discussion.

I thought it was a little too early for lunch anyway so I am letting this go, given that I think some of these points are of value to the FDA staff. I know you, Ed, and Dr. Cara would like to make some comments.

DR. SAUSVILLE: Yes, I would actually like to ask Dr. Goldstein. Clearly, the results that were both hinted at and potentially are now achievable using the new immunosuppressive regimens are very attractive in terms of using pancreata as the source of islets, but given the scale of need that I think one of the early speakers showed with the little pieces of pies for the potential availability of islets, do we really feel that that is where the field is going to end up or do we feel that entire new technologies of delivering islets or holding islets in various devices are going to actually extend the promise of this to the relatively large epidemiologic population that would potentially benefit from them?

DR. GOLDSTEIN: Joan Harmon put up the slide of everything else. So I refer you back to Joan's visual. Our position is absolutely clear. We feel that the most progress in the near term can occur with human islets, period. We are happy and willing to support everything else that was on Joan's slide and people have to, you know, figure out how they want to do that and proceed.

With specific regard to xenotransplant or capsule and capsulated approaches to fool the immune system, we would be delighted if one of those many people who have told us that that is going to be ready tomorrow morning would appear with data or with the research grant application that we could fund, etc., etc., etc. So, I won't bore you with the details, except to say that our portfolio of research funding in xenotransplant and capsulated models to accomplish the same task is pitifully small, probably under one million dollars which, given the amount we are willing to provide -- and what is driving that, from our point of view, is lack of scientific progress. So, I don't know how to predict that. I will take any version, however, and my public comments to people who want to study capsules and alternative source material is do it. Send us research applications; we will be happy to look at them.

So, the bottom line for us is that the shortage of human islets and pancreas material doesn't bother us today. We are actually mounting a public effort to join many others in organ donation enhancement efforts which we think everybody would like to have and need anyway and that, at the very worst, everything that occurs here in the next three to five years will be practice for what is absolutely necessary to actually implement anything else you are going to do anyway. So, even if it only works for a thousand

people we think it is well worth the investment because you will need to have done something exactly like that to gain the experience and knowledge necessary, technique, etc., for whatever next thing comes along.

DR. SAUSVILLE: So, then I would offer that as a key point in response to Dr. Auchincloss' comments because I think that transplant per se is probably actually not going to address the epidemiologic need. It should be done. It is an important opportunity. But ultimately one has to generalize the technologies so that, as was stated, what we are experiencing is practice for being able to get this to the larger population that needs it.

DR. CARA: I have a comment that I would like to make after Dr. Harmon's and Dr. Goldstein's talk, and that is that one of the most frustrating issues I think for pediatric endocrinologists is that many of the drugs that we use have not been tested or approved for use in children. And, I would strongly encourage you, as we discuss issues related to islet cell transplantation, to think about children in forethought rather than as an afterthought. It would be wonderful, for example, to develop an advisory committee of pediatric endocrinologists that might be thinking about applications of islet cell technology, islet cell transplantation technology for children, or something along those lines. But I think any ways that you can think

of for having some sort of pediatric component to the sorts of issues that you have talked about is critical.

DR. SALOMON: Dr. Ricordi?

DR. RICORDI: I would just like to make a comment related to Dr. Auchincloss' comment. I think it is very important that we address the general area where islets will be regulated because, to me, islet is not a cellular transplant and is not a transplant like you have when you address modified cells, proliferating cells or cells that can be stored in a repository prior to surgery like bone marrow or blood bank. Some people may actually consider islets close to organs. It is a very complex, delicate, multi-cellular structure with 1000 to 2000 cells per cluster. You may be responding to the same law of ischemia and revascularization as in whole organ transplant and, as we saw from the initial experience emerging from the different centers and approaches. The best series of transplants so far performed are with strategies exactly like in organ transplantation. You process the cells. The patient is ready to receive them and they are infused immediately after preparation.

So, I see FDA's regulatory component as critical to bring the field together and establish what are guidelines for comparison, product release criteria, how we can better address safety, and post-release criteria to

retrospectively see what we can improve. But, at the same time, it would be very dangerous to consider an islet transplant like other cellular transplants or, even worse, like tissue transplants, like bone marrow or transplants that are stored for a longer time. So, if islet transplantation evolves to a form of cell transplantation, like having expanded cell lines, then you will have another, much more complex series of regulatory aspects. But, for the time being, we are still very much closer to an organ transplant.

DR. SALOMON: We have been through these arguments with xenotransplantation, that there are always concerns by some in the field that regulation would not be done in a way that would allow them the kind of flexibility to go forward in a new area, and I think that is an issue that the FDA is very sensitive about. I think you ought to understand that is why you are here today -- the FDA is trying to communicate the idea that they do not want to be characterized as preventing this field from moving forward in a reasonable and safe manner.

On the other hand, as Dr. Goldstein lists eight or more islet preparation centers, I think our recent experience from our colleagues in gene therapy who have done an excellent job single-handedly setting back clinical research a few years, the public is not going to cut the FDA

or the NIH any slack at all. If I set up an islet transplant center on my own in the University of "Buxtebehuda" somewhere, no one is going to cut anyone any slack if I start putting islets in the patients that are of poor quality or even dangerous and there is a complication, and there could well be a complication, and let's face it. So, I think that is sort of the line where you are going to have to accept the fact that when the complication happens everybody quickly turns to the NIH and the FDA and says where were you? What did you do? Joan?

DR. HARMON: I would like to address Dr. Cara's earlier questions. Especially for the treatment of Type 1 diabetes, we will most likely have to start thinking about treating children. About three or four years ago the NIH made it mandatory that any clinical research, specifically any clinical trials, must consider the use of children. Please remember that the definition of child at the NIH is from birth to 21. So, it is possible to perhaps begin utilizing the protocols that have been addressed this morning on adults to begin with and then move to an 18-21 year old age group, and then slowly worked down. I don't think anyone in the room would want to start using islet transplantation on younger children than that at the present time, until it is all worked out.

DR. GOLDSTEIN: Dan, I wanted to follow up on your

remarks. We fund outside of the United States significant amounts of money, numbers, etc., and we are looking toward the FDA et al. to tell us how to set standards and do this in a rational manner so that we can actually tell people outside the United States that we would like studies done in an FDA-equivalent fashion where appropriate. We would also concur with the need to protect and do things in whatever is the best manner, and we would like somebody else to decide that for us.

DR. SALOMON: Thank you, Bob.

DR. SIEGEL: We brought you here --

[Laughter]

-- to figure that out for us.

DR. AUCHINCLOSS: I think it is becoming clear with the discussion that lines are being drawn here. I think you understand that I disagree absolutely with your statement and I agree entirely with Camillo. I think the FDA should be very much involved in the regulation of an islet product facility but the product, islet transplantation, should not be called a product and should not be regulated by the FDA, in my view.

DR. RICORDI: Actually, I agree completely with Dan's statement and with you. I don't think that we shouldn't regulate at all processing. I think the safety issue for islets is different in that we will have this

quality control in this product, and this is what we are here to discuss, to make it as safe as possible but, at the same time, remembering that an islet is not a cell that you can cryopreserve and leave for a couple of months while you are sure that everything is perfect because you may have problems intervening with ischemia as in organ transplantation.

DR. AUCHINCLOSS: But, Camillo, I don't have any problem with the FDA wanting to know whether this is a good islet or a bad islet. That is what the FDA should be in the business of and they can help the field out enormously by forcing that to happen, which it hasn't adequately so far. But do you think the FDA should be in the business of deciding who should be the recipient and what the conditions of the clinical trial are?

DR. RICORDI: No, but what I am saying is not very contradicting. I mean, there is something in between those two statements.

DR. SALOMON: I am going to end that here. Maybe we should have gone to lunch at 12:30.

[Laughter]

Seriously, I think that this is excellent. I mean, this is what the committee is supposed to do -- to disagree, to get individuals to take a position and to examine critically the dynamics of those different

positions, and that is what is starting to happen. I hope that is what we can foster in the next few hours. Jay?

DR. SIEGEL: I would just like to clarify a couple of things in response to that last comment. The legal authorities of the FDA are based in legislation which deals with the regulation of products. So, the suggestion that it would be nice if we could regulate the production but not call it a product -- that would probably be difficult to figure out how to do. There are certain aspects of the production in terms of communicable diseases that there are other legal authorities to regulate. But it is, in fact, the legal framework and the FDA's framework to regulate on the basis of products.

However, I have to say when we regulate cellular products there should not be an assumption in this group that we fit them into the same holes that we fit drugs. We are very aware, for example Dr. Ricordi, that many of the products we regulate, many of the cellular therapies including not only those that look like organs but those that almost anybody would agree are a manufactured product -- it has maybe genes put into it, expanded, whatever -- many of them cannot suitably be fully tested for infections, for contaminants, for function, and that is readily accommodated through the method of regulation. So, don't necessarily jump to the conclusion about what it means to call something

a product. We are here to find out from you what is the right way to regulate this field.

Secondly, in response to your comment, Hugh, about -- I think you said -- would you want the FDA to determine who should be the recipient or what would be the right conditions for a clinical trial, we play a very active role in areas that we regulate in clinical trial development. Generally the position is, except where safety issues arise, that we don't tell people you can't do certain types of trials, or you can't treat certain types of patients. Rather, our area is to advise on what is the nature of a trial that will provide the types of evidence that are necessary to make the determinations that are necessary in order to satisfy our public health goals. So, what will be the trial designs; what will be the endpoints, what will be the measures that will answer the critical regulatory and scientific questions that are there. And, I think -- I hope that you will find that as we answer those questions, using the advice of this committee and other groups of experts, in fact, as I think has been the impact of our input in other areas, we are not restricting the options of clinical trials that can be done. It can certainly help focus clinical trials towards addressing clinical questions that need to be answered.

DR. AUCHINCLOSS: I think the difference in our

point of view will emerge as we come to the particular questions because many of your particular questions deal with an issue of is islet transplantation a good thing for the patient. That is the outcome that you want to know. I want to know that also, but I don't think it ought to be your question. Your question ought to be is the islet that went into that trial a good islet.

DR. SIEGEL: Well, I guess that is a fundamental difference because I can't imagine how you would know it is a good islet unless I know it is good for the patient.

DR. SALOMON: Wait a minute, I am going to take the chair's prerogative now to end this and to go to lunch. But I think there are plenty of interesting things and I have no concern that you will not return to this when it is appropriate. Thanks. I want everybody back at one o'clock, please. Thank you.

[Whereupon, at 12:10 p.m., the proceedings were recessed for lunch, to be resumed at 1:10 p.m.]

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

DR. SALOMON: Could everybody take their seats so we can begin the afternoon session? I would like to acknowledge that two more members of the panel have joined us in this afternoon's session, Dr. Sherwin from Yale University -- welcome.

DR. SHERWIN: Thank you.

DR. SALOMON: And Dr. Kathy Zoon, Director of CBER at the FDA, has joined us.

So, starting off, we have Darin Weber from CBER, presenting us an FDA view of the regulatory framework to procurement, processing and characterization of human islets.

Applications of FDA Regulatory Framework to Procurement, Processing and Characterization of Human Pancreatic Islets

DR. WEBER: Good afternoon. I hope you had an enjoyable lunch.

[Slide]

Again, my name is Darin Weber. I am a product reviewer in the Division of Cellular and Gene Therapies and, hopefully, this session will be somewhat educational in terms of providing our regulatory framework of regulation of cellular and tissue-based products.

[Slide]

The basic goal of this presentation is to describe

the regulatory review process which is employed by the Food and Drug Administration, and specifically the Center for Biologics, for cellular and tissue-based products and how this framework can apply to allogeneic islets.

[Slide]

So, this is an overview of what I would like to talk about this afternoon. That is basically divided into three main parts, the regulatory framework for cellular and tissue-based products and in this part I am going to describe the laws, regulations and guidances that the FDA uses to effectively regulate this field. The second part will be CBER's regulatory approach. Basically, I will describe how biological products such as islets are regulated by CBER and, hopefully, it will be clear at the end of the talk that the regulation of biological products is founded on science and law to assure the purity, potency, safety, efficacy and ultimately availability of these products.

[Slide]

Before we go too much further, I want to keep in everybody's mind that FDA's primary objectives are, in terms of the regulation of investigational new drugs, foremost to assure the safety and rights of all subjects in all phases of the investigation and, secondly, to assure that the quality of the scientific evaluation of the investigational

product is adequate to permit an evaluation of its safety and effectiveness.

[Slide]

When I speak of a regulatory framework, again, what I am talking about are the laws, the regulations and the guidances that we all are using at the FDA to regulate this area. Just to back up and clarify what a law is what a regulation is and what a guidance is, laws are statutes that are enacted by Congress and, obviously, require an act of Congress in order to form a new law, to modify that law or in some cases to repeal that law. Regulations, again, are agency rules that are promulgated to describe how the law is going to be applied and are, in fact, binding like a law and are enforceable. The third particular aspect we use for our framework is the guidance documents. Guidance documents, as the name implies, are guidances. They represent our current best thinking. They are not binding on you or the FDA. What that means is that alternative approaches are acceptable if they meet the regulatory requirements.

Going back to the point of the slide, this represents our regulatory framework. The two major laws are the Food, Drug and Cosmetic Act and the Public Health Service Act. Out of those two laws have come several regulations. Probably the most important is 21 CFR 312 -- CFR is the Code of Federal Regulations. So, 21 CFR 312

describes the general IND regulations for both drugs and biologics and covers things like safety and effectiveness.

Part 610 of that same code covers the biological product standards and, in the case of some cellular and tissue-based products Part 800 may become important if they are combined with medical devices, maybe in an immune barrier for example. So, if that happens we have a combination product of a biologic and a device. So, those regulations also become important.

Finally, Part 1270, which covers tissues intended for transplantation, are particularly relevant here because it describes prevention of transmission of communicable diseases from a donor to a recipient.

[Slide]

Continuing on with our regulatory framework, in 1993 the Food and Drug Administration published a Federal Register Notice basically stating that they intend to regulate somatic cell and gene therapies under the investigational new drug regulations.

In 1997 there was the proposed approach to the regulation of cellular and tissue-based products, which was an approach that only came about after real extensive discussion and dialogue with both industry and academia.

Now, under the proposed approaches come several documents. 1998 saw the proposed rule for the establishment

registration and listing which basically asks that manufacturers release types of products registered with the FDA so we have a way to communicate with them.

In 1999 there was the donor suitability determination. Again, that basically covers appropriate types of communicable disease screening to prevent the transmission from a donor to recipient.

A third document which is currently under development is called the good tissue practices, or GTPs. So, GTPs are going to encompass proper handling, processing, storage and labeling of cell and tissue-based products. It will also include requirements for record-keeping, reporting and establishing a quality program.

Finally, the last bullet here is that in 1998 there was a guidance for industry and guidance for human and somatic cell and gene therapy, which again are guidances that represent the agency's current thinking about regulation of a specific area.

So, the take-home message from this slide and the previous slide is that FDA's regulatory framework for cell and tissue-based products wasn't formulated overnight. It has been evolving for most of the last century and is continuing to evolve today, and we are here to listen to some more of that this afternoon.

[Slide]

So, let's move on to the second part of my talk which, again, is to discuss what the Center for Biologic Evaluation and Research regulatory approach is. The mission of CBER is to protect and enhance the public health through regulation of biological products and related products according to statutory authorities. So, for the next few slides I am going to describe some of those tools that we use at the FDA and at CBER to effectively do this, to make informed, science-based decisions within this framework.

[Slide]

As this slide shows, the backbone of our regulatory framework is the regulatory requirements, and I have listed again Parts 312, 610, 800 and 1270. Actually, there are other parts of the regulation that may come into play as well.

Secondly, the FDA actually follows its own guidances, such as the guidance for industry for human somatic cell and gene therapies. We also look at other relevant guidance documents, such as the International Conference on Harmonization which covers some technical requirements for a lot of biotech products.

Thirdly, of course, is why we are here today. Often when there are new or novel or unique preclinical, product or clinical questions the FDA often has the option of calling together a group of outside experts as part of an

advisory committee. The advice we receive from our advisory committees is frequently used to guide the regulatory framework as it applies to a specific class of products.

Additional tools that we use in the Center for Biologics is something called the step-wise approach to the application of the regulatory requirements. Really what that means is depending on the state of product development, you have to meet certain of the regulatory requirements and I will discuss that in a little more detail.

Finally, as a reviewer in the Division of Cellular and Gene Therapy, we use something called a CMC product review template. This template helps us guide our review to ensure it is consistent; to ensure that we meet all the regulatory requirements at the appropriate stage of product development. So, I am going to talk a little more in detail about most of these aspects on this slide.

[Slide]

In terms of regulatory requirements, for manufacturers of biological products there are basically four major regulatory requirements that must be satisfied during product development, and they are shown on the slide: product safety, product characterization; demonstration of control of the manufacturing process; and demonstration that you can reproducibly and consistently produce product lots. Especially for cellular therapies, it is going to be

important to address issues like proper dosing in order to get efficacious effect. So, again, I am going to be talking a little bit more about each of these four areas in detail in the next few slides.

[Slide]

In terms of product safety, we are talking about sterility testing, mycoplasma testing, pyrogenicity and endotoxin, as well as freedom from adventitious agents. It is important to note that, again, safety is a major concern, product safety in particular. So, we have the least amount of flexibility with regard to when during an investigational phase a sponsor is going to have to perform this type of testing. So, obviously, it needs to be in place by Phase I. However, the agency is somewhat flexible in the types of tests that must be done to satisfy these requirements.

[Slide]

The second area of regulatory requirements for biological products and the manufacture of them is something we call product characterization. We are talking about assessments of potency, identity, purity as well as product stability. For cellular and tissue-based products, stability is sometimes a critical factor. When I talk about stability I am talking about the ability to maintain a viable and functional product from the point of the manufacturing site to the clinical site. It could be

hundreds of miles; it could be a day or so. So, you need to provide us some data that shows that this product is going to work once it gets to the recipient.

Another issue for cellular and tissue-based products -- I alluded to viability but, of course, cell number or the amount of tissue available is also important because often for cellular and tissue-based therapies you must have a certain amount of tissue in order to get its expected clinical effect. This also goes in hand with the development of specifications. So, as you gain more experience we expect that you will tighten your specifications based on data.

[Slide]

The third area of regulatory requirements for manufacturers is control of the manufacturing process. This is especially important with cellular and tissue-based products since they are generally complex mixtures of several different cell types. So, control is going to help assure product consistency and safety.

The first bullet here is labeled cell bank characterization, which clearly is not necessarily applicable to islets at this point but at some point in the future it may well be, and since we are talking about general biological standards cell bank characterization would be an important part of control of the manufacturing

process.

Characterization of final product by testing for safety, identity, purity, potency, viability as well as others is going to help ensure that the product will meet specifications for lot release and is accepted for clinical use in humans.

Qualifying ancillary products, that is, those products such as enzymes or serum products, is another important consideration in assuring the control of the manufacturing process. For example, inadequate testing of ancillary products has resulted in the contamination of the final product with adventitious agents such as viruses which were present in the serum and enzyme preparations from animal sources. So, we certainly are very aware of that issue as well.

[Slide]

The fourth area is something that I alluded to earlier, the demonstration of reproducibility and consistency of product lots, which is really encompassed by what we refer to as current good manufacturing practices or GMPs. So, a definition of GMP is a set of current, scientifically sound methods, practices or principles that are implemented and documented during product development and production to ensure consistent manufacture of safe, pure and potent products. So, GMPs play an important role

in control and regulation not only of the product but all steps of the manufacturing process. Adherence to GMPs provides for quality and safety throughout the process and will ultimately lead to a reproducible and consistent performance of the product lots.

[Slide]

Some elements of the GMPs are shown on this slide. Elements of GMPs include detailed record-keeping, development of written procedures such as standard operating procedures or SOPs; institution of a formal quality control, quality assurance program, validation which includes assay validation, equipment validation and process validation as a few examples. GMPs also require a program for certification and training of personnel, as well as for environmental monitoring.

[Slide]

So, for the past several slides I have probably hit you with a lot of information about these four major areas in terms of manufacturing, and I am going to try and summarize this in a graphical slide that hopefully will not be too overwhelming. We are trying to convey a lot of information here but essentially this is what we call a step-wise approach, depending what phase of the investigation you are at. So, the step-wise approach involves a progressive scale of requirements for product

characterization and compliance with GMPs which is going to increase as the setting moves from early phases towards Phase III.

But I also want to point out that even prior to Phase I you are going to have to have your product safety testing and some basic characterization information already in place before you are going to be allowed to go into humans. So, we recommend that by Phase III you are fully adherent to both full characterization as well as full GMPs in the manufacturing process.

When I am talking about the step-wise approach I am talking about refinements in the manufacturing process. We don't expect that you already have a manufacturing process initially developed, but you are going to refine it; you are going to improve your assays; your specifications as well as addressing facility issues.

[Portion not recorded due to electrical interference.]

[Slide]

So, three major areas include pancreas procurement, such as methods for harvesting and handling; processing of the pancreas and islets and how demonstrate control and consistency of manufacturing; appropriate characterization of islets such as demonstration of safety, identity, purity, potency, viability and others as needed.

I am going to talk about each of these specific three areas in more detail in the next few slides. But the real point I want to emphasize here is that the agency believes that you must have control over each of these three areas, beginning with pancreas procurement through release and transport of islets from a manufacturing site to the clinical site.

[Slide]

The first step in the manufacturing of allogeneic islets begins with the procurement of a pancreas. As with any allogeneic product, testing of the donor for adventitious agents is required to prevent the spread of communicable diseases to the transplant recipient. In many cases, if not all, pancreata are harvested with the intent to perform whole organ transplant, therefore, the organ harvesting, handling and preservation methods typically used may not be suitable for successful isolation of islets since islets are known to be more sensitive to ischemia and other handling aspects, such as time and temperature, than are whole organs.

In addition, each pancreas, like its donor, is unique. There will be differences in organ age and size which may or may not impact the ability to obtain sufficient yields of islets, and there may also be other undefined parameters that could impact islet quality. So, procurement issues certainly represent safety concerns but also

manufacturing consistency concerns as well since a poor organ is going to yield a poor islet preparation.

[Slide]

The processing of the pancreas into islets is another area of regulatory concern. Islets are going to be obtained from pancreata by partial digestion of the organ through a combination of enzymatic digestion with highly purified collagenase combined with mechanical dissociation. So, islet yield is going to depend, again, on organ age, organ size, as well as the duration and the conditions of the dissociation used. Thus, the islets isolated from each organ will differ somewhat in yield, size distribution and, depending on whether they are over-digested or under-digested, overall viability and function may also be impacted. The final composition of the islet preparation is also going to depend on what additional purification steps are used and how that purification is done, and whether or not culture is done as well. Obviously, if enzymatic activity from one batch to the next is different the product quality is going to be affected. So, this points to the need to completely understand and control all aspects of the manufacturing to ensure that a reproducible process is used to ensure a consistent product. So, islet processing encompasses the control of the manufacturing process and consistency and reproducibility. However, safety can also

be impacted by improper processing such as introduction of microbial contamination or other adventitious agents.

[Slide]

Finally, islet characterization, like any cellular or tissue-based product, is going to be important to be able to demonstrate that you can safely and reproducibly manufacture the therapeutic product. This is typically done by characterizing the product and establishing specifications for lot release based on those characterizations. That is, the final product must meet certain parameters in order to be used clinically. This includes what safety testing has been performed, like sterility and mycoplasma and endotoxin. How do you know that you have islets? What kinds of identity testing has been done and which ones are appropriate for islets? We know islet preparations are very complex mixtures so identity is a concern -- what else is present in the islet preparation? And, that goes back to purity as well as, again, islet versus non-islet components. How do you know the islets are functional and viable? Again, those are critical issues not only for making sure the islets are going to work but if they are functional and viable when you finish manufacturing them, they still need to get to the recipient. So, shipping, again, is another issue and that is called product stability. Finally, do you know if you

have enough islets to give the intended effect? Clearly, a cellular and tissue-based product requires a minimum number of cells or tissue to give the clinical effect. An equally important question might be what if you have too much? Is that an issue? Is that a concern?

[Slide]

In summary, basically, as I said, the goal of this talk was to describe the regulatory review process which we employ here, in the Center for Biologics, for cellular and tissue-based products, and the take-home message is that we believe that manufacturing of allogeneic islets begins at the stage of procurement of the organ and continues all the way through processing, final lot release and transport to the recipient.

So, in summary, the FDA would like the advice of the BRMAC about procurement, processing and appropriate testing and characterization of allogeneic islets that will ensure patient safety and demonstrate control and consistency of the manufacturing. That concludes my presentation.

DR. SALOMON: Thank you, Darin. I won't ask for questions at this point since I think Darin's whole point was to introduce the questions that we will actually spend the rest of the afternoon on. So, if there are questions and I certainly hope there are, that is the whole focus of

this afternoon's session.

At this point, by protocol, I would like to remind our audience that this is a public hearing. We have not had any formal requests for presentations from outside but you are more than welcome at this point to come to the mike and address the committee on anything that is pertinent to the questions Darin has outlined. So, I would like to take a second at this time to invite anyone from the audience who would like to make public comment.

[No response]

Okay, I would also like to say that in the interest of continuing this as a public meeting, if during the evolution of the discussions that go on now someone from the public would like to come up, I would like to encourage you. I don't want anyone to feel uncomfortable. The idea here is to be inclusive and certainly not exclusive. So, please feel free to join in. Make sure that I recognize that you are there and come to the podium.

Committee Discussion

If it is okay, then I would like to start with this afternoon's questions. I have a document and I would like to make sure that we are all talking about the same thing. It is entitled background information and it consists of six pages -- Oh, I got some things in one format; I didn't realize that it came to the committee in

another. Let me repeat, it is under pancreatic islet product questions, and the first question is organ quality -- source material for islets. Let me put it this way, if anyone on the committee doesn't have that in front of them, I might suggest that you notify us, raise your hand and Gail and her staff will make sure that you get it. I am going to assume everybody has it in front of them.

So, to initiate the first one, I think that there is a piece of briefing that I have that I would like to read briefly, just because I think it is a good intro into where we should go in this set of discussions on organ quality. This is an FDA impression so if you, guys, don't go with this then your comments are appreciated. Historical data from the International Islet Transplant Registry in published sources indicate that certain practices in pancreas procurement, handling and organ allotment are detrimental for preparation of suitable islet preparations. The FDA is concerned that these practices may result in the use of substandard pancreata resulting in substandard and potentially inconsistent islet preparations. So, to ensure high quality islet preparations can be consistently made, the FDA would like discussion on the point that only the highest quality pancreata should be used.

We sort of got a little bit to that when I queried you. You might now know where I was going with that

question. When Dr. Shapiro presented the data from Edmonton he was talking about a percentage of pancreata that were not suitable for whole organ pancreas transplantation and I was trying to get you to tell me what percentage. So, keep that in mind as an issue. I also would like to note discussions I have had with Camillo Ricordi and Bernhard Hering about the current practice of how, in the actual operating room, pancreas is procured with respect to multi-organ procurement. I believe, and they believe, that that also institutes a significant problem for the quality of the pancreata available currently for processing of islets. So, that is all I wanted to say on that.

Let's turn to this first topic. Based on data in the International Islet Transplant Registry, most investigators recommend that in addition to standard infectious disease screening pancreata be excluded from use for clinical preparations of islets based on the following, and I won't read them but it is a list of donor age, warm ischemia, cold ischemia, history of diabetes, serum lipase, etc.

So, the first thing that they would like us to discuss and provide recommendations on is regarding the appropriateness of each of these exclusion criteria, which I guess are up here now. For example, are restrictions on minimum and maximum donor age appropriate? I would also

welcome, following the line I already started, discussions about how whole organ retrieval affects the quality of the pancreas as well. So, I am going to open this now to the committee. Mr. Benedi?

MR. BENEDI: Yes, my question is for the whole committee. I was taking notes through the presentations this morning, and it deals with what you just mentioned, given that there are so many people on the waiting list for whole organ transplants and given the quality of the organ that needs to be harvested to have, like you say, islets that are good enough for transplantation and for them to work, what criteria are going to be used to take one from a recipient who would receive a whole organ and decide it would be used for islets only?

My other point too is that if this is the case, I think one of the studies pointed out that islets that have been used for transplantation aren't as protected from immunosuppressant drugs as if they were inside the whole organ. I think it was your study that pointed that out. So, are we exposing the islets to harm by the immunosuppressant drugs and jeopardizing those who could receive a whole organ, and are we missing an opportunity to save lives?

DR. SALOMON: We have had a number of discussions about how to separate the discussion in two days, and I

apologize to everyone that you take a two-day discussion of one subject and you split it up. Obviously, that is artificial. So, my apologies. Try to make a judgment here. The judgment we made was that today we are going to talk about the product on the way to preclinical -- safety, quality, manufacture. Tomorrow we are going to start with discussions of preclinical animal models and go on to specific questions about immunosuppression, etc. Now, I know that every time we get into this the conversation is going to evolve and we are going to but up against this clinical and you, guys, are going to be mad at me for stopping you there, but I will a little bit, but not a lot.

So, the questions you asked were wonderful. Save them for tomorrow because those are two issues that are critical in how you go toward a clinical trial and who is an appropriate candidate, and that specific question will be addressed again tomorrow.

MR. BENEDI: My apology, I thought you were alluding to that difference of whole organ as not islets. I apologize. I will save it for tomorrow.

DR. SALOMON: Yes, Carole?

DR. MILLER: As an oncologist, I have a question about the malignancy. Is that active malignancy or history of? Pancreas is a relatively uncommon site of metastases for most non-GI malignancies, except for lymphoma of course,

but does this exclude anybody with skin cancer or with a glioblastoma who has no chance of having any metastases to the organ? And, I understand that that generally reflects the policy of anybody with a history of malignancy, but is that really necessary in an organ such as the pancreas?

DR. SHAPIRO: We used exactly the criteria as for any whole organ transplant. So, if there is a history of malignancy that is limited to the brain, which has zero risk of systemic metastasis, we would accept that. For whole organ liver, kidney, whole pancreas transplantation, then we would use the same organ for other transplant. But I don't think we would compromise whatsoever in terms of using an organ that might potentially risk transmission of malignancy to a recipient because it is just an islet.

DR. SHERWIN: I wasn't here earlier so I may be off base but I was sort of surprised that the cut-off begins at 14. I would have guessed that it would be easier to isolate islets of people that were younger, and I would be a little bit concerned, although I am not an expert, that the older you get the harder it might be to isolate islets. So, I would have thought that the lower end would have been lower and the high end might be lower as well. So, I am just curious about that.

DR. SALOMON: Camillo?

DR. RICORDI: I think that is our proposed cut-off

for some of the pilot trials using allo islets for transplantation, and are mainly related to the consistency to achieve purified islet preparation with well-cleaved islets. As you go to younger and younger donors you have a higher incidence of less cleaved or mantel islets and it can be more difficult to purify the tissue. But I actually wouldn't exclude it at all. I think there will be a whole new set of transplantations using no lower age limit. Actually, we are actively investigating in this direction because you have a much higher potential for regeneration and you may have to decrease very much the need for purification because you may have the total volume of islets that you may have to infuse. It actually could be within the safety range to avoid portal hypertension.

DR. SHERWIN: Yes, I would assume that your method was set up for adults. It is sort of like when you deal with different strains of mice or rats, you have to change your isolation procedures for that specific situation. I assume if you focused originally on adult islets, using the same techniques might not work as well in children, but I would think -- just the point you are raising, that it is more likely that you won't need the same level of purity and you may have the opportunity to promote regeneration. I would think it would be a big mistake, personally.

DR. RICORDI: I completely agree and, actually,

the automatic method is set to disperse cluster of cells from any pancreas, and it actually works better on young donors than on older ones. But it is the purification steps -- you may have to do modifications in the next steps of purification but tissue dispersal is actually ideal for young donors and I completely agree with your comment.

DR. SALOMON: Jeff?

DR. BLUESTONE: Camillo, one of the other reasons why I would imagine the younger the donor is potentially more problematic is how many islets you can get out of a donor who is younger. Is that something that is perceived as being problematic, or are there more than enough islets in the youngest donors?

DR. SALOMON: Bernhard, do you want to answer that?

DR. HERING: Well, it is not only the number of islets; the insulin secretory capacity is important and there is more data indicating that the insulin secretory capacity is strikingly different with age, and any donor older than 45 or 50 may show a decreased insulin secretory capacity and no proliferative capacity. There is one document in the literature, one patient received islets from a 2.5-year old donor pancreas and this was actually the first patient who ever became insulin independent 12 months after transplantation, presumably after some proliferation.

Of course, there is no direct evidence to support this. Basically, we don't know but experimental data indicates that insulin secretory capacity is determined by the age of the donor. That is why I would rather favor a younger donor than an older donor.

DR. SALOMON: Bernhard, I just have one question. Part of what I think we are asking is what kind of data do we have right now for the number and the mass of islets as a function of age from, let's say, a 2-year old to a 14-year old?

DR. HERING: The available information would suggest that the number of islets you can get per donor pancreas is much higher with an older pancreas, the total number of islets that you can isolate.

DR. SALOMON: Just to be clear now, the total number you can isolate or the total number if you sectioned the pancreas and calculated the number of islets by volume?

DR. HERING: In addition to the number, the configuration of islets is different. In a younger pancreas you may have single cells scattered throughout the pancreas. With increasing age you have well-defined, solid islets present in the pancreas that can be isolated using techniques that have been developed, and can be purified with available purification techniques.

I completely agree with what Dr. Ricordi mentioned

before, the techniques must be adjusted but it is basically possible to proceed with younger organs if you adjust the techniques.

DR. SALOMON: Dr. Sherman?

DR. SHERMAN: Another issue, history of diabetes -- I assume that means Type 2 perhaps because obviously it will be easier to pick up Type 1. But about a third of the people in the United States have Type 2 and don't know it. So, it is conceivable that you may end up with people without a history of diabetes who actually have Type 2 diabetes. So, I think it is important to think about that in the equation and to take that into account. I recognize that a lot of the donors have stress and their glucose levels may be elevated at the time you remove the pancreas but I think some consideration has to be -- you know, just put in a check mark to think about that issue because there are a lot of people in this country that do have Type 2 diabetes and it is occurring at a younger age now than ever before. So, I think, particularly in the obese population, particularly when you are dealing with high risk populations, it is something that one should consider.

DR. HERING: The beta cell itself may be intact to a point where you can transplant and succeed.

DR. SHERMAN: That is right but these islets may not be as good. In other words, when you are subjecting a

patient to a procedure you want the optimal outcome. You know, we have never really studied mild Type 2 diabetic islets. Obviously, part of the problem with beta cell function relates to the metabolic milieu that the islets are placed in, but it may be more complex than that. For example, they have amyloid deposits in the islet; there are other things that may, to some extent, interfere with function and it is conceivable too that the capacity of those islets -- we don't know enough about diabetes to know whether they are really normal islets. So, I think it is important to think about, and if I was being transplanted I would not want to have islets from somebody with mild Type 2 diabetes.

DR. HERING: Well, I think I couldn't agree more with your point. One strategy that has been discussed today and has also been detailed in the briefing material that we received is to start with the highest quality of donor pancreata, then establish the principle feasibility and document the benefits is probably the right strategy to take, and once you have established this you may discuss how to extend donor criteria. I think this is a reasonable approach.

DR. SALOMON: Camillo?

DR. RICORDI: Yes, I believe this is an important difference to make, that some of these criteria are selected

based on the fact that we have to do an initial pilot trial and prove the principle of equivalence like with the results that can be obtained with a pancreas transplant. Then, it is important not to introduce new, untested variables that may lead to failure of your initial trial. But I would never exclude a mild Type 2 pancreas in the absence of severe beta cell dysfunction as a potential alternative source of insulin-producing tissue for expanding the number of organs available from human donation sources. I would keep the two issues separate -- what we need in the beginning to test and what could be the potential of other sources.

DR. AUCHINCLOSS: I think it is important to recognize that organ procurement agencies have worked out a far more detailed list than this of criteria for what would be a reasonable pancreas donor for whole organ pancreas. That includes things like how high does the blood sugar go under periods of stress, etc. I would simply adopt in general terms those guidelines for which are acceptable pancreases and then turn to the experts on islet isolation and ask what additional features would lead you to not want a pancreas, or what things that we exclude a pancreas for whole organ transplantation do you not feel need to be exclusion criteria.

DR. RICORDI: In some cases this could highlight

areas where you can access organs that are not suitable for whole organ but would be suitable for islet separation, like in the case of whole organ transplant high BMI donors or like obese donors are not considered generally suitable because of the peri-pancreatic fat that could lead to complications. I understand your concern. The problem is, is this a non-diagnosed Type 2 patient, but experience-wise some of the best islet preparations can actually be obtained by high BMI kind of donors. So, we may highlight some categories of donors that are specifically suitable for islets without creating competition with allocation of organs for whole organ transplants.

DR. LEVITSKY: It sounds to me like you are isolating islets from OB mice or something like that. They are probably quite large. I guess I would like some data which I haven't heard. You are talking about all the criteria for age and body weight, but has any laboratory that has really experienced a human islet transplant actually plotted the data? Have you got it plotted so you know the correlation with age and the correlation with transplant, and if you do have a younger age but that means you get fewer islets and smaller ones but they have more progenitor cells, is that better or worse?

DR. LAKEY: In 1996 our group in Edmonton published a paper in Transplantation reviewing the variables

in the multi-organ donors that affect our ability to isolate the islets. There were six factors that were deemed to significantly affect your ability to isolate the islets. Donor age, body mass index, and the blood glucose in the donor prior to death were all positively correlated with islet isolation recovery. Duration of cardiac arrest, who actually did the procurement and the duration of storage prior to islet isolation were identified as negative factors precluding islet isolation.

I guess other factors that we have identified include hospitalization stay in excess of four days, the uncontrolled blood glucose which I mentioned and warm ischemia. If there is any warm ischemia, it very much affects our ability to isolate the islets.

DR. LEVITSKY: Can I just ask, that means that if you have a higher BMI or you are older it is easier to get the islets or harder?

DR. LAKEY: Yes.

DR. LEVITSKY: It is easier to get the islets? Are those better islets or are they not better islets?

DR. LAKEY: No, in our study we looked at the functional viability of those islets, and we found a significant decrease in the functional viability of the islets isolated from those donors less than 18 years of age as compared to those donors that are greater than 50 years

of age. So, the islets from the younger donors had a higher stimulation index compared to the islets from the older donors, yet, the ability to recover the islets was higher in the older donors as compared to the younger.

DR. LEVITSKY: Well, me thinks I hear a paradox.

DR. BLUESTONE: I was actually going to ask about a paradox too, although I can ask but I can't talk about it today, until tomorrow --

[Laughter]

-- so, what I am hearing is that Hugh, perhaps being the most extreme, says let's put the most stringent criteria possible on the donation, getting the best possible pancreas to work with without necessarily knowing that all of those criteria are critical for whether you are going to get good islets out the other end or not, and that just abuts us then right up against the issue of when do you do pancreas transplant and when do you do an islet transplant, which I am not allowed to talk about until tomorrow.

But, I think if we are going to have a discussion of what the criteria are for islets we have to keep in mind the fact that the more stringent the criteria are to make it look more like what the criteria are for a pancreas transplant, the further those two areas in transplantation abut each other. We might at least want to be careful not to make the restrictions so stringent if we don't have the

data, such that we force a reduction in the number of available pancreases rather than making it the most appropriate pancreases.

DR. SALOMON: That is a good point. To Dr. Levitsky, I think one of the things you have to understand is that the mechanical, enzymatic processes involved in isolating these cell clusters could be favorable in an older pancreas, right? But that doesn't mean that clusters that come out are as functional as in the younger pancreas.

DR. LEVITSKY: Well, that was actually the point I was trying to make. I mean, there was a reason why OB islets were isolated first. Because you have insulin resistance, you get a big, fat islet but that doesn't mean it is a better islet. I am concerned that by setting these limits you may be isolating islets that look great but do they work great? I don't know. It sounds like you say they don't work as well.

DR. SHAPIRO: It would be important to emphasize that the multivariate analysis that Dr. Lakey carried out was carried out with older collagenase enzyme blends and now, with the availability with liberase, that may change somewhat the characteristics of an ideal organ donor. So, we shouldn't necessarily exclude donors based on those previous characteristics. Would you agree?

DR. LAKEY: Yes.

DR. RICORDI: And, also the testing to assess functional integrity of younger islet preparations may not be adequate as they are for more adult islet preparations because, for example, if you go to the extreme of fetal islets they may require four months or three months in vivo before they start properly functioning, and if you go to neonatal islets and younger islets, they are surrounded by a ring of exocrine tissue and when you put them in an in vitro infusion system they might not be as responsive to glucose variation as you would expect from any adult islets. So, I wouldn't buy completely that younger islets are less powerful than adult or older islets. I actually think that will become a very important source in the future of insulin-producing tissue for transplantation.

DR. CHAMPLIN: Is there any test that you can do in vitro to assess the functional viability of the cells, other than trypan blue? I mean, some way to test the quality of the islets that have been collected.

DR. SALOMON: Before you get into that, I think that is going to come up in a few minutes. It is a perfect question, it is just going to come up in a minute or two. Carole?

DR. MILLER: Is there any evidence that high dose Decadron to the donor, as treatment of increased intracranial pressure, damages the pancreas at all?

DR. SHAPIRO: I have no evidence.

DR. SHERWIN: I bet it does though because it does interfere with beta cell function. Even though you make more insulin, it actually is an inhibitor, has an inhibitory effect on beta cell function and the likelihood is that it is not good.

DR. MILLER: Well, that could be a reason for the increased blood glucose having an adverse event because that could be a marker potentially for Decadron, or stress -- a lot of these people -- you get them from patients who are brain dead because of a closed head trauma, etc. and my understanding at least, and I am not this type of transplanter, is that many of those patients actually get Decadron.

DR. SHERWIN: It still is making it very insulin resistant which increases your insulin production but, at the same time, it is negative on the beta cell as well and, consequently, the compensatory response is not as good as it should be.

DR. AUCHINCLOSS: My first question is about the warm ischemia. You mean ischemia prior to the onset of cooling? How are you using the word warm ischemia here?

DR. LAKEY: At the time of procurement.

DR. AUCHINCLOSS: Again, before or after cross-clamping?

DR. LAKEY: Before cross-clamp in the concept of non-heart beating donors.

DR. AUCHINCLOSS: So, non-heart beating donors have not been good donors in your experience?

DR. LAKEY: That is correct, from our limited experience.

DR. AUCHINCLOSS: Jeff's second point, these criteria that exclude pancreases for whole organ pancreas are really not all that stringent. They had to do with high blood sugars in response to stress and evidence of active pancreatitis, things that by and large the islet people would want to exclude also.

Camillo brought up one example which I thought was quite fascinating, the moderately obese people who are candidates for islet isolation who are often turned down for whole organ pancreas transplantation. I agree with you, it would be nice to identify more of those but I have a fear that we won't be able to hear of more examples of organs that are turned down for whole organ pancreas that the islet isolators would like to have. Are there any?

DR. RICORDI: Well, younger donors, but now this limit keeps getting less and less defined. It used to be that less than 15-year olds were not considered for whole organ but now more and more centers are using younger and younger donors also for whole organ transplants.

But I think you made an important point on the warm ischemia because the only warm ischemia that we have on records from pancreas procurement procedures is at the cross-clamp time, but we don't have any information on actually how long the pancreas has been sitting there maybe without local cooling before it was actually procured. Those are all potentially very important variables in improving the quality of the pancreas available.

DR. AUCHINCLOSS: I agree that they are important variables. Just recognize that the term warm ischemia in the transplantation and organ procurement business does not ordinarily refer to the time prior to cross-clamping when there is warm ischemia or down time. It doesn't apply to the time after cross-clamp to organ removal. It refers to something quite different at the other end of the operation. So, if you start looking for "warm ischemic" times you might get data that has nothing to do with what you want. I agree that there is a whole lot of data that you would like to have.

My very last point on this subject I think is a general statement but, again, I would challenge the committee to disagree with this. That is, there is no firm evidence that leads us to know of any criterion that you can use to predict which islets are better for human transplantation and which ones are not.

DR. SIEGEL: I seem to recall somebody stating this morning that lacking significant numbers of patients with durable insulin independence or other outcome measures you really can't, at this point, validate what will predict that. You might look at what will predict numbers or insulin secretion but not that.

DR. SALOMON: It is always hard to know how long to let these discussions go on to try to get to five other questions this afternoon, but I think we have generally heard a pretty good discussion of this first point. Would it be fair to summarize this by saying that in general we hear the idea that you have to set some sort of limits perhaps at this early time so that based on your empirical experience up until now these ranges of excluding under 14 and excluding over 60 represents your best in the lab experience right now with getting fairly reasonable quality islet preparations, but that there continues to be concern, I think on everybody's part including the islet purifiers, that there may not be such rigid reasons not to go under age 14 or to go over.

Yet, to temper those, we have heard from others suggesting that as one stretches the limits on either side of these criteria guidelines for the product may actually change. So, you might need a certain set of guidelines for islets from donors over the age of 50 and another set

entirely for those under the age of 14.

So, would that be a pretty good summary of this first part of the discussion? Again, I am only summarizing for the sake of the FDA staff. So, please correct or add.

DR. BLUESTONE: Well, what I heard from Hugh and over here in the last comment is that one has to be careful that the criteria, whatever they are, aren't so arbitrary as to be limiting. It is the same comment as I made before, that you need to have a good reason to set criteria and if there really isn't a good reason, then one has to be careful because whatever criteria you set will place on the community a set of restrictions. So, I haven't heard any compelling argument yet for the age criteria, period. I have actually heard arguments on both sides. So, it is not obvious to me why you would set the age criteria if there is no data to support it.

DR. SALOMON: I specifically was saying that there was no arbitrary reason for either of these age limits so I agree with you. And, I think we have to be careful there; I don't think we have to worry so much today that the FDA is going to leave here with a set criteria and everybody's going to have to deal with them. I think that what we are trying to do today is give them some guidelines, give them some discussion. I think we are telling them that you could set some criteria in the first studies based on the evidence

of the people who have been doing it, that they are at least comfortable within these criteria but that there are certainly good reasons to think that we could go the full gamut of ranges, that there is no absolute here.

DR. SIEGEL: In addressing this, I would like to clarify something. It is correct that we are not eager to set criteria in areas where there are no data, and it may well be that approaches that are not currently considered the norm are, in fact, the optimal approaches, but underlying this question, perhaps not quite as explicitly clear, are a couple of areas of concern that we do want to make sure are addressed. One is in order to move from a state of lack of knowledge to a state of knowledge a couple of things have to be decided, and one is that we need to know what sorts of things need to be routinely measured. To some extent, the reason we don't know about all of this impact is that not everybody is measuring the same things the same ways.

I guess the other issue, sort of implied by Hugh's comment, is that if you are going to do an experiment to look at one or two factors you probably want to keep the other factors relatively standardized in an area that you think is relatively optimized so you can get good data on those factors.

So, we are trying to get a feeling for are these

the right factors and are there others. I heard about body mass index, for example. Is that data that should be collected, and what are norms of practice, more than what is the only allowed practice at this point in time?

DR. MILLER: From what we heard this morning, the plan for this is to look at reproducing initially some very good data that, regrettably, we didn't get to hear about this morning. So, I think the test that is going on now is to see whether you could take one center or institution to go to multiple other centers. So, I think it is imperative that you don't change two things at once. So, you have to use for whatever is initially going out the same criteria for the donor selection that were used in what is the best available data. It would be different if you had 100 percent insulin independence and you could say, okay, now we are going to whittle it down. But I think for the first experiment you have to try and reproduce as closely as possible what has worked in the past and then, if it works in the centers you could say, okay, what don't we need. So, I would caution at least initially of changing anything about what was done.

DR. AUCHINCLOSS: Can I make the comment that we who were participating in that trial feel very strongly that that is true. So, we will, in fact, try to emulate what Edmonton is doing for its isolation protocol. That makes

sense as a trial. But the FDA is trying to set guidelines that are more general than the first trial, and the only two criteria that make sense to me beyond standard organ donor criteria are that the islets should look like they probably don't come from a Type 2 diabetic and that there not be substantial pancreatitis because I think those guys would tell you that they can't get islets out. Now, if they tell me otherwise we will drop that one.

DR. SHAPIRO: These are guidelines for optimal islet isolation and, clearly, donors should not be excluded if they fall outside of these limits and we have a number of donors that we used for transplantation successfully, in Edmonton, the oldest of which was 71 years old. I mean, we wouldn't expect to get good islets from that but we did.

DR. AUCHINCLOSS: Is pancreatitis okay?

DR. HERING: Pancreatitis is certainly okay. The islet autografts are successful because we can say they are isolated from a pancreatitis pancreas. Those islets definitely work.

DR. AUCHINCLOSS: It turns out there is another one out there where you can get some organs that the whole organ people don't want.

DR. SHERWIN: That is graft pancreatitis, isn't it? When you do autotransplantation, that is chronic pancreatitis. If you have acute amylase elevations you are

talking about acute pancreatitis.

DR. HERING: This amylase may be related to the administration of starch as part of the treatment of the donor in the ICU, and not because of pancreatitis.

DR. SHERWIN: Sure, it is a non-specific test. Right. But the issue is whether acute pancreatitis would be a problem, not chronic.

DR. RICORDI: We have cases of the graft pancreatectomy with acute pancreatitis where we extract islets from a pancreas with acute pancreatitis and infuse them in that patient and they function immediately.

DR. SHERWIN: It is amazing actually --

DR. RICORDI: Actually, it is helping the digestion. If you think what we are doing in the process, we are infusing an enzyme blend --

DR. SHERWIN: Well, it helps the digestion, surely, but the problem is you have all these cytokines that are released --

DR. HERING: But maybe this is a good mechanism as well because islets have faced inflammation already and have built up defense mechanisms and may survive even better. So, you can argue either way. There are no data to support this. I guess that is why the statement has been made over and over again that we should not limit ourselves too much but for any given clinical trial I think it is a good way to

limit inclusion/exclusion criteria to really address a very specific question, but in general there are no data to exclude a pancreatitis pancreas.

DR. SHERWIN: I am just surprised because if you expose an islet to a cocktail of cytokines the islet dies, not immediately but over a few days. I just think in the early stages of this therapy we want it to work and I think anything that potentially -- at least experimentally you can induce pancreatitis in animals and see what the outcome of those islets would be. If, indeed, you could convince me that those islets function perfectly normally I would accept that. But I would want to have more data before I would want my patient to be exposed to islets that might be damaged.

DR. SALOMON: I would like to pick that one up. I don't buy that either. I mean, for all the reasons Dr. Sherwin said plus from an immunobiological point of view, it is concerning. You did make one interesting point, the idea that the islets may have some sort of compensatory response that might protect them from apoptosis or that sort of thing after the transplant, but I would like to see the data for that. But I would say if we are really trying to give some guidelines I would draw the line on taking acute pancreatitis. You guys can do science on that but I don't see that as a clinical part. That would be my personal

opinion.

DR. CARA: I actually have another question --

DR. SALOMON: Maybe they want to respond.

DR. CARA: Okay.

DR. SHAPIRO: I would just comment that you shouldn't necessarily exclude serum amylase as a marker of acute pancreatitis in the trauma situation because often the high amylase can be related to direct trauma to the salivary glands.

DR. SALOMON: That is fair, so let's make sure that we don't give the wrong message on this specific point. Serum lipase greater than 500, that shouldn't be on this list, guys?

DR. SHERWIN: I think the lipase would be a lower number than that but I don't remember the exact number.

DR. SHAPIRO: I have never see a donor with a low lipase. They are all high lipase.

DR. AUCHINCLOSS: We are getting mixed up again between your job of designing good clinical trials, which we will do and we will use islets that are the best quality islets you can come up with, compared to the FDA's job of regulating islet procurement. There is no reason to have pancreatitis on this list.

DR. RICORDI: Actually, if you put pancreatitis as an exclusion criterion, it means we couldn't have islets any

more from failing organ pancreas transplantations and be able to reinfuse them as has been done.

DR. SAUSVILLE: I really feel that why I recognize concerns that could be raised about pancreatitis -- I am seeing a disconnect here between the people who presumably do this for a living and what we might preconceive of as the best science. So, I mean, I return to the point that if the folks who are proposing to go forward with this multi-center trial feel comfortable, within the context of that trial, using pancreatitis-derived pancreases or pancreata, whatever the correct plural is, I guess I could be persuaded on that point because I think it becomes then an endpoint as you get experience as to how you do.

I would be more interested in the discussion that came up before about what level or evidence of prior diabetes is going to go into this because you can have ultimately pretty subtle changes described as evidence of diabetes, or you could actually require full-blown prior clinical history. That seems to me to be a somewhat more problematic thing to put on the list.

DR. SHERWIN: I just think that you have to show me the data. If you can show me the data that patients with pancreatitis have a great outcome, or no different than any other islet, then I will accept it. But I think you need to show the data, and show it to the FDA and convince the

people there that that is okay because I still think that if you subject someone to an islet graft instead of a pancreas graft, you have to do the best you can, and you need the data to show that. I may be wrong, but if you have the data I would be happy to accept that view.

DR. SALOMON: Jeff?

DR. BLUESTONE: I just want to support the way Bernhard put it because I think it really is the way, which is that we need to make good islets and safe islets to put into people but we shouldn't design the clinical trials, and where the exclusion and inclusion criteria can have the biggest impact is on the precise clinical trial -- keeping the variables, asking the questions and things like that. I don't see why, unless there is a clear documented example where a criterion is detrimental, we should exclude it a priori. It just doesn't make sense.

DR. SHERWIN: I think it does if you receive the islets. I mean, I think it doesn't theoretically but if it was my child I would want them to have what I feel theoretically would be the best in the beginning stages.

DR. BLUESTONE: And I don't disagree, but if the islets came out of a patient and you were blinded to whether it was a pancreatitis patient or not but all the other criteria which we are talking about -- the insulin secretion capability, the number of islets, the other quality controls

were all absolutely fine, why would you a priori, just because it came out of an acute pancreatitis patient, say, well, it can't be any good?

DR. SHERWIN: Well, first of all, if you have acute pancreatitis your beta cell function is not normal in vivo. Now, it is possible that when you take the islets out of that pancreas you might be able to reverse the phenomenon. I am not saying it is not so; I would just like to see experimental data to show that, and if I don't have that data I just -- you know, I am just giving the other side, but it just theoretically doesn't make sense because in vivo those islets don't function well. One of the complications of pancreatitis is diabetes.

DR. SAUSVILLE: Yes, but this returns to the Decadron issue that came up before. I mean, we will stipulate that prior Decadron exposure likely is going to induce "abnormalities" at some level of beta cell function. But if you exclude patients who received Decadron, then you are out of business because the nature of the patient population is going to do that.

DR. SHERWIN: I agree with that but I think there is a level of magnitude difference between steroids and a pancreas full of inflammation.

DR. SALOMON: Okay, Camillo?

DR. RICORDI: I think we are confusing two sets of

issues. One is the multi-center trial that is going to take probably four patients per center, and in this setting I don't believe anyone will select acute pancreatitis or take any chances because the centers that will show failure in two consecutive transplants will be in a serious problem of reassessing their ability to purify and obtain islets. In this case there may be more strict criteria, but what I would be very worried about is if you say a pancreatitis donor cannot be a suitable donor for transplantation in general, again, because there is now clinical experience. Actually, this morning one of the best category lists was pancreas from pancreatitis that reversed instantly the diabetes from graft pancreatectomy when you have to remove the pancreas because of the pancreatitis. Maybe that may not be an instant exclusion criterion, like younger donors, but I completely agree that I don't think any of the centers participating in this initial multi-center trial will try their luck and say, okay, let's try an 11-year old or pancreatitis for these initial two or three patients.

DR. SALOMON: I think that we haven't settled this pancreatitis issue but if the FDA is happy that they did get a balance of opinion, I don't believe that we want to get into voting at this point. Right?

DR. SIEGEL: I do want to clarify, not because of Dr. Ricordi's comment but an earlier comment just so

everybody is on the same page, we are focused only on criteria for allogeneic donors. So, were we to indicate that there should not be pancreatitis, that would not impact the ability to do autologous transplant in pancreatitis recipients.

DR. RICORDI: I wasn't referring to allo.

DR. SIEGEL: If you are studying a recipient of allo islet cells, is it acceptable that those allo -- and this is the question that was being addressed here, you know, should that only be restricted to non-pancreatitic islets or should it be broader? I am not arguing for a specific answer, I just want to make sure that we are all focused on that.

DR. BLUESTONE: You have to understand the last category that I was talking about was the situation in which you would be working with allogeneic islets because it is a patient who got an allogeneic pancreas, has pancreatitis and then they remove the allogeneic pancreas, make the islets and put it back so it would be an allogeneic islet transplant.

DR. SIEGEL: Oh, I see.

DR. SALOMON: A comment from the audience?

DR. OLACK: It is Barbara Olack from Washington University. Just for clarification for a lot of people, I have been involved in probably 1200 human islet isolations

and I can remember one pancreatitis isolation that we have done in those 1200, and the case, like Camillo was talking about, is also a very specific, uncommon isolation point of pancreases. So, I think the question, even though it is scientifically relevant, is not realistically relevant.

I have one other comment. As far as the criteria that were listed up there, if Edmonton's protocol is at this point the gold standard, and we know gold standards change, are any of the Edmonton criteria different? You had mentioned the age; you had a 71-year old. What about your ischemia time? Your cold ischemia time? That is something that was brought up.

DR. SHAPIRO: Just for the purposes of that trial, we tried to optimize every step in the process. We don't know how long we can use cold ischemia. We know for whole pancreas transplantation we can go out to 30 hours. We prefer not to do that. But for islet isolation, for the moment, we have tried to minimize that and tried to keep it at least under 12 hours and in many cases, because of the local donors or donors that are procured by our team, the cold ischemic time is around 3 or 4 hours.

DR. SALOMON: Dr. Cara?

DR. CARA: Are there any other medications, other than the issue of dexamethasone or steroids, that you would particularly be concerned about a potential transplant donor

having received, and would you be worried about the transplant donor perhaps having cystic fibrosis?

DR. HERING: Those are all very important questions that can be discussed on a case by case basis. I think the general picture is that we have no data to exclude use of any donor beyond accepted criteria in solid organ transplantation. Any clinical trial that is designed, I guess, should discuss lists of criteria that serve the purpose of this particular clinical trial. I accept that there are many questions that could be studied and many drugs or clinical settings that could favor or disfavor the isolation of islets, but at this point in time I guess every clinical center is addressing donor selection very carefully and we will generate data, but at this point in time we don't have any scientific basis to exclude any given donor, besides accepted donor criteria in solid organ transplantation.

DR. SALOMON: Kathy?

DR. ZOON: Yes, I find the discussion actually very interesting because there seems to be a variety of opinions and, while I am not going to pose a question right now, I think it is important tomorrow when we look at the preclinical information to understand what animal data is out there, and how that substantiates or doesn't substantiate the variables that we have talked about today,

and has the appropriate research been done in animals prior to proceeding to humans as the investigational model.

DR. SALOMON: One question that I know the FDA is interested in, and it was raised by someone on the panel -- I believe it was diabetes history; I know Dr. Sherwin kind of segued into it a little bit. Before we leave this donor organ criteria, what is it that the committee wants to tell the FDA regarding how much history should be gathered on the patient? Let me just throw out an argument -- a 50-year old guy, 110 kilos, with mild postprandial hypoglycemia.

DR. AUCHINCLOSS: Centers vary but a general rule of thumb for solid organ pancreas transplantation is that if your donor in the ICU, on steroids with lots and lots of fluid gone because the pituitary is knocked out, gets a hyperglycemia requiring insulin treatment you probably don't want that pancreas. Okay? So, that is a rough rule of thumb. Is it perfect? No.

DR. SHERWIN: It is not unreasonable really at this point. I mean, my guess is that you would probably like it a little better than that but I don't see a reason at this point to stop. But I think that these questions need to be studied experimentally so that we really know what we are doing beforehand.

DR. SALOMON: Now the 110 kilogram guy was put on a little DiaBeta about two years ago and he is doing great,

and he tapered it off about six months ago and then he got hit by a car.

DR. SHERWIN: I probably would not use those islets at this point, and I am sure you would -- I don't mean it in a negative way because I don't know the answer to the question, but I just think that those islets have had some insults, and from a number of different glucotoxicity, lipotoxicity, amyloid deposits, blah, blah, blah -- although they still function, those islets probably are not going to give you as good a result. They might but I doubt it. So, I would be careful at this stage, and if you could take DBDB mice and transplant islets and show me that those islets work just as well or better, you know, perhaps I would back away but at this point I just think that you want to have the best results in the beginning.

DR. SAUSVILLE: But then the point that comes up from this morning is that in the event that this clinical experience fails we have put the person back on insulin. In other words, the downside of not proceeding is potentially missing a really important opportunity. The upside is that if it doesn't work --

DR. SHERWIN: No, there are other treatments of diabetes. I mean, if there weren't any other treatments of diabetes I would say you are right.

DR. SALOMON: Again we have started to do what I

said we were not going to do today, this is a real clinical trial issue. It is okay, I mean I knew this was going to happen. We discussed this with the FDA staff.

DR. SHAPIRO: I would just point out that the ITN funded multi-center, prospective trial will record and define some of these variables and criteria and will provide very important data for this discussion in the future.

DR. SALOMON: The question that I raised at the beginning and I wanted to make sure that I offered it for comment is that currently, because of the competition between whole organ pancreas procurement and pancreas procurement for islet preparations, there is a potential for pancreata for islet preparation to be substandard. How much concern do you have for that, and what kind of guidelines, if anything, need to be considered on that one?

DR. HERING: I think this is a very important issue and we have to learn how to interact with the UNOS kidney/pancreas allocation committee so that the islet transplant community has access to choice organs for initial studies. So, we are dealing with two steps. One is proof of principle in multiple centers and the second one is increasing the availability of islet transplantation once the concepts have been developed. So, I agree. We have to learn how to interface and the UNOS committee is very receptive to our proposals, and I think with better and

better data and better protocols, such as developed at Edmonton, we have access to more choice organs.

DR. SAUSVILLE: I think that question actually verges towards something that came up this morning, and is it now appropriate to discuss it or will we talk about it later this afternoon, and that is that I would be very interested in extending the criteria for acceptable islets away from simple morphologic criteria into some functional assays that may actually provide the scientific evidence that Dr. Sherwin might like to see as we make these different preparations. When shall we talk about that?

DR. SALOMON: Yes, we will get into that in a few minutes. So, let's hold that. That is a key point.

DR. RICORDI: Can I just make a comment? The number of donor variables that we are considering if we are considering Type 2 subliminal diabetes or drugs that can interact will go way beyond the ones that were listed on donor age, organ size, ischemia or ischemia time. You have all the variables introduced from cause of death, duration of treatment in the intensive care unit before organ procurement, drugs used, and we can probably come up with a list of 50 or 60 variables, all interacting with each other for several hundred combinations, and this is why it is so difficult to exclude a priori or to define which one is the best condition. That is why I would suggest not to get too

much into the definition of pancreas conditions besides the general safety rule of organ donations. The issue of pancreas for islets is really critical also in consideration of all these variables.

DR. SALOMON: Well, in ending this first part, I would like to put to the NIH and to the FDA, for the record, that in parallel to defining and calling for the highest quality islets in these preparations I think it is incumbent for you to join with the islet purification groups and the islet transplant groups with UNOS and straighten out some of the procurement issues that obviously are not on the table today. I think we cannot emphasize enough how critically what is happening in multi-organ procurement of a pancreas organ is affecting the quality of the islets and the quality of the pancreases these guys have gotten. We are going to need some help since there really is a competition right now between the whole organ programs and the islet programs -- unless somebody disagrees with that.

DR. RICORDI: Actually, I think it is a very important point because right now we are in a situation in which many very suitable pancreases or ideal pancreases may be lost. There are organ procurement organizations that by choice do not procure pancreases because it is so much trouble to try to allocate them. So, I think it is not something that even FDA can solve with all the power of the

FDA. It would require a coordinated effort of several organizations to see how we can actually maximize the quality and harvesting of pancreas because we must begin to create an awareness that the pancreas is a potentially life-saving organ and cannot be wasted, and that procuring the highest quality pancreas must be a priority for every organ procurement organization, not just an option that they can do if everybody is in the right mood and there is adequate support. These are very important issues and I don't know if this would be a site where we could really address them, but it could be a beginning.

DR. SALOMON: Yes, I said that is not on the table now but I think we have to point out that a quid pro quo today is that if we help you define how to optimize and characterize the quality of islets, something that makes these guys a little bit nervous, the quid pro quo I am pointing out is that you should join us in making sure that the message gets out clearly to UNOS that this has to also happen on the table where it is really important, which is the operating room table.

The second direction we are going to go into is entitled appropriate types of identity testing. An islet equivalent is defined based on both insulin content and morphology and size. However, a beta cell is only one of several other cell types needed to constitute an islet, for

example, we have the glucagon and pancreatic polypeptide and somatostatin producing cells. Should measurements of these molecules also be made? Are there additional assessments that should be used in addition to an islet equivalent? Does everyone like islet equivalence? I have never been completely comfortable with that, parenthetically.

DR. SHERWIN: I guess I will start. My own bias, but I have much less experience, is, one, that the function of the islet needs to be assessed better than we do at the present time. Morphology is valuable information surely but I would assume, just because of my own bias which may not be correct, that perfusion of islets to look at function would make sense to me.

The other question is in terms of how we assess viability of cells. Sometimes they may look normal but in a couple of days they are really not very normal and, obviously, once you have set up apoptosis and motion it may take a little bit of time before the cells actually stop functioning. So, it might be nice to have better ways of assessing mitochondrial function, whether apoptosis exists in these islets and whether there is a functional test that might be done. Those would be the kinds of things I would think about. I am less concerned about other cells, personally. Even though my area of interest is the alpha cell, I don't know yet -- you know, I don't think we know

enough to say that that kind of assessment would be very valuable. I tend to doubt it, although that may be wrong.

DR. AUCHINCLOSS: I guess I would ask a question of the experts. My impression is that isolated beta cells are less good than isolated islets, but we have no evidence to suggest that that is so because of the presence of the other cell types as opposed to disruption of architecture. Is that true?

DR. RICORDI: It is in part true. I think the reason we still would like to characterize the content of beta cells is to have an idea --

DR. BLUESTONE: Well, Hugh, the one thing you should remember is that Danny Pipeleers isn't here and he might actually argue the opposite, that getting isolated beta cells is the best way to go. So, I think that is not a fully --

DR. AUCHINCLOSS: I think we are going to end up saying the same thing -- there is no reason to think that the presence or absence -- so let's move on to the next question.

DR. SAUSVILLE: No, wait, to pick up on a point that Dr. Zoon made, it seems to me that this is an ideal question where animal models could be quite informative. I mean, I just can't believe that people haven't thought this through or maybe even have some conclusions on this.

DR. SALOMON: I agree with you but the problem with the models is that when one dissociates the islet into its constituent cells you down-regulate gene transcription for endocrine hormones and you lose the function really quickly. So, it is a very difficult study to do. It seems like it would be real easy to just dissociate an islet, do, let's say, a flow-cytometry separation for insulin, positive beta cells, and then transplant only those as opposed to some kind of mixtures but those kind of studies actually don't work.

DR. SAUSVILLE: I am actually asking a much more rudimentary question. In other words, the process here is that there are going to be islets isolated and then given back to people in some site, and we heard this morning in the Edmonton protocol that it is to liver. It would seem that some criteria for how islets performed in a model, just going that far, could then allow this statement of how much or what level of function we might want to regard as the standard islet, as it were.

DR. SALOMON: Do you want to comment on that?

DR. HERING: The first topic that we are discussing right now is cell identity, and we are not discussing potency, viability or other issues. I guess for identity the available assays that we have are the DT zone staining and histology determining the cellular composition

of the graft. So, this is what can be done in this direction. I completely agree, we have to look into improving our potency assays but for identity we only have a few assays that are, I guess, well established.

DR. SALOMON: I will take the chair's prerogative to now move potency into this identity question because I think that they go enough hand in hand. So, I think you are back to what Ed is asking you, and that is identity and potency. I don't know of any data suggesting why today you should measure glucagon levels or pancreatic polypeptide levels. So, I want to make sure that is clear.

DR. SIEGEL: I would accept Hugh's comment certainly that we don't have data that suggest they need to be high or low but as far as your comment that you are not aware of any data as to why you should measure them, one might respond, well, as long as we continue not to measure them, then we can ensure that there never will be any data as to why we should measure them. So, what I am trying to do here is to make clear what the question is, which is not should we set specifications here or throw out islets because they are high or low in glucagon or not, but is it useful to collect this information or to consider it as part of an assessment of a quality pancreas? Should this be the type of information that we ask or require be collected, or is it, as many other things we could measure, too peripheral

to be worth requiring except for those people who specifically want to study it?

DR. AUCHINCLOSS: That is a superb way of phrasing the question. I mean, it does make it clear to us what it is you are asking because, again, really FDA playing such a critical role by forcing us to get the information that we have not obtained over the past ten years.

DR. SALOMON: Jeff?

DR. BLUESTONE: Yes, I think it is a good segue into what I was going to raise, which is when you start putting potency into it, I agree with Dr. Sherwin that, of course, the more we can learn about the function of the islets the better, but there is a clear question about whether or not we need to be doing this prospectively or retrospectively at this point. One of the things the Edmonton protocol does is it does culture the islets, and that provides opportunity but --

DR. SALOMON: I think a correction, Jeff, there is no culture done.

DR. BLUESTONE: That is right, I am sorry.

DR. RICORDI: But you can culture an aliquot.

DR. BLUESTONE: Right, that would make it retrospective and my point is that we don't want to put so many criteria on functionality right away that we force people to culture if, in fact, the best protocol right now

does not require culturing even though other people do culture. So, I would agree with the FDA's comment that we don't want to lose data; we want to generate data. We don't want to put too many restrictions on what is going to be considered a functional islet before we put it in so that we force a certain type of protocol in the process.

DR. SALOMON: Bernhard, do you have a comment?

DR. HERING: I can only assure you that I would be very, very interested in learning the answers to all the questions, and we are very grateful for the support that the islet transplant field is receiving right now to address the questions. Our proposal is to establish as many reference laboratories that are well-equipped and positioned to help address questions and so bring more experts into the field. Whenever we do a transplant we send an aliquot to reference lab A that is studying the cellular composition of the transplant that was actually performed. For example, as we are talking about identity, the Edmonton group has established a very wonderful assay to study the cellular composition so at the end of the day you know exactly what is the percentage of alpha, beta, delta, pp cells etc. that were transplanted. This information is available. This is how we like to approach this so that we have the data at the end of the day to address all the questions. It may be beyond the expertise of any given lab but there are

reference labs that are interested in studying this, and this is how we like to approach it.

DR. MILLER: It seems like some of the questions are mixing what is research and what is needed for the development of guidelines for the clinical use of these products. I think it is important from both a funding standpoint as well as a regulatory standpoint that you separate the two. As Bernhard was just saying, those are things that, you know, the people who are doing these really want to know but that is research and I don't think that should be included into your guidelines or what is required for centers who are going to do that. We always want to encourage research so we know more for the future but I don't think that that is an appropriate guideline.

DR. SALOMON: Right, and that actually goes back to what I was saying, that I think in general right now, aside from clearly some value in the research, there is no data that I am aware of, and again I look to the audience for other input, that we should be asking anyone to be measuring, as criteria of an islet preparation, glucagon, pancreatic polypeptide or somatostatin.

DR. BLUESTONE: I am sort of in the middle on this. I am not that extreme actually. I think we have been asked over and over again where is the data and not being able to come up with it. So, if we ever want to get the

data I think we -- we, the FDA -- need to at least be somewhat proactive in making sure that at least a minimal level of data is accumulated. Although we don't want to design people's experiments for them, I think you certainly want to ask groups who are going to participate in these early phases, as part of the guidelines, to collect data that will help the FDA and others come up with better criteria in the future. So, I wouldn't want to say, you know, you don't have to do anything in terms of retrospective analysis. I think some retrospective analysis should be part of the guidelines.

DR. SALOMON: I totally agree with that.

DR. RICORDI: I think there is a difference between research and this in between stage of collecting data, and I agree with Jeff that it is very important that we take this opportunity to learn retrospectively what may have been important for viability and functional assessment. Right now there is no single assessment that per se can give us a prediction of what will be a good islet transplant, whether it is an in vitro perfusion assay -- there are perfusions that work very well and don't work in vivo and vice versa. But the gathering of all this information together will allow us, hopefully, to retrospectively see what components put together make a requirement for a successful transplant. The research component is beyond

this, like all the new tests for apoptosis or like if you have elements where you can take a sample and see which cells are dead today and which ones will be dead in six hours, etc., and maybe have a procedure that may replace in vitro testing for insulin release. That is the research component. There is still an incredible need to develop new things.

DR. SALOMON: Right. But, Camillo, what I want to get back on track here is what isn't acceptable. I don't believe it is to say, well, we have no idea; suggest there are no criteria; we are going to do islet transplants in human patients and we will do research but don't ask us to set any criteria for the product. I mean, maybe you want to start to argue that, and that is what you should be doing if you believe that but I don't believe that. I think that we have to come to grips with some reasonable criteria for a quality islet preparation with all the caveats that are scientifically appropriate right now.

DR. RICORDI: And you know there has been an entire workshop dedicated to this subject and there are consensus papers that set up some very imprecise guidelines. There is a consensus paper co-signed by some 14 investigators from different institutions and these are the general criteria that are not perfect, but like islet equivalence that was established several years ago are

imperfect. It is an imperfect way so that you can compare islet preparations, say, if you normalize your islet count for hypothetically equivalent islets of a diameter of 150 microns and you add this to morphology so that you know which cell you transplanted to some kind of functional assays and you may have a picture. We are not saying study everything that is in the catalog for testing biologic systems and one day we will get some information, but something in between.

DR. AUCHINCLOSS: I was actually thinking back to Jay's question of forget about whether this should be criteria for an acceptable islet but should we gather the data. I was just going to make the comment that amongst the kinds of data that you might want to gather, information about the alpha and the delta cell would not be the information that I would think was terribly important, but you might get some more expert opinions from the committee.

DR. NOGUCHI: To try to focus us back on what we are doing here and this is a scenario -- in a year if we come back and meet I don't think any of us would be satisfied with having this same discussion. Here we have asked specific questions because we are wondering if these are appropriate things that you should be looking for so that you can be making incremental progress in a reasonable amount of time. If, in fact, they turn out to be the kind

of research development that needs to be done in a different arena, that is fine but we are asking you as the experts in the field that surely after this time you have some ideas about what is important and what isn't in the case of clinical trials, and that is what we are looking for, that kind of advice.

DR. SALOMON: So, picking up on what Dr. Noguchi said, can we go back to discussing a series of specific islet function tests, whether it be from dithiazone staining, insulin content to clamp insulin release assay, and just try and put that into perspective for the FDA in terms of how practical that would be, where it would be valuable and where it wouldn't be?

DR. SHERWIN: I was just going to comment before about the point you made. My guess is that it is not the highest priority to look at the other cells, even though I am interested in the other cells. If I was going to bet, it is probably not the highest priority. It may have a mild effect but, given all the other things going on, probably it is not the most important. There is no problem in getting retrospective data but that would be my bias.

In terms of the other issue that you raised about testing, and I am naive in that area because I don't work in it, but it just seems to me that there are tests that are evolving that allow us to predict whether cells are going to

die, and my guess is that islets that function in vitro are better than ones that don't, even though it is true that a functional islet, when you put it in, may get exposed to an environment which kills it anyhow. So, my guess is to optimize that you would like some way of rapidly assessing function at least for the future, even though it is a retrospective analysis. Even if you want to do things quickly, you can rapidly do some in vitro assessment and then do your study, and then retrospectively look at the data. I would think that area would be far more important than the glucagon side of it.

DR. SALOMON: Again, just so we can get to specifics, let me divide it up into descriptive assays and functional assays. Start with descriptive assays, of the following, which do you want to do? Do you want to do insulin content, dithiazone staining, the islet equivalence alone or some other descriptive assay?

DR. HERING: We have two sets of criteria, product release criteria and product post-release criteria. I guess we are studying all the different things within this trial and in other trials, and we will collect this information, and types of assays as far as potency is concerned are glucose-stimulated insulin release in vitro using static incubation or dynamic perfusion assay. Then you can move on and do insulin biosynthesis studies which are difficult and

demanding. Then you have your in vivo diabetic nude or SCID mouse BO assay. Those are the potency assays that many labs have established, and this is the type of information that will become available as product post-release data.

DR. SALOMON: So, if you had a preparation in which you counted the islet equivalents and it turned out that you had a reasonable number of islet equivalents but they were all 50 microns or less, would that be as good a preparation as the equivalent number of islet equivalents though less islets per se though they were all 150 micron structures?

DR. HERING: I think it would be difficult to accomplish a suitable islet mass with islets less than 50 in micron diameter. This is virtually impossible. To have 5000 or 10,000 islet equivalents per kilogram body weight available with islets less than 50 micron in diameter, this is something that may not happen.

DR. SALOMON: Can we make a criterion, Bernhard, that if a certain percentage of the islet mass is less than 50 microns that might not be an islet preparation suitable for a clinical trial at this early stage?

DR. HERING: I think there is no evidence. Again, you could argue maybe islets that are smaller have more access to oxygen and may engraft much better and survive much better. I think this is a very general point. We will

collect the data. We will make every effort. We have reference labs; we have expertise in the field of islet cell physiology that we have available now. The question is to what extent and in what detail should that be part of an IND application, whether this should be mandatory or optional. I think it is in our very best interest, and we will deliver the data. It is a question of what type of mechanism should we have to make sure that the data will be available in one year.

DR. SAUSVILLE: So, what islets would you not use? Let's put the question that way because I am disturbed that I am not hearing any box that you can fit things into or out of that will or will not be the subject of clinical investigation.

DR. HERING: We would not use islets as long as the islet mass is considered completely inadequate. If it is a single-donor protocol, anything less than 5000 islet equivalents per kilogram is considered inadequate. If you are doing a multiple donor transplant, sequential transplants, anything less than 4000. That is the first point.

The second point is the viability because this is an assay that can be done on site, immediately, anything less than 50 percent or less than 70 percent would be considered inadequate.

The third point is Gram stain positive would also be considered unacceptable. Those are the three criteria that we have in place, and it can be discussed whether it is an immediate transplant or whether this is a transplant after a two-day culture period. This is what I guess we consider the minimal requirements.

DR. SALOMON: So, Bernhard, if you had an islet preparation that equalled 5000 islet equivalents per kilo but had 30 percent non-viable percent that would fall into the 5000, would that be a non-usable preparation?

DR. HERING: This would be acceptable as long as the viability is in excess of 50 percent or 70 percent and you have the islet mass that would be considered acceptable.

DR. SALOMON: So, even though you would only be putting in something like 3000 or 4000 or even less, 2500 islet equivalents per kilo in actual viable cells?

DR. HERING: You are asking the question what is the minimum number of viable cells that you transplant.

DR. SALOMON: Yes, well, you answered it then.

DR. SAUSVILLE: To pursue the point, the definition of an islet equivalent is what?

DR. HERING: This is an islet having a biomass of an islet with a diameter of 150 microns.

DR. SAUSVILLE: So, the 50 micron issue then becomes more of the criteria, right?

DR. RICORDI: Equivalent islet is considered an islet with a diameter of 150 microns, which has a volume of 1.767145 cubic microns, and then you normalize all your counts divided by 50 micron increment to that equivalent islet, with an imperfect algorithm, meaning if you have a 50-100 cluster you need 6 of those islets to make 1 because the volume is connecting with the cube of the array like in a sphere equation. That is imperfect because islets are not perfect spheres. But if you have bigger islets you have a positive factor in your total count. So, the reason this was introduced is because in the early days of improvement of islet isolation technology you could have a tube with 100,000 big islets and then shake it 3 times and make 200,000 islets.

[Laughter]

So, if you don't normalize to average islets you could keep improving isolation technology just having a mechanical, more disastrous procedure. There have been groups reporting 20 million islets from a human pancreas, just counting fragments. So, it was the first, very primitive way for guys from multi-centers saying let's try to normalize our count to some equivalent islet. It will probably be replaced by a better analysis system to count the volume in 3-dimensional appearance of islets.

DR. AUCHINCLOSS: The comment that we are not

hearing what defines the box of good islets from the bigger box of bad islets is right on the mark because the fact of the matter is, I believe, you could spend all afternoon here talking about what defines a good islet and the information that does so is essentially not available. That made the conversation, at least as I understood it, that we began this afternoon not all that interesting because, no, that is not an adequate criterion for excluding islets, no, no, no, but they have asked us now another much more interesting question, which is what data should we start getting so we will know what defines a good islet.

We have heard about some good stuff about the functional in vitro assays, but there are two other approaches that you do want to consider. One is new imaging techniques, and I think there is perhaps something there but I don't have a clue what it is. The other is gene expression, are anti-apoptotic genes on or off in this islet preparation? Presumably in the course of the next to or three years you will be able to define a panel of 25 genes that you could quickly determine -- hey, this is a good islet or this is a bad islet.

Now, the fact of the matter is that no one of us, or few of us, will have the resources to that routinely on every islet isolation prep. But I would think that it would be perfectly reasonable for the FDA to require that every

islet isolation set aside one aliquot to go to some separate specialized facility, say funded by the JDF --

[Laughter]

-- to determine is this assay a good assay for picking out a good islet.

DR. BLUESTONE: Hugh, if you back up a step, couldn't you pick three or four criteria not knowing what the best assay today is going to be? For instance, you could say that part of the guidelines are that you have determine your percentage of apoptotic or dead islets? Today that may be by one technique and then a year from now there may be another technique. But we want to know what the viability both immediately and after 24 hours is. We want to know that. We want to know their dynamic function. We want to know their static function, and we want to know their dynamic function. Today that may be by looking at insulin, tomorrow it may be by calcium, and we need to work on that. But are there four or five criteria that we could say we just really need to know that we get that data, and then these guys can tell us what the state-of-the-art today is and what we are moving towards. It is my sense that knowing the number of alpha cells wouldn't be among the list, but knowing the absolute number of beta cells and the volume of beta cells might be on the list. So, can we come up with a list of five or six general criteria and then

worry about what the best state-of-the-art is to get that data?

DR. AUCHINCLOSS: Yes, I agree but my point is that I think the only number I would use to exclude an islet prep is viability. The cells are dead; don't put them in. Okay? But I don't even use the 5000 islet equivalents anymore because that means just putting in two, three or four transplants so it is not even a total cell number. Viability, yes. Is there anything else after viability that you can use to look at a prep in front of you and say I am going to use it or not?

DR. BLUESTONE: You are blurring a point, Hugh. I agree with you up to the point where you said, well, just put in three or four. It is okay. Maybe that is part of a clinical trial. We will discuss that tomorrow. But the issue now is defining the quality of a preparation. So, the prep that you can put 5000 in is a damn more valuable prep to me than one where you have to do five islet preparations from five different pancreata. I don't think we disagree on that.

DR. AUCHINCLOSS: Anything else besides viability?

DR. SAUSVILLE: The Gram stain issue.

DR. RICORDI: The total volume of what you are infusing for a safety consideration. I think the reason we estimate purity -- I don't actually have any criteria for

purity or minimum purity required, and it may be that unpurified or partially purified islet transplants may work better than islet purified. But what we are trying to define is a range of an acceptable volume that you can infuse safely in a patient in the portal system without having risk of portal hypertension or severe complications. So that is why we would still like to look at the total volume and not just the islet number or the beta cell content. Viability, of course, I agree. If someone does a fluorescent dye test and sees that more than 50 percent of the cells in the preparation are dead, nobody would feel comfortable infusing that preparation. But I think there are very general guidelines at this stage. I don't see any specific, more sophisticated test that has been validated yet.

DR. AUCHINCLOSS: Jeff, I think we are making three lists. One is things that would exclude a preparation at any center right now. The second is tests that every center ought to be able to do itself that seem like reasonable tests that might turn out to be predictive in the future. Third would be your wish-list of tests that would get done somehow.

DR. BLUESTONE: Yes, and obviously one and two are essential and the FDA should focus on one and two. Number three is going to be a constant moving target because one

man's ability to isolate RNA effectively is not going to be the next person's. So, I would say we could focus -- you are focusing on number one which, to me, I thought was actually pretty straightforward. We don't know enough to do much to exclude, except something like a dead islet doesn't work.

DR. AUCHINCLOSS: Yes, but they told us a couple of other things.

DR. BLUESTONE: A couple of other things but I think number two is what I was trying to focus on, which is what are the things that we really could ask now as a source of guidelines to make sure that any islet center that is going to get into the business thinks ahead of time that they have to be able to develop the expertise to do it. I think that we want to have enough of those criteria that we learn something in the end when something is done. I have read Bernhard's protocols and it takes 15 islet equivalents just to do 3 of the assays. So, again, you can do everything under the sun but there must be a subset of things that we could all agree on that would give us enough information that a year from now, or whatever, we are going to maybe be able to have a conversation that is a little bit more sophisticated than this one, and I am trying to get a handle on what those four or five things are, not what the 15 or 20 things are.

DR. SALOMON: Following on that discussion between Jeff and you, can you comment on what kind of things, like for example insulin content, which is a static assay that can be immediately determined, would help you?

DR. RICORDI: I don't think it would help us but I would rather do a static incubation after overnight culture and put into aliquots in low glucose and high glucose and measure the stimulation index.

DR. CHAMPLIN: What is the standard for viability testing? Are you doing assays of apoptosis or is it dye exclusion?

DR. RICORDI: Right now it is just dye exclusion, but we are working with a combination with apoptotic markers and also a different time because some of these tests are too late a marker to be introduced probably immediately after isolation but there are more and more sensitive tests being developed. But there is nothing that has been standardized in islets, or validated. But viability exclusion dyes are dithiazone, isolation of islets, determination of the total volume, assessment of viability by exclusion dyes and then the day after static incubation for insulin release and measuring stimulated insulin release and getting an index of stimulated versus basal is another test that is generally performed.

DR. SALOMON: Jim, I know you have a limit on what

data you can share with us, but you have now had a series of patients, albeit small, that have had success. Do you have any data based on, let's say, dynamic insulin release assays such as the ones we are discussing now that might correlate?

DR. SHAPIRO: We have been using static incubations. They are all post hoc. If we have an optimal pancreas where the yield is high, we go and transplant that immediately so the post hoc analyses tell us down the line if the islets worked, and in all the cases the stimulation index ranges from about 2 to about 7.7, which I think is the highest.

DR. SALOMON: Have you had any that didn't work, that had below a stimulation index of 2 and that worked in vivo or didn't work in vivo?

DR. SHAPIRO: Not that I am aware of, no.

DR. SHERWIN: I just think that is a very critical component. It is very important to assess the function of the islet because islets may be on their way to die and you don't know it but their function will be impaired as a result. So, it seems to me that there are simple -- as you did -- functional assays that can rapidly be -- I am sure we can get to the point where ultimately you can get answers very quickly with an insulin assay. It is not there yet. So, it requires retrospective analysis but ultimately, if this was a critical criterion, I am sure there would be ways

of developing insulin assays that are much quicker that might not be quite as perfect but would be adequate for your purposes. So, I think the development of a rapid insulin assay would be something that would be important but right now I would retrospectively look at function and I would retrospectively use mitochondrial dyes and tunnel assays as what we can do now in retrospect while you are doing your regular viability. So, the criteria should be, I think, assays of function beyond death at this point and dynamic insulin responses as being the two issues that need to be addressed in retrospect while you are moving along in the initial stages, just focusing on viability.

DR. AUCHINCLOSS: I am sure your comments make lots of sense a priori, but my understanding, again not being an expert in the field, is that the functional tests of islet secretion by islet preparations have not correlated very well with function in vivo in experimental models, but I am going to turn that comment back to the experts to see what they say. Has the data actually supported what Dr. Sherwin said?

DR. HERING: So far there is no data but maybe this is because other donor criteria were not defined to the point that you could actually generate the data. It is also because one given center may have done maybe just two or three transplants and it is completely unlikely that you can

identify any single assay that is predictive. So, we should learn from our past mistakes and collect the data but it is because of the history of islet transplantation, maybe some anecdotal transplants in one given protocol, maybe two or three cases, and then the centers moved on to the next protocol so there was probably no way that you could identify parameters.

DR. KENYON: We did start about six, seven months ago to do the static incubation and try to compare it to the post-transplant outcome, and we started to see a trend that there was a correlation if you got some stimulation in the static incubation that the transplant did work but it is not 100 percent. So, I would say there was a trend that it correlates but it is not 100 percent and we haven't done a lot of animals that way yet but, obviously, we are interested in this question and we are studying it.

DR. CARA: It seems like we are narrowing down some of the criteria that you think are important in terms of the actual transplant material itself, the islet cell transplant material itself. Is there anything in the recipient that you think might be worth looking at? For example, anti-islet cell antibody titer, age, medications, etc., etc.? I wouldn't want to just focus on the transplanted material only. I think there are probably issues that need to be looked at in the recipient that I

want to make sure that we are not going to overlook.

DR. SALOMON: I think tomorrow we are going to talk about those kinds of measurements in the recipient as part of a clinical trial design, unless you are talking about something that you would measure in the recipient within 24 hours of the transplant that would be a measure of the islet quality, which is kind of an interesting idea.

DR. WEBER: Dr. Salomon, can I just intercede? Perhaps I failed in my presentation to make it clear that many of the things that we are asking about, like purity, potency, viability, those are actually regulatory requirements. So, all of these are going to be important to address at some point in this conversation, and we do appreciate your discussion but we would like to get some feedback on some of the specific issues, if we can, as we go through.

DR. SALOMON: Actually, I thought we handled one with specifics, and I was going to take two, figuring that we are pretty close to getting done here, albeit I don't know if I can cut off discussion, and I will summarize it again and answer these two specifics, and if you don't have the specifics then you need to tell me, but I agree with that as a point.

DR. SAUSVILLE: Although one thing I would point out, I mean in this definition of islet equivalence based on

insulin content and size, I really only heard morphology and size; I didn't hear that you actually use now insulin content for defining that islet equivalent. Is that correct?

DR. HERING: Yes.

DR. SAUSVILLE: So, that is at least one point that can lead to a change here.

DR. NOGUCHI: I think if we try to go through the regulatory terms it will help because the first question is, after you do all the isolation, how do you know you have an islet preparation? What are the tests you did? Or, if you had another person coming in and claiming that they could make islet cells through a different method you would certainly ask them, well, how do you know they are islet cells?

Then the second question, when you get into terms of viability as a measure of the quality of that preparation, if it is only half viable you would rather have one that is three-quarters viable.

Then, the third and more difficult issue is potency. How assured are you that if you actually give the product it may have any effect in the patient? So, that starts to get into some of the functional assays.

But in terms of the first one of identity, I think Ed brings up a good point, above and beyond morphology and

perhaps staining, do you really care if there is insulin or not if you want to be assured that it is an islet cell?

DR. RICORDI: Well, the reason we don't count insulin content at the time of isolation is also because this is probably the worst time to assess potency because you are doing through the death, organ procurement, cold preservation, several hour processing with mechanical enhancement of an enzymatic digestion, plus steps of purification and multiple washing, etc. So, that is why we rely more on the next day, the post-release criteria, the stimulation index of insulin release from those islets when challenged with low and high glucose levels when you give the aliquot of islets a chance to recover after overnight culture, and that is our best measure so far of potency before going to biologic testing, like diabetic nude mice in which you can transplant an aliquot of islets.

So, to respond in part to Phil's question about how you find out if someone can actually get islets, this is the morphologic characterization of your product and is clearly something that has to go beyond just DT zone staining and the visual impression that you have during islet processing, like if someone has to publish a new method for islet isolation or an improved procedure you would ask them to have also immunohistochemical characterization of the cells that you retrieve. But here,

again, we are at the two levels of pre-release criteria and the post-release criteria.

Right now we know that there is a pretty good correlation, like if you visualize islets by DT zone staining, this doesn't tell you exactly what the percentage is of beta cells, if it is 82 percent or 76 participant beta cells, but it gives you a good estimate of islet content. If you see in your final product that you have only 5 percent of the cluster of cells that are staining with DT zone you know that you are in trouble with your final islet preparation. If you achieve like 70 percent or 50 percent or 40 percent, then for safety considerations, that is when you go and look mainly at the total volume of the islet that you are planning to infuse in these patients and whether you are within the safety range, typically less than 10 cc islets to tissue, and maybe we can bring it to less than 8. But those are mainly safety considerations.

Then you have viability. You can do instantly a Gram stain to make sure that this is a safe infusion to perform. But then, if you want to really know that new groups or that new methods have islets, you could ask them to characterize their product before they start a trial, which would include product release criteria that are less stringent, but you can say are you sure? Can you document that with your islet isolation method you can actually

obtain islets which you see are viable at day zero is actually still viable after overnight culture? You know, you can ask some more questions as general criteria of validation maybe of an islet isolation facility or of a new method that is proposed. But for a standard procedure I think we are still at the step of testing that is a little more primitive.

DR. SALOMON: What I want to do is try and bring this part together and then take a break, and then come back and start with question three, which is viability. So, can we establish a specific answer to the question of what are minimum criteria for the identity of an islet in the context of defining a product? You made a lot of generalizations, Camillo, but can we be more specific? What would you say would be a minimum set of criteria that I had to show you a, b, c, or whatever before you would believe I had purified an islet?

DR. RICORDI: Before transplant?

DR. SALOMON: Before transplant.

DR. RICORDI: Before transplant the only release criteria that we can ask, if you want to transplant islets within two hours, is that these are DT zone positive particles and you characterize your islet number and your percent purity based on staining with dithiazone.

DR. SALOMON: Okay, let's start there. Within a

two-hour period I have to demonstrate to you that I have dithiazone positive cell clusters of a specific size and mass -- right?

DR. RICORDI: Normalized by islet equivalent --

DR. SALOMON: Normalized by islet equivalent --

DR. RICORDI: Characterized with all the different clusters so you know how many of those 50 micron islets you have.

DR. SALOMON: Right, and we will get to viability in a minute. So, that is the first thing within two hours. Now, by the next morning what else do I have to demonstrate to prove to you that the quality of the product that went into the patient were islets and were of high quality?

DR. RICORDI: The next morning or the day after I think it would still be very valuable to have the confirmation by immunohistochemistry of the beta cell content, with the option if someone wants to see all the other cell types --

DR. SALOMON: Okay, that would be histochemistry?

DR. RICORDI: Yes.

DR. SALOMON: Okay, that is one. How about insulin content, or insulin content per beta cell which is a little different than insulin content per IEC? I am trying to be real specific now.

DR. RICORDI: Yes, the insulin content that is

done the day after is insulin release, which is a better test because you actually have a secretion test. We measure stimulation index, stimulated versus basal release by the transplanted islets. Then you normalize or you do per DNA content so you have a measure of DNA and insulin release from that aliquot.

DR. SALOMON: All right, so if you do insulin release per microgram of DNA, then you can make a rough calculation back to your IECs, then you still are short of insulin content per beta cell or number of beta cells per islet but that may vary enough between donors that you don't see that as a measure. Am I paraphrasing you right?

DR. RICORDI: I mean, I wouldn't have any problem collecting at the end on the aliquots that you are doing the stimulation and having another set of data that says insulin content per DNA of that aliquot.

DR. HERING: As I have said many times, we are collecting the data, and we are doing this, and I think insulin DNA ratios is acceptable and we do it. Also, we all understand it is not a perfect answer but we are not arguing here. We do this because it may give us some information.

DR. SHERWIN: Just a question, when you do the functional assay when do you get the results? Isn't it after the patient has gotten their transplant?

DR. SALOMON: Yes.