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DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

BIOLOGICAL RESPONSE MODIFIERS ADVISORY COMMITTEE

TWENTY-SIXTH MEETING

OPEN SESSION

VOLUME II

Pages 1 thru 274

Bethesda, Maryland
March 21, 2000

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at

AT

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Tuesday, March 21, 2000

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Holiday Inn Bethesda
8120 Wisconsin Avenue
Bethesda, Maryland

MILLER REPORTING COMPANY, INC.
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C O N T E N T S

P R O C E E D I N G S

DR. SALOMON: Good morning, everybody. I guess we are sort into the swing here; right? There is nothing formally I have to do this morning except begin by introducing Jay Siegel for an admin update.

BRMAC ADMINISTRATIVE UPDATE

DR. SEIGEL: Thanks. I am not exactly sure what an admin update is, but what I wanted to do was just have a couple of minutes to follow up on a letter that I hope you all received within the last couple of weeks to give you a little bit of background about where we are heading with this committee.

We have some, I won't say directions, but a slight shift in focus that I think is going to be really exciting. Based on yesterday, in fact, I am quite sure that it will be extremely beneficial to the agency.

About a year ago, as a number of people, members of the committee, rotated off, I was in a quandary about exactly who to suggest to replace vacancies as they filled because the committee has had a rather broad breadth of topics it has been addressing ranging from issues with hematopoietic factors to hematopoietic stem cells, transplantation therapy issues and so forth.

I consulted with Dr. Zoon and with the Division Directors, Phil Noguchi and Karen Weiss who are here, and

others whom you have met at various meetings, about how would be the best way to utilize the expertise on the committee and to utilize the committee and what sorts of expertise best to emphasize on the committee.

Perhaps to understand where we were, if I could take just a moment to mention historically that when this committee was started--when would that be, the late '80's, sometime--its original focus, like virtually all other FDA advisory committees, at least those for drugs and largely in biologics, was on product approvals.

It was a reasonably broad spectrum of product applications, interferons for hairy-cell leukemia and Kaposi's sarcoma and hepatitis, interleukin-2 for cancer. Around the early '90's, we began to realize a couple of products. One was that while biological therapeutics had been focused largely in the areas of cancer, we were seeing a great deal of application in sepsis and arthritis and gastrointestinal disease and it seemed next to impossible to try to have that sort of breadth of expertise on a single advisory committee.

We were also aware that our sister center, the Center for Drugs, had advisory committees in each of those clinical specialties. Kathy and I and our colleagues decided to move to increase our consultation with those committees in most clinical areas and to retain the focus of

this committee in hematology, hematologic malignancies and some other areas of oncology.

With that, the numbers of meetings deceased by we also started increasing, at that point in time, around the mid-'90's--we always had some, but we started increasing the number of meetings that were not focused on product approval but were focused on critical scientific issues and product development.

We had a number of meetings on xenotransplantation, in utero therapy with hematopoietic cells, extracorporeal liver-assist devices, use of PCR for hepatitis, and points most recently in that category, a meeting that many of you were at that we all benefited greatly from was a first of what I hope will be a series of meetings on immunogenicity of biological therapeutics, what should be studied during their development and how the products should be appropriately labeled.

So, with that as a background and with those many very informative and successful meeting, as I talked again with Dr. Zoon, Noguchi, Weiss and others, we realized that those were extremely valuable, those meetings that really got at the heart of critical issues in new areas of development generally early in product development before we were faced with a large database that was pretty hard to modify even if it didn't quite capture the endpoints or the

data that we most want to see.

I think we have decided that there were a lot of areas where we needed greater focus in product development particularly in the fields of cellular and gene therapy, in the areas of transplantation, tolerance inductions, a lot of immunological areas, microbiology, product purity and safety from contaminating virologic agents, in particular; cell biology--assessing cellular functions and mechanisms.

When we started putting together a list of the types of questions that we could bring to this committee, we not only realized that, in these areas, there were a large number of critical questions but also realized that these were not only areas in which we needed advice but in which there was a need for a forum for public discussion because we were developing approaches, scientific and regulatory approaches, to new classes of products and there needed to be public airing and, where appropriate, public input on more of these questions.

So, with that in mind, I would say it is not an overhaul but a subtle shift in focus, but an important shift in focus, of the committee. We made a decision to call on your expertise more on the scientific issues critical to product development in such fields. So our having made that decision, we are really pleased to welcome Dr. Bluestone as our first appointment, having made that decision, and to

have yesterday's meeting as our first meeting with that focus in mind.

I think that yesterday's proceedings bear out, as I said at the beginning, that this should be a very exciting direction. The other minor shift that will probably occur in the committee is that, although the committee is chartered to have, what is it, up to thirteen or fourteen members, I think we have realized that, even with this what I am calling a focus, it is hardly really a focus in the sense that there is such a broad spectrum of issues there, we really have benefitted, and I think yesterday was, again, a good example of this, from bringing in significant numbers of experts in the specific area under discussion.

I particularly enjoyed, as I hope you did, the interplay between a group of you who have highly relevant expertise in areas of development of cellular therapies, immunology, cell biology, microbiology and the knowledge base of dealing with product development and FDA regulations in advisory committee and interacting and combining and synergizing with people who may not have some of those expertises but, obviously, had tremendous expertise specifically in the area under discussion and I look forward to today's discussion.

So I think the other thing that we will probably be doing is keeping the number of the committee maybe two or

three people smaller than it had been, giving us a little more room to still have manageable discussions and invite additional experts to interact.

So I am making this announcement both to keep you informed, to find out if you have any questions and, also, importantly to solicit from you, either now or any time you wish, by e-mail or other mechanism, to the committee staff or myself any suggestions you might have for topics you think it would be wise for us to discuss in this forum at future meetings.

Thank you.

DR. AUCHINCLOSS: Jay, I understand entirely and I agree entirely and then I will give you a "but." The "but" has to do with maintaining the level of interest of your committee members. Part of excitement of serving on this committee is that, at the end of the day, in many case, there is a real-life decision, a vote yes/no.

There is a tension associated with that that really kind of focusses both interest and, of course, the specifics of the decision that is being made. I understand your interest in having broader discussions. I just remind you that, from our point of view, to lose the sort of case-law kind of approach to things would be disappointing if you gave it up entirely.

MR. SIEGEL: I appreciate that. I don't think we

intend to give it up entirely. I think a couple of the meetings of this committee in the last couple of years have focused on anti-IL2-receptor therapies and therapies coming to approval for immunosuppression for transplantation.

We had a meeting--well, it was of the xeno advisory committee that focused, as you summarized, in Epicell. I think that there will be a number of specific product issues and product decisions that will come forward as well many of which, as we use the Epicell case, as kind of a case in point to set standards for other areas in development.

Hopefully, a lot of fields that we are focusing this emphasis on, in the not too distant future, will be leading toward product approvals.

DR. AUCHINCLOSS: The Epicell case, I think, is a case in point with part of what I am saying there where I think that if you look carefully at what the committee recommended with regard to Epicell, I think it is actually in conflict with its general principles under Topic I. There is nothing like having the real thing in front of you to get people to say what they really mean.

DR. ZOON: I can make a couple of comments on this because I think it is very important to look in the context of the broader issues of FDA advisory committees, I think especially in biologics, looking at our TSE committees,

which is the Transmissible Spongeoform Encephalopathy Committees, the Vaccine Committees and our Blood Committees.

These committees will deal, like yourselves, with product-specific areas but, in many instances, their major value in public health is actually looking at data early on, identifying show stoppers early on, so when you do get to the point of looking at a product, there are no surprises, not to the committee, not to the FDA, not to the manufacturers, not to the public.

Our business here is to make an orderly transition of science from the development of policy through product approval. Now, you don't get rid of all surprises, but I think, in the outside world, a consistency of scientific soundness in framing policies and procedures as we go forward in these complex areas of science, is critically important and this committee plays an enormous role in helping us get there.

So I would say, while it may take away some of the flash at the end, I don't think that is necessarily bad because I think it is good public policy to have good consistency and develop scientifically sound frameworks early on in product development.

DR. AUCHINCLOSS: I understand entirely your point of view and the other half of what I was talking about is that you will lose some of the enthusiasm of your committee

if they come and talk early in the game and in the abstract. It just isn't as--you don't feel as important.

We got to NIH-type advisory committees and future task forces and all that kind of stuff and we usually feel like, at the end of the day, we have done absolutely nothing. So it is just a practical matter. To keep your committee as highly talented as you want it to be, involves having them advise you on real-life decisions sometimes.

But we are talking very much a matter of degree. There is nothing that you are saying that I disagree with.

DR. SALOMON: Just picking up on what Hugh is saying, when you decide, let's say, to do one of these scientific--like islet transplantation, for example, or pluripotential stem cells of gene therapy which I think are two topics, by the way, that ought to be on the list to come now--there are people who have come to you already for pre-IND discussions if not actual IND discussions.

Why couldn't they be encouraged to come and present? I do agree with what you are saying in terms of this focus. Even the last meeting, the Epicell really did provide a frame that focused the committee on some very specific sorts of topics.

I don't think we need to lose what you are talking about, Kathy, in doing that.

DR. ZOON: Usually, the way we do this is by

having a product as a case study and then the policy issues are discussed around a case study or a series of cases that reflect the point. It is not usually esoteric and in a vacuum. It is usually having something that is under development of a series of products under development where you would ask the sponsors to come and present.

So it is related to real product and real-product issues. I didn't mean to imply that that was not the case. So most of these are linked to products, very specific products and then those are used to discuss the more generic issues surrounding the policy.

DR. SALOMON: But I guess what you and I are saying is that when you actually confront the people, the sponsors of these specific projects, they know they are on the line. They know that they have had to think it through. If you grab any one of us on any day and say, "Let's talk about gene therapy. Let's talk about islet transplantation," yes; we have our opinions. We have an experience that we draw upon.

But we all know, and we would be the first to admit, that we haven't nailed it. We only nail it when we put ourselves on the line and put a protocol forward or something like that. So if you bring a group of sponsors in, they really are going to say, "Okay; we have thought exactly on how we are going to purify islets, exactly what

the room is going to be, exactly this, exactly that."

I don't think you want to lose that.

DR. MILLER: Do you intend to continue having members of this committee, then, when the products actually get to the other committees, be part of that committee? I was on a few ODAC. There is not a whole lot of, unless they have changed, hematologic or bone-marrow transplant expertise on that.

I just think that if they are going to be, then, doing all those, that either they need to pick up additional hematopoietic-malignancy people or biologic people.

MR. SIEGEL: Absolutely. I am not sure that we would want to take the hematopoietic replacement or stem-cell therapies over there. I think yesterday was an example, in fact, of how experience in that field, which is a little bit more developed, was, actually, an interesting background and informative in terms of dealing with pancreatic islet-cell therapy.

So that specific decision has not been made, in fact, but the answer would be, nonetheless, in general, yes. I think, whether it is an Arthritis or an ODAC or a Gastrointestinal or Cardiorenal Committee--those committees have the clinical expertise, although I agree that the ODAC does not have a great deal in hematologic stem-cell therapy but, often, not the kind of fundamental background in many

of the issues that we are facing in product-approval time such as, say, the immunogenicity issue. So we would intend to continue.

DR. ZOON: Just to highlight, we can supplement this committee with experts from the CDER committees, or vice versa, committees depending on where we think the expertise is mainly situated.

So that we look at as a combination and we look at consistency of findings. But we have the flexibility in this to look at the situation and decide that the committee--we also can invite--I was telling Dan earlier, once you are on an FDA advisory committee, you are there for life as either a consultant or an invited guest.

We would very much look forward, as issues go forward, even as things may cycle over the years, to invite people back that have been involved to look at consistencies in these areas. So we do have flexibilities in our approach, depending on the nature of the condition and the expertise.

DR. NOGUCHI: I would just add, too, that one thing Jay didn't mention but, with the current gene issues evolving, it is quite clear that the BRMAC committee can't handle all the issues. So we will be also bringing specific gene therapy protocols and issues here as well.

One question, as an example, is for adenoviral

vectors. We see that they have fairly high levels of toxicity that seems to be well tolerated in oncology patients. But in other patients with metabolic diseases, it may or may not be the most appropriate choice.

We can bring questions such as, really, that question, are adenoviral vectors, at any level, really appropriate for other than cancer. So I think we can, certainly, both bring specific cases as well as general topics that will, in fact, shape and help to direct the field in a safer and more efficacious manner.

DR. ZOON: If I could just add one thing to that; I think we look forward to this committee getting very much more involved in gene therapy. As part of our proposal with the department and NIH to address some of the recent gene-therapy issues, we have also proposed that some of the gene-therapy safety conferences be linked to the BRMAC.

So we would be involved maybe having, one day, a safety conference on an issue that we thought was important in the safety of gene therapy and link that to the BRMAC's normal committee schedule so that we can have those issues discussed in a forum where we bring scientists in to discuss the issues and then, the next day, have our committee to deal with specifics on issues.

So I think there is going to be a lot more excitement and a lot more interest in some of these evolving

areas and we are looking forward to having the committee contribute to this.

DR. SALOMON: My only other comment is that I hope that we don't lose the component of hematopoietic stem-cell transplantation in the committee as it evolves because I think that some of the future directions we are going in, whether it be stem-cell therapy or gene therapy, and cell therapy, after we get into the arguments of what is a tissue and what is a cell, which I got into with Camillo yesterday, I think it is going to be very important.

MR. SIEGEL: There is no question a significant amount of the gene-therapy work we are seeing is using hematopoietic stem cells, manipulated, expanded or not, as a vehicle for gene therapy. There is also no question that newer cell-device, expansion devices, culturing pancreatic cells, culturing neonatal cord blood cells is really part and parcel of the same class of issues. I agree.

DR. SALOMON: Okay, guys. Good. Thank you very much, Jay.

Phil?

TOPIC III - UPDATE CBER RESEARCH PROGRAMS

Division of Cellular and Gene Therapies

DR. NOGUCHI: Good morning.

[Slide.]

One of the very important extra duties that BRMAC

serves is a review of our scientific programs. I would just like to, very briefly, go over some of the reorganization of the Division of Cell and Gene Therapy and show you how our newer programs in stem-cell biology fits in.

[Slide.]

This committee has, in various aspects, reviewed the Laboratory of Cellular Immunology. At the present time, we have Dr. Ida Bloom, who has focused on some of the immune processes involved in xenotransplantation and Carolyn Wilson, who has been working on porcine endogenous retrovirus.

To augment this area, including that to focus on gene therapy, Paris Byrd left recently and we are now recruiting for a tenure-track person with expertise in adenoviral vectors.

The Laboratory of Molecular and Developmental Biology, in a way, splits some of its work between areas of immunity of plasmid vectors, both where you wanted immune response and where you don't want immune response, some developmental programs of Malcolm Moos. Deborah Hirsh is a new tenure-track person. Steve Bauer has been looking at the interaction of stromal cells with hematopoietic cells and their development.

[Slide.]

Dr. Raj Puri directs a program of tumor-cell

biology and is responsible for the cellular-tumor vaccines that we regulate. Tom Eggerman has been working on some very clever approaches to delivery of antisense compounds through liposomes that is finally starting to emerge as a major field.

We have also non-lab-based reviewers. Dr. Joyce Frey heads up this particular endeavor and also serves as my deputy for cell and gene therapy.

[Slide.]

The newest and last area is in the area of stem-cell biology. Dr. Liana Harvath has recently joined our group moving from the Office of Blood. But, as she has moved over, she is also bringing over all the issues and the work that she has done in hematopoietic stem cells.

Here is where we hope to extend some of the types of approaches being used in hematopoietic stem cells to things like islet cell transplants, umbrella INDs, development of external standards, heavy and very interactive industry and academic involvement with the process.

Dr. Gerry Marti has had decades of flow cytometry standardization and experience and a research program in chronic lymphocytic leukemia. Don Fink is our newest addition to this who came from the old Division of Cytokine Biology.

While the focus of the researchers that we currently have in stem cell biology has been mainly on hematopoietic cells, we know that there is going to be a very large and emerging area of nonhematopoietic stem cell biology. In fact, the next planned BRMAC meeting will be on the area of neural stem cells and whether or not they may prove to be useful therapeutic modalities.

I think you will find that particular next meeting to be quite interesting

I think, at this point, I would like to have, then, Don Fink just briefly review some of his recent efforts.

DR. CHAMPLIN: While you are fixing the projector, Phil, I was just going to ask, with Liana Harvath coming over, changing areas, does that change the regulatory framework for hematopoietic transplantation in any way?

DR. NOGUCHI: No. The approach that has been outlined and that Liana has been presenting and working with you and others will remain the same. It is our intent to continue that.

MR. SIEGEL: Liana is now integrated into the group and the team, including Phil and myself and Kathy. As of a few months ago, we were hoping to renew an effort to really finish, or at least advance, the regulatory framework in a way that made sense and provided further clarity to the

field.

I must say, many of us have been somewhat distracted by events in the gene-therapy field and has that the work back a little bit, but we are meeting later this month to continue that effort and her transfer into this division was significantly motivated by a desire to be able to coordinate development of regulatory policy and strategy.

DR. CHAMPLIN: Does that mean that will be sort of in your office as opposed to the Blood Products group? Or is that an oversimplification?

MR. SIEGEL: Most stem-cell products have been in the Office of Therapeutics--I shouldn't say that; not most, necessarily that are in use, but many that have involved use of growth factors, use of devices to select against tumor cells, select for CD34 cells and whatever.

It is likely that--perhaps Kathy can speak to this more directly, but it is likely that more of the stem-cell issues will be dealt with in my office; yes. I don't remember exactly where the rules are, but more of the less manipulated stem cells are moving into my office with Liana.

DR. ZOON: I think if you are interested in that, that is something maybe we can give you an update on at one of the future meetings of its status and how that is organized because I think it is an important question with some personnel switches.

If the committee would like an update on how the center is managing stem cells, we would be happy to do that.

DR. FINK: Good morning, committee members. 8 o'clock is a nice time. I am glad to see you all awake.

[Slide.]

I am going to give you a Reader's Digest synopsis, really, of what I presented at my site visit back in September of which Dr. Hugh Auchincloss was the chair. Up front, I would say, and he asked me this morning, I, personally, have sent a letter to each of you, I believe, who are committee members which had information that I felt was not in the briefing package that might be useful in your deliberations.

Dr. Noguchi is aware of that. Dr. Seigel has seen that comment. So you are free to talk about it. It is not out of bounds. Those issues are quite fine. So I have no trouble with that. I will come back to that at the end to tell you that some of those actions have actually been implemented which was a positive outcome of all of this.

What I presented--at that time, I had spent most of my tenure here in the Division of Cytokine Biology and, as you know, cytokines are therapeutic proteins and recombinant proteins. My area of expertise and regulatory oversight was in neurotrophic factors in the treatment of neurodegenerative diseases.

That basis was how my research program had been developing. So what I am presenting to you in terms of that is what had been done in the lab up to that point.

The model that we are using in the laboratory is a cell-culture model to look at neuronal differentiation. The cell line is a PC12 cell line, which is derived from a fetochromocytoma. I would consider this to be something akin to a neuroprogenitor. It works nicely in that you can add a trophic factor such as nerve-growth factor and it differentiates and becomes a functional neuron.

Also used were various variants, mutant cell lines, which have dominant negative expressions of signal-transduction molecules to verify certain observations that were made pharmacologically.

For the purpose of comparison, I will simply state that my expertise had been in nerve-growth factor for much of my training and then I have become interested in pituitary adenylate cyclase-activating polypeptide. So I run these things in parallel to find similarities and differences. They work in quite different fashions.

So what I am going to do--I am just going to do overheads that I have to show you just to summarize the bullet points for you so you will be familiar with the findings. Then I will tell you a little bit about what I have done to progress the research in the last couple of

months orally and then any questions that you may have, in particular, I will entertain at the end.

Basically, in terms of the research findings, using this cell model to look at neural differentiation, we are measuring neuritic outgrowth. We found in the laboratory that the PACAP, which works through a seven-trans-membrane protein through g-coupling mechanisms, was able to elicit a neuritic outgrowth response similar to what you would see if you added the neurotrophin nerve-growth factor.

We were able to deduce that these are independent phenomena and that the PACAP does not activate in crosstalk into systems that somehow activate Trk A, thus resulting in a borrowing of pathways in that manner.

In contrast to what we know about nerve-growth factor, if we use dominant negative expression systems and, number 2 at the bottom src and ras, which we know are involved in the signalling of nerve-growth factor, when we use our dominant negative cell models, we find that PACAP is able to work independent of those and is able to drive significant signalling through the cell in the absence of activation of those particular molecules, which makes it clearly distinct from nerve-growth factor in that regard.

At this point, we decided to use varieties of pharmacological antagonists to probe this in greater detail

and, in particular, looking at protein kinases A and protein kinases C which are known to be activated by PACAP and to see whether or not we could inhibit, in any way, the responses that we had been observing.

[Slide.]

On the top bullet point, you can see that, by using various pharmacologic inhibitors selective for the various pathways, we found that, in particular, if we use protein-kinase-C antagonism that we were able to inhibit a downstream signalling molecule called Erk which is, we know, to be very important to nerve-growth-factor activity in terms of inducing the neuritic response.

Similarly, when we antagonized protein kinase C and inhibited Erk, we also found that PACA was blunted. So now we have kind of found a focus where we can coalesce and we are converging on a point in this cascade where NGF and PACAP might be coalescing their efforts to result in a final outcome which was neuritic outgrowth.

Continuing those studies, we were able to observe that, in addition to the Erk and PKC dependence that protein kinase A was not involved, thus eliminating one arm, at least, of potential signalling for PACAP as being involved in this morphologic neurodifferentiative response that we had observed.

Finally, the area that we are continuing our most

active study is really in the upregulation of a Trk A receptor. Trk A is important for neuronal differentiation and survival of various nerve-type cells. In the model, PC12-6.24 cells, we have a system where we have overexpressed human Trk A.

What we have found has been very striking and very entertaining, actually, is that we have maybe a potential area to look at regulation of this neurotrophin receptor that has not been discovered or discussed extensively in the literature. We have found that PACAP treatment will remarkably upregulate the expression of the Trk A at the protein level as well as its phosphorylation status, which is indicative of its activation.

Now, 624 may be something akin to using sort of an induced expression system because we find that with PACAP, we from a basal level to over 200-fold, 300-fold, increase in this protein level. So it is quite robust, but it also has some interesting features.

It appears that receptor has been sequestered. It is not available to surface because the increase in overall binding of NGF, for example, to surface is only about three-fold to four-fold. So while there is probably some endogenous separation there, it is quite interesting to use as a marker.

We have extended those studies. We did a time a

course and we find that--we started cutting the time to exposure. If you only expose for 15 minutes to PACAP, do a wash-out and come back 24 hours later, you still have the robust signal. So it is almost like there is an on-switch and it is very intriguing.

So that really summarizes the gist of what I had presented at that time. Since then, I can say that, on the basis of the site-visit report, that we have been able to actually do a little novel kind of experiment within the purviews of research. I am now associated with the laboratory of Dr. Kathy Carbone who is in another office but works with neurotrophic viruses and has a laboratory that is quite extensively focused on, fortunately, the cerebellum.

The cerebellum, it turns out, is where you have the neurons, the cerebellar granule neurons which are PACAP-dependent early on in development. Her model is using a bornavirus which, interestingly, affects the cerebellum resulting in the death of cerebellar neurons following neonatal treatment injection of the virus into the CNS.

So we are developing--this is a new evolution, but we are developing a strategy to look at what is actually happening in terms of expression of PACAP--they have some suggestion that it is modulated by the virus and that may be representing a compensatory mechanism to try to protect these neurons--and then look for expression of various

neurotropic-factor receptors such as Trk B.

I also have a collaboration going with a colleague in Israel where we have an extensive program looking at in vitro modeling with the PC12 cell line at hypoxia and glucose deprivation representing a stroke model. We have some very interesting data which suggests that PACAP, by itself, is able to protect PC12 cells in this deprivation model from apoptosis by about 20 percent.

NGF is only about 35 percent protected, but the two of them together, it is almost complete protection against hypoxia and the glucose deprivation. So there is some nice signalling crosstalk that we can look at in that model.

Finally, I have a collaboration now with Dr. Ann Marini. She is at Walter Reed, very close by. She does work with primary cerebellar granule neurons and is very intrigued by the possibility that, A, in glutamate toxicity, she can use PACAP as a protectant and, B, that I am in interested in that because we can use PACAP in her model which is primary neurons to look now at upregulation but second neurotrophic receptor, which would be Trk B.

Finally, let me just bring this all to a focus in terms of stem-cell biology and the recent presentation at CBER by Dr. Catherine Verfaillie at the University of Minnesota who is developing adult stem cells.

One of the important features of that model for getting these cells to replicate in culture prior to differentiation is to use forskolin. If you recognize forskolin as a cyclic-A-and-P activator, you can begin to see how PACAP could be a physiologic modulator if, in fact, these cells express receptors for it and are coupled through adenylate cyclase.

We anticipate the possibility of obtaining some of these cells from her in order to investigate this particular approach. Within the laboratory of stem-cell biology, along with Dr. Liana Harvath, we are currently working together to try to look at the issue of cellular migration which she is an expert in and which is a very important feature for what will happen following the implantation of stem cells, particularly neural stem cells because they will, in fact, need to migrate to their proper location in order to establish connections.

So this could be a very fruitful and productive interaction. With that, I will conclude. If anyone has any particular questions they would like to ask, I would--yes?

DR. SAUSVILLE: In relation to that last point, your PC12 cells--you say PACAP is PKA independent? And yet, in these stem cells that you just alluded to, you state that they are stimulated by forskolin and you implicate it in the potential role of PACAP.

So, does that imply that in different cellular contexts, PACAP could either be protein kinase-A or cyclic-A and-P-directed and, in another cellular context, independent of that?

DR. FINK: That is absolutely correct.

DR. SAUSVILLE: How do you see that coming together from a unitary mechanism of PACAP?

DR. FINK: Let me just try to confuse you a little further on that regard because we have tried to probe this specifically using our upregulation of the receptor model as indices. There are aspects where, in certain cellular--let me backtrack--cellular contexts where the PACAP is mitogenic and, with cyclic-A-and-P activation, does, in fact, drive mitogenesis.

However, in those cellular contexts, it is not coupled efficiently, if at all, through protein kinase C. So, it may be that you have a preponderance of activation of one system that is, in the absence of another input from a secondary cascade such as protein kinase C, allows it to over--not overexpress but to predominantly express that particular phenomenon.

In PC12 cells, and this is also the case with NGF versus EGF, you have molecules that seem to activate similar cascades and yet lead to, really, diametrically opposed or different phenomenon.

All the players in that are not identified but it is clear that there are now capacities to activate distinct molecules in different discrete cascades that have crosstalk inhibitory effects. So, in that context of cyclic A and P that you suggested, in this case, it appears that, while the cyclic-A-and-P event is there, in terms of the neuritic outgrowth response, that is being driven more predominantly by other players, presumably protein kinase C and Erk activation at this time.

DR. SALOMON: Thank you, Donald.

Division of Therapeutic Proteins

DR. ROSENBERG: Good morning, everybody.

I would like to start out by showing you the organization of our division and where the people who have been site-visited fit into the division. I would like to then tell you about some of their regulatory activities so that you can understand how their scientific endeavors benefit the regulatory mission of the agency.

[Slide.]

We are the Division of Therapeutic Proteins. This division was constructed or generated last year, in October. It is composed of the former Division of Hematologic Products and some personnel from the Division of Cytokine Biology.

I am the Deputy Director who is the Acting

Director. There are four laboratories within our division. First there is the Laboratory of Immunology. Drs. Donnelly and Petricoin who were reviewed are in this laboratory; the Laboratory of Gene Regulation lead by Dr. Ed Max and Dr. Gibbes Johnson is located within the laboratory. Dr. Kathy Zoon is located in the Laboratory of Chemistry that is led by Blair Frazier.

[Slide.]

What products do we have and what scientific programs support the regulation of these products? We have an amazingly wide variety of products. Starting with the cytokines, Dr. Donnelly has been responsible for regulating IL1. Recently, he has taken on regulation of IL2 as well as IL2 fusion toxins. He has had long-term responsibility for regulation of IL4, IL10 and IL12.

Regarding the interferons, there is a wide variety of interferons and many groups are involved in their regulation. So we have Dr. Zoon's group and, primarily, within that group, Mr. Joe Bekicz has been responsible for some of the interferon alphas. Dr. Chip Petricoin, as well, has been responsible for the regulation of interferons and it is Dr. Zoon's abiding interest in interferons that qualify her laboratory as regulators of these products.

Dr. Petricoin has had a long-standing interest in interferon signalling although his direction has changed

recently. The interferon gamma is also regulated by Ray Donnelly who has primary responsibility for it.

Interferon beta is handled by Dr. Gibbes Johnson who has a long-standing interest in signalling by growth factors in general. Dr. Chip Petricoin also has regulation of interferon omega and interferon tau.

[Slide.]

In terms of receptor antagonists, of which we are seeing increasing numbers, Dr. Petricoin is responsible for antagonist to TNF. Ray Donnelly is responsible for receptor antagonists to IL1 and IL4. Regarding the lists of enzymes that you see here, which we recently acquired since the reorganization of the divisions, Dr. Johnson is responsible for the uricase.

There is a group of miscellaneous products which are difficult to classify. Dr. Petricoin is responsible for lactoferrin and relaxin, Dr. Johnson for ICAM-1.

[Slide.]

Regarding tissue growth factors, Dr. Gibbes Johnson has primarily responsibility for platelet-derived growth factors, vascular endothelial growth factor and hepatocyte growth factor. There are others in the division who are responsible for the remaining factors.

Regarding the growth inhibitors, Dr. Donnelly is responsible for mammastatin.

[Slide.]

So, having shown you what their regulatory aegises are, I think that I would just like to briefly touch on the nature of their laboratory programs so that you can see, in a nutshell, how their laboratory programs address regulatory issues. So we will start with the laboratory program of Dr. Donnelly.

[Slide.]

The scope of his research really focuses on the mechanisms by which cytokines cross-regulate signalling by other cytokines. He uses a monocyte model in this regard. He has most recently focused on the role of the SOCS proteins. These are suppressors of cytokine signalling. He is endeavoring, in his future studies, to try and define the mechanism by which these SOCS protein inhibit cytokine-induced signalling.

[Slide.]

In terms of the laboratory program of Dr. Johnson, the primary interest here is in signalling through EGF receptors and the role of the SHP-2 phosphatase in this signal transduction pathways. So his studies mostly focus on mutational analyses of the erbB receptor, tyrosine kinase--

[Slide.]

--as well as looking at the role of the SHP-2

phosphatase by generating mutants and characterizing the activity of these mutants in vitro and in vivo.

[Slide.]

The laboratory program of Dr. Zoon is focused on the structure/function relationships of interferon alpha. It involves extensive characterization of newly generated hybrids in terms of what domains are essential for antiviral activity, antiproliferative activity, competitive binding activity and signal transduction pathways.

[Slide.]

The future goals include examining the interferon binding characteristics, characterizing the signal transduction pathways and really delving into the structure/function relationships as they apply to antiproliferative activity.

[Slide.]

Lastly, the laboratory program of Dr. Petricoin, who I think may be a victim of the bad traffic this morning, so I will just go over these a little more slowly, although, in his former years, he was focused primarily on interferon signalling, he has recently gone off in a very exciting new direction involving the use of proteomic technologies to identify new proteins.

The power of this technique is enormous with regard to many different areas. So his goals are really to

use this technique in many settings. One is to identify new proteins that track with normal and diseased cancer cells in following human solid tumors, cancers of the prostate, breast, ovary and esophagus.

He wants to use the proteomic technologies to identify signal transduction pathways and to potentially identify new phosphoproteins in the setting of solid malignancies, the erbB2 positive and negative breast cancers, normal versus premalignant versus tumor breast epithelium, normal versus premalignant versus invasive prostate epithelium with low metastatic potential versus invasive, so, really, to try and get a correlate of what proteins are involved, perhaps in malignant transformation or in transformation to a more highly malignant form.

[Slide.]

He wants to use the proteomic technologies to identify biomarker profiles from body fluids for early disease detection. This would clearly be of enormous benefit in a variety of malignancies, many of which are not detected until late stages of disease. So he wants to examine the nipple-fluid aspirates from volunteers in breast-cancer patients to see if there is something that can be picked up there as a precursor or malignancy.

He wants to examine serum from normal volunteers and prostate-cancer patients and also the ascitic and cystic

fluid from borderline non-invasive and invasive ovarian-cancer patients and he further wants to use these proteomic technologies in a way that I think is very exciting for the agency in general which is to identify biomarker profiles for early toxicity screening using, hopefully, serum, which is clearly one of the easiest fluids to get.

So, for instance, looking at whether one can detect the early toxicity of adriamycin-treated patients, the cardiac toxicity; also to look at pre- and post-treatment of vasculitis and cardiotoxicity-inducing agents in rat models.

That covers the presentation.

DR. SALOMON: Thank you, Amy.

DR. BLUESTONE: Amy, for the proteonic work, is that being done using a mass-spec approach, at 2D-gel approach? Is it in collaboration with a company or how is that being--

DR. ROSENBERG: He has gone to using the SELDI which is a very high-powered technique for identifying proteins. It is not terribly quantitative at this point but it is qualitative and very sensitive in terms of picking up all protein forms.

There are a variety of chips that can be used with this to really detect proteins of different molecular

weights. If he were here, he could tell you better.

DR. GOLDMAN: If I may add, SELDI is, in fact, laser desorption so it is like MALDI. It is mass spectrometry.

DR. CHAMPLIN: Can I ask a question? What are you envisioning for this Office of Protein?

MR. SIEGEL: The Division of Therapeutic Proteins?

DR. CHAMPLIN: Yes.

MR. SIEGEL: Basically, we reorganized in October, which was the start of this fiscal year. The reorganization can be described, I think, as having two major characteristics. We went from laboratory-based product divisions to three. That, among other things, largely reflected the fact that our program has significantly downsized, particularly its research aspects, over the last four or five years.

But also what was really the critical guiding feature, and this is really to the point of your question of this reorganization, was to reorganize the product-review divisions more along the lines of product classes which shared common concerns regarding methods of production, methods of product testing and manufacturing control.

So, whereas in the past, we had cellular therapies in all of our product divisions and protein therapies in all of our product divisions, and antibody therapies in at least

two of the four product divisions, we kind of, by refocussing them, have created a situation where it is easier to insure consistency in manufacturing and standards as well as to develop policy and guidance in a concerted way.

So that is how the products, some of which were in cytokine biology and some of which were in hematologic products, kind of got merged into the Therapeutic Proteins Division.

DR. CHAMPLIN: I was struck by how broad-based--everything from growth regulatory molecules to l-asparaginase to biomarkers. All of biology comes down to proteins in the end and so it really is everything.

MR. SIEGEL: That's true and that is true of all of our divisions now. The clinical diversity, of course, as you all are probably aware, we have both a preclinical animal models group as well as a clinical group that is together in Karen Weiss's division that is basically organized by clinical specialty that works with all of these product classes.

But it is true from the basic pharmacology and basic science that, just as in the Division of Monoclonal Antibodies and in the Division of Cell and Gene Therapy, the Division of Therapeutic Proteins has a diverse group of products.

We have been aware that, in reorganizing, you can organize along clinical disciplines. You can organize around product class. You can organize along scientific disciplines or mechanisms of action. Whatever you do has advantages but requires that you pay careful attention to a large number of cross-cutting issues and interactions. That is where we are.

DR. SALOMON: I am getting a little concerned about time here, so if we can keep on going. Dr. Johnson?

DR. JOHNSON: Thank you.

[Slide.]

I thought, today, I would spend my five minutes just bringing the committee up to date on some recent progress that we have had since the site visit. My laboratory is interested in signalling by the erbB family of receptors. Ligands are comprised of four receptor tyrosine kinases which interact through process of a ligamerization when the receptors engage a very large family of growth factors which are structurally related to epidermal growth factor.

[Slide.]

ErbB1 is actually the epidermal growth-factor receptor, EGF receptor, and there is erbB2, erbB3 and erbB4. This is an old slide, but actually, all of the erbs have been shown to interact in a ligand-dependent manner.

[Slide.]

There are actually two projects which are ongoing in the laboratory. One is trying to understand the function of the EGF receptor tyrosine kinase activity and signalling by the EGF receptor.

[Slide.]

As you might guess, the way we have addressed that is by mutating the EGF receptor kinase activity into an EGF receptor kinase-inactive mutant form. Just to kind of describe some recent progress in this area and put it in a nutshell, what we found is that, actually, the EGF receptor kinase activity is not essential to EGF-induced signalling in many cell types.

This appears to be due to the fact that there is an erbB2-EGF receptor heterodimer which signals in the EGF-dependent manner. The signalling is not dependent upon the EGF receptor kinase activity. This heterodimer can actually activate two pathways which are essential to growth and differentiation of cells--that is, the mitogen-activated protein-kinase pathway or MAPk and also the AkT kinase pathway which is thought to be involved in cell survival.

The heterodimer is incapable of activating signal transducers and activators of STATs--signal transducers and activators of transcription, also known as STATs 1, 3 and 5. We have actually set up two model systems to study the

signalling by the heterodimer. One is in NR6 fibroblasts and the other is in 32D myeloid cells.

The biological response to EGF in these two cell lines is actually different even though biochemically signalling appears to be identical, in the fibroblast, EGF can elicit proliferation. In the 32D cells, all we see, really, is a weak survival and a delay in the onset of apoptosis.

[Slide.]

A second project in the laboratory is trying to understand the molecular basis for the role of the protein tyrosine phosphatase SHP-2 in signalling by the erbB receptors. Several years ago, we were able to show that SHP-2 plays a positive and a central role in mitogen-activated protein kinase activation by the entire erbB family of receptors.

We are trying to identify how SHP-2 functions in that regard and what are the targets for SHP-2.

[Slide.]

One of the ways that we have been addressing this question is to generate a constituitively active form of SHP-2, express it in cells and see what pathways and transcription factors we can turn on in the absence of receptor activation.

We have generated several constituitively active

forms of SHP-2 that, in vitro, show at least a ten-fold greater activity relative to the wild type and preliminary experiments in intact cells show that we are able to turn on a number of specific transcription factors in the absence of receptor activation.

With that, I will end and answer any questions if there are any.

DR. SALOMON: Is there a SHP-2 knockout?

DR. JOHNSON: It is lethal. There actually is a SHP-2 knockout where a defective form of SHP-2 still appears to be made and so it is missing one of its SH-2 domains, the protein that is made. It has been useful even though there is death in utero. They have been able to establish some cells and use them in some studies.

Whether that mutant form of SHP-2 has any signalling capacity is not exactly clear. So there is not a knockout where there is no protein, that I am aware of.

DR. SALOMON: I had a follow-up question. We are interested in SHP-2's association with the CXCR-4 receptor. Have you done anything with putting it into a T-cell or any other cell and looked at migration?

DR. JOHNSON: Putting in the constituitively active forms?

DR. SALOMON: Putting in the ten-fold constituitvely active--

DR. JOHNSON: No; not in those cells. We are mostly interested in fibroblasts and epithelial cells. But SHP-2 appears to be doing a lot of different things in different settings.

DR. SAUSVILLE: So, in the model system that you described of the EGFR independent activation, the kinase independent activation of the MAP kinase, does that require, though, an active erbB2 tyrosine kinase domain or is it actually independent of both?

DR. JOHNSON: It appears to require erbB2 kinase activity although, at times, we have seen a very weak signal generated by just heterodimerization where the EGFR is kinase inactive and erbB2 is kinase inactive. But is very weak relative to the erbB2 kinase active heterodimer.

DR. SALOMON: Thanks very much. Very interesting work.

Next is Dr. Donnelly.

DR. DONNELLY: Well, I am pleased to say that I was not a victim of this morning's inclement weather, although I was worried about this about thirty minutes ago sitting out on the interstate. I am pleased that I was able to make it here.

[Slide.]

As Dr. Rosenberg mentioned previously, the research in my laboratory is primarily focused on exploring

the mechanisms by which certain cytokines and how cytokines cross-regulate the actions of one another using principally monocytes and, to some extent, murine macrophages as targets for the actions of these cytokines.

In particular, we are focussing on how interferons can inhibit IL4-induced signalling, conversely how IL4 can inhibit the activation of monocytes and macrophages in response to interferon gamma stimulation

Just very quickly, I would like to show you a couple of slides that illustrate this second point--that is, now IL4 can inhibit interferon-gamma-induced responses. There are a number of genes that are interferon-gamma-inducible in both monocytes and macrophages. They include genes such as the high-affinity FC receptor of IgG, the B7 isoforms CD80 and CD86, ICAM1, IP10 and iNOS.

[Slide.]

This is simply to illustrate that when one looks at the effects of interleukin 4 on induction of certain genes that are interferon-gamma-inducible, in this case the FCgammaR1 gene. This is an RNA analysis of RNA from monocytes that were stimulated either with interferon gamma alone, which gives a significant level of FCgammaR1 gene expression.

However, when cells are preincubated with

interleukin for, there is virtually a complete inhibition of the ability of interferon gamma to induce expression of this gene.

[Slide.]

This inhibitory effect of interleukin 4 on interferon-gamma-inducible gene expression in human monocytes correlates with an inhibition of activation of the transcription factor, Stat 1, which is the principle interferon-gamma-inducible transcription factor. The inhibitory effect is not immediately apparent, so here this suppression of activation of STAT 1 requires that the cells are preincubated for at least 60 minutes or so before this effect becomes apparent and, thereafter, is fairly complete.

More importantly, we had found that this induction of inhibition by IL4 of interferon gamma's ability to activate STAT 1 and to activate expression of interferon-gamma-responsive genes correlates with the ability of IL4 to induce expression of a novel gene known as SOCS1, or suppressor of cytokine signalling 1, which is one member of a family of genes which now numbers seven or eight.

IL4 activates expression of SOCS1 in human monocytes. The induction of SOCS1 mRNA which, it is quite apparent, at 60 minutes correlates with the inhibition of STAT 1 activation.

[Slide.]

If you over-express SOCS1 in a myeloid cell line, in this case M1, this is, again, an RNA analysis of total RNA, a panel of M1 cells, either the parental line or M1 transformance that over-expressed SOCS1 or SOCS2. When these cells are stimulated with interferon gamma, the parental cells, you can see that there is strong induction of FCgammaR1 and mRNA.

Forced expression of SOCS2 did not inhibit the ability of interferon gamma to upregulate FCgammaR1 gene expression. However, forced expression of SOCS1 markedly inhibited the ability of interferon gamma to upregulate FCgammaR1 gene expression.

[Slide.]

The SOCS proteins encoded by the SOCS genes act by two principle mechanisms. In future experiments, we are focussing in terms of how IL4 can inhibit interferon-gamma-inducible gene expression specifically as to the mechanism by which SOCS1 inhibits expression of interferon-gamma-inducible genes.

I am not going to go through this in any detail in the interest of time, but, suffice it to say, when expressed, the SOCS1 protein interacts specifically with the receptor-associated JAKs which thereby blocks the ability of the kinase to phosphorylate the intercellular domain of the

cytokine receptor.

For example, in our studies, we are interested in the interferon gamma receptor complex. Alternatively, other members of the SOCS family, in this case Cis1, act by binding directly to the phosphotyrosine motif on the intercellular domain of the cytokine receptor and, again, block the ability of the latent STAT from docking and, in turn, becoming activated by the receptor-associated JAKs.

[Slide.]

On a more general level, the importance, I think, of understanding how cytokines can cross-regulate the actions of one another, and the role of the SOCS genes and the SOCS proteins in mediating this inhibition is very important. In terms of understanding how the balance of cytokines produced by either Th1- or Th2-type T-cells in certain disease states predisposes to certain pathologies.

For example, it has been generally considered that, in many chronic autoimmune diseases such as rheumatoid arthritis or multiple sclerosis, that there is an increased frequency of Th1-type T-cells, increased production and activity of interferon gamma which may, in fact, disrupt the normal balance of Th1 versus Th2.

Conversely, cytokines such IL4 and IL13 produced by Th2-type T-cells normally control and prevent excessive activation by interferon gamma.

It is also worth stating that IL4 is being tested as an antiinflammatory agent in certain autoimmune diseases and the molecular basis for that may, in fact, involve a role for the activation of the SOCS genes in this process.

Let me leave it at that and address any questions.

DR. SALOMON: Thank you. Very well done.

DR. BLUESTONE: I have a question. I may have missed this. If you overexpress constitutive STAT, have you actually been able to bypass the inhibition of IL4 in any way by overexpressing any of these downstream targets?

DR. DONNELLY: By overexpressing the STATs?

DR. BLUESTONE: Something that will actually bypass--as I understand it, the IL4 inhibits. If the IL4 inhibits and you have got a number of readouts of that inhibition, STAT inhibition as well as the SOCS inhibition, I am just wondering if you can overcome the inhibition by bypassing that part of the pathway.

DR. DONNELLY: We haven't actually designed experiments to deliberately attempt that. I think, in theory, one could use a dominant negative SOCS to, perhaps, overcome the inhibitory effect, something of that sort.

DR. BLUESTONE: Right.

DR. DONNELLY: We haven't done those--

DR. BLUESTONE: I am just trying to get a sense of which of the effects you see are direct effects of the IL4

and which of them are downstream consequences of the IL4.

DR. DONNELLY: Very simply put, IL4 activates STAT6. STAT6, in turn, activates expression of SOCS1 which then feedback-inhibits--well, certainly can feedback-inhibit IL4-induced signalling; that is, the same cytokine that elicits its expression but we have found that it can also cross-regulate the activation of a cytokine.

It signals through an unrelated receptor, in this case, the interferon-gamma receptor. We are specifically looking at the mechanism of action by which SOCS1 inhibits, for example, interferon-gamma signalling. We have now, in fact, been able to aminoprecipitate and show a correlation in terms of a reduction of tyrosine phosphorylation of the receptor, itself.

We are also hoping to, very soon, be able to show a physical docking of SOCS1 on the receptor.

DR. BLUESTONE: On the interferon-gamma receptor.

DR. DONNELLY: That's correct.

DR. SALOMON: Thank you.

I guess Dr. Petricoin is not here. So, Kathy; you're on.

DR. ZOON: It is a pleasure to be able to present today. I know Jeff was looking over. He goes, "Now, where do you belong in the organization?" It is a bit confusion.

[Slide.]

I am in the Laboratory of Chemistry in the Division of Therapeutic Proteins in the Office of Therapeutics. So I am in the organization based on the scope of the responsibilities of my lab's research program as well as the regulatory responsibilities which is slightly weird, but that is the way it is right now.

My research in the laboratory involves the interferon alpha's structure and function. This is an area that I have been actively engaged in for twenty-five years. One would say, "Gee, aren't you tired of studying interferon alpha?" I wish I could say I was. But each time, I think, "Well, maybe I should do something else," something interesting pops up and there is a lot yet to discover and to really understand how interferon is working.

So this has still been a function of my laboratory. I have to, one, give credit to the members of the laboratory. Mr. Joe Bekicz is here. He is sitting in the back. Renchu Human, who is an research scientist who is also in my laboratory. And recently, as a result of some of the recommendations of the site-visit team, I have hired an ORIS fellow, Hannah Schmietzer, to do some of the studies that were recommended by the site-visit team.

I will discuss some further activities that we have been doing as a result of the recommendations of the site-visit team.

[Slide.]

What I am going to present in the first two slides are what I believe are the scientific significance of the work we have been doing on the interferon project. We have isolated twenty-two components of natural human interferon alpha. In doing so, we have, then, in the purification process, determined their biological activities and their physicochemical structural properties.

In doing so, we have identified a number of important areas. One is that there is a distinct spectrum of biological activities associated with each of these components. In saying that, we have looked at their antiviral properties and their antiproliferative properties and a number of immunomodulatory assays.

In summary, they each have a distinct combination of antiviral, antiproliferative and immunomodulatory properties.

From a scientific and interesting point of view, there are nine interferon--at least nine genes and probably in the order of fifteen to eighteen interferon alpha genes that have been identified, located in human chromosome 9. The question really that still is of great interest to the field, why has nature made this redundancy in interferons and what is their roles and responsibilities in eliciting their biological activities under a variety of different

stimuli and in compartmentalization in terms of the types of tissues that they may be produced by or cells that they may be produced by.

I think we are getting clues based on these studies and some others that will contribute to this area. We have also identified some very important cell-binding properties. In our examination of the interferon alphas, the predominant interferon alpha, one that has been cloned and has been used therapeutically, is interferon alpha 2.

In our system, we have identified two alpha-2-like components. In looking at competitive binding experiments using radiolabled interferon alpha 2, we have found a number of interferons, in particular, that have interesting properties, one being a component "o" which has a very high antiviral activity, an extremely high antiproliferative activity but competes poorly for the alpha 2B binding site.

So this has led us to propose either that there is a multicomponent receptor for which there may be a unique component attached to the clearly defined interferon alpha receptor 1 and alpha receptor 2 that have already been defined or there may actually be a distinct receptor that we still have not determined.

So these studies are clearly important and underway. I will discuss some studies that we have recently started as a result of some of the recommendations of the

site-visit team.

The third is in our structure-function looking at the different gylcoforms of interferon alpha. We find three. Alpha-2-like interferons in natural cells are glycosylated, which is an interesting aspect of these. Those are o-linked sugars. There is alpha 14 which is one of our components which is also glycosylated at asparagine.

We are currently continuing these studies using MALDI TOF mass spec to get a better handle on the particular structure, those glycoforms.

[Slide.]

We have also been involved in protein engineering where we have taken two interferons, one the alpha 2 which is the dominant form in lymphoblastoid interferon and, two, we have cloned what was our component "o" that gave the interesting biological characteristics of high antiproliferative activity, high antiviral activity but poor alpha-2-B binding.

We have made a variety of forms of this. What this has allowed us to do is to identify critical regions in the molecule with respect to binding and antiproliferative activity. What we have found is that the amino terminal portion of the molecule is very important in the binding domain of the receptor.

The binding has a higher affinity with the

interferon alpha 2 domain at the amino terminus. We have also found that the C terminal end of the molecule is very important for the antiproliferative activity. In fact, we have assessed that one particular region from 75 to 95 is also very important in the antiproliferative activity.

Taking our hybrids and making various versions, we have found that not only in the region 75 to 95 are there critical amino acids but additional amino acids subsequent from 95 to 166. In doing some site-directed mutagenesis, we have found two tyrosines that are extremely important in the antiproliferative activity, one at position 86 and one at position 90.

In addition, we have been doing some receptor signal transduction experiments. We have been doing these in collaboration with Chip Petricoin from our division. What we have found, in looking at a variety of systems, is that using our hybrids, the interferon pathway may not be solely determined by the activation of STAT1 and STAT2. We have recently published.

Right now, this is an area that the lab will be pursuing in greater depth. I am, right now, looking and recruiting and talking to various individuals to pursue this area in greater depth which was also a recommendation of the site-visit team.

[Slide.]

This summarizes the mission relevance of the work on interferon. As Dr. Rosenberg outlined, interferon alpha is a product that is regulated by the FDA. It is a licensed product. It is also still under IND for a variety of studies. It is licensed for everything from hepatitis B and C to AIDS-related Kaposi's sarcoma.

We are very interested in understanding the structure/function for two reasons. One is so that we can get a better handle on the activities and effectiveness of interferons using different clinical situations or potentially used and, two, for safety profiles of these products because, by managing and understanding toxicities of these interferons, one can eventually look at the possibility of engineering an interferon specifically that enhances its effectiveness and decreases it toxicities.

So those studies have as its basis for what is some of the underpinning of our work. In addition, the work we are doing--the lab has been very important interesting the development of methods and standards for interferon alphas. We have worked with the National Institutes of Biological Standards and Control in the United Kingdom that makes standards available throughout the world.

Our methods and our interferons have often been the lead in making determinations on some of these standards, so that work has contributed much to the

interferon alpha standards world.

Also, the uses of new technologies such as protein engineering will enhance the expertise to review new cytokines and interferons. These techniques are currently being used not only for interferons but other products as well.

[Slide.]

So where are we going in the future? As part of some of the recommendations of the team that came to visit us in December, we were recommended to further study the binding of alpha 21 and the hybrids to Daudi cells and characterize the receptor binding studies.

We have hired a post-doc to do this. Those studies are underway and the data is in the process of being collected. We also plan to further study the binding characteristics using soluble interferon receptors. This work is being done in collaboration with the Weissman Institute and Gideon Schreiber. We have already got a fair amount of data looking at the dissociation contents using the soluble IFNR2 receptor.

These studies will be very important in looking at the interactions of our different interferons with not only IFNR2 but IFNR1. We have all the variants of the interferons. The Weissman has all the hybrids and mutants of the IFNR1 and IFNR2. So, by studying the characteristics

of these, we may get further insight into the structure and function and interaction of the interferons with their receptors.

We are also, again, engaged in intracellular signaling pathways. Right now, this work is really being generated in a number of areas. We are hoping to narrow some of the important pathways by looking at our hybrids in some of these microray chip technologies maybe to identify more a subset of signalling pathways that might be more advantageous to study.

We have a number of recommendations by the site visit that we are considering. Right now, our first priority is to look for an excellent candidate to help us with these studies.

We are also looking at the role of the carboxy terminus of interferon alpha maximizing the antiproliferative activity. There are specific regions from 95 to 166 in the alphas, particularly in alpha 21, that we will be doing some site-specific mutagenesis in to really further define what are the critical amino acids to elicit the antiproliferative activity.

I already mentioned our work on MALDI TOF looking at the carbohydrate structures of those glycosylated interferons. We are also studying with a number of people in the Center for Biologics and in the National Institutes

secondary and potentially tertiary structures of some of the interferon alphas so we can understand the three-dimensional interaction between these species ultimately with their receptor.

The NMR studies are being done with Darren Freeburg in the Office of Vaccines and the circular dichroism studies are being done in conjunction with Peter McFee from the National Institutes of Health.

[Slide.]

Finally, in looking at some of the biological functions in the next slide, we are looking with Dr. Kathleen Clouse's lab the effects of our hybrids on HIV infection, of primary macrophages and T-cells. We have some very exciting results with some of our site-directed mutants having extremely high antiviral activity against HIV that looks very exciting.

We are repeating those studies to make sure that those data are reproducible. We are working with Dr. Eda Bloom and the Division of Cellular and Gene Therapy to better understand the effects of interferon on natural killer-cell activity and we are also pursuing a number of other immunomodulatory activities to have a better sense of how interferon alphas are affecting the immune system.

I would like to thank our site-visit team. They gave a lot of excellent advice and, hopefully, we will be

at

able to follow those through and get the results that might be expected.

Thank you.

DR. SALOMON: Thank you, Kathy.

Again, in the interest of time, I think we should take the next half hour and go quickly into the closed session.

[Whereupon, at 9:40 a.m., the proceedings were recessed, to be resumed at 10:15 a.m.]

TOPIC I (Continued) [10:15 a.m.]

Islet Transplantation/Preclinical Animal Models

DR. SALOMON: I would like to welcome everyone back to the second day of the BRMAC's considerations of islet transplantation. Today, as promised, there are no more limitations to the discussion on clinical issues as there were yesterday. Apologies to everybody for that artificiality.

So why don't we just get started. We have to finish this first session at five minutes to 12:00 which sort of gives us an arbitrary finishing point, but there are some of us who need to check out, including me.

The other thing that I want to announce to everybody is Rosanna Harvey who, if you don't know Rosanna, please note if you can make travel arrangements with Rosanna at least in any break that we have--well, we won't have any break until five minutes before noon, but if you can make any arrangements you can for travel with Rosanna.

Then, with that introduction, and I hope I am not missing anything, I would like to introduce Karen Weiss from the Division of Clinical Trial Design and Analysis to present an FDA introduction to this morning's events.

FDA INTRODUCTION

DR. WEISS: I will be very brief. I just wanted to welcome everybody back to the second day's discussion, to

thank the members of the BRM, the Endocrine and Metabolic Drugs Advisory Committee for joining us as well as all the experts taking time from their busy schedule to come here and discuss this extremely important topic with us.

As everybody now knows, today's discussion is supposed to be focussing on the preclinical and the bridge from the preclinical to the clinical. We have arranged the afternoon for a series of questions based on topics that have come before us at the FDA with these types of therapies.

In almost every one of these, we have a number of questions with respect to issues in terms of clinical trials as well as whether or not preclinical models can help sort out and address some of these questions.

So we are very much looking forward to your advice and discussions this afternoon. I wanted to start the session, then, by introducing Dr. Lauren Black, a pharmacologist from our division, who will present a brief overview.

Open Public Hearing

DR. SALOMON: Just one minor thing. I guess because, again, based on the format of the public hearing, we have not had anybody officially request time at this point but I am reminded that I should, again, ask if there is anyone in the audience who would like a five-minute

period to address the committee before we get started, you are more than welcome to step up now.

Lauren?

Animal Models of Islet Therapy: Utilization for Clinical Trial Design and Safety Assessment

DR. BLACK: I would like to thank the members of the advisory committee and the distinguished guests that are here for participating in this session on animal models of islet therapy.

[Slide.]

I will be presenting the FDA perspective on utilizing these models for clinical-trial design. Specific comments on the model attributes and results generated in these models will be addressed in the upcoming two presentations.

Islet therapy models are viewed at FDA in the context of the larger field of solid-organ transplantation. Transplant therapy models have been very helpful in prospectively designing clinical trials of investigational immunosuppressant drugs and biologic therapies and, more recently, of combined drug use and immunomodulatory strategies such as donor-lymphocyte infusions.

[Slide.]

Preclinical-model data are generated to advance our scientific understanding and are utilized to support the

rationale for new clinical investigations. In this context, animal models are used to assess the clinical utility of procedures such as in identifying promising immunosuppressive regimens, identifying effect islet doses or administration methods or to ask if there are durable effects of significant sequelae of effective treatments such as reductions in disease-related morbidity.

In these aspects, well-designed animal trials can contribute on the benefit side to the evaluation of clinical risk/benefit assessment as was mentioned by Dr. Zoon and people yesterday morning.

[Slide.]

Animal data also serve an important role in supporting the safety of investigational data and are used to determine safe doses and administration methods, for instance, by examining surgery or infusion adverse reactions.

Additionally, the dynamics, nature and dose-response relationships of the toxicities are evaluated. The nature of the toxicity may raise added concerns when events are hard to monitor clinically, are irreversible or are sudden in onset. Animal data are compared with the proposed clinical protocol and utilized to guide choices regarding clinical monitoring, endpoints and schedule, the appropriate patient inclusion or exclusion criteria and are

utilized to suggest appropriate adjunctive therapies.

Risks identified in animal studies often lead to modifications in clinical-trial design.

[Slide.]

To support clinical trials, animal models should be chosen to be clinically relevant. Islet allografting could be performed in an intact, healthy animal but the islet metabolic function would likely go unchallenged in the presence of a healthy pancreas.

It is an advantage for this field that allotransplantation is feasible in outbred diabetic animals in a manner highly analogous to human allografting.

[Slide.]

The proposed clinical strategy and its perceived risks and departures from knowns will influence the choice in animal model and the degree of biologic comparability needed between the animal and the patient. The more relevant the animal model, the greater the degree of confidence that an absence of safety problems in animals provides strong safety support for patients.

In counterpoint, where models are viewed as poorly comparable, clinical trials such as those perhaps tolerization therapies may be subject to more protocol restrictions.

[Slide.]

In brief, animal models for diabetes range from inbred NOD mice to pancreatectomized non-human primates and, very rarely, natural models of adult-onset diabetes. No one animal model could be expected to generate a perfect predictor for human outcome. The next speakers will highlight the utilities and limitations of each model.

[Slide.]

As in all thorough scientific investigations, there are attributes of preclinical study design and conduct that can provide the most convincing support for the safety of an IND. The study should provide a basis for comprehensive analysis of animal responses to all aspects of treatment, overall health, drug regimen and islet-induced toxicity and disease or disease markers are all needed to be monitored in detail.

Study design should also permit objective assessment of results, using prospectively designed protocols, randomization and blinding wherever possible to achieve this end. From the perspectives of data integrity and CBER review, the CFR outlines good laboratory-practice standards and requires that reporting of animal studies for INDs allows assessment of all aspects of animal safety.

In order to achieve this, animal-study reports need to be detailed and fully tabulated to include both group and individual results.

[Slide.]

There have been a number of shortcomings in preclinical aspect of INDs submitted to date for islet therapy and similar to those for other cell-therapy fields. These include incomplete datasets and designs that are incompletely comparable to proposed clinical protocols.

The concern is that, as proposed clinical therapies move further from those with which we have current clinical experience and preclinical experience, these inadequacies in preclinical support could become limiting to clinical development or miss an opportunity to predict clinical adverse reactions.

[Slide.]

In summary, clinical strategy and preclinical study design should be closely integrated. While recognizing that some information can only come from clinical trials, some data useful in clinical-trial design can be gathered preclinically. These data could include regimens for islet-only transplant.

In the past, animal models animal models have demonstrated utility in predicting clinical drug toxicities and could generate data to aid in reducing islet toxicity or rejection. In contrast, many questions remain for animal-model us of immunomodulators such as for tolerization approaches.

Current autoimmune animal models for these therapies have been open to criticism on account of clinical comparability. New research on the human pathophysiology of diabetes may be needed to bridge current gaps in our understanding of the models and human disease and the utility of models for therapeutic testing.

Lastly, to improve their utility in clinical-trial assessment, designs for preclinical studies of islet therapy should involve a more detailed concurrently tracked approach to both safety and activity monitoring. Consideration of the best model species, high comparability of animal and human trial designs, and more detailed documentation would increase the scientific validity of the preclinical evaluations and increase the impact of these investigations on advanced and clinical therapy of diabetes.

Thank you.

I would like to mention that this afternoon you will be asked to address a number of questions that involve both clinical and preclinical designs. They will highlight the role of preclinical models and the development of clinical protocols and you will be asked to comment on areas in which there are unaddressed clinical questions.

Concomitantly, we will discuss how these might be addressed in preclinical models. Please consider where we need to develop new animal models and how those models might

aid in identifying immunosuppressant regimens, appropriate measures of islet activity, evaluating the immunogenicity if islets or the impact of multiple transplants on sensitization, establishing the best methods for isolation, defining quality for the best methods of delivery or determining the impact of peri-implant type glucose control or the effects of the animal models in determining islet potency or the best determinants of dose.

At this time, Jack, would you like to come up? I would like to introduce the next two speakers who have been invited as experts to provide detailed information on the models currently in use for islet therapy.

Jack O'Neil has developed and studied dog, pig and rodent models to evaluate artificial pancreas, encapsulated the allogeneic islets and allogeneic islet therapies in both biotech industry and academic laboratories. Jack will give a broad overview of the non-human-primate models. He will cover dog and pig models of islet therapy as well as rodent.

Immediately after Mr. O'Neil, Dr. Norman Kenyon will address the non-human-primate models of islet therapy. Dr. Kenyon is investigating immunointerventions in three different primate models of islet therapy and will discuss the relevance of these models in developing clinical strategies.

Animal Models of Islet Transplantation

MR. O'NEIL: Good morning

[Slide.]

I would like to thank the FDA for giving me the opportunity to participate in this meeting as we embark on one of the most exciting times and, certainly as we heard yesterday from both the Juvenile Diabetes Foundation and the National Institutes of Health, one of the best finance times of islet transplantation.

This presentation will cover some of the preclinical animal models that are utilized in clinical islet preclinical studies and development.

[Slide.]

The major obstacles to successful clinical islet transplantation faced today is to treat insulin-dependent diabetes in the type-1 patient and the autoimmune destruction of the islet graft. Above and beyond that, you have the immuno-attack that would accompany any allogeneic tissue transplantation.

Currently islet transplantation is performed only with a preexisting kidney islet transplant in most cases and the conventional immunosuppression used to protect the kidney graft has been demonstrated to be cytotoxic to the subsequent beta-cell graft and, lastly, the insufficient supply of allogeneic tissue has encouraged us to seek alterative sources for beta-cell replacement therapy.

[Slide.]

The preclinical animal models used to investigate clinical islet transplantation have a major limitation in that transplantation incident animal models, there is not a sufficient for the autoimmune destruction of the graft. Currently, rodents are the only animal models available for this. Most importantly, the NOD mouse, the BB rat and then a model that I will talk about a little later is the humanized autoimmunity transfer recipient.

[Slide.]

Basically, what I am going to do is go through each one of these obstacles and try to list the animal models that would be appropriate in early development and later end-state development towards clinical studies.

The immuno-attack of the islet graft can be looked at in immunocompetent rodents, humanized immune-transfer recipients and dogs and pigs in the non-human primates.

[Slide.]

The conventional immunosuppressive regimen that generally is used in kidney transplantation in order to protect the islet graft is best studies in large animal models where the immune system is most closely similar to the human with the dog, the pig and the non-human-primate models.

[Slide.]

In the insufficient supply of allogeneic islets, although not directly under the scope of this particular meeting, can be addressed with the same animal models looking at immunocompromised rodents in early preclinical studies and then moving up to the larger animal models as you get closer to clinical development.

[Slide.]

Basically, the methods that can be used for all of these studies to evaluate both the safety and the efficacy of preclinical transplantation are listed here. Generally, when preclinical studies are undertaken in the laboratory, efficacy is the primary concern of the investigator but I am sure the FDA would certainly like us to implement safety measures of these preclinical studies as well, starting with the rodents and all the way up to the larger animal models.

For safety, by performing physical exams, a veterinary checks on the animals measuring body weight, blood chemistry and hematology. During the study, you can get an idea on what the therapeutic effect to the recipient is and then, in the post-transplant period, to do a necropsy and look at major organ systems, histopathologically, to see if there are any adverse effects to other organ systems in the recipient animal.

For the efficacy, it is much like the clinical islet transplant where you are looking at the blood glucose

which an exogenous insulin requirement of the patient, C-peptide secretion, hemoglobin A1c levels, response to secretogogue challenge looking at blood glucose, insulin and C-peptide secretion and then followed up, post-transplant period, with histopathology of the islet graft.

[Slide.]

First, the animal model for transplantation, a method to chemically induce diabetes in immunocompromised or immunodeficient rodents with aloxan and streptozotocin, as we heard yesterday. It is a very useful model in looking at an islet function in the absence of the immune system. Alloxan and streptozotocin were demonstrated in the '40's and the '60's to produce diabetes in rats and in larger animal models with a cytotoxicity to pancreatic beta cells resulting in insulin-dependent diabetes characterized by glucosuria and excessive weight loss and hyperglycemia.

[Slide.]

The advantage to this model, as I said, is to evaluate the islet function in the absence of the immune response and the toxicity that is associated with conventional immunosuppressive agents, as an accessible, cost-effective animal model to be used in preclinical development of therapeutic strategies.

The limitations of animal model is that there occasionally is return to the spontaneous disease following

the induction of diabetes with diabetogenic agents. The dose, the severity of the diabetes and the preclinical outcome in these animal models have been shown to be strain-dependent.

[Slide.]

For each one of the animal models, I would like to go through what I call clinical comparability where we look at some different aspects of the rodent or the animal model to the clinic, the surgical methods and the islet dose. As far as organ procurement and islet isolation and then the administration of islets is not comparable to the human situation for these immune-compromised animals, immunodeficient animals, there is no immunosuppression necessary.

The clinical induction is not an autoimmune disease and does not allow for the evaluation of therapy response to an autoimmune attack. C-peptide blood glucose and IVGTT and body weight, all these can be measured, but there is currently no method to correlate the results to the engrafted cell mouse.

The histopathologic assessment of the graft can be performed much like it can be in the clinical setting.

[Slide.]

The spontaneous non-obese diabetic mouse is a very important animal model as it represents the best animal

model for the immune response in the islets in the type-1 diabetic setting. The mouse was derived from an outbred strain in the 1980s and has been extensively inbred since.

Insulitis occurs in all animals starting at about four weeks of age and diabetes some time after twelve weeks with a predominance of diabetes in about 80 percent of the females and only about 40 percent of the males. The diabetes is characterized by glucosuria, excessive weight loss, hyperglycemia and ketoacidosis. Without insulin therapy, it is lethal.

[Slide.]

The advantages of this animal model is that it is an autoimmune model of insulin-dependent diabetes. It is a cost-effect animal model and it has extensively characterized disease etiology. There is also the availability of immunological reagents were are not necessarily the case in the large animal models.

[Slide.]

The limitations; it has been criticized for representing only one individual with type-1 diabetes because of its extensive inbreeding. There are numerous interventions that are successful in influencing the onset of the diabetes in this animal and, unfortunately, many therapeutic strategies which prevail in rodents fail when applied to larger animals and to humans.

[Slide.]

The clinical comparability is much like that of the immunocompromised rodents with the exception of the NOD mouse, disease etiology share many clinical morphological and immunological features with the human disease and, most importantly, autoimmunity.

Both immunosuppression and immune stimulation can prevent the disease in the animal making therapeutic strategies developed in the NOD mouse sometimes not relevant to the clinical setting.

[Slide.]

The humanized diabetic immunodeficient mouse is an animal model that has been extensively studied over the last decade or so and, basically, with the immunodeficient environment of the animal, you can transfer human lymphoid cells into the immunodeficient animal and generate the humanized mouse to evaluate immune responses to allografts.

Two examples of this type of animal model are the SCID mouse or the RAG knockout mouse.

[Slide.]

The advantages are that you can evaluate the mechanisms of islet-graft rejection. There is the ability to manipulate the cells that are transferred into this recipient and, therefore, the immune system of the recipient, and there is the potential to compare allo with a

normal patient and autoimmune responses with the transfer of lymphoid cells from a diabetic patient to the islet graft.

The limitations are the degree of the engraftment and the susceptibility of the animal models to graft-versus-host disease.

[Slide.]

The BB rat was a spontaneous mutation of the Wistar rat in the '70's and it has been extensively inbred since. Insulitis occurs at about four weeks of age and diabetes after eight weeks. Prevalence is equal both in males and females and the insulin-dependent diabetes is characterized by glucosuria and excessive weight loss, hyperglycemia and ketoacidosis.

[Slide.]

The advantages, again, is that it is a spontaneous autoimmune disease. It is an accessible cost-effective animal model. It has, again, extensively characterized disease etiology and the availability of the immunological reagents.

Limitations are that the animal is T-cell deficient to start with. It is prone to infection, has to be raised in SPF or VAF facilities and there is really no real advantage compared to the insulin-dependent diabetes found in the NOD mouse.

[Slide.]

The clinical comparability would be shared with the other rodent models with the exception that the diabetes does share many clinical, morphological and immunological features with the human disease.

[Slide.]

As far as the dog used as a preclinical model for islet transplantation, there are basically three types of diabetes in the dog. There is the spontaneous diabetes. You can also induce with the chemical induction with streptozotocin and alloxan and perform a total pancreatectomy to achieve insulin-dependent diabetes.

[Slide.]

Spontaneous diabetes in the dog is reported by the veterinarians to have an incidence of about between 1:200 to 1:800 animals. The most common cause of the diabetes is reported as pancreatitis with diabetes secondary to a chronic pancreatitis.

There has been one dog breed, the keeshond dog, that demonstrated a high incidence of diabetes at a young age--that was reported in the '80's and hasn't been heard from since--as well as the familial form of the diabetes in a colony of golden retrievers that has left the literature since the '80's.

[Slide.]

Chemically induced diabetes with alloxan and/or

streptozotocin; dogs are more sensitive to the non-beta-cell toxicity of these agents and, generally, by combining the two agents, you can miminize the toxic effect of each. The dogs following induction are suspectable to severe hyperglycemia, have to be monitored very closely following the chemical induction.

[Slide.]

Pancreatectomy-induced diabetes in a dog is a fairly straightforward surgery and results in insulin-requiring diabetes. It is essential to supplement the dog's diet with pancreatic enzymes due to the exocrine deficiency caused by the pancreatectomy.

[Slide.]

The advantages of using a dog model is that it is an accessible and well-established animal model. They are easy to handle and train. Most facilities in an academic setting, they do have housing available for dogs. The diet and metabolism resemble that of a human and it is a cost-effective large laboratory-animal model.

[Slide.]

The limitations are that there is no consistent source of spontaneously diabetic dogs. The chemical induction is associated with a high rate of mortality. Pancreatectomy results in brittle diabetes, digestive deficiency and may compromise other organ systems.

As with all allograft settings, there is the inability to differentiate the contribution with the islet graft to the native pancreatic function.

[Slide.]

For clinical comparability, the pancreas procurement is generally optimized in the preclinical setting using a healthy donor in a single organ procurement. Transplantation has been achieved in the liver by laparotomy and the transhepatic method and has also been transplanted in the renal and the splenic sites.

The dose studies support the clinical data in that the allograft survival and insulin independence are directly related to the total islet-cell mass transplanted.

[Slide.]

The immunosuppression requirements in a dog are generally higher as compared to the human to achieve the same therapeutic effect. Spontaneous diabetes in a dog is usually related to the destruction of the islet secondary to severe pancreatitis and is not an autoimmune response as seen in type-1 diabetes patients.

Some genetic disposition of diabetes was reported in the '80's but has not been reported since. Diabetes induced by chemical agents or pancreatectomy is certainly different from the autoimmune disease experience in type-1 patients.

[Slide.]

Tissue typing can be done to look at matching and mismatching. It has been reported, but the data is limited and is basically restricted to a few labs that have that technology. Again, C-peptide, blood glucose, IVGTT, body weight, can be measured but, again, there is no correlation to the engrafted islet mass. Histopathologic assessment can be performed much like it can be in the clinical setting.

[Slide.]

Diabetes in the pig; again, three different types of diabetes in the pig. There is spontaneous diabetes, chemical induction with alloxan and/or streptozotocin and total pancreatectomy.

[Slide.]

Spontaneous diabetes, there is a line of Yucatan minipigs that spontaneously developed diabetes. It is a type-1 diabetes with hyperinsulinemia and hyperglycemia. There is an insulin resistance, especially during gestational lactation. They develop angiopathies and other complications similar to the human disease. However, there has not been any extensive reports on that model since the 1980s.

[Slide.]

The chemically induced diabetic pig with alloxan and/or streptozotocin results in diabetes characterized,

again, by hyperglycemia, polyuria, glucosuria and weight loss. A partial pancreatectomy can be supplemented with these diabetogenic agents that result in insulin-dependent diabetes.

[Slide.]

A total pancreatectomy-induced diabetes is a technically challenging surgery due to the close association with the vasculature in the pig of the pancreas and the vasculature. Diabetes within the first week is characterized by fatal hyperglycemia of not treated and then, again, the removal of the exocrine function of the pancreas and it is essential to supplement the diet of the animal with supplemental enzymes.

[Slide.]

The advantages are that the anatomy, physiology and metabolism and diet are similar to human. It is a relatively inexpensive animal model to purchase and it does have the unique ability to evaluate the MCH disparities with the NIH minipigs.

[Slide.]

The limitations of the animal model of the pig islet isolation is that it is probably the most technically challenging procedure. It is probably no coincidence that there are very few labs that have had successful preclinical studies in porcine islet transplantation.

They are difficult animals to handle and they are not easily trained. Because of the difficulty of the close association with the vasculature, this is a potential for incomplete pancreatectomy. Pigs are very susceptible to post-operative infection due to the nature of the beast, and the inability, again, to differentiate the contribution of the drug graft versus the native pancreas.

[Slide.]

The clinical comparability for the pig is that, again, the pancreas procurement is generally optimized with a healthy donor and a single-organ procurement. Transplantation sites have included the spleen, the liver, the kidney capsule by laparotomy.

Dose studies support clinical data again that the graft survival and insulin independence are directly related to the total islet mass transplanted.

[Slide.]

Immunosuppression, again, in the animal model is generally higher to achieve the same therapeutic effect. Spontaneous diabetes and diabetes induced by the chemical agents and/or pancreatectomy are certainly different than the autoimmune attack experienced in type-1 diabetic patients.

Minor and major histocompatibility matching can be studied in the partially inbred NIH swine model

[Slide.]

Again, the C-peptide blood glucose, all the clinically relevant parameters, can be followed but, there, again, is no correlation to the engrafted mass and histopathological assessment can be performed.

[Slide.]

As far as future optimization of preclinical animal models in islet transplantation, I think we need to appreciate the limitations of each of the existing models when trying to apply those strategies to the clinic; to better characterize the etiology of spontaneous diabetes in large animal models; to closely mimic the clinical situation for organ procurement, islet isolation and subsequent transplant.

Certainly, one of the limitations is to develop methods to quantitate engrafted islet mass and correlate with graft function post-transplant and then to exploit any large animal model of autoimmune diabetes to develop one that more closely resembles the human disease.

[Slide.]

Progress in preclinical islet transplantation over the past has been basically cured "by the decade." In the 1970's, rodents were cured. In the 1980's, dogs were cured. In the 1990's, pigs and non-human primates were cured.

[Slide.]

Hopefully, 2000 is the decade of the human.

Thank you very much.

DR. SALOMON: Thank you. Well done. I think we can take a few minutes for questions but I am going to try and stay on time here so we can be done by noon.

DR. AUCHINCLOSS: Jack, after listening to your talk, and thinking about it from my own point of view, would you agree with this; I can think of using strep-treated mice for experiments, NOD mice for experiments, non-human primate for experiments.

I also think there are a very small number of indications for using pigs. The two I have encountered are demonstration that pig islets are working by using an allograft transplant and I have seen at least one experiment where taking advantage of the inbred NIH pigs was an advantage.

But, other than that, I can't think of any reason to go to any other animal models. Do you think that is true?

MR. O'NEIL: No, not necessarily. I think the field has learned a lot from using the dog as a preclinical animal model. Certainly, the islet isolation and the transplant and the maintenance of the animal is much easier for most laboratory settings and I think we have learned a lot from the dog model as far as applying that to the

clinical studies with different immunosuppression regimens that are necessary and different isolation techniques, and so forth. So I think that the dog does add value.

DR. AUCHINCLOSS: Why would you do a dog instead of a non-human primate?

MR. O'NEIL: The dog is less cost-prohibitive, I think, if nothing else.

DR. AUCHINCLOSS: Less costly, less relevant.

MR. O'NEIL: Excuse me?

DR. AUCHINCLOSS: But what you get in lower cost, you lose in less relevant; no?

MR. O'NEIL: I don't know if you can clearly say that one animal is more representative of the clinical situation than the other. They both have their limitations.

DR. KENYON: Hugh, I would like to respond to that, too. I think I understand your point, but one of the advantages of the dog--we actually use both models at the DRI--and, because it is less costly--and they are easier to handle. It is not just the cost.

You could study some initial variables in the dog and then, once you have narrowed them down, try them out in primates in order to move a little bit more quickly. The high-dose donor bone-marrow infusion studies that we have done, we have been able to study dogs more quickly and do more experiments with bone-marrow infusion than with the

monkeys which is much more labor intensive and time consuming, in addition to the cost.

DR. HERING: We are studying new islet-implantation sites, novel islet-delivery systems extensively in pigs. I think this is a good model to study this in and I am not sure whether the non-human-primate system is definitely more predictive than the pig system. I am not aware of any studies to demonstrate this.

So think there is reason to continue. We also think that studying novel immunosuppression regimens can certainly be done in pigs, does not need to be done in primates, necessarily.

DR. BLUESTONE: Jack, it seems to me that a lot of what has come down here and what makes this different than every other transplant setting we have talked about is this autoimmune issue and how focussed should we as a community, should the FDA be as a regulatory agency, on the fact that, with the exception of the NOD mouse and BB rat, which has all its own problems, we really don't have, whether it be dog or pig or monkey--it doesn't matter--we do not have a model that, in a very fundamental way, mimics what is happening in the patients we are proposing.

How important is this issue in dominating all of this discussion about, aside from where you put the islets, but a discussion about immunemodulation and

immuneregulation.

MR. O'NEIL: I think it is critical. As was discussed yesterday, the attempts to try to develop autoimmune animal models in the non-human primate certainly would be welcomed by the field. In addition, I think it may be important for us to get a message out to veterinarians and tell them that we are desperately seeking models of autoimmune diabetes in large-animal models, just to let them know what to look for, and to try to develop strains from that pool of animals.

DR. SALOMON: Can we follow that up just a little bit in terms of discussion of the committee in that if we think about the mechanisms of autoimmune diabetes, obviously, there is this whole field of trying to come up with ways of trying to understand better what the immunologic events are and how you break tolerance in that compartment and then how you create injury.

At the same time, of course, we have got another group of people working on islet transplantation. Jeff has asked an interesting question and that is, to what extent are these two fields overlapping. Can we talk just a little bit more specifically about in what ways would an islet allograft--I don't think we should go into xenografts right now--and islet allograft be affected in terms of its survival and function in a target organ by anything that has

anything to do with autoimmunity.

DR. BLUESTONE: There is no doubt that there is overlap. All of that is true.

DR. SALOMON: I wasn't tying to say there wasn't. I just wanted to get it out into discussion, particularly as there are members of the committee who are not experts in databases.

DR. BLUESTONE: But if one is, even in a more fundamental way, asked a question, and maybe here is where the pigs might be an advantage, for instance, what is fundamentally different about the autoimmune response than the allogeneic response.

One of the things that is potentially fundamentally different is the stage in which the response is being studied. It is clear in an autoimmunity response, you are studying a secondary response as a minimum in these patients, a long-term memory response and a highly established response, a response with a humoral component as well as a cellular component.

Yet, very little of what we talk about in our animal models are using presensitized or highly sensitized--now, granted, we don't have an autoimmune model; I understand that. But we don't even use highly sensitized animals for the most part for our animal models, something that can be done, for instance, in the pig system where you

can have MHC sensitization; right--a clear knowledge of what the MHC is going to be for sensitization which you can't do, maybe, in the beagles or something. I don't know.

You can use spleen or something to sensitize, a skin graft or something like that. All of those are possible, but, to me, we have to be thinking a little bit more creatively about how we take--granted, we should be talking to every vet in the world and pull out our great autoimmune type-1 animal, but if that is not going to happen quickly, are there ways that we can enhance the current models that we have that might actually have some more similarities to the autoimmunes. I don't know.

DR. CHAMPLIN: The antigens involved in an autoimmune response obviously are not alloantigens. They are not MHC or minor antigen, discrepant antigens, which are likely very involved in graft rejection. So there may well be some overlap ultimately in the patients, but I would think that you have two problems; one is to try to develop a system that performs pancreatic islet-cell allografts in an unperturbed recipient and then, secondarily, dealing with the ongoing problem of diabetes and whatever ongoing immune response you would have against the transplanted tissue.

So those are two separate but equally important questions in the ultimate solution.

DR. SALOMON: If we look at the experience with

whole-organ allotransplantation, pancreas allotransplantation, aside from I believe it is two reported cases--correct me if I am wrong--but it is really a minority. Is there any evidence of recurrence of an autoimmune diabetes leading to injury or destruction of these whole-organ allografts?

So I go back to the question of what, if any, data do we have that existing mechanisms in a diabetic patient that induced the autoimmune diabetes at one point in their course have anything to do with the survival and function of an islet allograft--

DR. BLUESTONE: But then you have to realize that, also, as long as you keep a type-1 diabetic on high doses of cyclosporine, their diabetes doesn't get worse, either. So, to say, therefore, autoimmune diabetes is the same as allograft rejection because cyclosporine inhibits both doesn't well--because you are saying they are not getting their recurrent allo while they are on cyclosporine.

They are not getting their autoimmunity while they are on cyclosporine? They are on drugs that are inhibiting and might inhibit both but that doesn't mean that both are the same. It just means they are both inhibitable by the drugs that we are using in our patients.

So, if we are going to move forward, hopefully, into therapies which are changing fundamentally the immune

system so that we are not working with long-term immunosuppressive therapies, I think that that particular piece of data may or may not be relevant to the long-term effects on the immune system.

DR. SALOMON: Again, I have no agenda and I am not pushing any scientific hypotheses yet. I am just trying to get some of these issues on the table. My point is that an alternative hypothesis is that autoimmunity is not--mechanisms inducing islet autoimmunity in the patient could potentially have absolutely nothing to do with the survival and the immune reaction to the allograft because, again, the antigens are being presented in a different MCH context and/or those mechanisms have been burnt out years before you go ahead and do the allograft.

I am not saying I have any data, either, that that is true but there seems to be--I am not certain that I go along with this tacit assumption that studying islet transplantation in non-autoimmune diabetic models is a limitation.

DR. BLUESTONE: I will say one more thing and then I will stop. I think that is ridiculous. There are lots of data. Camillo has got data. Everyone has got data. But when you try to suppress in an NOD mouse, an animal that is already diabetic, the same drugs that work absolutely perfectly in an NOD mouse where you have actually switched

the MHC so it is not diabetic work much, much better than they do in the autoimmune animal.

So I think that it is so unlikely that the autoimmune response doesn't play any role in this--the reason I am being so strong about this is that I would hate to come away from this discussion thinking that allo is all we should be caring about here and we shouldn't be caring about the auto response.

I think the auto response is absolutely involved in this thing and it doesn't burn out and be gone in these autoimmune patients. The antibodies are there.

DR. SAUSVILLE: I guess the question that comes up, then, is would you then choose, if you were going to recommend to the FDA which model they would potentially base the design or advise potential sponsors in terms of using immunosuppressive regimens, do you feel, therefore, that that should bias the selection of what animal model would be most relevant then?

DR. SHERWIN: There is no question that ultimately--you are going to have to approach it from both sides initially and then work--ultimately, there is no question. I agree with Jeff, there is absolutely no question that autoimmunity is a key player in the problems of islet grafting.

It is so highly unlikely that it is not an issue

so I think, ultimately, we need to have, to be effective, autoimmune models, either human models which are, I guess, the proof of the pudding or other--we need to focus much more--ultimately, it is much easier to do allografts and it is much harder to transplant in an autoimmune model across--doing allografts in autoimmune models.

I think that ultimately that is where we have to go. I could just say that there are other potential ways of developing autoimmune models that are more relevant to the human situation--I mean, just an example. We have models with human HLA transgenic animals, DQ8, DR4 animals, that get spontaneous autoimmune diabetes and it is due to T-cell--you know, T-cells. It is an autoimmune model.

So it is conceivable that one can manipulate the genetics of mice or even rats to develop humanized autoimmune models that have some relevance, at least, to the human condition.

DR. AUCHINCLOSS: I am going to make the prediction here that there will not be any adequate autoimmunity model, that we will never find a monkey or a supply of monkeys in sufficient numbers with type-1 diabetes to be useful.

I am going to suggest that I think all of the SCID-adoptive transfer models of human autoimmunity are close to worthless and I am going to suggest that all of the

mouse autoimmunity models, even the humanized mice, have terrible limitations.

While we should use them, the fact of the matter is we are never going to find the answer to this question without testing it in diabetic patients.

DR. SHERWIN: Nobody is arguing that. But there are steps to take it to a human and I think that one can learn a lot from autoimmune models even though some of the answers may not be relevant to human.

I think it would be a big mistake to totally ignore animal models and not strive to develop animal models that are more appropriate to the clinical situation.

DR. CHAMPLIN: There are a lot of limitations, particularly in trying to develop immunosuppressive therapies in animal models and then translate, then, to man, obviously major differences between species and in effects of steroids, major strain differences in one mouse strain and another, at least as we see it in bone-marrow transplants. Fludarabine had fundamentally different metabolism in different species and so the effects in humans are far different than they are in animals.

So, although animals are certainly extremely important in providing leads, ultimately, as you are trying to develop an immunosuppressive regimen that works in human patients, there is no substitute for testing it and

developing it in human patients.

So I agree with Hugh's comment; even if you had a perfect animal model, translating the therapy from that animal to the human being isn't the direct translation, that one has to do a lot of work within the human system.

DR. SHERWIN: But you are dealing with, perhaps, the diseases that are different from diabetes in the sense that we have other forms of therapy that are alternative and relatively safe. So, in the equation, even though I am very strongly in favor of doing human islet transplantation, very strongly in favor of it, one has to take into account the fact that there are alternative approaches that, in many people, work very successfully.

They are improving continuously. So one has to take that into account when doing more invasive procedures.

DR. SALOMON: That will be important this afternoon when we start to discuss what patients and what kinds of clinical trials, specifically, should be done.

DR. BLUESTONE: I think there is a middle ground here. I think, first of all, we haven't solved the allo problem so it is not like we have solved half and we only have half to go. We still have the allo problem and animal models are probably very important in doing that.

That is number one. Number two is, I think Hugh is right, but I would state it differently. I would say the

chances are we will probably solve it in humans before we get the models up that we want to get up but that doesn't mean that the models, all along, haven't been helping provide a road map for us.

So, although they may not give us the ultimate dosing, and they may not even tell us--but they are telling us where we are, where we are on target, which therapies are on target and are moving us in the right direction, and which therapies are not on target.

So where I see the question that I was asked before is is there an animal model that should be used by the FDA as sort of the gold standard for saying this drug is going to work in humans, I think the answer is absolutely no. But is there information that will be learned from the animal models which will help inform us that the therapies we are ultimately going to try in human beings have a better chance of working and, therefore, should be approved in an IND. I think the answer is absolutely yes.

So, to me, since there is no perfect model, then I think we are best off keeping our options open, really not sitting here and saying there are good models and bad models and no models, there is information that we need to learn. The allo response is important and I wouldn't be surprised if we don't learn something in a number of these other models which at least sets us in a direction that we are not

currently going in.

DR. RICARDI: I would like to comment that, to me, I agree with Dr. Champlin completely that the best model to test these new therapeutic approaches is the clinical setting and the model is the human and not the preclinical. But I complete agree also with Jeff in the fact that you need this basic model and NOD for screening the development of new tools like all the customary blockers and all the new monoclonal antibodies may be tested.

But there are a series of situations where it is either impossible or not practical, like anti-CD3--there are agents that do not cross-react with non-human primates. There is the Edmonton protocol that has been developed--these wonderful results have no animal model that prove the concept of the potency of what turns out to be the most effective way to prevent rejection of islets in an autoimmune background, like the trials in Pittsburgh with FK506 would be that with the requirement of an animal model because of the toxicity of the tacrilimus in dogs that actually block development towards clinical application.

So I think we have to be very careful, meaning that we need an animal model. We need to develop better animal models for autoimmunity in large animals. This is a science kind of problem and concern, but I would not necessarily require any preclinical model of proof of

principle or potency of what you want to demonstrate in a pilot clinical trial as a requisite for an IND for a clinical islet transplant because you may have this information from clinical trials in other diseases, just from experimental models that may not provide direct evidence, and the NOD mouse is a great model for basic research but is a fairly different disease than type-1 diabetes in humans.

It is very much a violent onset and it happens in a few weeks and it is completely different. There are, like, 127 ways right now to prevent diabetes in NOD mice and none in humans. There is probably different relevance even though I agree it is very important.

DR. SALOMON: I also just wanted to stay on record as, despite Dr. Bluestone's strong opinions, I am not at all convinced that a mechanistic link between autoimmune and alloimmunity, between the mechanisms that destroy islets in a diabetic patient and the mechanisms that challenge a successful allotransplant are really very well connected.

I think that that is a very interesting area for research. There are a number of examples of autoimmune diseases that burn out. I have transplanted many lupus patients, very example, who have had the hell immunosuppressed out of them and then actually completely resolved their disease once they get kidney failure.