We have an online research reporting database whereby each principal investigator provides a progress report, future plans, and, in some offices, that also includes a budget request. We also collect other relevant information such as presentations and publications. Other output might include things like employee invention reports or other tech transfer-related information.

That has been reviewed by the lab chief, division director, the associate director for research and the office director, and they look in general at the relevance of the work for stated office priorities, productivity and track record of the scientist and the quality of the work and the feasibility going forward. Then funding is allocated in accordance with that review outcome.

We also have a cyclic review that is every four years. That includes an external review which is the subject of today’s discussion, which is where we bring in a panel of scientific experts working in the same field as the individual scientist under review. We call that a site visit. And we convene those site visit teams as subcommittees to a parent advisory committee.

The report then becomes part of a much larger package that also goes to an internal peer review committee which is called the promotion, conversion and evaluation

committee, and they look at not just the research accomplishments but also the regulatory accomplishments and the portfolio of the individual scientist as well to make decisions on whether or not they are ready for conversion to being permanent or for promotion, or if they're still on track at the assigned grade level.

The site visit report that you will be looking at today is actually a draft report from the site visit team. You're being asked to look at the report and being asked whether or not you're willing to accept it as written. You also have the option to provide amendments to the report. You can also send it back to the site visit team if you feel there's a major issue that needs to be addressed.

Once it's approved by the full advisory committee, it's used in a variety of ways. As I mentioned, it goes to the internal peer review committee, the PCE, to support personnel actions by the PIs who take the recommendations and the scientific directions posed by the report very seriously to improve their own research program, and of course by management when looking at resource allocation decisions.

I wanted to just finish the last few minutes with two topics about what's new. One is an issue that has come up actually in multiple different site visits, which is the issue of having some kind of mentoring for our principal

investigators. We actually looked at this. It was led by Sue Epstein here in OCTGT who looked at a variety of different institutions and what they were doing to do peer mentoring. From that, taking into account the size and diversity of the research here at CBER, we decided that the best way forward is to have a voluntary monthly meeting that is open to all principal investigators.

We ensure that each month there is at least one or more senior investigators willing to participate as an informal mentor, and it's an opportunity for the younger scientists to talk to more senior scientists about various issues relating to management of their research laboratories. There's always the opportunity for informal individual mentoring as well, but I think this is a good way to get started.

The second thing is our move to the White Oak campus, and this is something that happened in the summer of 2014. We came to what is called the Life Sciences and Biodefense Laboratory here in White Oak. I wanted to just show you a few pictures to give you a sense of space, what it looks like. As you can see in the top two photos, that's an external photo. On the right is a break room area. There are various pantries and break rooms throughout the building. And the bottom two photos are of a particular BSL-2 level laboratory with individual enclosed space for

supporting tissue culture or other specialized needs.

We built into the facility a number of characteristics to help support science and to help us move forward with emerging science, including an expanded imaging capability in the vivarium, transgenic derivation capability. We also built into it expanded space to support certain core technologies. And, importantly, we also have a significantly expanded space for working with BSL-3, or risk level 3, pathogens including insectariums for BSL-2 and BSL-3. And we also have specialized suites to support things like microarray and PCR, NMR and mass spectrometry.

This is just a few pieces of high-end equipment that we have been able to acquire over the last few years. We have an Illumina HiSeq in our core facility. We have a flow cytometry core that has both analytical flow and live cell sorting capability, and, most recently, we have obtained the ability to do 12 parameter cytometric measurements. We have high resolution NMR, up to 850 megahertz, as well as high resolution confocal mass spectrometry and so on.

I'll finish where I started which is with a large thank you to both the site visit teams who participated and to you today for your input, which is really critical for us to ensure our high quality research and fulfilling our regulatory mission.

Again, thank you for your patience with my scratchy voice and I am happy to answer any questions if you have them.

Agenda Item: Q&A

DR. CRIPE: Thank you, Dr. Wilson. I have one question. You mentioned the research reporting database. Is any of that database public?

DR. WILSON: No. This is an internal database that we use for managing our research portfolio, making budget decisions and things like that. But part of the research program that is public is we have a publications database which is on our website, and then each principal investigator has a one-page summary on the Web that describes in very general language sort of an overview of their research program. And, of course, everything that we do we encourage our scientists to publish, so not only is the listing of presentations on our website but we try to publish as much in the peer-reviewed literature as possible to get information out to the public.

DR. CRIPE: I came up with a second question. The beautiful core facilities you have with all that nice equipment -- is that used by investigators on a fee-for-service basis charging their grants or their budgets, or is it just freely available?

DR. WILSON: It’s a mix. Right now, we have what’s

called a biotechnology core facility and that is fee for service where the individual principal investigators are charged based on the services they order. Our two more recent core facilities, which are flow cytometry and confocal, so far we've been funding those off the top, if you will, but we're looking into models to move towards a more fee-for-service type of approach. It's more challenging with confocal, as you probably know, so that is a little trickier to figure out, but we're trying to think through some of those questions.

DR. CRIPE: Does anyone else on the call have questions for Dr. Wilson?

DR. BUGBEE: This is William Bugbee in San Diego. I have a question regarding the researcher-regulatory concept. How do you manage conflict of interest in that scope where a researcher might be faced with regulatory questions in a similar area that they're trying to publish and develop novel products?

DR. WILSON: In general, most of our research really isn't aimed at developing novel products but is really developing the science to support evaluation of products. In that regard, we don't view that as a conflict of interest.

However, when there are those instances where there is a potential conflict of interest because an

individual might be developing a new product that could be in conflict with anything they're regulating, we do have a mechanism for looking at that carefully. We document it in a memo. We are in the process of tying that to a regulatory database to make the managers who make regulatory assignments aware of that potential conflict of interest so that people can be very thoughtful and careful about how they assign regulatory duties to an individual.

DR. BUGBEE: I have a question about one of the people we're reviewing today, but I'll bring that up when we get to that detail. It's about tissue banking and rapid assay. Thank you for your response.

DR. WILSON: That might be more appropriate to discuss in closed session.

DR. BYRNE: My question was when new projects are initiated within this life cycle it seems like there's a four-year review. Are those peer reviewed, and how are they budgeted and what is roughly the annual budget per investigator in the branch?

DR. WILSON: Let me answer the peer review part first. The peer review I talked about is every four years. It is two parts. One is the external peer reviewers that come as part of a site visit team, and the second is the internal peer review that's done in conjunction with personnel actions or evaluation for making sure that

they’re still working at the same grade level if they have already been converted but maybe aren’t quite ready for a promotion.

Again, the specifics regarding personnel actions are more appropriate to discuss in the close session, so if you have specific questions around that piece we can come back to that in closed session.

Regarding individual budgets, that varies depending on the nature of the science that’s being done. It varies depending on whether or not the individual is bringing in additional funding from other sources. In terms of your question, it’s very hard to give an average number that would really be informative.

I may defer to Raj if he wants to provide some general information about his division. Maybe that’s more informative than the center as a whole.

DR. PURI: This is Raj Puri; I’m the Director of the Division of Cellular and Gene Therapies. I can provide you some perspective about the budget.

First of all, the salaries of the staff come from the center and FDA. The post-doctoral fellows were supported also, in the past, from intramural targeted research grants. Our PIs wrote and succeeded in getting those. Also, the division assigned targeted funds that we give out to fellows as support for the folks.

Generally, for the lab support, we give out close to I would say, between $50,000 to $70,000 per year per PI. That does not include the cost for the lights and telephone bills and others, so this is direct research funding for staff. As you heard from Dr. Wilson, we have significant core programs that our staff will provide their funding from the lab to get their peptide synthesis or oligo-synthesis and so on and so forth. So, in the ballpark of $60,000 to $80,000 per year, and this number changes depending on the budget we might have.

DR. CRIPE: Any other questions?

Janie, I think you probably noted that Drs. Bugbee and Byrnes joined the call after our roll call.

DR. KIM: Yes.

DR. CRIPE: In the absence of any other questions for Dr. Wilson we'll move right on to Dr. Puri for this overview of OCTGT and research programs.

DR. PLUHAR: Excuse me, this is Dr. Pluhar. I just wanted to say that I am here, also.

DR. CRIPE: Thank you.

Agenda Item: Office of Cellular, Tissue and Gene Therapies (OCTGT) and Research Programs, Raj Puri, Ph.D., Director DCGT, CBER

DR. PURI: Good afternoon. Before I begin I'd like to introduce myself. I am Raj Puri, Director of the

Division of Cell and Gene Therapies in the Office of Cell, Tissue and Gene Therapies I would like to thank the committee for your time and your efforts in reviewing the briefing document, the site visit report and the evaluation of the research program in the Division of Cell and Gene Therapies.

In my discussion this afternoon I will talk about the organizational structure of our office, the office mission and activities, office regulatory portfolio, and I'll touch a little bit on research, regulatory scientist and researcher reviewer model, and the research management, some of which you heard from Dr. Wilson. I will end my discussion with providing the summary from the Tumor Vaccines Biotechnology Branch -- that will be the research summary -- followed by Dr. Steve Bauer, who is the Branch Chief of the Cell and Tissue Therapy Branch who will provide a summary for his branch.

I will talk about the organizational structure of the office, the office mission and activities and regulatory portfolio, and I will touch upon the regulatory scientist and researcher reviewer model and the research management. I'll finish up my discussion summarizing the research program in the Tumor Vaccines Biotechnology Branch followed by Dr. Steve Bauer.

Our office is headed by Dr. Celia Witten, who is

here in the audience, and it has three divisions -- the Division of Cellular and Gene Therapies, and, in collaboration with Division of Clinical Evaluation, Pharmacology and Toxicology, we do all pre-market review of regulatory files submitted to our office. The folks in the Division of Human Tissue are responsible for protecting the human tissue safety.

Slide No. 4 talks about the OCTGT mission. The mission is to ensure the safety, potency and effectiveness of cellular tissues and gene therapy products for the prevention, diagnosis and treatment of human disease.

Our office regulates products which are shown in slide 5, and that includes cell therapies, cancer vaccines, immunotherapy, gene therapies, tissue and tissue-based products, Xeno products and combination products where the cells are -- gene-modified cells are mixed with the devices which are used to select the cells and tissues and donor screening tests.

Slide 6 lists the regulatory portfolio of our office. We have more than 1500 active INDs and IDEs and master files, and our staff reviews more than several thousand amendments per year. They provide consult reviews. So far, we have 12 licensed products and a growing number of products in advanced stages of development, meaning they are in Phase 3 trials, and the BLA review.

We review devices by the various regulatory pathways and have other activities as listed on this slide. I'll expand upon them a little bit in the next slide.

Slide 7 lists those activities. Our staff reviews, evaluates and takes appropriate action on product applications and amendments or BLA license applications and supplements for our products. Our staff invests a significant amount of time in pre-pre-IND, pre-IND, and preIDE submission advice and in meeting with the sponsors or teleconferences in pre-IND meetings.

Our staff participates in collaboration with the Office of Compliance in inspections of manufacturing facilities for compliance and, in some cases, including court cases. We develop policy and procedures governing the premarket review and evaluation of our product class in keeping with the provisions of the Public Health Service Act and the Food, Drug and Cosmetic Act.

Slide 8 talks about our staff develops a number of FDA guidance documents for the regulation of our products. We provide consult and education to various offices within CBER, FDA centers, government agencies, and sponsors. Our staff organizes and writes briefing documents, develops questions and makes presentations at advisory committee meetings.

We do a significant amount of community outreach

as we develop the cutting edge area of medical research. The outreach includes a variety of professional societies, academic institutions and patient advocacy groups. We have partnerships with the standard-setting organizations -- NIH and global regulatory authorities from all around the world in cell therapy and gene therapy areas.

Our staff participates in counterterrorism activities and performs research to support the review and progress towards safe and effective medical products development.

Slide 9. Our staff has been very prolific, as you can see. In four years we have published a number of guidance documents that are very useful to sponsors who are trying to develop a clinical trial for their novel products and also to sponsors whose products are already in development who want to take them to the marketplace.

Slide 10 lists the research priorities of our office. Our research priorities are aligned with the center research priorities, which are focused on chemistry, manufacturing and controls; pharmacology, toxicology and product rationale; participation in various government FDA-wide and DHHS-wide initiatives; and improvement of the microbial safety of human tissue products.

Slide 11 shows the organizational structure of the division. The division has five branches. I would like

to recognize Dr. Kin Benton who is the Deputy Division Director with me. The two branches on the left, Gene Therapy Branch and Cell Therapy Branch, are full-time regulatory scientists. They review the applications, develop the policy and guidance document, and they perform quite a bit of outreach effort.

The staff in those three branches on the right side and in the middle are research regulators, and these folks also do regulatory review and outreach and policy and guidance document development, but 50 percent of the time. The other 50 percent of their time is spent performing the FDA mission-relevant research.

Slide 12 lists the current research areas in the Division of Cellular and Gene Therapies. That includes virology. We have expertise in various different viruses and viral vectors, expertise in immunology, cell and developmental biology, cancer biology and immunology, biotechnology. We have quite a bit of resources within the division. We have expertise in the areas of genomics, flow cytometry, proteomics, transgenic and whole genome sequencing, and we have a number of recruitments available that are not only used by our office colleagues but are also available to others in the center and the FDA.

The next slide talks about the MSC consortium. Many years ago, DCGT formed an MSC consortium of seven

principal investigators who set out to investigate the product characteristics of a model cell type. In this case, they chose MSC, multipotent stromal cells, and this cell type is very popular. There's a large number of clinical trials currently ongoing. The idea here was to link the product characteristics with the clinical outcome, or biological outcome, in the animal model. You will hear some of it from different PIs which were presented at the site visit, and Dr. Bauer will touch on this. This consortium is chaired by Dr. Steve Bauer who will discuss a little bit about this in his presentation.

Now I'm going to move on to slide 14 which talks about the researcher reviewer model. Our products are diverse and rapidly evolving. They use new regulatory paradigms which are developing rather than established. These novel products raise extraordinarily complex issues; therefore, DCGT seeks to foster a cadre of researcher reviewer scientists who perform regulatory review, identify regulatory science-related research needs to enhance and promote product development and patient safety. They perform research in key areas to support the FDA mission and help sponsors solve product development problems to advance OCTGT products to the marketplace.

We are not trying to reproduce industry, academia or NIH expertise here; however, we need scientists in key

areas that can engage in constructive and informed evaluation of files and productively interact with industry and academia including their cutting edge scientists.

Slide 15 will discuss the types of researcher reviewers we have in the division. We have principal investigators. Currently, we have 11 PIs and two are being recruited. We have staff scientists who are tenured researcher reviewers. They support the PIs program. They do both research and review. We have technical staff who do primarily research and some do a limited amount of review work.

We have staff fellows who are FTEs, and these fellows could be in this position for up to four years. They do research and review and they support the PI. And we have commissioner's fellows; these fellows could be selected for two years. It could be in the lab and also do the review work. And we have IOTF fellows, which is under the collaboration with the NCI Interagency Oncology Task Force, and we have fellows who are assigned to us from NCI for two years who do both research and review work.

The postdoctoral fellows come to us from the ORISE mechanism, Oak Ridge Institute for Science and Engineering. They are recruited through that mechanism, and these fellows support the PIs program. The resources are provided to the PIs who are expected to lead the research

program, the FDA mission-relevant research program, in their labs.

Slide 16 talks about the responsibilities of the PIs. In addition to product reviews, PIs participate in inspections, and the regulatory mentoring of the PIs is provided by their branch chief and the regulatory branch chiefs as well. They participate in the policy development, guidance document development and advisory committee discussions. They do outreach in addition to pre-submittal advice in the regulatory space.

They also do scientific and regulatory talks, refereeing, and editing of journals, chairing sessions at scientific conferences and scientific collaborations. They manage their lab activities, do training, mentoring, supervising, publishing papers, grant writing and leveraging and collaboration, and expert peer reviews of theses or research articles.

Now I’m going to talk about research management in the division on slide 17. You heard from Dr. Wilson that each PI is required to submit an annual report at the CBER research central database. This research summary is assessed by the branch chief and the office director. Each year, in addition, DCGT asks all the PIs to provide their research and regulatory accomplishments in terms of the number of papers they published that include regulatory

articles, review articles, the regulatory files review, any chairing, any license applications and special circumstances.

Dr. Sue Epstein, who is an Associate Director of Research in our office, takes this data and crunches the numbers and categorizes these folks in the high, medium or low category and we give them a reward for an additional budget, which is a small amount but we give it out recognizing staff for their excellence.

A number of years ago, DCGT began mentoring of the PIs program, and you heard from Dr. Wilson that this program has been implemented DBER-wide, which is a very useful program for particularly the new and also not-so-new PIs.

Our research programs have been reviewed externally in addition to advisory committee. Our MSC consortium project was reviewed by CERSI -- Center for Excellence and Regulatory Science Initiative -- under the umbrella of the Office of the Commissioner. In Maryland we got input and evaluation from the world’s expert in the field of MSC. You heard that we present at the site visit every four years and this report is presented to the full committee, which is being done here today, for your recommendation.

In addition to that, we have an internal review

committee which you heard about from Dr. Wilson, and the Promotion and Conversion Evaluation Committee. This committee reviews the scientific presentation of the PI, national and international recognition, site visit report. First and last are their papers in high-quality peer-reviewed journals, their regulatory contribution and CBER service work. In addition to that, they undergo cyclical review every four years to ensure they are performing at their grade level.

Now I'm going to change gears and talk about the research summary in the Tumor Vaccines Biotechnology Branch as the Branch Chief of this branch, including my own research program.

My research program is geared towards the development of safe and effective cancer vaccines and immunotherapy products. On slide 20, I have listed some of the important public health challenges and the scientific and regulatory challenges.

Cancer is the most difficult public health problem. A variety of different products are under development to target cancer. It's important to identify a target that is specific for cancer, not in normal cells. There are a large number of cancer vaccines and immunotherapy products that are being tested in the clinic. Often, cancer vaccines are not defined antigens; they are

peptides with a mixture of cells, antigens or gene-modified tumor cells. Therefore, identification of appropriate identity and potency tests which are critical for product development are often challenging.

The animal models of safety and efficacy are important to determine safety, starting dose, frequency of dosing, dose escalation and prediction of response in clinical trials. Thus, identification of appropriate animal models of human disease may help guide these parameters. Similarly, immune markers or other markers of response and safety are rare. These biomarkers may predict clinical outcome, good or bad, and may be identified in preclinical animal studies as well as in early phase clinical trials.

To address some of the challenges, in slide 21 we have listed three different program areas being done in my group. The first specific program deals with the characterization of tumor-associated cell surface proteins that could be antigens or receptors that can be used as a target for cancer therapy or can be used for identity and potency of these products.

The second program area deals with establishing various animal models of human disease or human cancer that can be used to assess the safety and effectiveness of vaccines and development of biomarkers for cancer vaccines, immunotoxins, cell and gene therapy products.

The third program area was established about 10 years ago. Our group established an interagency agreement with National Cancer Institute and we established a microarray program in CBER. Under that collaboration, NCI funded us very generously and we purchased a large number of equipment to begin the microarray program at CBER. Through that program, we have published a number of articles and mentored and trained and educated a large number of staff in CBER, FDA, as well as USHHS and NIH.

The last reporting period, our group has used the microarray technology in characterizing cellular cancer vaccine such as dendritic cells. We have looked at and identified cancer stem cells in head and neck tumors, and a staff fellow in the group who is part of the MSC consortium has used this technology in characterizing MSC to identify potential markers of quality of the cell type.

Slide 22 deals with the first project program that was established since the discovery of the Th-2 derived cytokine receptors in solid tumors. We were the first to identify the Th-2 derived cytokine Interleukin-4 receptors in human cancer, and over the years we have published a large number of articles on identifying this receptor in human cancer and also unraveling the structure, the signal transduction of this receptor and how it is different from that of immune cells.

In collaboration with the National Cancer Institute and Bob Criteman, we have developed an Interleukin-4 pseudomonas exotoxin that has been tested in various preclinical models and also in clinical trials. Currently, it's being further investigated for brain tumors.

In the last reporting period we have reported on thyroid cancer, anaplastic thyroid cancer, as well as in bladder cancer. These articles have been published.

In the late 1990s, a new cytokine was cloned called Interleukin-13, and we were first to identify the receptors for Interleukin-13 in renal cell carcinoma, followed by a number of studies we have reported on a variety of different cancers. Similar to the Interleukin-4 receptor, we have unraveled the structure of this receptor and signaling through this receptor and targeted this receptor with IL-13-PE immunotoxin. In the last reporting period we have studied the structure and function of this receptor and targeted this receptor in pancreatic cancer as well as adrenocortical cancer.

In collaboration with NCI, we have just completed a Phase 1 clinical trial using IL-13-PE and targeting adrenocortical and pancreatic cancer, and this article was published last year.

In the next slide we talk about a variety of

different human cancer models that we have established over the last four-year period including this model of human cancer in immune-deficit animals and xenograph models, and the mouse tumors in the immune-competent mice model -- three tumor models that we have established to be used in cancer vaccines and immunotherapy projects.

To conclude my research program, we have reported that human tumors express high levels of IL-4 and IL-13 receptor in cancer compared to the normal cells, and the structure of these receptors is different in cancer compared to that of normal immune cells and normal cells.

The Interleukin-13, we have reported can signal to IL-13Rα2 by AP-1 pathway, a novel observation. IL-13Rα2 is uniquely expressing cancer, and we have found that IL-13Rα2 is involved in cancer invasion and metastasis. We have reported that the IL-13Rα2 chain is a tumor antigen. A cDNA vaccine followed by boost with the protein-enhanced antitumor showed effects in three mouse tumor models and, in combination with IL-13-PE, immunotoxin targeting the IL-13Rα2 chain with the vaccine, we have shown a mediated robust antitumor response.

Lastly, we have identified potential surrogate molecular markers of antigen presentation in immature dendritic cells, and that marker is being confirmed by

using other peptides. In the previous studies we used the HPV E7 peptide for the studies.

Now I'm going to move on to Dr. Michail Alterman's work. Dr. Alterman is a PI in the Tumor Vaccines Biotechnology Branch. He is an expert in proteomics technology, and his program goal includes development of mass spec-based proteomic tools for qualitative and quantitative measurement of cellular products, cell substrates and vaccines.

Slide 26 talks about the power of mass spectrometry. Mass spec measures the molecular mass of the protein. Molecular mass is a major physical chemical feature that can be measured for all components of a biological mixture and uniquely identify and distinguish them. MS provides a direct quantitative and qualitative assessment of components in drug products and drug substances.

This technology can be used in identifying the product quality, batch to batch control, product identity, purity, potency, stability, comparability and identifying impurities and contaminants, and also the structure characterization for sequence and high-order structure.

Dr. Alterman, in the last 40 years, in slide 27 lists his completed projects. Since his previous site visit, he has characterized a unique proteomic signature of

human multipotent stromal cells. He has developed a protein marker panel for characterization of human induced pluripotent stem cells, and this work was done in collaboration with the scientists at National Institutes of Health.

In collaboration with the scientists at Office of Vaccine Research and Review in CBER, Dr. Alterman's group has applied mass spec technology in looking at quality control of influenza virus as well as flu vaccine. Finally, his group has done a mass spec proteomic analysis of human liver cytochrome P450 enzyme.

The major conclusions of his group are listed on slide 28. He has identified the largest to date proteomic dataset of culture-expanded MSC identifying more than 7,700 protein groups. The results obtained highlight the extent of donor-to-donor proteomic heterogeneity. The quantitative proteomics of proteins expressed at different passages -- Dr. Alterman's group identified additional differentially-regulated proteins panels of 150 proteins.

A panel of potential pluripotency markers for hiPSC was selected and validated based on their proteomic data. A quantitative comparison revealed that 300 proteins that were overexpressed in human embryonic stem cells and two human-induced pluripotent stem cell lines compared to their parental cells where they were derived from.

In the next slide, I will talk about the research summary provided to me by Dr. Shyh-Ching Lo. Dr. Lo heads the Tissue Microbiology Lab in the Tumor Vaccines Biotechnology Branch, and the goals of his group are to develop new technologies in the evaluation of innovative methods to assess the safety and quality of human tissues intended for transplantation.

There are an increasing number of human tissues recovered from cadavers and processed as grafts by the industry and used by the medical practice each year. This lab was established to help assess the technology and HCT/Ps in order to prevent the transmission of communicable diseases.

This lab was established in the Division of Cell and Gene Therapies in collaboration with the folks in the Division of Human Tissue to enhance both the safety and availability of high-quality human tissues. The scientific capability of the micro program was designed to bring wide expertise to be able to assess the bacteria, fungi, viruses and protozoan parasites.

The specific aim of the microbiology lab, slide 31, is to establish the required scientific capabilities to directly support the regulatory needs for tissue safety; to adopt new technologies for rapid detection of target high-risk infectious pathogens with high sensitivity in human

tissues; to develop highly effective genome sequencing capability for identification and characterization of various microbial agents using NGS sequencing technology; and to explore new scientific approaches for detection and characterization of previously unknown or newly-emerging infectious pathogens that can threaten the safety of human tissue and public health.

Slide 32 lists the accomplishments and a summary of this lab's activities. They have established the lab capability of clinical microbiology for detection and characterization of microbes. They have developed a rapid, highly sensitive technology -- for example, RT qPCR -- to assay the targeted high-risk microbes including bacteria, fungi and RCDAD relevant communicable diseases and disorders and viruses.

They have developed high throughput genomic sequencing capabilities for detection and characterization of established and previously unknown infectious pathogens, and they have identified pathways and biomarkers associated with the injury mechanisms in cells and tissues by NGS sequencing technology.

Lastly but not least, I would like to thank Dr. Sue Epstein and Dr. Celia Witten for their support, and the entire Division of Cell and Gene Therapies for their outstanding work on a daily basis. And I would like to

thank the subcommittee as well as the full committee for reviewing our research programs and providing your insights. Your input is very critical to fulfilling our regulatory mission.

I'll stop here and take any questions you might have.

Agenda Item: Q&A

DR. CRIPE: Thank you, Dr. Puri, for the excellent overview. I have a question. You cited early on the number of regulatory reviews, et cetera, which seems quite robust, 1530 active. I'm wondering how you prepare for growth and plan for capacity given that this is an emerging field with a lot of new companies and products coming down the pike. Are you able to model?

Part of that question is how far are you behind? What are your metrics in terms of timing to get reviews done now compared to other divisions? Do you anticipate that there's going to be a backlog soon?

DR. PURI: That is an excellent question. We have, as you know, the Prescription Drug User Fee Act. Under that act we are expected to complete review of all the INDs submitted to us within 30 days. If we do not complete on time, the sponsors can go ahead and start their clinical trials, which we don't want.

Our staff works very vigorously in meeting 100

percent of time the expectations, and that is the expectation of our staff, to meet the regulatory time lines.

Similarly, we have time lines established for the license applications. Under the program they have 10 months to review the BLA submission, and if it's an accelerated path, then we have 6 months to complete the review.

This is challenging, getting enough people to do the work. We are creative in trying to promote and help recruit Commissioner's fellows and IOTF fellows. We always try to make a case for additional support to be able to keep up with this challenge.

I just want to let you know that we're striving to meet all the deadlines as we are given those files. Going forward, we continue to make a case. Dr. Witten is here and our center -- they are all aware of our needs. We continue to make the plea for additional resources to be able to meet those challenges.

DR. CRIPE: Great. Thank you. Other questions?

DR. ISACSON: This is Dr. Ole Isacson. I have a question relating to your organizational chart, which is slide 11. There you have five different branches. I'd like to understand better how you coordinate the different branches. For example, how do you distinguish the work between the Cell Therapies Branch and Cellular and Tissue

Therapy Branch?

Perhaps you can also give us some scope on how many PIs are in each branch. And perhaps thirdly, the question of how you integrate those five branches.

DR. PURI: The folks in the regulatory branches -- the Gene Therapy Branch and Cell Therapy Branch -- are full-time review scientists. Their full-time job is to review the regulatory applications, develop policy and guidance documents, interact and do a lot of outreach efforts.

The number of applications they have to review is twice as many compared to the folks in the other branches, for example, in Cell and Tissue Therapy Branch. The Cell and Tissue Therapy Branch folks do 50 percent of the time their review work. Their INDs are assigned by the Cell Therapy Branch Chief, in this case, Dr. Steven Oh. He carries a chart with him and looks at how many files have been assigned to individual staff in both branches and tries to apply the standard of meeting 50 percent review by the Cell and Tissue Therapy Branch.

The files are assigned based on the expertise. We try to do our best to match with the expertise of the individual PI and their staff, but it’s not always possible. We could not include and have people in all different areas of products that we evaluate.

Similarly, in the Gene Therapy Branch, Dr. Denise Gavin assigns the files to staff in all three branches depending on their expertise. If the file is on cancer vaccines or gene therapy, she will assign that to folks with the expertise in these branches. Again, the same criteria she applies to trying to have half of the files in the research regulatory branches and then in the full regulatory branch.

Does that answer your question?

DR. ISACSON: Yes, thank you very much.

DR. ZOVEIN: I was just curious as to the focus on looking at MSCs. I assume a lot of the products that you all are evaluating probably use that as a main tissue or cellular source. I assume that's probably one point of focusing on that cell type, but I was curious whether you have done comparisons with other possible cellular carriers that may be either less heterogeneous or less difficult to define.

DR. PURI: If I understood the question correctly, you're wondering about the source of MSC and the heterogeneity of MSC?

DR. ZOVEIN: No. It's more the rationale for the focus on MSCs versus other cell types or comparative analysis of different possible cell types.

DR. PURI: I am going to have Dr. Steve Bauer

answer that question.

DR. BAUER: This is Steve Bauer, and I'll be talking about that topic a little more in depth in just a few minutes. But I can say right now that MSCs are one of the more common cell therapies that we regulate in this division. We think that some of the issues of cell characterization that come along with MSCs are illustrative and widely applicable to other types -- not all, but many other types of cells that we also see here in the division.

We hope that the research we do on MSCs is widely applicable.

DR. CRIPE: Do you ever have to go outside your division to get expertise for the regulatory reviews? Are you free to tap other groups?

DR. PURI: Yes, we do. A number of times we have special government employees. For a particular file we bring SGEs from our advisory committee as well as additional and the world's expert. A number of times we invite patient advocacy groups to our regulatory meetings with the sponsors.

DR. WILSON: And we also consult our colleagues at FDA, so a lot of times these could be combination products, or it could be people in drugs or devices that we need, too. So we also do that.

DR. CRIPE: And are there areas that you think

you're lacking? For example, CAR t-cells or NK cells? Do you need to have research programs in those areas -- ?

DR. PURI: Right. Thank you, Dr. Cripe, for bringing up that issue. CAR T-cell is a very popular cell type. We have more than 106 INDs currently in-house, and this is a rapidly developing field.

I probably neglected to mention that we are trying to recruit two PIs in our division, and one of the PIs will have some expertise in the CAR t-cell project as well.

We have a Commissioner's fellow who was recruited in Dr. Gavins' branch, and she is helping support looking at the regulatory files and identifying commonality between CAR t-cell products and learning from them, scanning in the field to see what else needs to be looked at as the field is rapidly developing.

So definitely there is a need for this. We are trying to build the support internally. Also, we are trying to have a contractor who can help us out in looking at the data of different files and see what are the safety signals and how we can assess them and compile them together in collaboration with the NIH Recombinant DNA Advisory Committee that can help facilitate those comments and questions for the field to address them as they develop those products.

DR. CRIPE: Excellent. Any other questions from the committee? Great. We are doing well on our time.

Next on the agenda is Dr. Steve Bauer who will be giving us an overview of the research in his branch.

Agenda Item: Research Highlights – Cellular and Tissue Therapy Branch, Steven Bauer, Ph.D. Chief, CTTB, FDA

DR. BAUER: Before I get into the slides I just want to add my thanks to all the people on the site visit committee who came and worked so hard to give us input and evaluate our research, and to you folks on the advisory committee as well for your efforts and giving us valuable feedback.

I also want to thank my colleagues in the Cellular and Tissue Therapy Branch in the Division of Cell and Gene Therapies, the office, and FDA and the Center for Biologics Evaluation Research for the generous research support that we've received over the years.

Today, what I hope to do is give you a very high level overview of some of the research efforts that go on in the Cellular and Tissue Therapy Branch and were presented at our site visit on November 19th of last year.

The first slide has a list of the PIs and the staff fellows who presented at our site visit. Just to give you a feel for what people in this branch review, it covers a lot of the waterfront that we see in the division

including cell therapies, gene therapies and devices. In the arena of cell therapies, people in my branch are actively involved in stem cell applications, adult cells and combination products, and gene therapies and a variety of devices as well.

Some of the challenges that face this field are that the markers and the characteristics that many of our sponsors use to characterize different types of cell products don't seem to adequately be predictive of cell state and cell fate, and I'll be spending a lot of time on that theme. It's one of the major issues that our research addresses. There's a relatively poor understanding of how cells interact with their microenvironment and where they go and what they do after they're actually administered, post-transplantation.

The research approaches that are used rely on complementary systems including xenopus, drosophila, mouse and human cell lines. We look at gene protein cell and tissue interactions and how they influence normal development. The overriding theme is that if we have an increased ability to understand how different signaling pathways relate to cell developmental biology, we think that will represent a new and perhaps better way than just relying on a few cell surface markers and gene expressions in general as a way to characterize cell products. So these

are generalities and not true across the board, but things that we encounter often as we look at the regulatory science challenges for cell therapy.

I, of course, was one of the presenters and talked about the MSC Consortium, and then I'm going to briefly touch on the research of Heba Degheidy, a staff fellow in my lab, on the issues around flow cytometry standardization and quantification.

The MSC Consortium, the intent there is to take one of the kinds of the cells that we often see. A few years ago, we published a survey of the kinds of information that come along with cell characterization for MSCs that we actually see in regulatory applications that come before our part of the FDA. I think these illustrate very strongly this idea that the kinds of markers that people use, first of all, there's a great variety of the quantitative aspects of how those markers are used, and they haven't by and large resulted in a great deal of success in the clinical application of MSCs. So they fall under that category of adequate markers predictive of cell state and cell fate.

In the MSC Consortium, our goal is to develop strategies and illustrate that there are perhaps other ways to go about product characterization. Again, we think this can be applicable to other types of cell therapies that are

currently under development.

The strategy of the consortium was to take MSCs from the bone marrow that we purchased commercially but came from the bone marrow of eight different human donors. This slide just shows the ages and genders were from 22 to 47 years old. These were all manufactured, so to speak, in my laboratory using one lot of serum to minimize variability, and then cells were cryopreserved at passages 3, 5 and 7, if the cell lines made it that far, by a variety of techniques. You might notice that two of the cell lines -- I think circumstantially, but in this case, two of the older donors didn't make it past passage 3 in one case and passage 5 in another case.

Once these materials were available, they were subjected to a lot of different techniques for kind of high throughput or medium throughput analytical characterization and also, at the same time, subjected to a variety of *in vitro* and *in vivo* bioassays. As shown on this slide, we made a large number of MSCs; we handed them out to the different partners in the consortium.

Brenton McCright in my branch has been working on *in vivo* and *in vitro* models of wound repair. My task was to develop quantitative differentiation assays, and then you'll also hear a bit about work in the laboratory of Deborah Hursh, who has looked at epigenetics and karyotypes

of these MSCs. And Malcolm Moos' lab looked at gene expression kind of on the population level and, more recently, on the single cell level with PCR and next-generation sequencing. We also had Dr. Puri's lab looking at microarray gene expression, and Michail Alterman's lab looking at proteomics.

We looked at a variety of different parameters of cell passaging and saw -- not shown on this slide -- that there were generally increases in cell size with passage. So, as you went from passage 3 to 5 to 7, we saw increases in sizes. We also saw decreases in proliferation. Those kinds of observations have been made by many other researchers.

We also saw decreases in adipogenic potential, and we developed a quantitative assay to assess that using automated microscopy and lipophilic dyes and DNA dyes to count the cells in an automated fashion, and you see here the results of looking at those eight different lines at passages 3, 5 and 7. You can see that the adipogenic potential of these cells also decreased with passage.

Adipogenesis is one of the hallmark characteristics of MSCs, and you can see that at the onset of passage 3 there were different levels of adipogenic capacity in the different cell lines that we looked at. This has been published, but I think this suggests a couple

things. At least with the conditions that we used in the adipogenic assay that we used, you see this differentiation capacity decrease with time in culture, and it's different between cells from different donors. But it also suggests that with these kinds of assays you can begin to see that there might be heterogeneity in subpopulations that are responsible for different bioactivities that you can actually measure.

We're also interested in another hallmark differentiation pathway which is osteogenesis and looked at alkaline phosphatase activity and mineralization. Again, not shown here, we also saw, in general, differences between these different cell lines and decreases with passage.

I did mention that cell size and proliferative capacity also decreased, and one of the ideas that we had was that perhaps morphology is related or is an indicator or perhaps even predictive of some of these differences we could see. Ross Marklein in the lab took that idea and developed a quantitative measure of osteogenic activity as reflected in mineralization and developed a computerized automated methodology to quantify mineralization after long-term osteogenic *in vitro* induction. That assay takes 35 days, as shown on the left.

What we did in addition was to look at short-term

morphological characterization after only 3 days and compared that to non-stimulated, just growing cells, and then used software called CellProfiler to extract cell-shaped and nuclear-shaped features and did principal component analysis on the resultant data and picked out parameters that, in some models, we could see had a very strong ability for a 3-day morphological signature to predict the capacity for mineralization. And this is in present stem cells, but this is one of the goals of the group, to look at predictive assays of some biological function, and I think we have gone a good ways toward reaching that goal with this particular approach.

I'm now going to switch to the work of Dr. Heba Degheidy. Flow cytometry characterization is perhaps the most frequently used and perhaps the most important cell characterization scheme because many of the cell types that we look at are defined by their cell surface marker profile, but you face questions of reproducibility between different cytometers and across different laboratories. So Dr. Degheidy, along with colleagues at NIST, and a former member of the group, Jerry Marty, have been working for years on developing strategies to better standardize flow cytometry.

The approach they have come up with is to take a variety of flow cytometers and look for the one that has

the worst technical performance in terms of electronic noise in the PMT detectors and then identify target values for a bead standard or cell standard, and then transfer those values to the other cytometers. If you do this, you can get very robust performance across flow cytometry platforms and in different laboratory locations. If you do that in conjunction with a cellular standard, you can use a quantity called antibody bound per cell and standardize, or use that as a way to calculate the number of antigens on different cell types.

This is just a schematic that shows that. If you have a standard with a known ABC value you can relate that to the median fluorescence intensity. Again, this was done with NIST and they have helped develop a standard for CD4 based on mass spec. Using this simple ratio formula you can calculate the number of CD4 molecules on different cells. But here we've shown that you can use this approach to standardize across four different instruments and get very similar values for CD20. This has been a nice contribution to cell therapy.

I'm next going to turn to Brenton McCright's group and briefly talk about Dr. McCright's development of preclinical mouse models for evaluating angiogenic therapies, among them being MSCs, and Dr. Prajakta Varadkar has been looking at a signal transduction molecule, protein

phosphatase 2A, and its regulation by a subunit during cardiac differentiation.

Dr. McCright has shown that MSCs can indeed enhance angiogenic sprouting in the aortic ring model where you take a thin donut-shaped slice of mouse aorta and expose that to MSCs, and if you look at the far left you can see outgrowths. You can digitally capture those images and measure them, and you can see a difference between those cells being present and the MSCs being present and you can quantify that.

In the efforts to date, we haven't seen differences between the MSC batches that have been looked at, but I think this assay could potentially be developed and added to the armamentarium of quantitative bioassays *in vitro* that might be useful for better characterizing some cell therapy products.

Now I'm going to turn to protein phosphatase 2A. This molecule is a heterotrimer, and there are a variety of regulatory subunits in this heterotrimer. These regulatory subunits have been shown to be regulators of cell growth and potentially tumor suppressors, so mutations in the 2A subunit are associated with tumors. The goal of the lab is to study functional requirements and consequences when some of these regulatory subunits have been activated in mice and cells derived from knockout mice.

This is one slide from Dr. Prajakta Varadkar's presentation showing that the knockout of this gene don't respond and don't enter into cell cycle arrest following a particular drug treatment, Nocodazole. If you look at untreated cells, you look at the G2M phase on the left, and on the right if you look at wild type cells after treatment, most of the cells enter mitotic arrest. But when this gene is knocked out, you don't see as much of the arrest.

This has led them to other potential molecular mechanisms and interactors with this subunit and PP2A enzymatic activity, and that's an *in vitro* assay that gives you insight into that particular signaling pathway.

This slide illustrates work that has been done on a knockout animal and the inactivation effect and what it does in heart development. What you see here is ultrasound of day 16 fetal mice, a histological section showing that when that gene is knocked out you get these ventricular septum defects that can be seen on Doppler ultrasound in histology. This kind of work is leading to insights in that particular PP2A pathway suggesting that monitoring that pathway might be illustrative of certain characteristics of cells for cardiac indications and perhaps others as well.

I'm going to move on to Dr. Deborah Hursh, who has been looking at cell communication in intact tissues in

a drosophila model and epigenetic control of MSC gene expression following cytokine exposure.

Dr. Hursh likes to refer to drosophila as little test tubes with wings, as illustrated in this slide. They are a very powerful way to study cell communication in intact tissues and you can very specifically alter genes or gene expression within different anatomical locations that form different microenvironments important in different stages of development. They have used genome-wide genetic screens to help identify participants in signaling and morphogenesis and, particularly, head morphogenesis. Using this approach you can look at markers that are predictive of different signaling pathway activity and predictive of cell survival.

Her particular interest and focus has largely been on BMP and its relation to head morphogenesis and, through genome-wide dominant interaction screens, has found a number of important partners that interact with BMP signaling. She concentrated on a Jun-kinase family member and showed that this was important in head morphogenesis and potentially between interaction of different layers within one of the imaginal discs involved in head development in drosophila.

Now I’m going to switch to the MSC part of her work with the consortium. There’s a large body of

literature that discusses how MSCs can be licensed towards an immunomodulatory phenotype through the action of various cytokines and, particularly, interferon gamma and TNF alpha. Two genes are known to respond to this induction phenomenon, IDO and TSG6.

So the question asked by Dr. Hursh's group was whether chromatin plays a role in MSC cytokine licensing. These two genes are activated in the presence of those two cytokines. They are thought to be very important in immunosuppression. The questions are: is chromatin modified in this process, and is it stable after you remove the inducing cytokines, and is it stable to things like freezing or culture recovery -- two steps that are very important in MSC clinical development.

In the field of epigenetic control there has been a large amount of work showing that chromatin signatures can distinguish regulatory elements and events, and that there are marks that affect histones on promoters, enhancers and insulators. Some of these marks are associated with activation and some with silencing of gene transcriptional activity.

Their approach was to look at the histone marks at IDO1 and TSG6, and, looking at a variety of different genes like GAPDH, kind of a housekeeping gene, and IDO and TSG, they saw that there were decreases in repressive marks

on a locus that was activated after cytokine treatment and there were increases in permissive marks at the IDO. The TSG locus, in contrast, didn't seem to respond to these.

So, learning about how cytokine treatment in MSCs -- or MSC gene transcriptional responses can be affected, again, can leads us to insights into different ways to predict activities of MSCs.

Dr. Malcolm Moos' group has been very interested in tissue specification and cell state and cell fate, in particular, looking at BMP signaling. I'm going to say a few words about that, also talk about single cell mRNA sequencing to identify and characterize MSC subpopulations, and the work of Sema Rosinbum and his lab looking at single cell RT-PCR analysis of MSCs.

Dr. Moos' group has looked at the BMP signaling pathway and various BMP family members extensively over the years and have previously seen that SMOC and another member of the BMP pathway interact at joint inter-zone formation in Xenopus. The question has been, subsequent to those observations, how does SMOC work.

SMOC seems to be an instructive extracellular matrix protein which modulates both BMP and Wnt signaling. It seems to inhibit BMPs locally but it expands their range, so the in-terminal part of SMOC seems to activate MAP kinase, which can inhibit the BMP signaling pathway.

The C terminus seems to bind heparin sulfate proteoglycans, and, through some elegant experiments, their lab showed that the SMOC can displace BMPs and then promote their diffusion, so extending the range of the BMP signaling. They are now looking also at how phosphorylation events can further explain the activity of BMP in this system.

I am now going to turn very briefly to the RNA seq and sort of the systems biology approaches that are being explored in Dr. Moos' lab. This illustrates an experiment in which MSCs at low and high passages were grown either under room atmospheric oxygen tensions, or low, more physiological oxygen tensions, and by doing single cell analysis or mRNA sequencing followed by high performance linear discriminate analysis you can classify MSCs into discrete groups. I think this has been a theme that I touched on earlier in the adipogenesis work that I showed, and now here, where it looks like there are discrete subsets with different activities, here is a way to pull out multivariate high-density data that might describe some of these subsets. Of course, this is a response to oxygen tension, but this kind of approach I think is going to bear a lot of fruit in the coming period.

Here you can see that oxygen tension and time in culture change the cell population in terms of some specific gene expressions. Again, you can see this idea

that between atmospheric early passage cells and atmospheric oxygen late passage cells you can see a difference in gene expression, but you can also see differences between lower oxygen and high oxygen in the passages again.

Now I'm going to talk to the work that Sema Rosinbum presented looking at single-cell PCR using the Fluidigm instrument. This represents the heat map. Each column represents a specific mRNA probe, and each row is one cell. Then you can see things like GAPDH in the middle and other markers of MSCs that seem to be fairly uniformly expressed, but you can see a great deal of heterogeneity in the cell population as well in terms of many other genes.

They have expressed this data in a nice way called a violin plot. If you look on the right you can see what are called housekeeping genes and all the cells seem to express them at similar levels. We call that unimodal gene expression. There are other genes which are expressed at either higher levels or no expression whatsoever, and again, I think this fits the idea that you can look at potential subsets of genes with different expression levels.

It's really the fraction of cells that are in different states that might give us great insights into heterogeneity and how to better tease out the different

populations that you might find in a heterogeneous population like MSCs. And you can also see that there are differences in the fraction of cells that express those particular genes. So, again, a very nice way to start to tease apart at the single-cell level this question of heterogeneity and how that potentially can lead to better ways of understanding our cell therapy products.

The research I've told you about in general addresses the cell therapy challenges using complementary approaches, cell-cell interactions, genetic interactions, screens, protein-protein interactions, organogenesis. And the significance for cell therapy is we think these findings may reveal cell product quality attributes that lead to improved characterization of cell-based therapies, methods to monitor manufacturing differently, perhaps the ability to choose donors differently, and form the scientific basis for policy development and guidance for sponsors.

With that, thank you for your attention.

Agenda Item: Q&A

DR. CRIPE: Great. Thank you, Dr. Bauer, for that nice overview. A lot of really interesting biology going on in your group.

I'll start off the questioning again. I get the mission relevance of a lot of the projects but I'm not seeing it for the PP2A - B56 gamma story or not really even the drosophila, figuring out gene networks in head morphogenesis. Can you help me understand the mission relevance of those two projects better?

DR. BAUER: Yes. I think our underlying idea is that signal transduction pathways that you can study in model organisms are going to be evolutionarily conserved and very important not only for those model organisms but also for our human-based cell therapy products. I think this has been accepted within our office and division as relevant and important for the reason I just mentioned.

DR. CRIPE: In other words, you would potentially test some of the cellular products and their effect on those pathways when they're given to model organisms?

DR. BAUER: I'll make a comment and then Dr. Epstein wants to make one as well. I think if you get information from one of these model systems that something might be of interest in a product, then you can manipulate not clinical products but in the research lab you can start

to look at those signaling pathways and you can start to manipulate them and test specific hypotheses about how those pathways are related to cell product characteristics and quality attributes.

DR. EPSTEIN: I just want to comment that we take a broader view of relevance. If we tried to link all our research to products that we regulate today, then we're going to, by tomorrow, be behind the times. There are all kinds of things coming our way that require an understanding of these types of developmental biology systems, the signals that are used to change cell fates and so on, so we intentionally take a broader view.

Some of the work is very directly related to a product. Some of the work is more generally related to preparing ourselves for the future.

DR. CRIPES: Fair enough, thank you.

DR. ROSE: I have a couple questions. Very nice work, Dr. Bauer, and the rest of your crew.

In looking at your MSC in passages, have you all done any what I would call more broad-based chromosomal analysis, G-banding? We know that in IPSC development there are chromosomal changes to lesions and such. Have you looked at that with respect to the rest of the analysis that you're doing?

DR. BAUER: Yes. Dr. Hursh's lab has used the SKY

technique for kind of higher throughput and more resolution in chromosomal analysis. I'm trying to go back to that slide of the overall MSC Consortium just to show that one picture. That group has looked at mutations that carry abnormalities that can be detected through this chromosome painting technology. A paper has just recently been accepted.

I'm going to ask Dr. Hursh to come to the microphone and maybe give you a little more precise detail.

DR. HURSH: Yes, of course, that was part of the basic analysis. All of the lots of MSCs that were done, different donors and different passages were examined by SKY, and this was just recently accepted at Cytotherapy. It should be out in the next couple of months. The bottom line was that we did see chromosomal abnormalities starting in the early passage, and some of them were clonally propagatable.

However, what we found was that after extended passage, up to passage 7, those chromosomal abnormalities sort of fell away. We were not picking them up by this assay, suggesting that they weren't giving any kind of replicative advantage.

We're extending this in other directions, but it basically forms all of the karyotype analysis of every lot that was examined in the consortium. Is that satisfactory?

DR. ROSE: Yes. I read that in the report. I was just wondering because I've seen reports, like I said, and maybe this is the big difference between MSC and IPSC and others, that it did propagate but did not give an advantage, so you might look at the sensitivity.

DR. HURSH: Yes. We are interested in that as well because basically, with the kinds of karyotyping, you're only looking at dividing cells, so, in another project in the lab we're actually using some other methods to look at genome stability that are not dependent on dividing cells, and we're actually comparing IPS cells to differentiated MSCs made from those IPS cells. So we're hoping to kind of tease out some of those questions with that approach.

DR. ROSE: Very nice. If I could follow up on the epigenetic work, histone modification obviously is in the forefront. Have you looked at that in relation to something like inactivation markers of whether it be DNA-hypersensitivity or other A modifications?

DR. HURSH: No. We really weren't set up to look at DNA modifications directly. That would be interesting to do but that wasn't something we really had the technology in house to do, so we went with histone modifications instead.

DR. ROSE: Okay. Thank you.

Agenda Item: Open Public Hearing

DR. CRIPE: Other questions? Last call for questions about any of the presentations today so far.

Hearing none, we reserved the next hour for the open public hearing session. I have been told we don't have anybody actually registered to speak. I guess the question is whether anyone has shown up as a walk-in who might want to come forward from the public to speak.

At this time, if anyone would like to ask questions from the public, please come forward. If not, that means --

DR. KIM: Dr. Cripe, in addition to no one registering ahead of time for OPH, nobody signed up, so we have no walk-ins also. We can conclude OPH.

DR. CRIPE: That means we are ready to adjourn our open session. Janie, any other logistics or comments that need to be made before we adjourn the open session and move to the closed session?

DR. KIM: Just that you can make that last statement closing the OPH session and then we can go to a brief break.

We will adjourn the open session and we ask that anyone not affiliated with the actual review -- if you are not a member of the committee, you need to vacate the room.

We will take a 10 or 15-minute break, and we ask

our industry representative, Dr. Dale Endo, thank you for calling in, but we ask that you kindly disconnect from the call, also.

DR. CRIPE: Let’s make it a 12-minute break.

(Whereupon, the open session adjourned.)