DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

BIOLOGICAL RESPONSE MODIFIERS ADVISORY COMMITTEE

XENOTRANSPLANTATION SUBCOMMITTEE MEETING

Wednesday, December 17, 1997

9 o'clock a.m.

Double Tree Hotel
Plaza I
1750 Rockville Pike
Rockville, Maryland 20852

PARTICIPANTS

Hugh Auchincloss, Jr., M.D., Chairperson
Gail Dapolito, Executive Secretary

MEMBERS

Jonathan S. Allen, D.V.M.
John M, Coffin, Ph.D.
Ronald C. Desrosier, Ph.D.
Martin S. Hirsch, M.D.
Richard Kaslow, M.D., M.P.H.
Nicholas W. Lerche, D.V.M.
Abbey S. Meyers
Claudia A. Mickelson, Ph.D.
David Onions, BVSC, Ph.D., MRCVS, FRSE
Prem S. Paul, D.V.M., Ph.D.
Daniel R. Salomon, M.D.
Harold Y. Vanderpool, Ph.D., Th.M.
Leroy Walters, Ph.D.

GUESTS

Antonio Benedi
William G.Lawrence, J.D.
Marian Michaels, M.D., M.P.H.
Robin A. Weiss, Ph.D., FRCPath, FRS

FDA

David Feigal, M.D.
Philip Noguchi, Ph.D.
Any P. Patterson, M.D.
Mary K. Pendergast
Jay P. Siegel, M.D.
Carolyn Wilson, Ph.D.
Kathryn C. Zoon, Ph.D.

C O N T E N T S

C O N T E N T S(Continued)

P R O C E E D I N G S

Administrative Remarks

DR. FREAS: Good morning. I am Bill Freas. I am the acting chief of the CBER advisory committee program. I would like to welcome all of you here to this meeting this morning.

The entire meeting today will be open to the public and everyone is welcome to attend. I would like to explain to those present in the audience that, despite the hard work of the organizer of this meeting today, Dr. Amy Patterson, she tried to make it an official advisory committee meeting.

However, to charter and establish a full advisory committee takes a considerable period of time. Therefore, today's meeting will be conducted as a subcommittee meeting of the Biological Response Modifiers Advisory Committee. This will be a subcommittee on xenotransplantation. This was listed in the Federal Register Notice.

There are two notable differences between a subcommittee meeting and a full advisory committee meeting,

and they are that there are only two standing advisory committee members present at the table today. As I go around and introduce the members at the table, I will point them out to you.

The next difference is the report that will be

furnished today by the subcommittee will be approved by the parent committee, the Biological Response Modifiers Advisory Committee. Other than that, you will not be able to tell any difference between a subcommittee meeting and a full advisory committee meeting.

At this time, I would like to go around the table and introduce to you the members seated at the table. Would the subcommittee members please raise their hand as their name is called so the people in the audience can identify you.

Starting at the end of the table on the audience's right-hand side is Dr. Prem Paul, Associate Dean for Research and Graduate Studies, Iowa State University. Next is Dr. Ronald Desrosier, Professor of Microbiology and Molecular Genetics, Harvard Medical School. Next is Dr. Richard Kaslow, Professor of Epidemiology, Medicine and Microbiology, University of Alabama at Birmingham.

Next is Dr. Nicholas Lerche, Associate Professor, California Regional Primate Research Center, Davis. Next is Dr. Martin Hirsch, Professor of Medicine, Harvard Medical

School. Next is Dr. Jonathan Allan, Adjunct Associate Professor, University of Texas Health Science Center. Next is Dr. David Onions, Professor of Veterinary Pathology, University of Glasgow.

Next is Dr. Harold Vanderpool, Professor of

Preventive Medicine and Community Health, University of Texas. Next is Dr. Claudia Mickelson, Director, Biosafety Office of Massachusetts Institute of Technology. Next is Dr. Leroy Walters, Director, Kennedy Institute of Ethics, Georgetown University.

Next is our Chairman and representative from the Biological Response Modifiers Advisory Committee, Dr. Hugh Auchincloss, Associate Professor of Surgery, Harvard Medical School. Next is the Executive Secretary for this Committee, or the designated federal official, Gail Dapolito.

Next is Dr. John Coffin, Professor of Molecular Biology, Tufts University School of Medicine. Next is Dr. Daniel Salomon, Director of Transplantation Research and Graduate Studies, the Scripps Research Institute. Next is Abbey Meyers, President and Executive Director, National Organization for Rare Diseases, New Fairfield, Connecticut.

Also seated at the table this morning are several FDAers who are here to coordinate presentations this morning. They are Dr. Kathy Zoon, Director, Center for Biologics Evaluation and Research; Mary Pendergast, Deputy

Commissioner at FDA; Amy Patterson, Medical Reviewer, Division of Cellular and Gene Therapy; Dr. Jay Siegel, Director, Office of Therapeutics, Research and Review; and Dr. Phil Noguchi, Director, Division of Cellular and Gene Therapy.

I would like to welcome everyone again. I would like to make sure that the members of the audience understand that Gail Dapolito, the designated federal official, will be your point of contact should you need to communicate with members of the committee. Please do not directly approach the table but, during a break or during the lunch period, see the Executive Secretary, Gail Dapolito, and she will relay your messages to the committee.

I now will read into the public record the conflict of interest statement for this meeting. The following announcement addresses the issue of conflict of interest issues associated with this meeting and is made a part of the record to preclude even the appearance of a conflict of interest at this meeting.

The following announcement is made part of the public record to preclude even the appearance of a conflict of interest at this meeting. Based on the agenda made available, it has been determined that all financial interests in firms regulated by the Center for Biologics, Evaluation and Research which have been reported by the

participating members and consultants, as of this date, present no potential for an appearance of a conflict of interest at this meeting.

Based on the agenda made available, it has been determined that all financial interests in firms regulated

by the Center for Biologics, Evaluation and Research which have been reported by the participating members and consultants as of this date present no potential for an appearance of a conflict of interest at this meeting with the following notations to preclude even the appearance of a conflict.

Dr. Hugh Auchincloss; a waiver was approved by the agency permitting his full participation in committee discussions and deliberations. Dr. Martin Hirsch reported that he consulted with a regulated firm on virological issues including testing materials for porcine retroviruses. He received a fee for his services.

Dr. David Onions; a waiver was approved by the agency permitting his participation in the committee discussions and deliberations. It was also disclosed that Dr. Onions reported that he is director of Q-One Biotech Limited. Q-One is negotiating with two regulated firms to conduct safety testing provided by Sanzino Transplantation.

Dr. Prem Paul disclosed that he consulted with a regulated firm on retrovirus screening in

xenotransplantation. He received a fee for his services. In addition, he reported a consulting agreement under negotiation with another regulated firm. Also, members of the subcommittee had disclosed consulting relationships with firms not on the agenda that may, in the future, be

regulated by FDA.

The following participants have no financial interests to disclose; they are Mrs. Abbey Meyers, Dr. Jonathan Allan, Dr. Ronald Desrosiers, Dr. John Coffin, Dr. Richard Kaslow, Dr. Nicholas Lerche, Dr. Claudia Mickelson, Dr. Daniel Salomon, Dr. Harold Vanderpool and Dr. Leroy Walters.

With regards to FDA's invited guests, the agency has determined that the service of these guests is essential. Mr. Anthony Benedi, Dr. Walid Heneine, Mr. William Lawrence, Dr. Marian Michaels disclosed no financial interests relevant to today's topics.

The following interest is being made public to allow the meeting participants to objectively evaluate any presentation and/or comments made by Dr. Weiss. Dr. Weiss reported that he received consultant fees from a regulated firm.

In the event that the discussions involve other products or firms not already on the agenda for which FDA participants have a financial interest, the participants are

aware of the need to exclude themselves from such a discussion and this exclusion will be noted on the public record.

A copy of the waivers are available by written request under the Freedom of Information Act. With respect

to all other meeting participants, we ask, in the interest of fairness, that they address any current or previous financial involvement with any firm upon whose product they may wish to comment.

So ends the reading of the word.

Dr. Auchincloss, I turn the microphone over to you.

DR. AUCHINCLOSS: Thank you, Bill.

We are going to begin today's meeting with some opening remarks from Dr. Kathryn Zoon. As you have just heard, Dr. Zoon is the Director of FDA's Center for Biologics Evaluation and Research.

Opening Remarks

DR. ZOON: Good morning and welcome. I would like to particularly extend my thanks to the subcommittee to address this very important issue today and thank you to the public for attending.

Xenotransplantation raises important questions regarding potential public-health benefits and risks of across-species transplantation. The recent reports by

patients et al. in Nature Medicine and Le Tissier in Nature describe the capacity of porcine endogenous retrovirus to infect human cells in vitro.

These reports exemplify the rapidly evolving nature of our understanding of the transmissibility of

infectious agents across species barriers. The dynamic nature of the potential public-health risks and benefits call for the implementation of the Xenotransplantation Advisory Subcommittee constituted with members possessing the expertise to address risk assessment and risk management and calls for continuing public awareness of new scientific data in xenotransplantation.

During the meeting today, the subcommittee is asked to address to following public-health issues concerning xenotransplantation. One is the development of appropriate assays for detection and identification of porcine endogenous retrovirus.

Second, limitations of current screening and diagnostic tools. Third, diagnostic testing and clinical care of patients post-transplant. Fourth, impact of test results on clinical-trial development and, fifth, patient education, informed consent, and eligibility.

The meeting is intended to provide an open public forum for discussion between members of the scientific and biomedical community and to provide advice and

recommendations to the FDA regarding public-health issues raised by xenotransplantation. The FDA and the Center for Biologics, in particular, appreciate the time, the insights and the perspectives of the committee members in addressing these important public-health issues.

At this point, I would like to introduce Deputy Commissioner Mary Pendergast.

MS. PENDERGAST: Good morning. I would like to join Kathy Zoon in welcoming you to this new FDA advisory committee and in thanking you for participating in the committee's work. The FDA cannot do its job of assessing the safety and effectiveness of new therapies without the dedication and commitment of outside experts such as yourselves.

Our need for advise is, perhaps, nowhere greater than in the area of xenotransplantation. Xenotransplantation offers the hope of treatments to many desperately sick persons. Yet, while we are mindful of its promise, we must be equally mindful of its risks and we must think of those risks quite broadly to include not just the patient being treated but the larger society in which that patient lives.

It is never easy to regulate a new technology. Indeed, the FDA has often led a new technology progress for a while before imposing any type of regulations. This

hesitancy is understandable. Many promising new technologies fade from view after a very short time and, for other new technologies, agency staff are hesitant to regulate when very little is known and sensible standards are difficult, if not impossible, to set.

It goes without saying that most physicians and companies would prefer to have as unfettered a field as possible so agency staff know that any efforts to regulate will not be met with universal approval.

These same factors apply here. There was no FDA regulation of the Baby Fay baboon heart transplant or of other xenotransplantation efforts that took place in years past. It is a technology that could have, but did not, fade away. Rather, because of recent advances in immunology, genetics and biochemistry, we are at the cusp of a possible explosion of new xenotransplantation efforts.

Yet, as is painfully evident from the porcine endogenous retrovirus issue you are going to discuss today, the science of xenotransplantation is still evolving and there is much we do not know about the risks from transplantation, thereby making the creating of a regulatory system different.

As usual, there are some who would prefer to have the agency leave their xenotransplantation efforts alone. However, the field will not stand still as we wonder what to

do and we believe that the public will be disadvantaged if we do nothing.

This disadvantage can come in several ways. First, if we are too afraid to regulate and, so, ban the technology outright, thereby depriving patients of possibly

effective therapy. Second, if we do not properly regulate and either personal or public health disasters occur. Third, if we extensively overregulate, thereby unnecessarily slowing down a promising technology.

We need your help in finding a middle path. But recognizing all the while that if the science is not there to let this technology go forward safely, then we have to have a courage to say no.

Our staff took a very important step towards finding that middle path when they spearheaded the efforts to create a public health service guideline on xenotransplantation. It is now our job to build on those efforts.

Because the science an public-health issues concerning xenotransplantation are so complicated, this is a technology where we will expect the very best from industry and physicians. In our jobs, we hear a lot of quibbling. Isn't a small trial sufficient? Do we really have to run so many tests? Does it really matter that we have invalidated this assay?

In this arena, we are going to expect and demand the best. The stakes are too high not to. Similarly, this is not a field where science and technology will be able to go forward behind closed doors. We cannot regulate xenotransplantation without full and open discussion by all

interested parties. And that will require a level of openness about the science and study plans that may be uncomfortable to some.

My advice is if you don't want your work in progress to be publicly discussed and assessed, then you had best find a new field. This is also going to be an area where extensive public discussion and debate will be needed. Patients will want treatments but we also have a duty to the public at large.

We will have to work collectively to figure out new ways to engage the public and get their ideas and concerns on the table as we move forward.

Finally, I think that today's meeting, when we discuss what to do in the face of uncertainty about porcine endogenous retroviruses is only the first of a series of discussions we will need to have as we face the inevitable challenge of creating and regulating science and medicine in the face of uncertainty.

I, for one, am incredibly pleased that we are able to call upon all of you to help us as we think through these

very difficult issues. There is an enormous amount of fire power around this table and it is hard not to be in awe of your expertise in so many scientific disciplines all of which are needed to discuss xenotransplantation issues.

Again, I would like to thank you for your

willingness to help us.

DR. AUCHINCLOSS: Thank you very much.

Open Public Hearing

DR. AUCHINCLOSS: We would now like to move on to the open public hearing. I would like to initiate that by asking Gail Dapolito if she would introduce us to the responses to the Federal Register announcement of this meeting.

MS. DAPOLITO: As part of the FDA Advisory Committee meeting procedure, we hold an open public hearing for those members of the public who are not on the agenda and would like to make a statement concerning matters pending before the committee.

We have two sessions scheduled on today's agenda for public comment, one in the morning and one in the afternoon. In response to the Federal Register announcement regarding this open public hearing, we have received requests for written submissions and requests for oral presentations.

Imuntran Inc. Novartis has provided a written

submission of public comment to the committee and that can be found in your blue folders. We have also received four requests for oral presentations.

Our first public speaker is Dr. Achilles Demetriou from Cedar Sinai Medical Center. Dr. Demetriou would you

please come to the microphone. I would like to remind those individuals who are speaking to please keep your comments to five or six minutes. We will be using the three-light system to help you keep track of your time. Also, we ask, in the interest of fairness, that anyone addressing the committee please state any affiliation that you may have with sponsors and products or competing firms and products under discussion today

Thank you.

DR. DEMETRIOU: Thank you very much and good morning. I am Achilles Demetriou. I am the chairman of the Department of Surgery at Cedars Sinai. I am a professor of surgery at UCLA. I work with Circe Biomedical who is the industry sponsor of the clinical trial that I participate in.

We have developed a bioartificial liver which utilizes porcine hepatocytes and we have used it to treat 37 patients at our institution with severe acute liver failure. What I would like to do is put the clinical situation into perspective and urge the committee to take into

consideration the disease which is being treated and the risks of the disease and assess the risk/benefit, taking that in mind, and recognizing that there may be a real risk with PERV transmission in the clinical setting.

Patients with fulminant liver failure, without

transplants, have a 90 percent mortality. With transplantation, survival is approximately 70 percent. However, 50 percent of patients never make it to transplant. These are patients who are candidates for transplantation but waiting around and they cannot get a liver. So we lose half of our transplant candidates while they are waiting for a liver to treat fulminant liver failure.

90 percent, if they are not candidates for liver transplantation will actually die. So we are dealing with a disease which is certainly lethal. So the theoretical risk, at this point, of PERV side effects or infection in the clinical setting needs to be put in that perspective.

I am not aware of a single clinical instance of transmission of disease from pig to human. There have been over 100 whole-liver perfusions in the literature. Again, there has never been a single report of transmission of the disease.

Secondly, if we are going to regulate future studies using porcine tissue, maybe we need to take a step back and look at the instances where porcine tissues have

been used to treat patients. For example, pig skin has been used for years to treat thousands of patients. This is cryopreserved tissue which is place on top of open wounds with direct contact between cellular elements.

Are we now going to notify patients who have been

treated with porcine skin grafts, thousands of patients over a period of years, or are we going to restrict notification to patients who participate in future studies.

So these are issues that are not simple and I think we need to address. Similarly, slaughterhouse workers who work with porcine tissues; is the USDA going to issue directives that these workers have to be following essentially HIV precautions in that setting. Again, that is something that needs to be considered.

All patients that we have tested that we have treated in our study tested positive for porcine antibodies in their plasma suggesting that there is massive contact, at least, with porcine tissues. So porcine tissues are all around us in things we eat and in things we touch or come into daily contact. Therefore, I think we need to put this whole thing in perspective.

Finally, gene-therapy trials are using retroviruses in which we deliberately utilize a retrovirus to correct a specific genetic defect of treated disease. Are we now going to expand the warnings to include

retroviral use in gene-therapy trials?

This is certainly a very complex problem. I think our major responsibility is to notify our patients regarding the risks of PERV transmission. I think patients and families need to have informed consent when they participate

in the studies.

I think the papers in Nature and Nature Medicine are important because they outline the risks and we need to follow it and we need to inform our patients and their families. However, that risk needs to be placed in perspective considering the very high morbidity and mortality of the disease that we are trying to treat.

Thank you very much.

MS. DAPOLITO: Thank you, Dr. Demetriou.

Our next speaker will be Dr. Barbara Potts from Tektagen.

DR. POTTS: Good morning. Tektagen is a contract service lab and we do do work for companies that have xenotransplant products.

[Slide.]

The list of animal retroviruses that may pose a health hazard to humans is really quite lengthy. I have just organized them here for you according to their morphology and family.

[Slide.]

The way to detect these viruses in humans, we need to amplify the virus. It is either by PCR or cocultivation. I am going to approach the cultivation assay and the possibilities and limitations of this system today detecting it in human PBLs. That is going to be my focus. I am

detecting the virus either by a reverse-transcriptase assay using either magnesium or manganese, which I am going to Mg and Mn from now on because I get them mixed up and also antigen-capture ELISA assay.

Just to look quickly at this, this is an assay that was developed by Michael Martin at the NIH because it gave us a high signal and a low noise level for HIV and, also, for Mn, there is a high signal and a low noise and fairly good reproducibility.

For HIV, we know that two times this negative control was ten infectious units and I know that two times this negative control for simian foamy virus is 20 plaque-forming units. So I set two times the negative controls as the arbitrary cutoff on this assay. It is very conservative.

[Slide.]

I am just going to march through the alphabet in these viruses real quickly. Porcine endogenous retroviruses are in all of the tissues, in porcine tissues. This is just a titration of the cells times 103 versus counts--this is a

P32 RT assay. You can see that for the fetal porcine test that there is a nice dose response.

At PK15, there is a nice dose response. Minipig kidney is a little bit lower and there is a nice dose response. If these cells are frozen and thawed more than

three times, this goes flat. So it is very unstable.

However, the supernatant, especially in the minipig soup, remains stable after many freeze-thaws. If we look at primary culture, we have 107 here porcine PBLs. We have about 800 counts. Again, this goes flat after three or four freeze thaws and porcine hepatocytes, really, this, perhaps, is noise.

Again this negative control cutoff is really arbitrary. It hasn't been established for this system. This is using Mn in an RT assay.

[Slide.]

If we go to Mg, you see that almost all of them are negative. However, what we have here is we have this really high signal in the minipig soup.

I have repeated, I might add, that all of these tests are a replicate of eight separate assays done by two analysts on at least three different days. This, I did ten separate assays because I didn't believe it but this is real. There is definitely an Mg signal coming out of the minipig soup and it is stable after many freeze-thaws.

I would like to hear some comments about that later. Again, you have your porcine hepatocytes are just really bouncing around here with the noise. Here you have your porcine PBLs.

[Slide.]

Just quickly mention the HTL-V group. There is P19 antigen ELISA that works for both PLV and STLV. I just wanted to point out this nice low noise and the nice dose response for P12 versus cell concentration.

[Slide.]

For the type D's, we have a good RT signal here, in negatives, and it is very low.

[Slide.]

I will just quickly go through this. The lentiviruses are all really very closely related in their RT and, consequently, we were unable to detect all of these viruses. Using these as positive controls, we were able to detect all of these lentiviruses in a fairly good reverse-transcriptase assay.

I wanted to present this first so that you believe that I can grow these viruses and detect them.

[Slide.]

The negative controls are nice and quiet.

[Slide.]

Simian foamys have nice signal, a little bit of

noise with the M. dunnis here but that is the murine retrovirus. But still we have a good signal here.

[Slide.]

So the real key question is can any of these animal retroviruses replicate and produce infectious progeny

in human PBMCs. The assay system, we used both select and random human PBMC donors because we know that that makes a difference for HIV-1 and HTLV-1.

I enriched for monocyte macrophages because some of these viruses replicate exclusively in monocyte macrophages and PHA stimulate for three days, maintained and IL-2. And virus infection was either by inoculation or by cocultivation with gamma-irradiated virus-infected cells. This was 10,000 rads.

Additional PBMCs were added at day 13. Cultures were maintained, splitting feeding two to three times a week for 20 days for inoculation, 30 days for cocultivation. Cells and supernatant were harvested at different times. I am going to show you the data from 20 days for the infection and 30 days for the cocultivation.

Samples were frozen and thawed only once in either an Mg or an MNRT assay were run on them.

[Slide.]

So the bottom line is that all of our positive controls replicated nicely. These are logs, TDIC 50 per ml,

and all of these were negative in both systems. The BIV, I saw a lot of CPE. It either may be non-protective infection or it may be another virus. Just because I think it is BIV, there may be something else in there but there is definitely CPE.

[Slide.]

Probably the most important is if you look at the simian foamy viruses, they all replicated to greater than 2 logs in the human PBMCs. I took this culture, then, back and titered it back into M. dunnis, which is the parental cell-line that I used to grow the simians, and I had 20 plaque-forming units.

So even though the RT was very low, we definitely could detect the virus. So this, I think, is a good circular result because we know that simian foamys can infect humans. Now we know they can infect PBLs at a very low level.

Also, BLV and STLV, I did detect transformation. I won't present the P19 antigen ELISA data today. All of the PERV are irradiated to PK15 cells, minipig cells, STs. And the porcine PBLs were all negative at 30 days.

Thank you.

MS. DAPOLITO: Thank you, Dr. Potts.

Our next speaker for open public comment was Dr. Bob Brown, University of North Carolina, Chapel Hill. Is

Dr. Brown here? I guess he didn't show up today.

We have a speaker who was scheduled for this afternoon. I wonder if Dr. Savill--are you prepared for this morning. Thank you. Dr. Corinne Savill from Imutran.

DR. SAVILL: Good morning.

[Slide.]

What I am going to try and address is one of the subcommittee's remits which is the impact of diagnostic screening on clinical-trial development in xenotransplantation.

[Slide.]

In order that the undoubted benefits of xenotransplantation can be weighed against the risk of retrovirus infection, we are proposing a risk analysis of this infection in the context of limited, closely monitored, clinical trials of xenotransplantation.

The risk assessment falls into three categories: firstly, to look at and characterize the expression and the release of the virus from our herd of transgenic pigs; secondly, to determine the infectious status of human recipients who have been subjected, as we have just heard, to treatment with living porcine tissue; and, thirdly, to look at our archive of primates who have been treated with solid-organ xenografts, either hearts or kidneys.

[Slide.]

Looking at the expression of the retrovirus in our herd of pigs, we are characterizing the variance which were actually released from our pig herd in terms of the tropism of each variant, whether it is infected for human cells of whether it is a pig-to-pig species to determine which type

of cell in these pigs expresses the retrovirus and, perhaps, most importantly, determining whether PERV-containing virions are released from cells from the organs both pre- and post-transplant into non-human primates.

If virions are detected, we will also look to determine whether they contain xenotropic variants of the retrovirus.

[Slide.]

As has been said already, living pig tissue has been used to treat human disease. We are looking into this and we have found many more than 100 patients already worldwide have been exposed to living porcine tissue. We are in the process of sampling these patients, collecting blood and cells for analysis and testing for potential PERV transmission, looking at the DNA level, the RNA level by RT-PCR on plasma and also testing to see whether these patients have made an anti-PERV-specific immune response.

The numbers in the brackets indicate the number of patients so far that we have identified and are in the process of drawing blood from.

[Slide.]

We now have an archive of well over 200 non-human primates who have received a solid-organ xenograft. We are testing those primate recipients by PCR for PERV and, if positive, we will have to look and determine whether this

represents a true infection or whether the test is picking up potential contamination by pig cells in microchimerism in these primates.

We will look by RT-PCR, if we find any positive samples, and we will screen all the primates for an anti-PERV antibody response.

[Slide.]

I think it is important to consider, and it may be something that is discussed later today, that not all xenotransplantation approaches and not all clinical trials may be equivalent in terms of retroviral risk. One should look at the length of exposure, the presence and the nature of any immunosuppression, the type of tissue, whether or not that tissue is encapsulated, for instance, and the number of patients who are proposed to be treated and, of course, distinguish between risk to individual patient and that which may present a public-health hazard.

[Slide.]

In our estimation, the studies that we are performing which will be key for the initiation of

extracorporeal trials, is the retrospective study of patients who have already been treated in similar manners with living porcine tissue and also looking to see, in our pigs, whether we actually see virion release.

In our opinion, if we detect positive PERV genome

in human cells in these patients or if we detect virion release containing xenotropic variants of the PERV retrovirus, we would consider that this is important to think again about our approach to this trial and to perform further risk analysis.

[Slide.]

When one considers the initial trials closely monitored of solid-organ xenotransplants in addition to the two studies I just stated, also looking at our archive with xenografted non-human primates is important to be considered. Again, we feel that if we detect positive-PERV genome in the primate cells, so true transmission of the infection, we would do more work and restudy these archives before we would actually approach a trial in humans.

[Slide.]

We also have a longer-term approach under way. We are characterizing the loci of the retrovirus in transgenic animals and mapping those loci which are associated with the production of the xenotropic virus. By carrying out this work, one will be able to analyze and make an assessment of

whether such approaches to either breed out or, if possible, if the technology advances, to genetically knock out the virus from such animals is technically feasible.

[Slide.]

So, overall, in summary, moving towards more

extensive trials in launch, we would propose a stepwise and cautious approach to the development, to take each step as it comes and with a safety follow-up of initial trials before proceeding.

This will enable us, and anybody else working in the field, to actually gain an increased understanding of the PERV infection as one proceeds towards a more extensive development of this technology and, also, in parallel, to work on longer-term approaches toward selective breeding or genetic knockout of the xenotropic form of the virus.

Thank you.

MS. DAPOLITO: Dr. Savill, for the public record, would you please state your affiliation.

DR. SAVILL: Sorry. Imutran Limited Novartis Pharma.

DR. DAPOLITO: Thank you.

At this time, I would like to ask if there is anyone else in the audience who would like to make a public statement to the committee. If you would, please raise your hand. I see no one.

Dr. Auchincloss, I turn it over to you.

DR. AUCHINCLOSS: Thank you very much.

We will move now to the open committee discussion. In preparation for doing that, I would like to introduce several specially invited guests of the Xenotransplantation

Subcommittee. These guests are Dr. Marian Michaels, Mr. Antonio Benedi, Mr. William Lawrence and Dr. Robin Weiss.

At this time, I would like to invite them to the table to participate in the day's discussions.

The next item on the agenda is the Chairman's introductory remarks, which I promise to keep very brief.

Introduction of Focus Groups and Presentation of Questions for Committee Discussion

DR. AUCHINCLOSS: First of all, I am not an expert on today's subject. I think I know a fair amount about xenotransplantation but I am not a virologist and, therefore, am here to learn about a subject that I do think is of enormous importance, of such importance that I really want to stress how much I appreciate the members of the subcommittee and the effort they have made to come here.

I know many of you have traveled long distances and, for every one of you, it is an inconvenience, at least, and we really appreciate it. I also want to call attention and offer our thanks to a number of people from the FDA. In particular, I think it is the drive and energy of Amy

Patterson who has kept this going, the FDA's effort, really, to grapple with the problems presented by xenotransplantation.

She is quite an extraordinary woman. They tell me that she puts together these reading books for us in just an

evening's worth of work, which is a terrifying thought because it is hardly an evening's worth of work to go through them.

But it is a tremendous effort that you have made and we really do appreciate it, Amy.

There are three other people who, every time we come to the FDA, make our lives so much easier including Bill Freas and Gail and Rosanna Harvey, and we really do appreciate, again, the efforts that you make on our behalf.

You understand, then, the content of my introduction otherwise is simply to introduce the fact that there will be questions that will be addressed to this committee during the course of the afternoon after hearing some data and presentations during the morning.

In essence, and I am not going to go through these questions in detail now, those questions have to do with first developing the assays for detection of porcine endogenous retrovirus; second, what kind of screening for patients is appropriate; and, third, questions related to the aspect of informed consent and selection of patients.

What I do want to do at this point is to simply highlight a process that we have used in preparation for this meeting and that is that members of the subcommittee and, in addition, other participants in this group have been assigned to "focus groups" in order to have a preliminary

discussion on each of these questions prior to this meeting.

So that has happened and you will hear brief presentations this afternoon from these focus groups. What I want to emphasize, however, about that process is that it was not designed to limit discussion; it was designed to initiate discussion. Whether you are a member of a particular focus group or not, we still invite every member of the committee to offer their views on every subject that we are discussing this afternoon and it is very important that we understand that process.

If I have not forgotten anything else, I think it is time to move on with the morning's agenda with an initial presentation from John Coffin on endogenous retroviruses providing us with the overview.

Endogenous Retroviruses: an Overview

DR. COFFIN: Thank you very much. I am very pleased and honored by the invitation to give this talk on this important subject. A little personal note; the study of endogenous retroviruses has been, really, a fairly major aspect of my career for the last 25 years and actually of a

few other people at the table as well, that was undertaken because it is really a fascinating aspect of how retroviruses interact with their host and provide some very interesting models for disease processes and, also, for understanding host virus evolution.

When I started off doing it, and I never imagined that we would be discussing these in the context of real applications and potential risks.

That said, let me move on to the first slide.

[Slide.]

The focus of the whole meeting today really is the risk that endogenous pig viruses might present in the context of essentially cocultivation of human pig tissues that occurs during xenotransplant procedures.

Let me just set the context of that quickly and then I will get on to talk about endogenous viruses in general and give some examples of things that we have uncovered from our experience, particularly with the mouse endogenous viruses.

I come from the position that at least some kind of infection of the recipient of a xenotransplant, albeit limited, perhaps, to some very small numbers of cells, is virtually an inevitable consequence of introducing tissue from any species that can, potentially, express infectious endogenous viruses into humans.

There is precedent for that based on experiments where human tissues have been transplanted into mice and where mice are capable of expression endogenous viruses, although they don't. But if those viruses can infect human tissue, then, in fact, very often, the tumor that comes out

of new mice that are transplanted in this way ends up producing infectious virus.

When I had my hat as chair of the IBC at Tufts, several times people came running around to me with human tumors that were producing these large numbers of tissues or human cell lines, and my first question was has it ever passed through a nude mouse.

The answer always is yes, actually, when you see that. So it is a very famous phenomenon that actually Robin Weiss wrote about very nicely in an earlier version of the retrovirus book.

[Slide.]

Retroviruses are really remarkable in their ability to form a permanent genetic association with their host. This is the only infectious agent of higher organisms that we know that is capable of doing this. Over the years, over our evolutionary history, our germ lines and that of all our mammalian, avian and probably other vertebrate relatives as well, have been under fairly constant assault by infection with these agents.

In fact, it is amazing that we tolerate that. A substantial fraction of our DNA, a half percent or more, thousands of elements, has come from infection in the more or less distant past of germ-line tissue.

Endogenous retroviruses form several distantly

related groups, as I will show you in a minute. About half of the extant retrovirus groups have been know to leave endogenous rows in their germ line and most groups are almost certainly derived--if you look at the patterns that these are in in species--from exogenous viruses that have infected the same species.

So endogenous viruses are not a precursor to the infectious retroviruses that we see. Perhaps, there are some elements that are such precursors, but all of the ones that we can see almost certainly are the consequence of infection of the germ line with infectious retroviruses and can often be induced.

A useful distinction is whether the endogenous viruses were recently introduced into the germ line or, in fact, entered there in the very distant past. Humans have, as I said, thousands of elements. But, as far as we know, every single one was introduced more than a million years ago.

So there is no genetic polymorphism among humans in any of these elements that have been discovered.

Furthermore, their infectivity and their ability to even make particles, in most instances, has been severely eroded just by the accumulation of random mutations in the absence of any counterselection over the evolutionary history.

[Slide.]

As I said before, this slide shows sort of what we now take as the correct retrovirus taxonomy or at least the current retrovirus taxonomy. Of the seven groups of virus genera that are defined here, four of these have been known to leave endogenous relatives in the germ line.

The one that is of the most concern today is the probably the largest of these that is in the most species, and these are the MLV-related or C-type retroviruses, mammalian C-type retroviruses.

These are all viruses that are fairly closely related in sequence to murine leukemia virus and much more closely so than they are to retroviruses from any other group. This taxonomy is based on sequence relationships of reverse-transcriptase.

[Slide.]

So, over the years, endogenous retroviruses have been associated with quite a number of rather interesting and famous genetic phenomena. I will just list a few here just to get us into tune of what we are talking about.

There is the famous human rumor viruses. Again,

it is very nicely defined by Robin in his chapter about fifteen years ago, often due to expression of defective human proviruses that are endogenous to cells, although no infectious agent and no definitive genetic association of any of these with actual tumor causality has ever been

established and these are probably sort of epiphenomena.

Endogenous retroviruses can recombine with infecting exogenous viruses to create new antigens, and the famous feline virus cell-membrane antigen is an example of that. That appeared in the study of cats some years ago.

Endogenous proviruses can often masquerade as developmental or strain-specific antigens. These are some famous examples in mice. Some endogenous viruses, although they not the ones we are most interested in, encode superantigens which are necessary for the efficient replication of the exogenous virus in the immune system.

This is the case of mammary-tumor virus. These superantigens, when expressed in endogenous viruses, actually inhibit the replication of the corresponding endogenous virus and probably make the animal somewhat resistant.

Endogenous viruses are actually a problem in gene therapy because they can be very efficiently transmitted with retroviral vectors. In fact, a few studies have shown that there are often more of these VL30s or MLV-related

sequences in vector preparations than there are of your vector. These can be very efficiently transmitted, and are not a matter of our concern today, but are something that should be kept in mind in gene-therapy applications.

They can repair defects by recombination with

vectors or other species or other defective infecting viruses and a lot of replication. They have come to the attention of the FDA before in the context of monoclonal antibodies and other types of biological preparations as possible contaminants.

I mentioned before the phenomenon where human tumor cells, grown in nude mice, very frequently come back producing very large amounts of virus. This is not a subtle phenomenon. If you take a thin section of these cells, these cells are loaded with virus.

Then the question at hand is can these be transmitted to xenotransplant recipients and, if so, of course, what are the consequences.

[Slide.]

I want to focus, now, on what I would like to refer to as the recent endogenous proviruses. These are ones that have been put into the species, into the germ line, of some individuals in a species subsequent to speciation. They are not found in all species. We know of none in humans, for example.

They are, however, common in some birds but not other closely related birds. The distribution of these is taxonomically erratic suggesting that this is a horizontal transmission effect. They are found in most strains of mice and most subspecies of wild mice. They are found in cats

but not dogs, as far as we know. And they can be found in some primates such as baboons and also in other animals, of course, such as pigs.

These are inserted recently. So if you look from one individual to another, there is polymorphism in the genetic location although different individuals and different locations can be very, very similar to one another.

Different proviruses at different sites can be more than 99 percent identical to one another. Many of these can be replication-competent or potentially replication-competent if they are allowed to replicate in an appropriate environment and if they are induced by some inducing agent. Endogenous proviruses are generally expressed at a very low level because of germ-line methylation but can be efficiently induced by agents that reverse DNA methylation.

It actually might be worth including this sort of a treatment in protocols to try to viruses out to give one the best chance in vitro to see what is there.

[Slide.]

So the recent endogenous proviruses of mice--mice, like all other species, also have a load of old ones that are like the human ones that I have mentioned that are largely defective. The recent ones include a fairly large

group of C-type viruses of which, per animal or inbred strain, there are about 40 to 60 members; the B-type viruses, the mammary-tumor virus, which have just a few members; and the A-type sequences which are not necessarily viruses in that there is no evidence that these get out and infect cells, but they are closely related to some groups of retroviruses and, genetically, it has been shown they can move around by a mechanism resembling retrovirus infection.

The numbers of these can be quite large, up to 1,000 proviruses in a given strain, and so the genetics of this, not surprisingly, has not been as well worked out as it has in some of these other groups.

[Slide.]

I want to focus the attention now on the murine leukemia virus related, the C-type ones, of which there are about 50 to 60 members, or 40 to 60, I guess, in a given inbred strain. Some years ago, Jonathan Stoye, when he was a post-doc in my lab, discovered that one can conveniently divide these into four classes based on what a really subtle sequence difference is and also differences particularly

focus on the enveloped gene which provide these viruses with potentially different host ranges, meaning they use different receptors.

A small class are the so-called ecotropic viruses which can use a receptor that is limited to mouse cells.

Then the larger groups are these others which use a receptor--actually, they use what is probably the same receptor on different species, but the receptor, itself, has been altered in mice so that this particular class of proviruses, the xenotropic proviruses, although it is present in mice, can no longer infect the species that it is found in.

This is almost certainly an adaption of the species to having this provirus, to protecting it from infection. In term, probably, we visualize the evolution. Some descendants of these viruses probably, then, evolved an ability to use now the receptor as it has been modified genetically in mice.

So these now can see the receptor in mice and in other species. These are limited to the receptor as it is found in other species but not in mice. Unfortunately, we don't have this receptor cloned yet so we can't flesh this speculation out with some actual hard data.

There are, also, some restriction-site polymorphisms and some sequence polymorphisms which allow

the synthesis of small simple oligonucleotide probes which allow these all to be distinguished in simple southern blotting or in PCR experiments.

So, using southern blotting with these sequence probes, one can identify the provirus composition of a given

animal or a given strain of animal.

[Slide.]

This is just the seven most popular strains of inbred mice probed with each one of these different probes. Each of these detects a unique set. There is no overlap between these maps. What these bands are junction fragments composed of virus sequence joined to cell sequence.

Almost all the viruses have the restriction enzymes that are used here.

We have to go back, actually.

[Slide.]

They have, say, this echo-R1 site in common. What makes the fragment different is the appearance of the next echo-R1 out here. So these detect proviruses not by their internal sequence but by their location in the genome. This is a very important point that people often still don't quite follow with these.

[Slide.]

So each of these represents the insertion of a provirus at a specific point and some ancestor of the

particular animal that you are looking at here. If you extend this analysis to about fifteen different inbred strains, what you actually find is the polymorphism, which is obvious here, is such that there is only one provirus, in fact, that is present in all inbred strains of mice.

So it would, in fact, at least in theory, be possible to design a series of genetic crosses which take out of mice--all of these, despite the fact that any given strain, if you add up all of these lanes, you have, as I say, 40 to 60 proviruses--it would, at least in theory, to design a series of crosses that removed all but one of them from mice.

It would take quite a long time and some fancy genetics, but at least it is a theoretical possibility.

Each of these proviruses, as I say, has a certain sequence that defines it and each of them probably is associated with certain kinds of biological properties. We don't know, for the most part, what they are.

Only a few of these, particularly one of the XMVs, are known to give rise to infectious virus and we don't know whether that is because a very large number of these are inherently defective or because they have some other properties where they simply don't replicate well in the host cell.

This really hasn't been investigated very well.

There has been very little work of cloning full-length proviruses and testing them for infectivity and things like that.

It is known, however, and I will come back to this, that if you take a virus that is of a type I haven't

shown here, an ecotropic virus, and the mouse expresses that, in the course of replication in the lifetime of an animal, you can get a series of recombination events which join sequences from these various elements and lead to a pathogenic virus.

[Slide.]

These proviruses are widely distributed. This actually doesn't even show all of the proviruses. It just shows the proviruses that different between two strains of mice, and you can see, however, they provide markers and they were actually useful for genetic purposes for this for a while--that are distributed across the entire mouse genome.

As far as we can tell, they are randomly distributed. There is a noteworthy cluster here near the FV1 locus, but we don't know the significance of that. No test for significant clustering has ever yielded anything with any statistical value.

So these are randomly distributed in the provirus. This may or may not reflect what really goes on in real-life

species because inbred mice are somewhat special. Inbred mice are derived by taking mice that have evolved somewhat independently and then recombining them.

So one of the things about these individual proviruses is that probably, individually, they are fairly

benign to the animal but, by bringing strains of mice that evolved independently back together again, we set up situations where you get pathogenic interactions that may not occur in the wild.

We don't know that for sure. In fact, we presently have a study going on where we are looking at the genetics of proviruses in wild strains of mice.

[Slide.]

As I said, endogenous proviruses probably often evolve in the direction of being fairly benign to their host. But, even in the mouse, there are several tumor pathogenic models that have been pretty well studied. The first deals with the fact that the insertion of a provirus into a particular locus is a genetic event that sometimes leads to a detectable mutation.

Several known mouse mutations were found to be due to the insertion of endogenous proviruses. Particularly these coat mutations, hairless and dilute, were caused originally by the insertion of a provirus into the gene and disrupting, in some way, the activity of that gene.

The expression of endogenous proviruses can also give rise to specific effects. I mentioned the superantigens before which lead to the deletion of specific subsets. Expression or replication of endogenous proviruses can give rise to tumors as in the model of the Akr mouse.

The Akr mouse was originally bred as a mouse that had a high incidence of thymic lymphoma at a fairly reproducible age. When that was studied in detail, it was found that this mouse, what one had selected for in generating this strain of mouse was a pattern of proviruses which gave rise to these so-called mink-cell focus-forming, or MCF, recombinant viruses that arise.

As a regular evolutionary event, and I will show you this in a second, in every single one of these mice, virtually in lock-step, you have this evolution of viruses that involves at least four genetic changes to the virus plus that insertion of the proviruses next to the "myc" gene and a few other genes in these cells.

Similarly, the mammary-tumor virus which doesn't need to undergo this kind of complicated recombination events but can, in some strains of mice, be expressed, infect mammary epithelium and give rise to mammary carcinoma in a fairly high frequency in certain strains of mice.

Then, as I said, there is the genetic alterative of provirus that goes on in the course of these events.

[Slide.]

This just shows an example of what the recombination events in the course of this evolution that occurs of viruses in mice. As I say, this occurs in every single Akr mouse virtually in lock-step during the early

life of this mouse, shortly after birth.

You can get, first, the expression and infection of mouse tissue with an ecotropic provirus of which there are only a couple in the mouse that is capable of giving rise to a full-length infectious virus and that, then, spreads around the animal which becomes viremic.

It is noteworthy, by the way, that the mouse, of course, inherits these proviruses and these proviruses are capable of giving rise to a virus which will affect, probably, a large fraction of the tissue of the mouse but yet you don't actually see that happening until about the time that the mouse is born.

In cell cultures from embryos, it takes a while even for cell cultures to start to show this virus. That indicates that the methylation that holds the virus in check is actually pretty tight because if one cell expressed a lot of virus, you would imagine that the whole animal would get infected.

But it doesn't happen for a while. But once that appears, you again get a rather rapid series of events in

which you acquire first either a new enveloped gene or partial enveloped gene or new LTR. This gives the virus, probably, a unique ability both to replicate in thymus tissue and to give some stimulation to the tissue.

This enveloped gene actually has site-stimulatory

effect on cell replication. You then get a reduplication event where the enhancer sequence is actually reduplicated or triplicated in some cases. Then, finally, you see proviruses that are integrated next to the proto-oncogene.

I will give you the source for this picture right at the very end.

[Slide.]

In addition to causing diseases, the integration of genetic virus, as I said, can have genetic effects. I think I have probably covered most of these already. You have the insertional inactivation of genes.

You actually can have insertional activation of expression. A famous case, one of my favorite endogenous provirus stories, and it is not really relevant, but salivary amylase in humans--the expression of amylase in the salivary glands in humans is driven by an endogenous virus LTR which is why starches, of course, taste slightly sweet to us and may well have had something to do with our cultural evolution. Most other species do not have that.

You can get, as I said, spontaneous malignancy due

to virus expression and replication. You can new antigens and superantigens. Endogenous viruses actually also provide very useful genetic markers and, also, markers for evolution. That is another thing we are doing in the lab.

[Slide.]

So, to get back closer to the chase here, one of the potential hazards of endogenous viruses in xenotransplantation--this is my own personal view, now, of course and this will be a lot of the focus of what the discussion will be later on, but I think it is virtually certain that at least some recipient cells will become infected in some significant fraction of patients.

This could be a very limited infection where you just get a few cells. It is likely that an immunocompetent recipient will reject the virus and the infection will go no further in many cases. In immunosuppressed individuals, or perhaps even in some competent individuals, one might get a spreading infection that, again, might or might not be resolved after immune restoration, the infection could, conceivably, lead to--malignancy is the most likely outcome because models of immunosuppression and other diseases really require extensive virus replication for long periods of time.

So that is a lower-order event whereas in models where you get malignancy, you have an infection early on.

Even if the virus is cleared, you can still have cells which have an inactivation that are waiting around. So this can occur long after signs of the virus are no longer present, at least in theory.

I think recombination--although the issue of

recombination of human endogenous viruses is often raised in this, I think it unimportant in the sense that, in the first place, it is likely to be a secondary issue. The important issue here is whether infection is occurring.

If infection is occurring, then recombination is actually a pathogenic mechanism. So one wants to know, A, is infection occurring and B, is there pathogenesis. If there is, then you can go back and ask about mechanism. This is a mechanistic issue. It is not an issue which should be right at the top of our thinking.

Furthermore, actually the recombination events I showed you in the mice are not essential in other animals for these things to occur. You can accomplish some of the same kinds of things, this is with point mutations of the virus, if the recombination partners are not available.

Of course, the thing that everybody really worries about is transmission of the virus particularly to contacts and so immunocompetent individuals. This is a very, I would argue, unlikely outcome but it is obviously where much of our thinking and discussion should focus.

Finally, I want to change the subject a little bit for those of you who are interested in learning more about this, and many of you asked me this question. I want to point out that there new Cold Spring Harbor book on retroviruses is actually coming out. It is being printed

right now. The first copies will be mailed out around the first of the year. This is really happening for those of you that have wondered about this over the last how many years.

Thank you very much.

DR. AUCHINCLOSS: We do have time later in the morning before lunch for a general discussion of the morning's presentations, but I would be perfectly willing to take a few questions at this point, if you like.

DR. HIRSCH: John, can you just briefly go over the history of transmission and disease production with either polytropic or xenotropic viruses from species to species? What is the example when these have been intentionally transmitted, say, a mouse virus to the pig or whatever.

DR. COFFIN: There has been relatively little work, actually, on that done of intentional infection. Maybe Robin has more insights into that than I do. I know Robin, some years ago, performed experiences with endogenous avian viruses where, in fact, he could show some

pathogenicity in other species.

I can't think of any examples where introduction of endogenous, say, mouse virus into other species--of the straight endogenous viruses, not necessarily recombinant virus, has actually given rise to any kind of pathogenesis.

But maybe I am blanking on something I should remember.

DR. WEISS: I agree with John Coffin. There is very little data on cross species, pathogenicity of pure endogenous viruses. But we do know that viruses such as mouse leukemia virus will cause disease in other animals. We had an example of this fairly recently in that replication-competent recombinant virus that emerged from a retroviral vector being tested for gene therapy delivery gave use to leukemia in monkeys.

Not only that, it happened to transfer some endogenous AL30-like elements, too. So I think if the virus grows in a foreign species to a high enough load, then there is no particular protection of that species.

The gibbonate leukemia virus which is reported as a natural virus of gibbons also has a recent mouse origin. The transfer probably occurred in recent historical times in gibbons in captivity. Gibbons are apes and it causes leukemia because it grows to a high viral load in those animals.

So I think there is no question that these types

of the C-type retrovirus that could emerge from rodents or other animals can be pathogenic in primates if they take off and become a rip-roaring infection in primates.

DR. ONIONS: I concur with Robin's last comment. There are data from another model in the cat leukemia virus

which is actually a naturally transmitted virus from cats to cats. These actually shed viruses in their saliva that are infectious both for canine and for human cells.

There is no evidence that these viruses, nevertheless, have infected either people or dogs. But there is a difference. If you take that virus that is capable of infecting canine cells and inject it parentally into newborn dogs that are also immunosuppressed, it is capable of causing disease.

I think this is important to bear in context of the route of transmission is important and reflects the comments that I think came from the floor earlier that one should be cautious of drawing analogies that we have been exposed to, to pig tissue in other ways, just in, say, slaughterhouses and so on, that this is a group that would be at risk.

That is not necessarily so. There is a vast deal of difference between casual exposure and true parental delivery of these viruses.

DR. COFFIN: FLV is one of the very few viruses of

this type for which there is a good model for horizontal transmission. But all of these viruses can be transmitted vertically from mother to offspring with some efficiency.

DR. AUCHINCLOSS: Any more questions?

DR. MICHAELS: Dr. Onions, is there any data,

then, or has it been looked at to see whether those newborn dogs which are now infected could then transmit from more casual types of contact with other dogs?

DR. ONIONS: These experiments were conducted in the 1970s by Rikard here in Cornell. If I remember correctly, the interesting feature was this was really before we had good molecular tools.

If I remember correctly, these dogs did not become viremic. Some of them developed tumors but most of them eventually became latently infected and there was no evidence--I can't be sure but I don't believe there was a tracer dog in there to check for horizontal transmission. But, certainly, these dogs were not shedding virus in their saliva.

DR. SALOMON: I apologize if I got this wrong, but you suggested that in the human that there was no evidence of recent endogenous viral integration which raises a very interesting point, then. Are you saying that something that seems to have been a very powerful evolutionary mechanism going on for millions of years for some reason, now, we have

reached some point in evolution in the human species that this is not happening anymore?

DR. COFFIN: I don't view it that way. There are many bird species for which there is also no evidence for recent endogenous virus infection. I view it as being more

a chance thing that the appearance of endogenous viruses in the germ line depends on an endemic infection in the species. That has occurred in some species, as in mice, for example, which probably do transmit exogenous virus around in the wild even now although we don't know for sure that is true.

DR. SALOMON: So you don't believe that would be an argument, then, that we would be more resistant?

DR. COFFIN: No; I don't think it is justifiable at all to argue from the absence of these infections. Human cells can be infected with viruses of this kind perfectly well if they have the right receptor utilization.

DR. AUCHINCLOSS: Thank you very much. We will have a chance, again, to return for discussion on these overall issues for the morning.

I would now like to ask Dr. Carolyn Wilson from CBER to talk to us about data that she has demonstrating the capacity of the porcine endogenous retrovirus to infect human cells.

Data Demonstrating Capacity of Porcine Endogenous Retrovirus

(PERV) to Infect Human Cells

DR. WILSON: Thank you. I also want to thank Amy Patterson for providing the opportunity for me to present some of the data that we have generated here at CBER on porcine endogenous retrovirus specifically.

[Slide.]

When we first started thinking about what the safety concerns are in the use of porcine xenografts regarding the presence of porcine endogenous retrovirus, it was already well known at that time that these agents existed, that they were capable of coding infectious retroviruses.

Therefore, because, as John already pointed out, this is an endogenous element that is integrated into the genome of the pigs that there is a potential to generate infectious porcine endogenous retrovirus in all pig cells.

What wasn't know was what conditions are required for activation of this virus.

[Slide.]

The approach that we took is outlined on this slide. This has been in collaboration with a number of investigators at CBER. What we decided to do was look in primary cells. We looked a peripheral blood mononuclear cells from two different breeds. I will be talking only about the NIH minipig studies today.

We mitogenically stimulated these cells and then looked for activation of viral expression as measured by the retroviral enzyme reverse transcriptase, abbreviated RT.

To then correlate any RT activity with the presence of an infectious virus, we then did a coculture

assay with different target cells and then looked in these target cells again for RT activity as a measure of spreading productive infection as well as a more sensitive and specific RT-PCR assay which we have developed for detecting porcine endogenous retrovirus.

[Slide.]

These are the activation results which we found. These are NIH minipig PBMCs which we cultured either with PHA and PMA, shown in blue, or PMA and a calcium ionophore, shown in red. On the Y axis is tritiated thymidine incorporation. This is a measure of reverse-transcriptase activity.

As you can see, at five days there is a clear peak of RT activity but this is transient. By day 8, it returns to baseline levels.

[Slide.]

In the coculture experience that we did, we directly exposed a pig cell line, ST, which is swine testis, and a human cell line, 293, which is human embryonic kidney cells, to either live PBMCs--again, these are NIH minipigs

which have been activated, mitogenically activated, or irradiated PBMCs.

In either case, we were able to see an increase in RT activity in the ST cells after about a two to three-week lag time. It took a bit longer going out to more like 40

days before we saw an increase in RT activity.

Actually, it is not shown in this slide, but if we go out to day 56, RT activity is as high as it is in ST cells. If we look at earlier timepoints with more sensitive RT-PCR assay, these are the results we have found in the human 293 cells.

At these early timepoints where we don't see detectable RT activity, we clearly can see evidence for transfer and expression of the retrovirus.

[Slide.]

So, because of the delay or the difference in the time course in the ST and 293 cells after exposure to PBMCs, we wanted to look at whether or not this represented different populations of virus that were being activated out of the primary cells. So the strategy which we have developed to study that question is, again--this is what I have already shown you--mitogenic stimulation of the porcine PBMCs, exposure to either a pig-cell line or a human-cell line.

These two cell lines are then used as chronic

producers of the virus--they are called here ST/NIH and 293/NIH since they are from NIH. They have virus in the mice later from NIH minipig cells--and then going back and reexposing ST and 293 cells--this is by a coculture assay using irradiated, lethally irradiated, virus producer cells-

-and then looking at the time course of viral infection by RT assay. So we are looking at the spread of virus in these cultures.

[Slide.]

On the left side are the results that we found when we used the pig cell line, ST/NIH, as the source of chronic virus producer. As you can see, by two weeks-- again, this is tritiated thymidine incorporation on the Y axis, we see evidence for a very productive infection. It is pretty much maximal output by two weeks in the pig cell line.

Human cells become infected but the kinetics is somewhat delayed. 293 cells, when we use human cells as a source of virus, shown in the right panel, human cells become infected and, although it looks like this is more rapid, I want to just point out this is a different scale and, actually, if you overlay these curves, they are actually fairly similar.

But what is quite striking is the pig cells are infected with much slower delayed kinetics. At day 32, this

is about 8,000 CPMs compared to 100,000 at day 15. This suggested to us that, in fact, we are selecting for different populations and viruses, that the phenotype of the viruses coming out of the pig cells and the human cells is, in fact, different.

[Slide.]

Just to sort of summarize what I have shown you so far, what we think we are seeing is when we activate primary cells, that what comes out is actually a population of naturally occurring varying viruses, that we are not activating a single porcine endogenous retrovirus, that depending on the cell line that you put that virus into, that there is a process of selection, perhaps adaptation, of accumulation of mutations and we can identify a human tropic virus.

Again, the issues of concern here are whether or not that would cause disease in the recipient, whether or not it would be transmitted to others.

In an initial effort to get a handle on whether or not this virus can cause disease in the recipient, we have extended our host-range analysis of this virus into a variety of human and non-human primate cell lines to try to look at what types of cells or tissues are susceptible to infection.

[Slide.]

This is the first table. I know this isn't a very pretty slide, but what we did is, again, using virus-producer cells, we did lethal irradiation and coculture with a variety of different target cells. We then looked at various time points after coculture by the sensitive RT-PCR

assay which is specific for the porcine endogenous retrovirus as well as by the more generic and less sensitive RT assay.

But the advantage of doing an RT assay is it allows you to actually determine whether or not it is a productive spreading infection.

In all cases where you see a positive and the number of days, that is the earliest time point where we see a positive signal. When you see a negative in all these tables I will be showing you, that is the latest time point where we tested where it was still negative. If it is negative, it has always been negative. It is not positive and then goes negative.

In this slide, these are a variety of non-hematopoietic human cell lines. We looked in cervical carcinoma cell line, colon adenocarcinoma, liver hepatoblastoma, astrocytoma, so a variety of different tissue types, as well as normal skin fibroblasts.

In all cases, at some time, we were able to detect an RT-PCR signal suggesting that the virus, in fact, does go

in and, perhaps, is replicating at a low level. At least it is getting expressed.

However, only in some of the cell lines were we able to detect an RT result, in the HeLA cell line and HepGW, the liver hepatoblastoma. All other cells at this

time, even in some cases being carried out for almost two months, are still negative by RT assay suggesting that these RT-PCR-positive results do not necessarily reflect a productive spreading infection.

[Slide.]

Then we looked at a variety of hematopoietic human cells. In this case, we looked at primary monocyte macrophage and primary lymphocytes. These are human. In both of these cases, we never saw a signal by RT-PCR or by RT assay. This is at day 38 these cultures were carried out.

In a promonocytic leukemia cell line, THP 1, we have gotten a signal by RT-PCR but, again, out to day 46, no RT activity. A T-cell line, PM-1, we see a signal by RT-PCR but never by an RT assay.

[Slide.]

In our analysis of non-human primate cells--and I apologize because we have also done this in cos cells and I forgot to put this on the slide--the results are the same as vero. But if we look at rhesus monkey, in this case it is

primary lymphocytes, and we see an RT-PCR signal. But this didn't translate into a positive RT result.

In a rhesus monkey kidney cell line, again, we see RT-PCR but never an RT. In African green-monkey cells, either the vero or the cos, we never see an RT-PCR or an RT.

[Slide.]

So, to summarize what I have shown you, then, we have demonstrated that mitogenic activation of primary pig PBMCs releases a type C retrovirus which infects human cells and we have done EMs. I am just not showing them to you for the sake of time.

We think that different virus populations are selected when primary isolates of this virus are passaged through pig or human cell lines. Some human tumor cell lines can be productively infected with porcine endogenous retrovirus but it is certainly not true for all we have looked at.

Cells of human hematopoietic lineages as well as rhesus monkey cells are susceptible to infection but not a productive infection.

[Slide.]

So what are the implications of these results? What I have done here is I have tried to highlight some of the issues that we think these results are important in the context of the questions that you will be discussing this

afternoon.

The first, obviously, is whether or not these findings with PBMCs can be generalized to other porcine tissues since, obviously, a variety of different porcine xenograft tissues are being examined for clinical use.

Secondly, what cell substrate is most appropriate for virus detection. It is clear that not all human cells are permissive. Also, going back to one of the earlier slides I showed you where the ST cells became positive before 293s, primary isolates may more efficiently infect porcine cells.

Third, we don't fully understand the implications of our positive RT-PCR but negative RT assay results, but one hypothesis we have is that this may allow for low-level replication, perhaps resulting in accumulation of mutations and development of a strain which may be more fully adapted for human cell lines.

Finally, our results with the hematopoietic cell lineages really beg the question whether human PBMCs are the best sample for patient monitoring and, if not, what type of cells would be more appropriate.

I want to thank the committee for their attention.

DR. AUCHINCLOSS: Thank you very much.

Questions? A small number of questions for Dr. Wilson?

DR. COFFIN: Carolyn, have you used PERT assays at all?

DR. WILSON: No; we haven't.

DR. AUCHINCLOSS: I'm sorry; could you explain that question just a bit.

DR. COFFIN: Yes; there is a very much more sensitive assay for reverse-transcriptase which is fairly easy to do which is being studied here.

DR. WILSON: I have talked to Keith Peden who is in our center who has been doing a lot of work on those assays. In his hands--now, this is more specifically for HIV. He has done direct comparisons of an RT-PCR assay versus a PERT assay. He claims that they are relatively similar in sensitivity.

DR. COFFIN: But you are comparing it with an RT-PCR assay. That is different. What you are looking for is RT activity in the soup.

DR. WILSON: Right; these are conventional RT assays. I purposely, actually, in this case, want to do conventional RT assays because I want to see a full-flown infection.

DR. COFFIN: But then that does get to the question that you got to at the end of whether there is any spread at all. For that, it would be a good idea to go to a very sensitive assay.

DR. WILSON: But I think that is what the RT-PCR--

DR. COFFIN: Oh; that is in soup. Sorry.

DR. HIRSCH: Have you passed our cells that are RT-PCR-positive and RT-negative several times? I mean, sometimes it comes out only after many passages.

DR. WILSON: Right. Well, some of these cultures, as you saw, I have carried out 30 to 40 days. What I plan to do sort of after the holidays is to continue growing these cells for longer periods of times.

Also, what I have done now is I have taken sort of a secondary 293 passage virus and I am repeating the experiment I have showed you with the ST and 293s. If you passage it a second time on 293s, now, actually, the kinetics on 293 actually does increase. Those curves are no longer overlaid.

DR. AUCHINCLOSS: At this point, the agenda calls for a coffee break. I think it is really more like a coffee gulp, since you have exactly ten minutes to have some coffee and be back to reconvene at 10:45.

DR. AUCHINCLOSS: There is an announcement from Gail.

MS. DAPOLITO: I would just like to make a short announcement about an upcoming xenotransplantation workshop that is going to be held at the NIH in January. There are handouts regarding that meeting on the table outside,

handouts and registration forms, for your convenience, if you would like to fill out the registration forms today and leave them with Ms. Harvey at the registration table.

Thank you very much.

[Break.]

DR. AUCHINCLOSS: We are going to resume the morning session with a presentation from Dr. Robin Weiss, again on data demonstrating the capacity of the porcine endogenous retroviruses to infect human cells.

Data Demonstrating Capacity of PERV to Infect Human Cells

DR. WEISS: Thank you for inviting me. It has been a short trip for me because I was in Washington downtown giving a lecture earlier this morning at the American Society of Cell Biology. If I start to talk about pig herpes viruses instead, it will show I am mixed up.

[Slide.]

Most of our studies have been published already. The committee has the papers in their big blue folders. So I won't go over too much of it and I will try and be brief so that David Onions has plenty of time, too.

Our work is a collaboration between members of my laboratory at the Institute of Cancer Research in London, and collaboration with Jonathan Stoye's lab. John Coffin has already mentioned him, a great expert in endogenous viruses in mammals, and Paul Le Tissier at the National

Institute of Medical Research in London, too.

[Slide.]

We published some eight months ago in Nature Medicine observations on pig retroviruses that were produced from two well-established cell lines in culture, so differs

from Carolyn Wilson's because she was looking at virus that came straight out of primary cells in normal pigs.

These are established cell lines, but it seemed, at the time we started this a couple of years ago or so, a sensible starting point because it has been known for over 20 years that these two cell lines, pig kidney line 15 and minipig kidney line, released viruses, retroviruses, into culture and they turn out to be endogenous retroviruses.

We found that the virus released from PK15 pig kidney cells would grow in another indicator line that Carolyn has already mentioned, the ST cell line of pigs, would grow in a mink cell line that is a favorite of retrovirologists and in kidney 293 cells which is why these have become a favorite cell for looking at transmission to human cells.

We did not get a take in a variety of other human cells including diploid fibroblasts. However, if we cocultivated the producer cell line with these human target cell lines, many of them became positive for productive infection--that is, they released particles that were

positive by reverse-transcriptase assays, though we use a PCR amplified one, what John Coffin called PERT or Walid would call something else, which is more sensitive.

There were a number of cell lines which were in our paper in the footnotes but not on the slide that were

not productively infected. But, among them, several had been infected. We could detect DNA provirus there and, by RT-PCR, which is, again, very sensitive for RNA transcription, you can show that there is some expression.

So if you grow cells that are actively producing the virus, pig cells, together with human cells, some virus transfers across and, in a variety of cell types, you can get a productive infection.

In the case of this particular cell line here, we only got infection of pigs amongst the ones we have tested. So, using John Coffin's terminology, we would say that this virus is ecotropic, that it is probably infectious for pigs and not for humans, whereas this one appears to be amphitropic, can infect both pigs and foreign species.

In fact, we suspected that this virus was a mixture and it might be a mixture of a pig-tropic virus and a xenotropic virus because, in early experiments where we used the PK15 virus to rescue a retroviral infector containing a marker gene, we found that there was no infectivity for pigs.

It turns out we were correct in suspecting that the virus produced from the pig kidney cells is a mixture, but we were incorrect in that it was a mixture of an ecotropic and a xenotropic virus. It turns out it is a mixture of two amphitropic viruses, both of which have the

capacity to enter human cells, one a little more strongly than the other and one of which is very efficient at reinfecting pig cells and the other is less efficient.

So we think we have three that are biologically active--that is, infectious viruses--from these cell lines. Two viruses from PK15 that we provisionally called PERV-A and B, and the PERV-C from minipig kidney cell line which is ecotropic.

They have different properties. They are all very closely related in genome sequence, if you look at the reverse-transcriptase or protease parts of the genome, which we have characterized as virtually indistinguishable. But when you look at the envelope, which is probably the major determinant of host range, their infectivity for different cells in culture, they turn out to be different.

[Slide.]

So lets look at the PERV-A and B, the viruses with envelopes that can deliver the virus to human cells. A sequence analysis indicates that they have a very common sequence across the transmembrane part of the envelope gene

as we would expect and the bottom part of the surface glycoprotein. These are very closely related in mouse and cat leukemia viruses, too.

But the two subtypes of the pig endogenous virus differ particularly in the regions that, by analogy to what

is already known about the mouse leukemia viruses, are the regions that interact with cell surface receptors and determine host range at domain A and B, the variable sequences in the protein-rich the domain here where they are quite widely divergent.

This divergence here has allowed us to prepare specific probes, PCR probes and the hybridization probes, that allow the differentiation between PERV-A and B and C so that we can distinguish these different virus infections molecularly and can distinguish how many genomes of each there are.

[Slide.]

If we look at the host range in culture, the susceptibility of cells in culture which may not be the same as in vivo susceptibility, we can show you here just A and B. In this case, we have made an artificial virus. We have taken a fairly standard murine leukemia virus vector for the gag and pol genes and have supplied it with the envelope genes cloned from the pig virus subtype A or subtype B and have then allowed it to rescue a betagalactosidase gene as a

marker.

What we find is that PERV-A has a high efficiency. This envelope will deliver with high efficiency into pig cells and mink cells but poorly into human cells. But is positive.

PERV-B delivers with fairly high efficiency to mink cells but poorly to pig cells and rather poorly to human cells, although the titer is twice that of pig cells. This is a positive control. This is an endogenous virus of cats.

So one of the conclusions here is that, in fact, these viruses, at least as cloned recombinant vectors, their envelopes are rather inefficient at delivering the virus to human cells. We think the suspectibility of human cells to these pig viruses is positive but is low.

However, if you keep recycling the virus in human 293 cells, the whole live virus, take off the supernatant and put it back on new cells and do that for six or seven passages, then the efficiency replication does increase. But it is not going up remarkably. So we think there is a low susceptibility but a real susceptibility for human cell infection as gauged in culture.

[Slide.]

We have also, and this took a lot of work, biologically separated the A and B viruses because they were

growing as a mixture in the 293 cells. So we took supernatant and we made serial dilutions on new human 293 cells and the PERV-B is growing at a higher titer.

But, by making a whole series of serial dilutions, a lot of microtiter wells, we were able to obtain 293 cells

that were singly infected with PERV-A or singly infected with PERV-B.

[Slide.]

If we took those cells that became chronically infected, so all the cells are infected, and we took a range of laboratory C-type viruses, mouse leukemia viruses, gibbon-8 leukemia virus, the cat endogenous virus, we could then ask whether these two viruses used common receptors to enter cells. This is a classical retroviral interference test where the cells are chronically infected with these viruses, each singly and a passage until all their receptors are saturated, you then come in and challenge them with a retroviral vector with a reporter gene that has the envelopes of the same viruses.

A plus means you get positive blocking of preinfection with the whole retrovirus blocks the entry of the marker virus, the vector.

What we can see here--I mean, a message that may or may not be important for health purposes, is that these two pig viruses that do have the capacity to place on human

cells, appear to be getting in by different cell surface receptors, as we might have expected from the sequence analysis, at least Jonathan Stoye said it was perfectly obvious but it is also nice to back obvious suppositions with actual fact.

[Slide.]

If we take the specific probes that distinguish between PERV-A and B and all other C-type viruses, and look at normal pig tissues--here are some abattoir organs and some ordinary peripheral blood lymphocytes from a standard Landroce Duroc F1 hybrid pigs, we find that both the genomes of the A subtype virus and the B subtype virus are expressed in normal tissues.

I just give them as pluses and minuses here. In fact, the PBLs and the spleens are richest for expression and we think that expression is mainly in blood cells and, of course, hearts and kidneys have blood cells in them. But we haven't done really careful cell separations.

This is just showing that there is some expression at the RNA level. It is not showing that these normal animals destined for the butchers and your kitchens are releasing live virus. We don't have that evidence. We are just saying is they have the sequences in them and those sequences can be expressed.

[Slide.]

If we take normal pig DNA from a variety of different strains of pigs, including these meat-strain pigs, the minipigs that Carolyn Wilson has already mentioned, Meishan Chinese pigs that were probably separately domesticated thousands of years ago, separately from the

European wild pigs, we find that there aren't many copies.

This is a southern blot along the lines that John Coffin showed from murine leukemia viruses where each band essentially represents a different exogenous viral genome somewhere in the pig DNA. Again, they are probably scattered amongst different chromosomes and the people interested in pig genomics are actually quite excited about using these as markers just as they were used for mouse genetics.

Our analysis would indicate that there are more copies of subtype A than subtype B and that certain bands are common across these widely different strains of pigs. Now, that is significant if we want to pose the question, can we eliminate these endogenous viral genomes by classical, conventional breeding. Can we take distantly related pigs and breed them and get out virus-free animals.

That may be possible but it doesn't look as if it is going to be easy. It is going to be a hard, long job. It has been done with chickens that have had not quite as many copies as these but, certainly, several copies and it

was successful.

So I think what we need now is an analysis of those pigs that are being used by the pharmaceutical biotech companies of these different strains of viruses and the systemic, systematical, careful cloning of these different

proviruses to see which ones have the potentiality to give rise to live virus.

Again, as John Coffin said, many of these may be, so to speak, dead genomes. They may be defective and not be able to give rise to live virus. It is going to be important to find which of them can give rise to live virus and can give rise to viruses with these envelopes that can infect human cells.

These specific probes, as well as specific probes we have developed in the protease region of the genome, we have tested out for sensitivity on human cells and primate cells. We have very sensitive nested PCR techniques that will only pick up pig sequences and not endogenous primate sequences.

We can get down to between 1 and 3 copies of the pig genome. I think Walid Heneine will speak on the CDC's experience with this, too. So, with these probes that are all in the public domain, one can use them to probe exposed humans and to see whether these genomes have got across and whether pig viruses can be detected in human tissue.

I think that will be useful for later analysis by a number of labs investing in xenotransplantation.

I will stop at this point. Thank you.

DR. AUCHINCLOSS: Thank you very much.

Any particular questions for Dr. Weiss before we

move on?

DR. HIRSCH: Robin, when you looked at the spleen, heart, kidney and PBL, does that mean you didn't look at other tissues or that other tissues were negative, is the first question.

The second question is we heard earlier this morning that several hundred people have been exposed to pig tissues in one form or another. Is it possible to use fixed tissue from some of these preparations with your probe?

DR. WEISS: Yes; it is possible to use our probe-- well, let me answer the first question first. This is Dr. Marty Hirsch asking. We went down to the abattoir and got a job lot of organs, and we have not done a more systematic analysis. I think David has so he might speak to that.

Do you want to wait for your talk or answer that one now?

DR. COFFIN: It is not my work. It is the work of Gilian Langford at Imutran. She has shown expression in very many different tissues including, I think, the ear. Our working assumption is that this expression is coming

probably from polymorphic nuclear cells which are positive wherever you look.

So we think that these are probably infiltrating cells. But we don't have formal evidence for that.

DR. WEISS: After analyzing human material, the

only experience we have had, and this is rather preliminary, concerned material from two kidney dialysis patients in Sweden who were exposed for a very short period by dialyzing their blood through a pig kidney on a slab rather than through a machine.

We have looked at prebleeds and serial blood samples afterwards up to two years out. Using our most sensitive techniques that get down to about 1 to 3 copies of pig genomes and using mitochondrial probes is, again, absolutely specific for the pig mitochondrial DNA rather than human mitochondrial DNA.

So far, we have struck a negative. We got a terrible shock because one of the patients, two years out, turned out to be positive. It was contamination in the lab. Even though the extractions were made in a different medical school halfway across London and then brought to our lab, we still, somehow, got contamination. It was both positive for mitochondrial DNA and viral DNA so it was pig DNA that got across.

So that is just a cautionary tale that you cannot

be too careful. Of course, in academic labs like ourselves, we are not as clean as sort of quality-controlled companies. But you can never be too careful. You have got to do it twice or three times in separately isolated systems.

But, in fact, from those two patients, the studies

have been entirely negative to date once we cleaned up our act. But I think the similar techniques, and many labs now have them down to this level of sensitivity, can be used for probing. Perhaps that could be taken up later in the day.

DR. COFFIN: A quick point. One has to be a little bit cautious about interpreting the same-size bands with one single enzyme as indicating the same provirus. Probably a few of those are not, based on our experience with the mouse.

DR. WEISS: Yes; John Coffin is absolutely right. These are bands in a gel and they don't necessarily mean they are exactly the same sequence.

DR. AUCHINCLOSS: Thank you very much.

One of the things I appreciate from our speakers is that you have all stayed so closely within your time allotments. Our next speaker, however, was given a time allotment longer than what we have listed him on the sheet, so when he speaks longer, he is still doing what we asked him to do.

David Onions will, again, be talking about data

demonstrating the capacity of PERVs to infect human cells.

DR. ONIONS: Thank you very much indeed. I would like to thank the FDA and, particularly, Amy Patterson. I think this is a very important meeting and I think it is going to be very important in our progression to the safe

introduction of xenotransplantation.

[Slide.]

This is the virus we are concerned about and, as has already been articulated, the real concern with xenotransplantation is not so much the concern for the individual patient, although that is a matter of concern, but the issue of whether the endogenous retrovirus from the pig could infect human cells and, either through a selection process that Robin mentioned in vitro or, possibly, less remotely, through recombination with human sequences, commented on by John Coffin, could develop viruses that could be transmitted on to the general public.

[Slide.]

About two years ago, now, Q-One Biotech, at the request of Imutran, sponsored by Imutran, looked at the expression of human retroviruses from the porcine cell line, PK15, and these were cocultivated with a variety of cells.

The way this was done is, perhaps, quite relevant. It was not using cell-free virus nor was it, in all instances, direct cocultivation, but used a system which

separates the two cells with a permeable membrane. I say this because it falls in between in terms of sensitivity cell-free virus and cocultivation.

These were, then, passaged for five passages in the usual way. If I could just summarize on the next slide.

[Slide.]

This just summarizes some of the data. We looked at a large number of cell lines and I am not going to dwell on this because the data that has been presented by Robin and by others and Carolyn are very, very similar. First of all, we found that would could get the virus from PK15 cells into Raji cells. These were positive by reverse-transcriptase and also positive by electron microscopy and by PCR, of course.

We found, however, that a large number of cells, like 293 cells, in our hands, using this transwell system, we could, in fact, but usually they were RT-negative; that is, it was an infection. The provirus was there, but the provirus was transcriptionally silent.

This is actually the norm out of our range of cocultivations we have now done with human cells. We normally see the second situation rather than the first situation. We are also, of course, all interested in finding a cell line that might be permissive for the range of these viruses to do cocultivation studies on patients.

Like Robin, we have found that mink cells can be infected reasonably efficiently by several of these viruses but, so far, in our hands, the feline cell line, an in-house cell line, has been the most useful.

[Slide.]

What we then did was to try and characterize molecularly these viruses that were getting into the Raji cell line. First of all, we sequenced, obtained the complete sequence--sequenced the complete genome, the consensus sequence of the virus that has been released from PK15 cells. You must remember, of course, however, these are a mixture of viruses but we are sequencing the commonest sequence in that population.

We independently sequenced the genomes of the two proviruses that have infected the human Raji cells. To preempt the next comments, this is identical in sequence to PERV-B commented by Robin. For a variety of reasons, we call this, under our nomenclature, PoEV2 and this one PoEV1. These two sequences, we will see in a moment, are distinct.

We then wanted to confirm that the envelope gene sequence that was present in this provirus in the human cells did, indeed, confer the ability to infect human cells. This is important because viruses can get into cells by wrapping their genome in the envelope of another virus so that genome A, for instance, could get into a human cell

being wrapped by genome B.

But, as Robin has shown, in fact, both viruses do get into human cells.

What we did was we took the envelope gene. We cloned the envelope gene and then mixed it with a variety of

murine leukemia virus genes to form a pseudotype virus. When this pseudotype virus is released, we can then ask the question how efficiently does this envelope gene which is derived from the pig-cell line confer the ability of this murine virus to infect human and other cells.

[Slide.]

So, this infers an ability to infect about 10,000 infectious units per ml on a standard 293 cell. On the other hand, the PERV-B, as it is, or as we would call it PoEV-2 envelope gene only allowed about 10 infectious units per ml on the same cell line.

So, again, these data, as Robin has already said, show that this particular genome, at least, confers the ability to infect human cells but it is very inefficient at getting into human cells.

[Slide.]

We also have looked at the potential receptor usage by PERV-B or PoEV-2, and, essentially, this is the same data as Robin with slightly different viruses. FeLV-B uses the same receptor as gibbonate leukemia virus as,

indeed, Robin showed, some years ago and, in fact, this data just shows that we can infect either the clone-4 cells that contain the FeLV-B genome or control cells that don't contain the FeLV-B genome. They can all be infected by amphitropic murine leukemia virus, xenotropic murine

leukemia virus, FeLV-B or FeLV-C.

This demonstrates that probably these are using different receptors to get into cells than the range of viruses shown here. So it looks like these virus is using a receptor distinct from these viruses.

[Slide.]

This is a Tom and Jerry cartoon that sums up many years of John Coffin and Jonathan Stoye's very distinguished work on the envelope genes of retroviruses. Just to make the point that upstream of--this is the major surface envelope of protein. As Robin has already pointed out, there are two domains, this VRA region and the VRB region, which are associated with diversity in a whole range of viruses, whether you talk about murine or feline viruses or, indeed, the pig virus.

These are domains that are variable and are believed to be associated or, indeed, one can demonstrate this is the case, are associated with envelope interaction.

[Slide.]

We now look here, what I have done here is--I am

sorry for the quality of this overhead. We have now sequenced four different viruses. I should say that these data are a concatenation of data from several groups. They are from my own group in the university, from Q-One Biotech, from Biotransplant, Pipia Bannerjee, and also from Jay

Fishman at Massachusetts General Hospital.

We put our collection of data together. These data have been in the public domain since the spring of this year, but we just pulled it altogether. This shows four distinct envelope genes that we can identify associated with pig cells.

These are called PoEV-1, PoEV-2, PoEV-3 and PoEV-4. The important point is that these are distinguished in two domains, both in the variable region A and the variable region B. PoEV-1 is the virus that we sequenced coming out of the PK15 cells and is identical to PoEV-2--that is, PERV-B-- through much of its sequence in the envelope gene.

But, in fact, there is a frame shift just upstream of the proline hinge which may well be associated with their distinct receptor usage of this virus.

PoEV-3 and PoEV-4 are, in fact, very distinct viruses. PoEV-4 is the same as PERV-A that Robin has commented on whereas PoEV-3 is a virus that has been sequenced by Jay Fishman from an inbred pig cell line. This may or may not be the same as the MPK virus. We must not

jump to conclusions and assume that they are the same, but it is possible that it is the same virus.

[Slide.]

I just wanted to comment on the general grouping of these viruses. These viruses are tightly group and this

group of viruses are related to murine, feline, baboon and, in fact, the gibbonate leukemia virus. If we look at the pulse sequences, this virus is closely related to gibbonate leukemia virus as it is in its envelope gene as well.

This is important because this group of viruses biologically behave in a very distinct way than the viruses like the lentiviruses or, indeed, the foamy viruses or the bovine and human T-cell leukemia viruses. Biologically, these are a very tight group in terms of their behavior.

[Slide.]

This now just puts these four different sequences in perspective. Down here is PERV-B, which is the virus commented on by Robin, which we know gets, from our data and from Robin's data, into human cells. This is PoEV-1. This is about 10 percent divergence in the envelope gene region.

PoEV-4, which is the PERV-A virus which is identically sequenced to PERV-A, is more distantly related but, as Robin has commented, also gets into human cells. PoEV-3 and the secuba isolate which has also been widely used, consists of two characterized isolates, secuba and the

virus isolated by Jay Fishman which is PERV-MSL.

The complete genome sequences of this virus and this virus are available. We have the complete genome sequence of both of these.

There are some quite, perhaps, important points

here. PoEV-1 is actually very abundant in our hands in PK15 cells in terms of its real experience. We have not yet demonstrated that this virus is able to get into human cells. You have to be careful when using southern probes because it is very difficult to distinguish this virus and this virus using a southern blot strategy.

But we have not yet shown this virus actually gets into human cells.

[Slide.]

Just to summarize a few features of these viruses. PoEV-1 is present in virions released from PK15 cells. It differs from PoEV-2--that is, PERV-B--in the VRB region through a frame ship. There is no evidence thus far that this virus infects human cells. There is no evidence thus far that this virus infects human cells. I am not saying that it could not but, so far, we have been unable to show that.

[Slide.]

PoEV-3, remember, is the most distantly related of these viruses, at least in the envelope gene sequence. It

differs from PoEV-1 in both its VRA and its VRB domains. It is a very different virus. It also differs in another interesting way for the retrovirologists. PoEV-3, I should say here, utilizes a proline transfer on A primer whereas PoEV-1 uses a glycine transfer on A primer. These are

sometimes features that are used to classify retroviruses and we normally put them in slightly different groups.

Ironically, these viruses actually have different tRNA primers. The reason, I don't know.

The U3 regions which are in the area of the transcriptional machinery of the virus--that is, the enhancer region of these viruses--are also very, very distinct. We have reported previously that PoEV-1 has an LTR enhancer that contains a number of motifs that look like it would be reasonably well expressed in lymphoid tissue.

PoEV-3, however, has a very different LTR structure from PoEV-1. We have not yet determined the host range of PoEV-3 and we must be careful in making analogies that this is the same virus as PERV-C or the MPK virus. We don't yet know that and Robin and I will have to compare some sequences and see if they are the same virus.

But, so far, we don't have any host-range data. It is also worth commenting that the MPK virus was reported by Lieber and Todaro to infect mink cells. So it is not entirely clear in my mind, Robin, whether that virus really

is strictly ecotropic. I think we need to maybe talk about that.

[Slide.]

But the important point is that PoEV-1 and 3 display high identity in other regions. For instance, in

the gag and pol regions, there is 90 percent identity in the gag region and 96.8 percent identify in the pol region. So, in certain domains, these viruses are very closely related.

[Slide.]

The relevance of this is this means that we can now use strategies to distinguish these viruses, first of all by using RT-PCR because the envelope gene is a spliced product. This means by using a primer down here, and we can find conserved primers or primers up here, which are distinct, we can actually distinguish between all of these viruses using an RT-PCR strategy

So we can distinguish four different variants using an RT-PCR strategy. We can use a similar strategy to identify virions in plasma or in other tissues to identify specific subgroup viruses.

[Slide.]

However, we can also use these criteria to ask questions what is going on in terms of pigs, in terms of primates, and in terms of people because these are the three groups of data we are going to use to determine the safety

of xenotransplantation, what of these four viruses are being expressed and is there data in primates that these proviruses are being expressed in the transplanted organ.

[Slide.]

Rather like Carolyn Wilson, using a very similar

protocol, and I think her data were very important in demonstrating you could isolate virus from primary cells, we have looked at the isolation of virus from Imutran's pigs using stimulated PBNCs. there is some difference to Carolyn. We see P production at day 3, but I don't think that is important.

You see the kind of stochastic nature sometimes of infecting cells. For instance, so far, we have not infected 293 cells. On the other hand, we have got the virus into Raji cells but, this time, there doesn't seem to be RT positivity and the virus is not spreading through the culture.

This time, however, we have managed to get into MRC-5 cells, which we haven't done before. We have also looked at number of primates because these may be useful models in terms of determining whether we can use these as models for the safety of xenotransplantation.

So far, and I should say these data are preliminary, we have not demonstrated infection in vitro in cynomolgus cells using virus from immunotrans pigs, but we

have demonstrated infectivity of a baboon cell line, the 2C1B cell line. This cell line is positive by PCR but is not positive by reverse-transcriptase.

[Slide.]

I mentioned earlier that the gag regions were very

similar. We have now expressed recombinant gag protein, the major capsid protein, and also taken whole virus to try and develop a western blot which is just under development at the moment. It is not yet complete.

This is a marker lane. I made this slide and I am at fault; this is the wrong position. It is not 30 kiloDaltons. It is actually lower than that. This is a peptide antisera that we have raised which is common to all the viruses that we so far have sequenced. This is a negative control which is a supernatant from a non-infected Raji cell culture.

This is a Raji-infected culture and it picks up a P30-like protein in this Raji cell line. We are now doing mass spec to sequence this. We are doing mass-spec sequencing to confirm that it is the virion protein.

This is actually using recombinant P30. Again, this peptide antisera picks up the recombinant sera. We are now beginning to go through a whole series of sera from people who might be in risk occupations. This is a normal serum, normal human serum. It is not entirely normal. It

is my Ph.D. student and she is far from normal.

But these are three sera that are quite interesting. They come from patients who have acute lymphoblastic leukemia, and they are a group of eight patients who were identified by the Leukemia Research Fund

Epidemiology Center in the U.K. These are all butchers or abattoir workers from the South Wales region of the United Kingdom and all developed leukemia in a relatively short time frame as well. Unusually, they were acute lymphoblastic leukemias. None of these are positive for the P30.

[Slide.]

I just want very briefly at the end--how do we put all these data together to think of the testing strategy. I just want to comment in two minutes about models that we have. This is a model from the cat which is a natural model. These viruses are transmitted from cat to cat. We know a lot about this virus. Many hundreds of thousands of samples, literally, have been screened from cats.

We have been able to identify--I say "we;" I mean the whole collective field has been able to identify a number of patterns of infection. The commonest is actually recovery. Most animals infected by this virus recover. They have a transient viremia with antigen, virus antigen which usually the capsid protein can detect and plasma

virus.

But, after a period of time, that declines and the animals recover with neutralizing and antibody to that P30 protein. That is the commonest pattern of infection. The worst kind of infection is a persistent infection which

virus antigen and, also, of course, plasma viremia persist for life. It is these groups of animals that die.

This is also true in the primates that have been infected with amphitropic murine leukemia virus, the studies Robin referred to. They fell into this pattern of infection before dying of leukemia or lymphoma.

We see two other modes of infection, however. One we call sequestered infection in which you get an apparent transient infection. But this time the virus is eliminated from the bone marrow in the blood stream but is confined to epithelial glands like the salivary gland or, perhaps, the mammary gland. We can detect in the circulation antibody; no virus, but we can, sometimes, not always, detect antigen of the virus which seems to leak back from the epithelial surface.

Finally, there is a true latent infection which, again, looks like a recovery infection but there is no virus in the plasma. But if we look in the bone marrow or we look in the lymph nodes, we can find viral sequences which tend to disappear through clonal extinction over a period of

time.

It is this pattern of infection that is probably the kind of infection that was seen by Cornetta in his studies when he put amphitropic murine leukemia virus into primates. I think, if I had to make a bet, and we shouldn't

in these kinds of meetings, this is the kind of infection that we most likely might see in patients exposed to PoEV, if we see an infection.

[Slide.]

Just to show the difference between these; this is a cat's mucous membrane. This is the nasal mucous membrane and the submucosal glands stained with an antibody to that P30. This is cat virus, I emphasize. These animals are throwing out 105 infectious units into saliva and mucous secretions.

This just shows that effectively all the epithelial cells become infected in these florid infections. This persistent viremic type of infection is very different from the kind of latent infection that is seen in other systems.

[Slide.]

What does this mean in terms of a testing strategy? This is the last overhead. Just to emphasize that if we are going to talk about using these collective data in terms of testing patients, then I think we have got

to account for all those types of infection; transient, persistent, latent and sequestered. What I want to draw your attention to is that we tend to jump looking at this population, peripheral blood mononuclear cells, but there are occasions when this is not the most useful site.

In a persistent infection, it is plasma viremia that is the critical determining event of disease. We occasionally see tumors developing in these latent animals that don't have a plasma viremia but it is uncommon. Tumors and other conditions normally develop in these animals that have high-titer plasma viremias.

But I also want to draw your attention to this group of infections, the sequestered infections, where there is neither plasma virus nor provirus in the peripheral blood mononuclear cells and we have to look for other criteria of infection like antibody or plasma antigen.

Thank you.

DR. AUCHINCLOSS: Questions for Dr. Onions?

DR. LERCHE: My question would be you said you expect the latent or the sequestered pattern to be most likely in human recipients. Would you expect the pattern-- or how would the distribution of these outcomes be influenced by the immunosuppressive regimens.

DR. ONIONS: That is an important point. In fact, studies in cats can switch the start of a latent infection

back into a productive viremic phase. That is certainly true and studies done in this country by Ed Hoover, I think, have very elegantly shown that.

I perhaps shouldn't speculate like this but I just think from the data that we are all collectively getting

from Carolyn and Robin and ourselves that this virus is not very infectious for human cells. It does get into human cells, clearly, but it is a sort of grumbling type of infection.

That, to me, would suggest that the patterns of infection we might see are those kinds of infection. Of course, there is a balance between what will happen in immunosuppression and I don't know.

DR. COFFIN: In the cases where there has been more than one sequence determination of a given type of virus, are those sequences identical to the base, because this addresses the number of possible infectious proviruses there are.

DR. ONIONS: Yes. We have two clones of PoEV--I could be wrong about this. One of them is identical in the envelope sequence to the sequence Robin has, the available sequence of PoEV. The other one has got one nucleotide difference but it is conserved.

DR. COFFIN: That is actually not good news because that suggests the likelihood of a second provirus

that is very, very similar but with similar biological activity.

DR. ONIONS: We don't know whether that has occurred during--

DR. COFFIN: Right; it obviously could be--in the

worst case--it is good news to see the identical sequence, potentially good news to see the identical sequence, but potentially to not see it.

DR. ONIONS: The PoEV sequence; I'm sorry, I just can't remember. The sequence that Robin, of course--PoEV-3. They are very, very similar. I think they are almost identical. I honestly can't remember. I will obviously talk to Robin about that and to Jonathan.

I should add these data are all available. They are all in public domain and have been since the spring in case people are wondering.

DR. SALOMON: There is certainly no quick answer to the question I have but a critical question that is on the table for this group right now is what kind of screening strategy we use, and that is relevant even to these retrospective studies that we have heard today are going on, for example, from Corinne Savill at Imutran.

So, as I listen to this, there are so many subtleties here. I want to make sure that I make sure that I understand what the different speakers are saying. What

cell and what screening strategy should we be using. I am not talking about PCR, now, or something but just what target. Is it peripheral blood mononuclear cells?

It seems to be pretty good, but how good is it?

DR. ONIONS: I think Carolyn Wilson made a point

that, perhaps, those cells were not particular set to an infection. That is a very important point. But you are also limited by what you can access.

My own view is that we certainly should look at peripheral blood mononuclear cells for infection, and that might be a latent infection. It might not be productive. It could be just the proviruses, though.

I believe we should also look at the plasma for the virus. Let me make just a suggestion. Perhaps this pigs might produce virions but they might fall into one of the classes of virions that do not infect human cells at all, but we would like to know whether they are there or not.

So we need to able to look at plasma and be able to distinguish which virus is present in the plasma. I would reemphasize that where we normally see disease, that is usually associated with a high-titer plasma viremia--not always, but usually.

I think that is the real worry I have is when you get that situation. So I would always want to look at

plasma. And I would also want to look at antibody. I know these patients are going to be immunosuppressed, but I would still want to look at antibody because that helps you define certain categories of infection, particularly infections where the virus might be lurking somewhere that you are not

sure. You can't find the virus in the blood stream but it might be in some other site.

DR. AUCHINCLOSS: Thank you very much.

I think we will move on to the next presentation by Michael Egan from Diacrin/Genzyme describing their data from clinical studies with their assays for detection of porcine endogenous retroviruses.

Data from Clinical Studies: Diacrin/Genzyme Assay and Clinical Study

MR. EGAN: Good morning. We also want to thank the committee and the FDA for the opportunity to present. Our objective today is to give an overview of our program but, unlike what is listed on the sheets you have, I will not be able to do this alone.

I am going to have help in the form of Alan Moore who is going to present our assay systems and our clinical data using those assay systems and then Dr. Steven Fink who will review our medical data from our phase I program.

My objective is to give you an overview of the program as well as to take you through some of the

manufacturing and quality-control issues so that you get an understanding of what we are trying to achieve.

[Slide.]

Our focus is the use of porcine field tissue for the treatment of Parkinson's and Huntington's disease. Both

of these diseases are neural degenerative diseases where very specific cell types are lost and, therefore, you have the capability of replacing them.

The current treatments for these diseases, in the case of Parkinson's disease is L-dopa which is clearly effective but only for a certain period of time and then it is efficacy wears off. There is no such treatment for Huntington's disease to date.

A lot of the basic science in this area was done using human fetal tissue and it has been shown that human fetal tissue used in Parkinson's disease patients can reverse their symptoms. Similarly, in our own preclinical studies, we have been able to show that porcine fetal tissue can work in Parkinson's and Huntington's disease models.

[Slide.]

To give you a bit of history, we began this process in our first meetings with the FDA in April of '94 and then it was approximately a year later where we treated our first Parkinson's disease patient.

I want to emphasize here that the Parkinson's

disease patients that we are focusing on are these late-stage patients. These are patients whose L-dopa is no longer effective. Our first Huntington's disease patient was treated in May of 1966.

[Slide.]

We continued this program and concluded the recruitment in October of 1996 for the Parkinson's disease and March of 1997 for Huntington's disease. There were 12 patients treated under each of these conditions so there is a total of 24 patients that have now been treated.

Since that time, we have continued meetings with the FDA and have also clearly, over the past several months, spent a fair amount of effort in collaboration with them and their scientists addressing the porcine endogenous retrovirus issue.

[Slide.]

The manufacturing here clearly has a number of parts to it. I am going to focus on the four major aspects to give you, again, the sense of what we are trying to achieve from the manufacturing and quality perspective.

[Slide.]

The first aspect is clearly the qualification of the animals. We go through an extensive screening that is done on an animal-by-animal basis in order to be admitted into the program and use as a source of tissue.

The screening consists of screens for parasites, bacteria, mycoplasm and viruses. These viruses do not include the porcine endogenous retrovirus because the data that is available now to look for that was developed later. We will talk about that in a moment.

[Slide.]

They are then transferred to a biomedical animal facility. [Slide.]

The objective of a biomedical animal facility is to maintain the health status of the animals once they have been screened. This biomedical animal facility has filtered air. You will see, through some of the photographs I am going to show you, that the personnel are required to gown as entry into that facility.

[Slide.]

Animals have extensive checks from the veterinary staff and then, very importantly, all of the procedures that are done on those animals in preparation for harvest are done under aseptic conditions.

[Slide.]

Here you will see an animal being prepped for surgery.

[Slide.]

Clearly, the staff is dressed appropriately. This is done by a veterinary surgeon where the intact uterus is

removed at the appropriate time during gestation. It is then double bagged and is ready to be transferred to an isolation facility.

[Slide.]

This isolation facility is run under GMP. That is

good manufacturing practice. These are a set of standards that are in place in the pharmaceutical industry to which we adhere.

[Slide.]

This isolation facility is dedicated. As I said earlier, we have all the GMP controls and, very importantly, the quality control and quality assurance is in place all during this process.

[Slide.]

Dedicated room.

[Slide.]

Again, staff in full aseptic dress.

[Slide.]

There they are unloading the uterus. This is the beginning of the process. This is done inside a biological cabinet where the fetuses are isolated.

[Slide.]

It is then moved into a cell isolation facility where, again, we have biocontainment. You will see the technician is appropriately dressed. This is a microscope-

outfitted hood so that all the microdissections are done within the biological cabinet.

[Slide.]

All of the quality assurance is in place such that every piece of equipment and every reagent that is used is

tracked.

[Slide.]

Air monitoring goes on continuously, both in the room as well as in the hood during the process.

[Slide.]

As far as final release is concerned, once the cells have been isolated, and you will see them right here, beginning with that intact uterus through all the processing.

[Slide.]

This is basically what you end up with.

[Slide.]

There is a quality-control step prior to their release.

[Slide.]

Then it is through a pass-through such that you never get the crossing of incoming product and outgoing product as part of that quality-control process.

[Slide.]

Therefore, our objective, overall, in this program

clearly is the production, the quality production of porcine fetal tissue. We have treated 12 patients in our phase I Parkinson's disease program and 12 as well in our Huntington's disease program. We have and we will continue to work with the FDA on the issue of porcine endogenous

retrovirus, and that gives me the opportunity to introduce Alan Moore, who has actually done all of our assay development and patient testing.

DR. AUCHINCLOSS: It is obviously very important that we try and stay in our time allotments here as we come towards the end of the morning. Clearly, this is the important part of the presentation in terms of the assays, so we want to focus on that.

MR. MOORE: I will try to cover it thoroughly but succinctly. I apologize for the overheads but some of these were wet as of last night.

[Slide.]

Our laboratory has been involved in the characterization of the porcine endogenous retrovirus of the porcine neural tissue. One of the things that marked this program, at least from our perspective, was the opportunity to explore samples from patients post-treatment. So it is not an opportunity that we have in a QC laboratory very often.

So our program included an in vivo component which

was the analysis of patient samples from the Parkinson's disease trial with the DNA PCR assay systems that were published by Robin and which Robin referred to. The development and validation of that DNA PCR was discussed in quite some detail with the CEBR scientists as well as some

of the advisory members of the scientific group.

Additionally, we launched an effort to conduct an in vitro characterization, a cocultivation study. That employed endpoints such as reverse-transcriptase and RT-PCR assays as referred to by some of the other speakers like Carolyn Wilson.

The goal was to look for potential virus capable of infecting human cells.

[Slide.]

Just to refresh the knowledge about the assay systems, the DNA PCR system was described by Robin Weiss and his coworkers. It was conducted in a QC setting following validation. The sensitivity of the assay system was between 0.02 and 0.1 porcine cell per 105 human PBMC. The specificity was confirmed by testing fresh PBMCs as well as cells integrated human retrovirus sequences.

The RT-PCR system was validated along the same lines. The sensitivity was shown to be capable of detecting 10-3 dilution of unconcentrated PERV material in a 5 microliter reaction sample. The specificity was confirmed

as previously described.

[Slide.]

The results using the DNA PCR assay for, it turned out to be 11 Parkinson's disease patients who are presented here. That patients ranged in post-transplant interval from

six months to 24 months. In all cases, we turned up negative results.

[Slide.]

We tested 15 samples. Some of those samples turned out to be Diacrin employees that were included as a laboratory control. I do have to echo Robin's comments about the care that must be taken in performing the PCR analysis. We utilized a five-room protocol and were able to prevent cross contamination with specific carry-over prevention mechanisms.

So, basically, the DNA PCR results for the patients were negative and the PBMCs were negative using the probe.

[Slide.]

The cocultivation study, and I apologize that this is a very busy slide, employed LGE and VM which are the porcine fetal neural cells. This was actually conducted in duplicate. The porcine cells were cocultivated with human 293 cells or STIowa cells, as Carolyn Wilson had mentioned.

In addition, monocultures of the neuronal cells

were maintained and the appropriate controls along with those including human 293 cells, STIowa infected with PERV, 293 cells infected with PERV, and PK15. In all cases, the cells were carried out for a period of 35 days which represented a significant population doubling level.

Then supernatants were passaged onto 293 or STIowa cells and then those cells carried out for an additional interval. Each time there is a chevron or an arrow as a sampling point, we were collecting samples for a reverse-transcriptase assay, RT-PCR analysis of cell supernatants and, where these may be negative, DNA PCR analysis of cells.

[Slide.]

The results obtained with the cocultivation study are presented in this slide. The porcine fetal neural cells cocultivated for intervals of 35 days with the human 293 cells were negative when tested using the RT PCR assay system.

Also, at day 49 in the supernatant passage component, these cells were negative using the DNA PCR so there did not appear to be integration of the PERV in those human cell populations. STIowa was employed and positive results were obtained with the RT-PCR and the DNA PCR analysis. This is, I believe, by virtue of the fact that the STIowa cells were, in fact, expressing porcine endogenous retrovirus.

The appropriate controls were employed. PERV plus human 293 cells did give positive results so infection was transmitted using the porcine endogenous retrovirus preparations. The same is true with the STIowa.

The monoculture cells of human 293, VM1, the

porcine neuronal cells were negative in both assay systems and, as noted, STIowa and PK15, as expected, would be positive.

[Slide.]

Additionally, the same samples were analyzed using the enzymatic RT assay. The human 293 cells cocultivated with porcine neural cells were negative after that cocultivation interval of 35 days. Positives were obtained with the cells cocultivated with STIowa. I think it is important to point out that these levels of incorporation were less than or equal to the STIowa control cultures, so those monocultures that are shown here were positive.

These were not any more positive than that. Again, the controls gave expected results PERV plus STIowa and PERV plus the human 293 cells.

[Slide.]

So, in summary, we can demonstrate, at least from the data in these studies, that the post-transplant patient samples which were from six to 24 months post-transplant were negative using the DNA PCR assay system. In the

cocultivation study, supernatant samples from the cocultivation were negative for PERV using the RT-PCR assay system and all of the human 293 cells from the cocultivation study with, of course, the exception of those spiked with the PERV, were negative for the DNA PCR.

I should point out as well that the RT assay results were conducted using the same setting as Dr. Carolyn Wilson presented before. Lastly, the assay systems would seem to be very relevant for appropriate monitoring of patients and the analysis of samples from clinical administration.

Thanks.

DR. AUCHINCLOSS: Before you step away from the microphone, are there any results on the patient that we know died at eight months from tissue--

MR. MOORE: No; we have not received samples for patient No. 12 for analysis.

MS. MEYERS: Were they collected? Were those samples collected? Was there an autopsy? Was there anything?

MR. MOORE: This is Dr. Steven Fink.

DR. AUCHINCLOSS: Dr. Fink, now, you have got sort of two or three minutes left in the 20 minutes. I have looked through your slides and they are largely the clinical data. Is there, in fact, information that will add to our

understanding of the assays?

DR. FINK: There is some safety and efficacy data from the Parkinson's phase I trial.

DR. AUCHINCLOSS: Particularly related to viral issues?

DR. FINK: No; this is all clinical data. It is general adverse event, safety reporting and clinical information from the neurological rating scales.

DR. AUCHINCLOSS: I guess what I am asking is for the purpose of this meeting, can you shorten the talk so that you stay within the 20 minutes and move on?

DR. FINK[: Yes.

DR. AUCHINCLOSS: Because I think we are concentrating on the viral issue here today.

DR. FINK: Okay. Let me just highlight a couple of slides.

[Slide.]

I would just like to highlight some of the safety and efficacy parameters in the xenotransplant trial that was initially started in Parkinson's disease. This is a trial in which 12 patients received 12 million ventral mesen cephalic cells implanted in three sites in the striatum.

Six of the patients received standard immunosuppression with cyclosporine and six patients received cells that had been treated with monoclonal

antibody to complex 1 of MHC. These patients were followed at three-month intervals for safety and efficacy testing.

[Slide.]

This slide summarizes all of the adverse events seen in the two groups. There were 232 adverse events.

There were more serious and non-serious adverse events in the cyclosporine group. The profile of these adverse events was often consistent with known side effects of cyclosporine.

[Slide.]

In terms of relationship to study treatment, investigators felt that in the antibody-treated group, there were no adverse events that were probably or definitely related to study treatment. The three adverse events in the cyclosporine group so judged related probably or definitely were all laboratory deviations in cyclosporine levels.

[Slide.]

This is the serious adverse events. I can just summarize this briefly by saying that with the exception of the one patient that was already mentioned who died of a pulmonary embolus, all of the other serious adverse events were resolved without sequelae.

[Slide.]

Regarding the patient that died, there was an autopsy performed and the opportunity was available to

perform a neuropathological analysis on that patient.

[Slide.]

From the safety and efficacy standpoint, I think two points are important. First is that using a specific porcine genomic marker using a repetitive element, specific

cells that were of porcine origin were seen within the putamen and those are indicated here in the purple.

[Slide.]

These are dopaminergic cells that were seen in the graft.

[Slide.]

When markers for inflammatory response were looked at, either for T-cells or MHC class II upregulation, there was an minimal or mild upregulation and infiltration of T-cells at the border of the graft site.

[Slide.]

This is the efficacy data. The column on the left is where you should look. At 12 months, the ten evaluable patients have improved in their Parkinson scores of 20 percent. This level of improvement has been seen in both the group that has received cyclosporine immunosuppression and in the antibody-treated group.

I was going to show a brief video of one of the patients but, with respect to Dr. Auchincloss' guidance document, perhaps I will pass on that.

DR. ALTER: That would be terrific. Thanks very much.

Back to Abbey's question.

MS. MEYERS: You evaluated for the virus in twelve Parkinson's patients, but did you look at the tissues of the

Huntington's patients?

DR. FINK: The Huntington's patients have had blood sampled and saved but we have not had the resources given to the other testing. We felt it was high priority, but we have not run those samples on these patients yet but we intend to do so.

MS. MEYERS: When do you intend to do so? Soon? A year from now?

DR. FINK: Soon. It is very high priority.

DR. AUCHINCLOSS: And the post-mortem tissue?

DR. FINK: The post-mortem tissue was saved and is available. That has not been looked at. I think we do need to consider some of the technical aspects of whether the assays that have been talked about can be actually applied to that tissue.

DR. DESROSIER: I am surprised that nobody has said anything about antibody responses to the pig virus. Can you tell us what is being done along those lines? Is that a fair question?

DR. FINK: Currently, we do not have that kind of

assay available. I think that there is a belief that this would be important and very useful in not only analyzing the patients that have been transplanted but in subsequent monitoring of these patients and others.

DR. SALOMON: We got into this at the Cross-

Species Infectivity meeting a few months ago. In this particular case, for example, we are seeking reassurance that we are not getting the infection in the clinical setting and these are very important studies.

The use of the UT93 kidney-cell line, though, is not biologically, perhaps, the most reassuring cell line used as a target. It does have cell receptors for some of the known PERVs, but there are multiple PERVs. We don't know that they have the same cell receptors of the others.

Moreover, in the clinical sense, neural cell lines or lymphocytes, would be the two kinds of cells most likely having access to tissue in a clinical setting would be more appropriate, I think, targets for testing these things.

So I think before we make conclusions like, well, this data shows that there is no risk, I think that we have to be very cautious about the studies that we are interpreting.

DR. ONIONS: Could I just extend that comment? One of the things that I am concerned about is if we rely on cell cultures to detect infectious virus--or, sorry, plasma

viremia on infectious virus, there are mutations where we do see this stochastic nature with the stocks of virus we know have high amounts of particles but their infective cells can be very stochastic on experiences on several occasions.

Say on four out of five occasions we get

infectivity and on the fifth occasion, we don't, with the same amount stuff. I think if we are going to look for plasma viremia, you look for the virus in other situations and you need to consider using electrical techniques which are not ideal, but, for instance, for plasma, by using RT-PCR.

DR. LERCHE: I have just a question on, again, the tissues that are being examined. If I understood the presentation correctly, it was buffy-coat cells in the patients? I was wondering if any opportunity was available to look at, for example, by RT-PCR or other methods in, say, spinal fluid, some other tissue or fluid.

DR. FINK: So your question is whether there is utility in looking for PERV in cerebrospinal fluid which is, perhaps, the most readily accessible fluid adjacent to the graft site. We have given this some though and I think, still, we are in discussions of the technical applicability of that in that presumably one would need to get cells from cerebrospinal fluid and there may be very few there.

DR. AUCHINCLOSS: I think it is going to be a very

important question for us to get back to this afternoon and definitely plan on doing so.

DR. COFFIN: I had a question regarding the sensitivity of the PCRs. The slide indicated a detection rate of 0.02 cells. I assume that that was based on pig

cells where there are 50 copies per cell or so for viral DNA. Is that where that comes from?

DR. MOORE: Yes.

DR. COFFIN: But an infected human cell can only be detected at 150, although at that sensitivity, you may only have one provirus. You have got to be a little careful here about what it is that you are actually seeing. That is a real problem, though, if you are thinking about detection of these, this differential sensitivity between the host cell that you would really like to be looking at and the much, much more sensitive detection by the same assay in the donor cells where there might be traces of microchimers and may make the assay impossible.

DR. AUCHINCLOSS: Again, I think this is a critical issue that we can come back to this afternoon as we talk about what assay is good enough.

DR. COFFIN: Could I ask one specific question? What was the denominator in those assays? You said the sensitivity is 0.02. You didn't see anything. I didn't catch how many cells were actually tested.

DR. MOORE: That was in 105 human cells.

MS. MEYERS: Did you save the tissue from the rest of the pig? Can you go back to that tissue?

MR. EGAN: We covered it briefly but part of this is that there is a regular sampling of all the various

tissues that are part of an archive system. This actually is part of a registry that is being developed. So the answer is yes, we do have those tissues available. So, it being an endogenous virus, the DNR PCR obviously is there. It is a question of whether it is expressed by the particular tissue that we are transplanting.

MS. MEYERS: Have you gone back to test that tissue?

MR. EGAN: It is there.

MS. MEYERS: It is there?

MR. EGAN: It is there because it is an endogenous virus. So you will find it in all the tissue. The question is whether it is active or expressed as people have been talking about.

DR. AUCHINCLOSS: We are going to move on to our next speaker, but I appreciate your flexibility in response to the Chairman's prompting.

From Barry Solomon, The Circe Biomedical assay and clinical data.

Circe Biomedical Assay and Clinical Data

DR. SOLOMON: Thank you, Mr. Chairman and advisory committee, for inviting me here. I am going to try to stay in the 20 minutes, I promise.

[Slide.]

We are going to be talking today about a liver-

support system that we have developed which utilizes porcine hepatocytes for the treatment of acute liver failure. Liver failure is not small disease. There were about 250,000 patients last year in the United States. About 50,000 of those died, most deaths from complications of chronic liver disease.

[Slide.]

With liver transplantation, we have been able to transplant about 4,100 liver transplants last year, but there are about 9,000 patients in the United States currently waiting for liver transplant and, last year, over 1,000 patients died while waiting for a donor liver. About 1,700 of these 4,100 liver transplants were emergency liver transplants which were required because the patient would die without that.

[Slide.]

There are two specific types of patients in which there is an acute need for liver support. One is the fulminant hepatic failure which is a very rapid onset of the liver failure leading to encephalopathy and coma. The liver

potentially can regenerate to provide necessary liver function. The morality of this group without transplantation is over 80 percent. Even with transplantation, considering all comers with FHF into the hospitals, it ranges from a mortality of about 40 to 50

percent.

The challenge is to provide time for liver regeneration of transplantation. Another group of primary non-function patients occurs after liver-transplant surgery in which the liver has not been able to kick in. There is no liver activity. There is no sustainable liver function.

These patients, essentially, have a 100 percent mortality without a subsequent retransplantation.

[Slide.]

From the basis of the National Center for Health Statistics in UNOS, we estimate about 2,000 of the acute liver patients last year fell in the FHF and the PNF range.

[Slide.]

Our solution has been to come up with an extracorporeal system which allows us to be able to treat liver failure patients with isolated normal porcine hepatocytes that are encased in a hollow-fiber reactor such that the plasma from the patient, once separated from the blood using a plasmapheresis system, is recirculated in an extracorporeal loop so the plasma passes through the center

of the hollow fibers and the hepatocytes are sequestered from the plasma outside the hollow fiber in a situation in which the membrane, which has about a 100 nanometer pore size, allows proteins, nutrients and oxygen to be able to perfuse those cells and allow those hepatocytes to function

and provide missing metabolic function for a period of time.

Our current protocol utilizes a six-hour treatment approximately once a day until the patient has been able to either recover, regenerate or, in certain instances, die.

[Slide.]

We have put together a machine, our HepatoAssist machine, which allows to be able to treat patients with the hollow-fiber cartridge in the intensive-care system. All the tubing sets, all of the ancillary materials are attached to the system so that they can be utilized the same way throughout all the centers in our study.

[Slide.]

Working with the FDA, we were able to begin a phase I clinical investigation in which we targeted severe acute-liver failure patients, end-stage with stage III or stage IV hepatic encephalopathy.

We have, in this phase I trial, treated 41 patients, 27 FHF, three PNF, nine chronic patients where they had an acute exacerbation of a chronic disease, and two cancer patients, these in compassionate opportunities. We

have utilized three clinical sites, the fundamental one with Cedars Sinai Medical Center which we have treated now 24 patients in our clinical trial under the direction of Dr. Demetriou, whom you heard from this morning.

Dr. Demetriou and his group were key in developing

this system, and the system that we are using today is based principally on the work that was done at Cedars Sinai Medical Center.

We have also involved the UCLA Medical Center with six patients with six patients were treated and the Paul Brousse Hospital in Paris where an additional eleven patients have been treated.

[Slide.]

All in all, we have had 96 treatments over six hours, as I said. The average number of treatments per patient was approximately 2.3 treatments. In addition to those 41 patients, an additional 12 patients were treated at Cedars Sinai prior to the IND under local IRB approval with a previous generation of this system, seven FHF, one PNF and four on chronic patients.

[Slide.]

All in all, these are the results of the 53 patients that we have seen. We have been able, bottom line, looking at survival of 30 days, to see about a 90 percent, 89 percent, survival of 30 days for these targets FHF and

PNF patients. For the acute liver patients, we see about a 47 percent survival.

Interestingly, there are six patients who are listed status 1 for emergency liver transplant that recovered and left the hospital without a transplant after

our bioassist treatment. Also, no patients died while awaiting a transplant.

[Slide.]

To put this in perspective, we compared this data to data that was generated at the Paul Brousse hospital by Dr. Henri Bismuth, where he looked at the same stage 3, stage 4, coma patients in FHF and PNF patients. He looked at 175 patients in his hospital where the average waiting time for a liver was about 1.3 days. He still saw an overall survival of only 67 percent.

However, when we look over here, we see that only 1 percent, or two of those patients, recovered and about 24 patients, or 14 percent actually died having been listed and waiting for transplant.

[Slide.]

How do we this? We deliver to the hospital our machine, the cells. Our hepatocytes are delivered in a cryopreserved state. The hollow-fiber biocartidges in a sterilized manner are also delivered to the hospital and the other disposables are ready at the hospital.

[Slide.]

Once the patient arrives, the cells go through a cell-preparation step, are seeded into the cartridge, put on the machine and the treatment can commence.

[Slide.]

But, to do this, we have had to develop a significant quality-control process that involves our animal facility where serological testing on the herd occurs prior to bringing the animals into our system. The animals, just prior to the excise of the organ, are anesthetized and weighed, blood and stool samples are taken.

We screen the herd for the series of viral contaminants before they are even brought into our facility.

[Slide.]

The excise suite, which occurs under a hepafiltered air shower--the preparations occur, and additional samples are taken.

[Slide.]

This is example of the surgical removal of the liver which at the time is filled with a transport solution and the liver is doubled back to the animal facility and brought into the cell-preparation facility in our GMP facility.

[Slide.]

There, we have perfusion, digestion, dissociation of the cells. The cells are washed. The cells are pooled, when appropriate and a precryopreservation suspension is used and they are filled into the bag.

[Slide.]

This is the teasing of the tissue bag in the GMP room. We have gowned people working under a hood.

[Slide.]

It is interesting to note that once the cells have been digested and are placed into a bag, the cells never are exposed to the environment again. Throughout the rest of the process, the cells are in bags and a completely closed system has been developed which allows us to transport these cells, wash the cells to the clinical site and put them into the final reactor without ever exposing the cells to air again.

In this particular case, we are looking at a cell washing. We have put them into the cryopreserved state.

[Slide.]

A very important part of our process is the cryopreservation of the cells. All of our cells are cryopreserved in order for us to be able to carry out extensive sterility endotoxin, standard viral for culture, mycoplasma, viability and function tests. It is interesting to note that from the time the organ is excised from the

animal until it is actually released to the clinical sites is at least 45 days for all of these data to come back.

[Slide.]

The cells are placed in special cryobags at this point. They are put into a specially designed cooling

chamber.

[Slide.]

The cells are stored after controlled cooling in liquid nitrogen for long-term storage. We have now checked the stability of these cells for a period of over three years, now, for function and viability and the cells are performing the same as the freshly isolated systems.

[Slide.]

So, in an instance, we have the animal, the hepatocyte as well as the final product all quality controlled prior to their being delivered to the clinical site. At the clinical site, they are thawed. When the cells are used, again, we do a cell account and viability. They are added to the microcarrier, seeded into the cartridge and there sterility and endotoxin samples are taken.

[Slide.]

At this point in time, we are ready to treat the patient. This shows the cells on the outside of the cartridge and the system is ready to go.

[Slide.]

We have looked at a strategy for our risk analysis for the curve. We see this in two particular areas; the patients, we have now begun a retrospective analysis for the evidence of PERV in the blood cells of patients treated

prior to or with the HepatoAssist system.

Blood samples are now taken of serum samples that we have stored so we are now in the process of identifying and contacting all of our patients that have been treated and looking back and seeing if we can get blood samples from them, samples which I will talk about in a minute.

Prospectively, we are planning to test and analyze the blood samples from patients treated with the HepatoAssist system for evidence of the PERV, both of these using the DNA PCR assay. From a product standpoint, we are looking at the cryopreserved hepatocytes. We are looking at the animal analysis of these hepatocytes for evidence of infectious PERV using both a standard RT assay and the RT-PCR culture assay.

We are also looking at an interesting alternative here which is the actual use of the system in the way it is being used and that is to run a bioreactor with both the cryopreserved hepatocytes or the hepatocytes thawed out or PK15 cells and being able to look at the ability for this PERV to transmit from the bioreactor into the perfused

plasma in the system and then, again, looking at RT-PCR culture for ability of this PERV to be transmitted.

[Slide.]

To date, we have treated 54 patients. I include one more patient that was treated under compassionate IND

last month at Cedars Sinai and there have been no instances of any clinical manifestation of unknown viremia for periods now ranging up to five years.

The peripheral blood lymphocytes of five patients which we have now identified and, after duly-informed consent, have taken their blood and they have been tested. Now, Allen Moore and his group, and recent results suggest that they test negative for the PERV infection using the DNA PCR.

These patients have been treated at various times with one treatment up to three treatments and it should be noted that the oldest treatment is now 3-94, which is about three-and-a-half years since the actual treatment date.

All but the 10-95 patient received a transplant. The 10-95 patient is one that actually recovered and does not have a transplant now.

[Slide.]

In summary, we believe the HepatoAssist is significantly different in risk profile compared to organs, themselves. Xenotransplantation, as well as whole organ, ex

vivo perfusion and freshly procured xeno tissue. One, he cells that we used were cryopreserved and allow for extensive QC evaluation prior to human exposure.

Two, the cells are not in direct contact with human tissue during or after the treatment. Three, the

cells are exposed for a short period of time via extracorporeally plasma-perfused membrane bioreactor. They have no evidence to date of infectious PERV in cryopreserved porcine hepatocytes.

We have sent samples for standard RT assay. We have retained hepatocytes from the hepatocytes used on our patients plus new patients and, right now, the data is not suggestive of any PERV in that area. We are still working on some validation issues regarding that particular assay.

[Slide.]

Number three, we have no evidence of PERV infection in humans either via the clinical symptoms of the unknown viremia for periods of one month to five years and no evidence of PERV infections in the five patients tested. Finally, the data from the phase I/II clinical study of the hepatic system indicated a good safety profile and preliminary evidence of clinical efficacy in the treatment of patients with acute liver failure.

Thank you.

DR. AUCHINCLOSS: Thank you very much. We heard

this morning, I believe, some information that suggests that the cryopreserved tissue might, in fact, not be infectious for endogenous retrovirus. Carolyn, did you want to make a comment on that?

DR. WILSON: I think the only thing maybe for

consideration is just that we don't know what conditions are required for activation and whether or not it is reasonable to try and explore some of those avenues.

DR. AUCHINCLOSS: Other speakers from this morning. Is there reason to think the cryopreserved tissue would be somehow safer? No. Shaking heads. I am seeing "no" all around the table.

DR. COFFIN: I have a different question. What is the nature of the barrier between the cells and the plasma. I mean, what it pore size?

DR. SOLOMON: It is a microporous fiber that has a pore size of about 100 nanometers, probably smaller after it has been exposed to the plasma. We are not making a claim that it is a total barrier to the virus, but it is certainly one that has a significant rejection of the virus, looking at a virus that potentially could be as large as 80 nanometers in size.

DR. COFFIN: Have you actually looked at this barrier with any viruses?

DR. SOLOMON: We are doing those studies now. We

are actually going to put PK15 cells into our bioreactor and look for that transmission right now. The studies are beginning right now. We have just completed the study design for that.

DR. HIRSCH: I have two quick questions. I am not

sure what is meant by clinical manifestations of unknown viremia, if you clarify that. The other one is getting back to what Ron asked earlier. Are there plans to look for any kind of antibody in all of these people. It seems to me that might be a more sensitive way of looking at things than--

DR. SOLOMON: We can do that. I am not aware of any antibody test that has really been developed right now.

DR. HIRSCH: Do you know if there are any contaminating lymphocytes or macrophage monocytes into the hepatocyte--

DR. SOLOMON: We have done studies on that. I don't know if there are any--at this point in time, we can't say that they are absolutely free of any red cells or cells in there. We do see a purification of the hepatocytes from a norm of 65 percent up to about 90, 95 percent. But there are probably other issues in there.

They will be sequestered and kept on the outside of the membrane. Those will not move across the membrane.

DR. DESROSIER: I didn't hear whether those were

fetal hepatocytes or adult--

DR. SOLOMON: These are, I would say, juvenile pig hepatocytes.

DR. DESROSIER: I didn't hear anything said about what infectious agents were specifically screened out in

those pigs. Are they reared in some of--are they gnotobiotic pigs, for example?

DR. SOLOMON: No. But they are herds of pigs in which we screen--I think I showed at slide that showed all of the different viruses.

DR. DESROSIER: Were there any herpes viruses on that list?

DR. SOLOMON: Pseudorabies.

DR. ONIONS: Hepatitis C wasn't on your list which is the virus with which we are all concerned now because of the porcine hepatitis C which is very closely related to the human hepatitis C.

DR. SOLOMON: We don't have that yet.

DR. PAUL: My question also pertains to hepatitis C because it has recently been reported it is very common and we have some work going on in collaboration with NIH at our university. I think this is a virus especially for this particular application which should be looked at.

DR. WEISS: With the type of perfusion you are conducting, one might expect that the human complement of

human antibodies might get through into the liver cells and cause a sort of hyperacute injection of lysis of the liver cells and that could, then, allow a lot of pig-liver DNA to come through.

I wonder if you are testing for this?

DR. DEMETRIOU: We have tested that and we don't see any evidence of cytolysis during the treatment. Theoretically, if we treat patients more than ten or 15 times, it is possible that that would be an issue. The largest number of treatments we have done so far is five. In those patients, we have not seen any evidence of cell damage, cell death, release of anything into the extrafiber compartment.

We do measure--all our patients, as I indicated this morning, had baseline levels of xenoantibodies, both IgG and IgM. We see an increase and the increase is higher in patients who are treated more than two times and then, about two or three months after the treatment, they come down to the baseline, the antibody does.

DR. SALOMON: I have a question. My understanding is, in other people's experience and I thought that you have also reported it, that after four hours of extracorporeal circulation, you begin having a significant loss of these cells and that starts at around two hours and continues and you lose over 80 percent of their viability by six hours

after perfusion.

People have talked about why, but the fact is there is a lot of cell death going on on the other side of the membrane; right?

DR. DEMETRIOU: Yes. Actually, we have not

recorded these data. But we do measure all possible--during the treatment so we can monitor if there is a change in viability. Usually, we see about a 30 percent loss of function at end of the treatment. Where we do tripon blue or something crude like that, we see approximately 30 percent cell death at the end of the treatment.

It depends a lot on the underlying disease. People with fulminant liver failure due to acetaminophen ingestion with very high levels of plasma acetaminophen, you see much higher levels of cell death.

DR. SALOMON: It might be interesting, then, to look for antigen, for example, in the patients.

DR. DEMETRIOU: We do measure porcine antigen in the patients. We measured more than seven or eight patients. It goes up and stays up for a couple of months. It usually does not go up very high over the baseline in patients treated only once. It certainly goes up in patients treated over three times. It stays up for about-- and also the anti-albumin, anti-porcine albumin, antibodies stay up for several months.

DR. AUCHINCLOSS: We will move on to our final speaker, Walid Heneine from the CDC.

CDC Collaborations and Surveillance

DR. HENEINE: Good morning.

[Slide.]

What I will be covering is the strategies we are using for the diagnosis of liver fractions. I would like to point out that to date they are limited to PCR analysis and reverse-transcriptase analysis. We are working to develop a serologic assay that will complement these tests.

The limitation with the developments serologic assays is the availability of antisera, so working on producing these antisera as positive controls. So you will be hearing from me on the PCR analysis that now we are using and the compartment we are tolerating because of its accessibilities, the peripheral blood lymphocytes. We will be discussing whether this is a good target or not.

But the assay, itself, we use amplification of two different virus regions. We have been careful in designing the primers and probes for these two regions that are known for the sequences. For example, the generated sequences in our lab were generated from the Shimozuma. This is the secuba variant virus.

For the pol, we have compared our sequence with the one published from Dr. Weiss' lab. We have genetic

primers and an internal genetic probe based on the two sequences.

Remember, as Dr. Onions mentioned, that the Shimozuma sequence is variant similar to the pol and the MPK. That may be different. In the envelope, we know it is

different from both A and B and maybe the C sequence.

We define the positive PCR on the basis of two different regions. I will tell you why we do that, because of region and so forth. In addition, we see positive, we need to look at the possibility of microchimerism. A positive PCR signal for the first sequence does not imply the presence of these sequences and infection of the human cells but it may also be due to the presence of pig cells and we need to have assays that can distinguish between these two scenarios.

We use PCR analysis of a porcine cell-marker sequence. We have at least three different assays developed, one on a low copy number, porcine-specific betaglobulin. We now have mitochondrial pig-specific sequences which should present a high copy sequence and, therefore, cannot be missed.

All these assays, as I will show, you, the key feature is that they should be highly specific to the pig and should not be active to human sequences.

[Slide.]

This is the algorithm we are now using in our lab. The PBL samples, after we obtain them, we lyse it and we put it in different aliquots. We test aliquot level 1 for both sequences, the pol and the gag. If it is negative, then we stop there. If it is positive, then we go back to a

different aliquot, and we test it again for the positive sequence, either pol or gag or both, if that is the case.

If it is negative, then we report it negative for third-class sequences and this could be interpreted as potential absence of infection. It is possible to stop there. This is a confirmatory positive. Then we proceed with the analysis for microchimerism.

We thought a little bit about a simple way on how to differentiate between presence of infection or presence of chimerism. This is what we ended up doing.

Again, we wanted something simple but effective. The way we are doing it right now is that we obtain peripheral-blood lymphocytes. We dilute these before lysing them for the analysis. We dilute them and then we test these different cell dilutions for the presence of the pig mitochondrial sequence and the PERV sequence.

This is a scenario which may reflect infection. If you have PERV sequences in dilution, the titer of it is much more significantly high than that of the pig mitochondrial and that would reflect that you have the human

cells that are infected with PERV and maybe some residual chimerism with pig cells as well.

Now, if both PRC titers of the PERV and the pig mitochondrial sequences are equivalent, then that may reflect the presence of pig cells only in your sample and

not the absence of human PBMCs that are infected with PERV.

[Slide.]

Again, for the pig mitochondrial series, we used the same algorithm. If it is positive, we go to aliquot 2. If that is confirmed, then we will go to what I have mentioned before in microchimerism studies.

[Slide.]

It is important that the assays are developed to look at in the normal human population to see if those sequences are floating out there or not. It will give us confidence about the basic viral activity in these samples.

So we have obtained normal U.S. blood donor samples and we have tested them for PERV, gag, we have the pol now, and for also the pig mitochondrial. All these samples we have looked at so far, 54 here and 69, were negative.

[Slide.]

So what do we do when we identify a sample which is positive for PERV sequences, meaning no evidence of chimerism and this is a true infection of the human cell.

Of course, we are not interested in this only to stop there. We would like to look at markers of expression, namely, do we see any evidence of virus that has been released in the plasma.

We can do that by our TPCR. We have those assays

available. We can also do it by Amp-RT which is an ultrasensitive PCR-based assay that looks for reverse transcriptase in the plasma. We have included here serology which was a very important marker, as I have mentioned earlier.

We also do sequence analysis. We are interested in looking at the divergence of the sequences that will be infecting those human cells. Of course, tropism, focussed on the envelope to figure out what kind of envelope the sequence is associated with these human infections.

[Slide.]

My last part is what are the data we have available right now from these studies. Again, we have an IRB-approved study protocol at CDC which is open for everybody and we don't charge for these testings.

We have looked so far at three humans that received liver perfusions extracorporeally. They are from three different institutions. One is Johns Hopkins, Dr. Klein. Another one is from the University of Wisconsin, Dr. Kirk. The third one is from McGill University, Dr.

Trechenkow and Dr. Tector.

The McGill patient, the sample was obtained approximately three months after the procedure. The other two were more than a year and a half, one year and a half to two years. All three patients were negative by DNA analysis

from the gag and the pol PERV sequence.

In addition, we realized the importance of animal models to address the important issue of transmission. We are doing collaborations again to look at transmission of these animal models.

This is an example of one collaboration with Dr. Allen Norin at New York State University. There is a type here. It was six animals rather than eight. I would explore this for the second slide.

[Slide.]

This is the information we have on these six bonnet macaques that received two skin grafts. One of them is from a CD59 transgenic pig and another is a control from a normal pig. These are 2.5 cm by 2.5.

They are divided into three groups. Group A didn't receive any immunosuppression. Group B received cyclosporine and steroid and Group C received, in addition to these two, irrigation.

I have shown to you time to rejection of the CD59 skin graft which was higher than the normal graft. So this

would give you an idea about the exposure time of these macaques. These are the three time points we have tested on these macaques. Day 13, Day 40, Day 81 after the grafts were put.

Again, all six animals were negative by DNA PCR

analysis of all the gag sequences.

[Slide.]

This is the example of the tests we did and it will serve two purposes, to show you sensitivity and specificity of the different assays. This is pig cell PK15, diluted in a constant background of DNA for 150,000 PBLs. This is what we use as our PCR here for 150,000 PBLs.

Again, here, we can see reliably these two assays detect the equivalent of 0.15 cell equivalent DNA from 0.15 PK15 cells. We have now plasmas for these gag and pol, and we will be determining the exact sensitivity of these two assays in terms of plasma copy numbers.

But, again, we go beyond the cell level because, as you have heard, those sequences are high copy, more than one copy per cell, and this is evidence of that as well.

This is the mitochondrial DNA sequence. Again, this is high copy and here we can go beyond the 0.15 and we see the strong signals associated with these dilutions.

This is a typical experiment. These are different animals, A1, A2, one type of experiment with other controls,

sensitivity controls. They include every experiment, and if this does not show up, then the experiment will be repeated. These are the negative results I have mentioned to you.

[Slide.]

I don't want to complicate your lives further. We

are focused right now on the porcine endogenous virus, but we should keep in mind that the virus in pigs have not been very well studied as much as simians and humans.

One has to keep in mind the possibility of the presence of another or a different retrovirus. We plan also to look genetically for expression of retroviruses and the presence of retroviruses in the plasma from these patients by looking for reverse transcriptase activity using the Amp-RT method.

If it is positive, of course, go to virus isolation, cloning and so forth. But also we will be using it on some of the PCR-positive individuals as a marker of expression of PERV and we may expand that to include it as a basic component of our algorithm.

This is the team.

Let me add here that we have initiated additional collaborations here. We will be starting looking at ten Swedish patients that have received flutin bacteriatic cells and, hopefully, we should have those data soon.

We also have additional collaborations of animal

models. One is with Dr. Colin Weber at Emory who is doing encapsulated bacteriatic eyelet cells in rhesus macaques.

DR. AUCHINCLOSS: Any questions?

DR. ALLAN: Your algorithm for PCR. The question I had is it looked to me like both PCR assays you used in

the first case and then this second to confirm are both the same assays; is that right?

DR. HENEINE: Right. We will be running both regions on every sample. If you have a positive on one, or on both sequences, then you will look at the second aliquot and confirm that positive result.

DR. ALLAN: The reason I ask is because it seems to me you may be biased for a negative result because, if you have got a positive result and then you run it again and you get a negative result, to me, that doesn't say that it is negative. It still could be positive. You just got a false negative. So I would want to go to a third one.

DR. HENEINE: We have addressed this and this is an important point. We have determined the rate of false negativity of these CRSs. I don't have the data here. It is being compiled. Usually, you do that by running a large number of reactions at your sensitivity levels.

We take the 0.15 dilution and we run about 90 or 100 seconds and you look for any times that have missed your detection system. So you can flag the negative by the rate

of your false negative results. That data is available.

DR. AUCHINCLOSS: Any questions from our last speaker?

If not, it is quarter of 1:00. I am going to suggest that the thing for us to do is go ahead and break

for lunch and then we will begin to discuss these issues during the course of the afternoon.

But, in return for halting the discussion at this point, I think we will try to reconvene here at 1:30.

[Whereupon, at 11:50 a.m., the proceedings were recessed to be resumed at 1:30 p.m.]

[Whereupon, at 1 o'clock p.m., the proceedings were recessed to be resumed at 1:30 p.m.]

A F T E R N O O N P R O C E E D I N G S

[1:40 p.m.]

DR. AUCHINCLOSS: We will resume the afternoon proceedings. We have another comment as part of the open public hearing.

Open Public Hearing (Continued)

DR. LEVY: Thank you very much. My name is Marlin Levy. I am a transplant surgeon at Baylor in Dallas. I have some research sponsorship from Nextram.

[Slide.]

My comment really addresses the issue of where I live which is at the bedside. I understand that the committee and the FDA is charged with public-policy issues which are of tremendous importance.

This is a 17-year-old man who came to our hospital, a 17-year-old youth, really, who came to our hospital in acute fulminary hepatic failure at the very first of October and who was dying and had no liver transplant available. No human transplants were available.

We placed him, as you can see in this picture, in

an extracorporeal circuit with a transgenic liver.

[Slide.]

This liver is transgenic for CD55 and CD59. We perfused him for six and a half hours during which the liver worked very well. The patient improved.

[Slide.]

He subsequently went on to receive a human liver transplant and has done very well. He is now 75 days both from his perfusion and his transplant. So, as I listen to your proceedings and I understand the evidence that you are weighing, I beseech you to keep in mind use like this one and others because I think they are very critical. It is very critical to think of them also.

Since this particular perfusion and transplant were done, we had one more opportunity to have another patient go this trial. But, by then, of course, the trial had been placed on hold and so we weren't able to do that. She was successfully transplanted. It remains to be seen what the outcome is going to be because she is neurologically quite handicapped at this point.

Thank you very much.

DR. AUCHINCLOSS: Thank you.

Any other comments from the public as part of the open public hearing?

We move on to the afternoon session. We have the

data on the table from this morning. In fact, however, those who have followed this field recently have been aware of most of the data that we saw this morning for some time. I don't know about everybody else, but it is far from clear to me, despite knowing the data, exactly what we are

supposed to do about it.

That is really the topic for this afternoon, in general. As I mentioned earlier, the discussions of three questions here will be initiated by presentation from the focus groups. In order to get the conversation going--I don't expect that to be very hard--but, again, the emphasis is that this is open for discussion by all committee members whether you are part of the individual focus group or not.

Again, our purpose here--this is not a federal regulatory body, this subcommittee. We are here to provide advice to people from the FDA and that won't necessarily be in the form of a consensus. We really want to hear all points of view and put those out in front of the people who have some very difficult decisions to make in this area.

With that introduction, we will move on to the presentation from the focus group. John Coffin, I believe, is first at presenting the response and the general introduction to Question No. 1.

Formatwise, what I understand we will do here is the focus group leader will make a ten or fifteen

presentation and then you, Amy, will lead us through the questions part by part as a larger group; is that how we are going to work this?

DR. PATTERSON: We can do that or we can project the questions; however the focus group wants to go through

them. But I think the strategy you have outlines probably makes the most sense.

DR. AUCHINCLOSS: Is that all right with you, Joe?

DR. COFFIN: It is okay with me.

Presentation of Focus Group Analysis of Question 1
Development of Strategies for the Detection
of Porcine Endogenous Retrovirus

DR. COFFIN: We were fortunate enough, actually, to be able to get together for a one-hour teleconference. So we were able to have some discussion and come to a consensus. I should say, starting off, we are taking a very stringent line on the consensus but I think it is a good starting point for discussion of these issues.

The questions that we were asked to address can be summarized as follows; first, should it be required--I am just giving a quick summary of them. I am not reading them as they are written in the book--it should be required that assays for porcine endogenous viruses in graft material be developed and applied and, if so, what assays, what are the important considerations of sensitivity and specificity,

should there be induction regimes, how long should cocultures go on.

Second, we were asked to consider the issue of biologic characterization of infectious agents; again, discussion issues of sensitivity and specificity,

infectivity for other cell substrates. The big question is hidden in there and that is the impact of the detectable virus on the suitability of xenotransplant material for use.

The final issue was how are these considerations affected by different types of uses, for example, consideration of transplant versus ex vivo applications or considerations of duration of use.

I should say, starting out, that the group was somewhat concerned also that we should not forget consideration of infectious agents other than endogenous viruses. But given the lack of information that we were provided concerning these other agents and the complexity of this specific problem and the specificity of the charge, we won't consider that issue any further. But we certainly shouldn't forget it.

Second, it is clear that our principle problem here is ignorance. We know very little about the distribution of infectious proviruses among pigs and essentially nothing about their potential for replication and pathogenesis in normal and immuosuppressed human hosts.

We are beginning to get that data, clearly, from what we have heard this morning but we have a long way to go and we can certainly only guess about their potential to be transmitted from one host to another.

Furthermore, there is no established animal to

study these issues. Clearly a major focus of clinical/preclinical studies should be to obtain this information. Again, we have heard a fair amount of research in that direction. That should certainly be continuing until we feel more comfortable that we have some basic understanding of what is going on.

It was noted that currently ongoing xenotransplantion trials in primates could and should be utilized to provide some of the necessary information--I think we have heard a little about this--as well as, of course, all the data from the human studies that have gone on.

Given these considerations, it was the consensus of the committee that human graft recipients should not be exposed to infectious virus either present and/or inducible from xenograft tissue until such time as animal studies or human studies provide a measure of confidence that spreading infection will not result in the recipient.

Stringent testing of the potential donor tissue will be required. Because the proviruses are inherited,

however, it may not be necessary--it probably won't be necessary--to test each donor organ or tissue prior to use. If it can be established for a given strain that the prospective donor animals have the same composition of proviruses as the test samples, which is the genetic effect

we are looking at, there needn't be a burden of extensive testing of each organ if you understand the genetic composition of the host relative to this particular issue.

Conversely, if a given tissue or a specific donor strain has tested positive for infectious virus, then that tissue must be assumed to be infectious in all other animals of the same strain unless we demonstrated genetically the responsible provirus is not present in the donor.

So, given those principles, our answers to the specific questions as posed are as follows: yes, appropriate tests for the release of PoEV infections, infections for humans should be developed and applied. That clearly is ongoing.

The development process should also include studies on the genetics of the proviruses since, again, it can be assumed that if the virus can be obtained from a specific organ from one individual, all individuals carrying the same composition of proviruses will have the same property.

Conversely, it can be established that, if a

tissue does not release virus from a sample of animals, then all donor animals with the same proviruses will also have a same property.

Testing should include in vitro cocultivation studies as well as follow-up studies in appropriate animal

models and long-term clinical monitoring of human recipients.

Second, the best sort of in vitro test is cocultivation with as sensitive an indicator line as can be found. It is not necessary for the cocultivation to be with a human cell line. The mink cells or the cat cells that Dr. Onions mentioned would seem to be reasonable--this requires, of course, a careful study to determine this--under conditions which will vary depending on the donor tissue.

I would think that, although we haven't heard much of this used, the subtoxic levels of inducing agents such as demethylating agents, such as 5-azatadine or bromodeoxyuridine, also are good adjuncts to that because that gives the best chance of seeing if anything in there can be induced. These are well known to be good inducers of endogenous proviruses in other animal models.

Filtered supernatant from coculture can be tested for infectivity in appropriate indicator human cell line. We have seen a lot of this this morning. I hardly need to belabor it--using specific PCR assays and so on. Certainly,

coculture is to be maintained as long as practical, particularly since we consider they are being used to clarify the situation with a given strain of animals rather than with a specific individual.

Recipients should then be tested for spreading, or

localized transmission of infectious virus. The next focus group will consider these issues. Certainly, any recovered infectious should be characterized by sequencing and to identify it as a novel or known agent.

As an aside, an issue that came up this morning. It will be important to get an idea of how many proviruses can release infectious virus. One way to do that is to sequence a lot of these and see if you get even slight differences. Even a single base difference, reproducibly in sequence from what is otherwise the same virus would provide fairly strong evidence that there are actually a couple of different proviruses that can give rise to essentially the same thing. It will be important to know that for future genetic analysis.

Any new agent with a novel sequence should, of course, be characterized by determination of host range for cells in a variety of species in part with the goal of identifying potentially suitable animal models. Also, infectious for primary human cell types should be tested, again to provide guidance as to how best to assess infection

in the human recipient.

Guiding by these results, infectivity and pathogenicity should be evaluated after infection at as large a possible of virus in appropriate newborn animals. Again, experience with retroviruses indicates most

pathogenesis models, especially with viruses like these, require the use of newborn animals in order to really see what is going on.

Injected animals should be monitored appropriately. They, obviously, should be thoroughly analyzed by PCR and by antibody studies. Until such studies provide some evidence that there is no spreading infection or pathogenic consequences in the models used, the bottom line sort of is the likely presence or inducibility of virus infections for human cells and donor organs should preclude its use in xenotransplant studies until there is good reason to expand and to go forward, given clean negative results in appropriate models.

In answer to the last question, although it was recognized that some applications such as short-term extracorporeal perfusion and introduction of small numbers of cells into sites such as the brain where virus spread is likely to be inefficient, that such treatments would have a lower probability of giving rise to spreading infection than others.

It was a consensus view that all procedures carried some risk of transfer of infectious virus and should not be performed if virus is present.

That is basically the bottom line of our discussion.

DR. AUCHINCLOSS: Before we go to putting the individual components of the question up, at least I am going to ask for a couple of clarifications on this and maybe other members of the committee do as well, and then we will go through the stages of your question one-by-one for the committee at large.

There are really two points that I wanted you to clarify for me, John. One is this issue of is there an animal model and, in particular, does pig into non-human primate constitute an adequate test of the infectivity, pathogenicity, of the pig endogenous retrovirus?

During the course of the morning, we seem to hear that infecting non-human primates with this virus was harder than infecting human cells, but we did see several examples where it appeared to be possible. But, on the other hand, would the tropism that you might find or not find and the pathogenicity you might find or not find in a non-human primate tell you anything about what would happen in the human?

Is there an animal model for this or is the

information going to come from clinical studies?

DR. COFFIN: It is a very good question. I think attempts should be made to develop some kind of an animal model, perhaps even in the mouse, just to get some handle on how you work with this or what you might want to look for.

I think the points that you raise are very good ones.

Starting off from scratch, there is no assurance that what you are going to see is going to be relevant. I think one thing we didn't appreciate, at least I didn't appreciate, when we had this discussion, was the size of the human population that is available for study already. Given that, I think I, at least, would modify somewhat and I see some nods from other subcommittee members, on going more toward detailed in-depth studies of human recipients.

DR. AUCHINCLOSS: A second point of clarification for you, John, is that it sounded like what you were saying is that if you can demonstrate infectious virus in a donor source that that source is out. That is what I thought I heard you say. That, I though, translated to pigs are out. Actually, I thought it translated to no further xenotransplantation.

DR. COFFIN: That is a possible outcome of this. That actually, I should say, in all honesty, is a more stringent position than I, myself, personally take but it was the consensus. It was the consensus of our group that

we should at least start with this point, start with this position.

DR. AUCHINCLOSS: Am I right in saying that this is a Catch 22, the only way of testing the issue is clinical trials. That was the point of my first clarification. But

no clinical trials should go ahead, would be your focus group--

DR. COFFIN: It was suggested that there be a graded sort of series of things as you get more and more comfortable. That is what I would suggest.

DR. AUCHINCLOSS: Is it not correct that we are all taking--is it correct? Are we working with the assumption that all pigs have an infectious endogenous retrovirus that can go into human cells?

DR. COFFIN: Until shown otherwise, I think we have to. I don't think we have any choice.

DR. HIRSCH: I am surprised by the recommendation. I think it takes an awfully draconian position.

DR. COFFIN: It does that.

DR. HIRSCH: That the virus is guilty until proven innocent and that the tissues are proven guilty until--

DR. COFFIN: You can't prove innocence here as in a court of law.

DR. HIRSCH: But the point being that the data I have heard this morning is that most pigs have this or all

pigs have this and all pig tissues must be assumed to have it. And yet it seems to have a low infectivity--

DR. COFFIN: Can I just work through the alternative because the alternative really is that you go ahead, obviously, with the extensive monitoring and,

perhaps, with some limitation on the kinds of things that are done initially limiting to more life-threatening conditions and so on until a better experience is obtained and work sort of through in that way.

What that means is that basically you don't pay any attention to whether the virus is in the tissue or not, because that is the alternative. You actually don't test. You don't bother because you assume it is there but you are not going to care.

DR. AUCHINCLOSS: There is a middle position. I would take that position that you don't care, because I assume it is there. I think the tests are worthwhile primarily to validate the test because what you really want to be testing later on is the patient.

DR. COFFIN: There is a second use for these, and that is what we have heard today so far, is to get as much information as possible to work later on.

DR. AUCHINCLOSS: So you can argue for testing without saying that because the virus is assumed to be there, you should never do xenotransplantation--

DR. COFFIN: I am in a slightly uncomfortable position here.

DR. ALLAN: I think part of the reason there is this rigidity and, obviously, it is a starting point--the real question is whether we have done enough homework in

terms of understanding what a pig endogenous retrovirus might do.

Obviously, you have got the non-human primates that have already been used in preclinical studies. Those really need to be evaluated and it sounds like people are beginning to do that. But you can do something very clearly and, even though Carolyn's studies would suggest that maybe it doesn't go into primates, I would probably suggest that three cell lines isn't enough to be able to tell.

I would think that probably you could find a non-human primate species, whether it is a macaque or baboon and we should probably use several of the species, is that you can do everything possible to set up an ideal situation to transmit a virus and to get to express in a non-human primate.

You can use newborn baboons and macaques. You can bolus them with large amounts of cell-free virus, also virus that has been put through a non-human primate to set up an ideal situation to get the virus to express itself in that particular primate.

If, in fact, the virus doesn't infect a baboon, it doesn't infect a macaque or it is only at very low levels, you may be more assured, going forward with these particular studies, that you may have less of a risk than you initially have thought. You have some sense of where your starting

point is.

If you just start into humans going blind, then I think you have got the problem. The real issue for me is that you are dealing with, and as John Coffin said, the question of whether the virus is infectious. He is starting at the point that it is infectious.

It may be infectious, but minimally infectious. If you look at a newborn baboon and you see that you have got a cell-free virus, it is being expressed and it is in high virus loads or something like that, you are going to be more careful about going further in humans because what you need to understand is that we can talk about pathogenicity and whether these viruses are pathogenic or not, but there is no assay, there is no way to determine whether or not pig endogenous retrovirus is going to be pathogenic in humans.

We have enough information from human retroviruses that suggests that their pathogenicities may only show themselves after many years and after a lot of people are infected.

So I think that if you can demonstrate that you

have got very little virus expression in that particular animal model system, it is going to give you a lot more assuredness that going forward is not going to be--or is going to be, maybe, less of a problem.

So the issue is what do you need to do. Can you

do it in parallel at the same time you are doing human studies or do we need to do a few non-human primate studies very quickly. We can argue that point, too.

DR. DESROSIER: The committee didn't start with a complete knowledge base but I think most members of the committee were not comfortable with the notion of knowingly putting in replication-competent pig retrovirus into people. I think that was our basic starting assumption.

We were not aware of much of the data presented this morning, particularly the numbers of humans who have already received either the brain implants or the hepatocyte treatments. So I think there is a wealth of information now in these people who have already been exposed to pig materials.

I will frankly say that I think it is entirely possible that all of these humans that we heard about this morning have been infected with pig retrovirus, but appropriate assays have not yet been used to demonstrate that.

I think if all of these people had been Herpes

simplex virus negative and we had knowingly inoculated Herpes simplex virus into these people, then it would not have been detected with the assay procedures that were used.

I think it is very important, very important, that sensitive, reliable antibody tests be developed and that

these people who have already been used in these early trials be monitored for antibody responses to the pig retrovirus.

I think that information is going to be enormously valuable in deciding how to go forward.

DR. AUCHINCLOSS: I am going to continue with general comments here for a couple more minutes and then we are going to tie ourselves down to the sort of particular questions. So let's go ahead.

DR. VANDERPOOL: I am not a biologist or a specialist in the areas in which most of the people this morning have spoken so far. I am primarily interested in ethics and history, the history of medicine. But it seems to me that the surprise we all had with the new information about the porcine retroviruses certainly seems, on the surface, to have entirely legitimated the FDA's putting studies on hold.

Yet, as I listened this morning, I kept saying, "Okay; should I get more scared or less scared, more scared and less scared?" I went back and forth more or less for

most of the morning.

And then I began to reflect, okay; there are xenotransplant trials and there are xenotransplant trials. Some seem to be less risky than others and, certainly, more efficacious than others. I just want to put a word in for

those trials that appear to be greatly efficacious for desperate patients and to say unless there are clear signs that there is real danger out there, that we ought to give those a chance to go forward.

It depends on how squeaky clean you want to be about harm and risk, but the challenge, as was introduced this morning, is to balance harms, conceivable harms, and certainly benefits. So I think we need to restore the benefit side of our deliberations as we go forward.

DR. WEISS: Unfortunately, I wasn't able to join in the long conference call that John Coffin conducted, but maybe that is an advantage. It gives me an individual opinion. As one of the guys who sort of set the hare running by showing that these retroviruses, that some of them can replicate in human cells, I still feel that it would be too draconian to say we have got to stop everything.

Just as we have heard from Dr. Vanderpool, there is a risk in every medical procedure. I think the risk/benefit calculations for the indication recipient--

well, if I was one of them, I would take the risk. So, to my mind, the big question before us is the most difficult to answer; what is the risk of human to human transmission if a recipient of a xenograft becomes infected with the virus.

I think C-type viruses are different from

lentiviruses. They tend to be less pathogenic when introduced into adults, although severely immunosuppressed adults is a very different matter from immunocompetent adults.

I think the probability on the transmission, of starting a human epidemic iatrogenically through xenotransplantation, is a remote one but it is a very devastating one.

So my opinion wouldn't be that we absolutely must not proceed in a single further patient but, rather, as we have heard already, that we should extract the maximum information that we can from the people who have been potentially exposed because they have been exposed to live pig cells.

We should get more information on that. We should try and develop animal models, imperfect though they are. We should proceed slowly. But I don't think we should go into reverse. That is just a personal opinion.

DR. AUCHINCLOSS: Amy, I am going to turn to you to put up the questions and we will really go through

question 1 piece by piece. But, before we do that, I am going to suggest two things that I think I have heard that maybe there is a consensus on at the table here.

Let me start with the one that Robin Weiss just expressed, that, at least in my view, the risk/benefit

analysis for the individual recipient is well within reason and not the issue, certainly not the retrovirus issue or even any infectious issue related to xenotransplantation. Frankly, if you compare it to allotransplantation, it is much safer.

So the issue is not the individual. The issue is public health. Is that something you would agree with at this table?

[Affirmative responses.]

DR. AUCHINCLOSS: The second thing I am going to suggest that there might be agreement on is that one must assume, at this point, that all pigs have an endogenous retrovirus capable of infecting human cells. Is that something we would all say?

DR. SIEGEL: Let me get a clarification of that, though, because there was a lot of discussion. I am not sure all the people were addressing the same issue. Maybe Dr. Coffin, you can clarify what your subcommittee was talking about. On the one hand, this committee, everybody sat around and said yes, all are infected.

But Dr. Coffin, I thought, said you shouldn't put in tissue in which you identify or can induce infectious virus. I am not a virologist, but it is not clear to me that just because all pigs both have the genome and, in some tissues, you can induce virus, that that will necessarily be

true of all products.

We have heard from a couple of sponsors who, to date, have cocultivated--they haven't induced with BRDU but have cocultivated and have been negative. Is, then, what you are getting at is it doesn't matter what they test or induce, whether it is negative or positive, we treat it as though there is infectious virus there?

DR. AUCHINCLOSS: Jay, that would be my answer is that I would treat all tissue as if it was potentially infectious. But let's put this as a question because it was one that I was going to ask of the committee.

There is, I think, the assumption, the way you phrased the issues in question 1, and, indeed, in the way you have presented your report on question 1, that some pig tissues are better than others and maybe some pigs are better than others and maybe some strains or lines of pigs are better than others.

Is that what you mean to imply and is that what the FDA believes?

DR. COFFIN: I don't know what the FDA believes,

but that is what we meant. We meant to leave the door open on that particular point until we have more information. It certainly is possible that one could identify or breed animals that, in fact, don't have infectious proviruses.

DR. AUCHINCLOSS: Do other members of the

committee want to comment on that?

DR. ONIONS: I really want to echo Robin Weiss' comments because, normally, I am very conservative on these matters. But I think one of the problems is often we use the HIV paradigm. These viruses are, biologically, very different.

To actually infect mice or cats with these viruses, there is an age resistance. Once you get beyond the newborn, you have to use very high titers of virus to establish a persistent lifelong infection, over 103, 104 infectious units per animal to actually establish an persistent viremia.

I think that the kinds of infection that we might see here, I don't exclude the possibility that we would see infection of human cells. But, to my mind, the real danger is the establishment of a persistent viremia which may also lead, then, to shedding of virus in saliva or other tissues. I think it is very important that we distinguish those two patterns of infection that might occur in patients.

Whilst I think the first might occur, I am not

convinced that the second will occur. I think what we have at the moment is a very good pool of patients, these 100 or so, or more than 100 patients, that have been exposed to porcine tissue who may help us answer that question.

DR. AUCHINCLOSS: Can you give us some words that

we can use to refer to these different--I have used infection in my own mind to mean viruses in human cells. Then there is a second level at which the viruses in human cells replicating and being shed; what do we call that?

DR. WEISS: John has just whispered to me and said productive. I would sort of call them either persistent productive infection or persistent viremia.

DR. AUCHINCLOSS: So there is infection, producing infection and then, of course, the issue of pathogenicity is still separate from that.

DR. WEISS: Sure.

DR. HIRSCH: I also wanted to ask John, I am willing to accept what you said that every pig has, potentially, infectious virus. But I don't think I am willing to say that every cell in every pig has potentially infectious virus. Again, you are assuming the cell is guilty.

It may be that a thyroid cell or a neuronal cell or a hepatic cell can't be induced to produce this virus whereas a lymphoid cell could be. I think we should accept

that possibility.

DR. COFFIN: In some cases, clearly, you can't put in pure tissue. You can't put in pure kidney cells if you are transplanting a kidney. In other cases, there is room, clearly, for substantial purification of cells.

DR. AUCHINCLOSS: My point is that there is an implication to the fact of testing which you may or may not mean to include, and that is that if you test and the pig tissue shows that it has got the endogenous retrovirus and you can induce it, then you shouldn't use it.

You should only use tissue that you can't demonstrate the virus because you believe that that is, in fact, better tissue. I don't believe that. I would love to know whether you people think that that is true. The tissue from pigs that you can't get the virus induced is better than tissue from pigs in which you can?

DR. SALOMON: I would also like to point out, just from the point of developing guidelines, until we formally prove that there are novel agents that we are not currently testing for, I would agree with our chairman's more conservative view that we ought to act as we do in the lab for HIV that all tissues are potentially infected and then use our best judgment and good scientific principles to move forward.

So I am not excluding your argument, Dr. Hirsch,

that there would be cells that might not produce a known virus, but we would never be able to formally exclude it at this early stage in the process.

DR. COFFIN: In some sense, our approach to this aspect of the answer was framed by the way the questions

were phrased.

DR. AUCHINCLOSS: Let's go to the questions. I think it is time that we tie ourselves to some words on the piece of paper and see what we really say in response.

Discussion of Question 1 by Subcommittee

DR. PATTERSON: The Center for Biologics at the FDA currently requires that sponsors of porcine xenotransplant clinical trials develop assays capable of detecting infectious porcine endogenous virus potentially present in their porcine xenograft products as part of the preclinical screening evaluation process.

The first question is a very fundamental one. Is it appropriate for FDA to require that sponsors of xenotransplant clinical trials develop assays to detect infectious porcine endogenous virus potentially present in their product.

DR. COFFIN: There are actually two issues here that Hugh separated. One is the research aspect of coming to understand what is there, what is out there to be obtained. And that needs to go on for a while, although

probably not forever. Clearly, I don't think anybody would disagree that that has to be done and should be required to get that information into the bag so that we can get an understanding of what we are facing here.

DR. AUCHINCLOSS: I think Amy thought this was a

"gimme." We are going to say yes, but we are not going to draw implications from it at this point. We are just going to say that good science says that you should be testing your tissue.

DR. SIEGEL: Let me clarify where we are on this because, when I do that, it may or may not be a "gimme." The position the agency has taken at this point in time is not a position on the recommendation which was just made which we will get to in a later question about what to do when a test is positive but that, in fact, we are at a state of technology now which calls for doing the assay, that there are sufficiently sensitive and informative assays that can lead to risk control and risk and risk containment, that those assays should be done on the tissues and on any patients that have been treated to date for whom adequate specimens are available prior to proceeding with further experimentation which is implicit in the word "preclinical."

That is why, at the present time, a number of these investigations are not currently proceeding because they are gathering the data that we have asked them to

gather. And so part of what we are asking the committee is not simply should these data be gathered but is it appropriate, while we have out there patients who have already received treatments who have yet to be tested in any way and tissues that have yet to be tested, is it

appropriate to continue to treat patients or should we get that information and then proceed.

DR. COFFIN: That is a very different question.

DR. SIEGEL: That is that question. It is just worded that way.

DR. COFFIN: We certainly read it differently.

DR. SIEGEL: I will take full responsibility for any confusion in the wording, but that is the question we need an answer to.

DR. COFFIN: It is no longer a "gimme" then.

DR. AUCHINCLOSS: No; it is not a "gimme." It is distinctly not a "gimme" and, in fact, we have been trying to get this distinction since we first began with this question. We are all going to say, all right; yes. Testing is a good thing. Fine.

Now, my own personal view would be testing is a good thing because you want to gather information and you want to prove that company is capable of doing testing that is sufficiently sensitive because what you are really going to want to test eventually is patients who received the

tissue.

But my own personal view would be that just because you got a positive on the test doesn't mean that that is tissue that you shouldn't use. Now, that is far from a "gimme."

DR. SIEGEL: The question now is is it okay for companies to continue to use untested tissue and not to have any way to test the recipients to see if they are viremic, whether they need to have those tests done before they proceed.

MS. MEYERS: Could I address that? During the last decade, all the blood-products companies didn't properly test their blood products for HIV and look what happened. So any company, knowing about this virus, who is dealing in xenotransplant products, who goes ahead and transplants tissues without testing for this virus will be liable.

So even if FDA doesn't stop it now, if the tissue is positive, then the courts will have to stop it later because that is what is going to happen when the first person gets sick.

DR. NOGUCHI: Abbey, you mentioned companies. A lot of these studies are done by companies but many are also done by transplant surgeons as part of the medical community. Do you see any distinction, because that is the

other thing that we have done. We have made no distinction in our clinical hold.

MS. MEYERS: There really shouldn't be a distinction, the way I see it, because if you don't test to see if the virus is there, then you are guilty, whether you

are a university or a company. If you do test and you find that it is positive and you go ahead an transplant, you are double guilty.

DR. COFFIN: The question is you stipulate the virus is there, which is what we are trying to do, and then move on.

DR. AUCHINCLOSS: That is the issue.

DR. COFFIN: You stipulate. You don't need to test it in that sense.

DR. MICKELSON: I would agree with you about the testing, in particular, not testing. But I think what that testing then does is allow you to inform the patient or recipient of the status of the tissue. An alternative might be, then, to allow the patient to try to make some sort of informed decision.

Presumably, the recipients at the moment are in an extremely life-threatening situation that these would tend to be bridging. Most of them are bridging transplants. They are not considered long-term therapeutics but possibly, at the moment, the way to look at the testing is a way to

sharpen in informed consent document with the proviso that, of course, efforts are being made to develop donor tissues that are getting freer and freer of these PERVs.

DR. MICHAELS: I think it is very dangerous to actually make the connection with HIV in testing because, in

fact, when the HIV testing was being done, HIV was recognized as a pathogen. This organism, even if the testing is positive, at this point in time, we don't know it to be a pathogen at all.

Now, I do believe the testing should be done. I do believe that follow up, and I certainly concur with Claudia's comment that it really leads to the informed-consent part. But I think that it is very dangerous. I think what we have heard from the retrovirologists today is that it is a very different virus.

MS. MEYERS: So you think the tissue should be transplanted even if there is a positive result and it does carry the virus.

DR. MICHAELS: I think that is something we are going to discuss. We are talking about the choice.

DR. AUCHINCLOSS: We are going to come to that question in a few minutes.

DR. ONIONS: I think there is confusion. What do you mean by positive. I would just like a clarification of what you mean by positive. All of these pigs, at least to

date until we really characterize the loci and determine whether it is possible or not possible to remove some of these loci, all the pigs that I have looked at and, I think, all the pigs that Robin has looked at, all contain the two viruses we know infect human cells.

So they all contain the provirus, the genetic information. I would strongly suspect that, at least in solid-organ transplants where you are going to be transplanting lymphoid cells, I would suspect, but do not formally know, that you can induce those viruses from those cells.

So I am not quite sure what you mean by positive. Do you mean that they are genome positive? Do you mean that they are expressing the virus? My third point would be, if you really mean that, how are you going to do that in the context of the xenotransplantation of solid organ. You don't have the time to take the organ, test the organ for production of virus--not in the presence of the genome, production of virus--and put it into a patient.

So I think we have to be very clear about the terminology here.

DR. COFFIN: Built into our response to the question was a comment that if it is true for one, you assume it is true for all, leaving open the possibility it might be possible to get some tissues that you could never

get virus from. But if you get it once, then you have to assume that you get it every time.

DR. AUCHINCLOSS: Back to the question, now, for a second because I need some help from you, Jay. We are saying yes, it is appropriate to require that assays be

developed. We are saying that we are going to deal a little bit later on in the questions with what to do when you get a positive.

What is the distinction, now, in this question that you want us to--

DR. SIEGEL: I guess one could argue that even if you know it is going to be positive, that having an assay in place, more than informing, informed consent, provides certain safety features.

DR. AUCHINCLOSS: I believe that.

DR. SIEGEL: You really know what sequences to look for in monitoring the patient. You may know which organs to look at because you might know what organs--

DR. AUCHINCLOSS: Precisely. It is good to have the assays in place.

DR. SIEGEL: So part of question is should the assays be in place and done before the--not necessarily in the specific heart, but in the product, in the type of tissue that is going to be transplanted.

DR. AUCHINCLOSS: Again, I would say yes. But I

think there is some narrowing to the yes.

If you have proven that your assay works, if you have proven that your source of pigs is positive or negative or a particular source of tissue from your source of pigs is positive or negative, I wouldn't imagine that you need to

prove that "every batch" be tested because the purpose is, again, not to define the virus. The purpose is to prove that you have the assay.

DR. VANDERPOOL: I agree with Marian that we are not assuming that this is an AIDS-type virus. We are just saying we need the data right now, and if later studies show that these are not as problematic as they might will become or appear, then the requirement could be eased.

I want to register one more objection to this question as worded and that is that the sponsors be required to develop assays. I think that the sponsors should be required to use assays or utilize assays and, in that way-- as it reads, it looks like every single sponsor needs to go out there and develop a particular series of assay.

DR. AUCHINCLOSS: Jay, how do you feel about that? Do you have to access two assays?

DR. SIEGEL: The sponsor is responsible for performing them. It shouldn't say developing.

DR. HIRSCH: Jay, I am still confused as to whether you are saying that company X who is doing this

procedure, there has to be a moratorium on any further patients getting this procedure until they have shown that using every conceivable assay that the previous recipients don't have infection. Is that what you are saying?

DR. SIEGEL: What we have told companies now, and

there is a letter in your book under Tab 9, is that before proceeding, they should have in place assays for both the product and for the patient and informed consent for the patient.

What we are asking you now is, is that an appropriate approach and then what you just asked me, what assays? Is it every conceivable one, what should they have to induce?

DR. AUCHINCLOSS: We are going to come to (b) right now. What tests, and what do we do with the results? We have made no decision that just because it is positive-- we understand that that would be a hold. I think, rather, our current situation is based not so much, as I view it, on a risk-benefit analysis but a risk-control analysis. We don't always accept a risk simply because it is overwhelmed by benefits. There are different types of risks. There are some that are intrinsic to a product. You are going to get them; if you are going to get the benefits you are going to get the risks. There are others that are controllable. To use what is probably a poor analogy, we, in blood, say if we

were to use less sensitive blood screening mechanisms for HIV we could probably still prove, and they were cheaper-- some company could probably come and say, you know, for every 1000 units we give we are going to save a lot more lives than are going to transmit HIV. But we would say that

is not adequate risk control because you can do better.

So, where we are now is saying, well, you can do these assays and these assays are going to be informative and potentially important in terms of assessing the arm and controlling arm in looking how to follow the patient, knowing what the potential are and knowing what to tell future patients, and since that can be done, and it appears at this point reasonable to do that, and since it is evolving so rapidly we need expert input as to whether, in fact, it is reasonable and how much is reasonable. I can assure you that if we ask them to do every conceivable assay by the time they are done there will be a better assay. Somebody will have a more sensitive one. You know that-- more sensitive strains.

We recognize it is an evolving thing and we are just trying to get at that. So, let's go to (b) because for (a) I think the answer is yes and (b) is fundamentally, all right, what assay? So let's go from there.

DR. PATERSON: Actually, if I may take the liberty, Carolyn had a quick question to clarify one of Dr.

Coffin's comments.

DR. WILSON: I just wanted to follow up on a comment you made before. You said if you did testing on a particular organ and it was positive you would then be able to assume that in all cases it would be positive. What if

it was the opposite, where the first few tests you did were negative?

DR. COFFIN: Clearly then you would have to come to some consensus or decision as to what you would consider an adequate sort of negative representation and the sampling, and that is something that would have to be considered separately.

DR. AUCHINCLOSS: Let's see what we feel about (b).

DR: PATERSON: Yes, question (b): Please discuss the types of screening assays most appropriate for accurate detection and identification of infectious porcine retrovirus in porcine xenotransplant products.

DR. AUCHINCLOSS: You have this broken down into (i), (ii), (iii). Do you want to go directly to (i)?

DR. PATERSON: Right.

DR. COFFIN: I think we have seen good examples of this today in some of these things being done.

DR. AUCHINCLOSS: Maybe that is one way of asking it.

DR. COFFIN: With the exception that I would like to see some induction studies included as well to see if we can get other things.

DR. AUCHINCLOSS: We have heard one statement that glaringly missing is antibody. This is tissue now at this

point, so we will come back to antibody when we come to the patients. I am jumping the gun. That is question 2. Sorry about that. So, stick with the tissue. So I guess we can turn to Carolyn and say, Carolyn, you have heard two companies tell you what they now do with their tissue. Is it okay?

[Laughter]

DR. WILSON: Well, I was going to turn it around. I think one of the areas that is being explored, and we heard data from Robin Weiss and David Onions about alternative cell substrates that may be more sensitive for detecting certain strains and whether or not we need to have more exploration in that area. Right now, we are currently recommending that 293 and ST cells be used because we have seen the most data on that and have the most experience. But if there are recommendations for other substrates, or whether or not we need to even explore that more fully, I think that might be useful to discuss.

DR. AUCHINCLOSS: So, ST is the positive control; 293 is the test cell. Is that what you are recommending at

this point?

DR. WEISS: But in our experience, which is limited, you know, the mink cells are clearly more sensitive to the 293 cells for detecting viruses then have the potential to grow in human cells, and from what David Onions

said, his in-house cat cells line is even more sensitive than that. So, I think we have to test human cells but if we know that there are other indicator cell lines that are sort of surrogate markers for infection and are more sensitive, they should be included.

DR. ONIONS: Yes, but I would be cautious because, again, our experience is limited because we see fantastic patterns, certainly on 293 cells, I wouldn't like to stand up and say it is more sensitive but, certainly, our experience echoes Robin's. That is, these two cell lines at least are more permissive than 293 or, indeed, Raji. In our hands, actually Raji is slightly better than 293 but, again, it is limited experience.

DR. AUCHINCLOSS: But Carolyn said Raji didn't work for her.

DR. WILSON: I didn't do Raji.

DR. AUCHINCLOSS: It was somebody else who didn't have a positive on that.

DR. COFFIN: It is important to keep in mind that the co-culture cell is different from the indicator cell you

eventually use in testing. You want to give yourself your very best shot at getting something out on the first round and then see what you get.

DR. AUCHINCLOSS: So, does somebody from the Committee want to tell the FDA what a basic set of assays

should be?

DR. ALLAN: I have just one comment on that, and that is with the RT-PCR. There were some studies that have been implemented. One of the things I noticed was that none of them have been standardized. I saw 10-3 and 5 microliters of concentrate and soup, and based on what we know about working with HIV and some of these other viruses, you really need to have better standardization. You need to know how many virus particles or what the equivalents are, RNA copies/ml, something like that, something that is more standardized rather than we just have this culture soup and we diluted it out and this is the sensitivity. So, that is very specific but may be helpful.

DR. COFFIN: Let me say as well that there is probably room here for development of some rather more efficient assays where one could co-cultivate with an VM1 or a cell line.

DR. ONIONS: I think we need to distinguish, again, between what one might test on the donor organ or the donor pig and what one might test on the patient. In the

donor pigs I think it is going to be very important, experimentally at least, to demonstrate variance produced in the organs in the whole pig and are they produced in organs that are being transplanted into immunosuppressed hosts, that is, find out whether you are actually producing

virulence in either of those two situations, and I mean virulence not RNA transcripts because we know we can actually detect RNA transcripts. But we do know situations in other species where we can find transcripts and no virus, clearly no virus. So, we need to define that, it seems to me.

In the patients, I think really, as I tried to allude to at the end of my talk, we need a battery of tests, at least in the first instance to try and identify --

DR. AUCHINCLOSS: I have already slipped on the patients once. Questions 2 is the patients, and I have drawn us in there and I am sorry --

DR. ONIONS: Right.

DR. AUCHINCLOSS: -- otherwise we will just get confused.

DR. SOLOMON: The issue that we had before, and we got into it with the cross species is where infectivity comes in. Again, I realize we want to be pragmatic, so you want to go to the incubator every day and pull out your mink cell line or your mutant 93 but these are not real and you

are really concocting it for a specific set of known viruses, which is okay but rather limited, I would think, at this early stage. I would think it is very important that we agree -- and here I defer to my infectious disease colleagues -- I would think it would be very important to

agree that certain primary cell lines, human cell lines, transformed, activated T-cell macrophages as well as possibly primary cell epithelial cell cultures be tested as well.

DR. ONIONS: Could I just make a comment? I think certain co-cultivation should be in when you look at these and you take them from the transplanted cymologous or baboon or whatever your species is. But I actually thought that once you put those infected cells in, there are other ways at looking for virulence, getting virulence not transference, and I think those should be introduced. We have done this in the past in animals. Robin's group have done very similar studies in another context where you then put these on gradients and then look for viruses at the right density. These can be very sensitive assay systems and I think it is worth considering those in these two contexts.

DR. AUCHINCLOSS: Well, let me ask all the members of the Committee. You could make your dream wish list of assays and they can be impossible for any reasonable company

to survive with. Have you just told me about the sort of assays that no ordinary company in this world could perform?

DR. ONIONS: I don't think you have to do this on every pig. Don't get me wrong, what I was trying to say is to try and find out what the parameters are in a number of

experimental situations. I am not saying you should do this on every xenotransplant at all. That would be impossible. In fact, my point earlier was that I don't think that is going to be possible. What we need to know is are virulents produced in these pigs normally in the line of pigs that you are using? And when you put them into, say, twenty transplants is there any evidence of virulence produced in these? That at least gives you some kind of idea of what is going to go into a patient with a whole organ transplant.

But I would also go back to the early comments that I think different transplants have very different risk factors. That is, bridging transplants carry a much lower risk than, say, a solid organ transplant. I think we also need to distinguish between these transplants.

DR. AUCHINCLOSS: Indeed, because at least one of the groups hopes to go ahead soon and put pig cells into the brain and hopes they will stay there very long term. So, it is not just bridge transplants that we are talking about at this point.

DR. ONIONS: Right.

DR. AUCHINCLOSS: What I am hearing from the Committee is that they can make you a list of some assay to do, and I am not sure that we want to sit down and write out all the details, but you and some of the experts here can do a reasonable set of assays that are doable and, you know, I

think from a practical point of view you would like to have that set of assays be doable, affordable and not going to change every two and a half weeks, or that the company be told that the rules changed last week and next week we are asking for something different.

DR. SIEGEL: One thing that Dr. Coffin and I guess the subcommittee mentioned is that there is some further discussion on induction. The comments were limited to BRDU. We have considered other types of induction. Should human cytokines -- is there any other dialogue?

DR. PATTERSON: We asked the Committee to discuss whether agents should be used to induce viral activation to enhance detection of virus.

DR. HIRSCH: I think that is going a bit too far, frankly, because I think from what we have heard this morning you probably will be able to induce virus from most of the tissues if you use enough chemical or mitogens or whatever. The same could be said if you took human blood, most of them carry EB virus latently; many of them carry CMV latently; many of them carry HHV8 latently and you can

activate them too with the appropriate chemicals. Does that mean we shouldn't be using these known human pathogens? I think that is going a little bit too far. I think it is important to know whether these pigs or these particular tissues have virus, but to take every one of them and induce

them, I think, doesn't tell us anything very useful.

DR. COFFIN: You are arguing against yourself, Martin, in a sense because EBV in fact is a real problem in transplant settings.

DR. HIRSCH: But it is not being tested --

DR. SOLOMON: EBV is tested on all transplant situations.

DR. HIRSCH: No, you don't. You don't try to induce EBV in every transfusion --

DR. COFFIN: But you know you can. The issue here is partly the research issue, the searching for things, and there, clearly, you want to give yourself the best chance of finding whatever is there and all induction does is increase in frequency events which will happen without it given enough cells and enough time.

DR. SOLOMON: So what are you going to do with the data when you have it?

DR. COFFIN: Presumably you will obtain a virus that you either know or don't know. If you know what the virus is, then fine; if you know what the virus is you

already know how to deal with it or you know what has been done before. If you don't know what the virus is, then you work it up.

DR. SOLOMON: How do you deal with the issue that really at this point there is no transplant, except maybe

the identical twin transplants, that doesn't engender an acute immune response or a chronic immune response? So there is going to be local infiltration of cells, release of cytokines and some inflammation, period, no matter how you concoct this system -- at least in the conceivable future. I mean, things could change five years from now. So, the idea of induction is something that is going to occur, at least with current technology, every time we do one of these transplants. Don't you think it is reasonable to come up with some sort of induction protocol? I defer to you if you say certain kinds of induction protocols get ridiculous, like ionizing irradiation maybe, but an appropriate set of inflammatory cytokines might be more acceptable.

DR. HIRSCH: I personally don't think you are going to learn anything that you don't know already.

DR. ALLEN: I would just say that since you are dealing with an unknown -- these are viruses that may or may not cause any disease so if you can actually characterize them -- we may be talking about unknown viruses; maybe not pig endogenous viruses but maybe something else you activate

that you get out of pig cells that you may be transplanting to a recipient that you didn't know about, and this is one of the ways you could be able to study that virus if you already have transmitted it. So these are just, like, new viruses we are talking about.

DR. MICHAELS: I think from a research interest that is reasonable to do, the inducible studies and look for other viruses. I am not sure that it, in itself, should be the issue that allows the FDA to say to move forward or not move forward, but I certainly think from a research area to try and push the system as much as the system can be pushed is reasonable.

DR. AUCHINCLOSS: Key point; absolutely key point.

We all agree, or at least I would agree that it would be nice to have induction studies; that the FDA should require induction studies in order for a company to proceed with the use of that tissue -- no, I don't understand why that would be the case. I can't imagine that we would be learning something that would make a go/no go decision, or that knowing how to do the induction studies makes you better at detecting the virus later on in the patient.

DR. SOLOMON: Well, I would agree with that only to the point where it was not long ago that Dr. Hirsch was making the argument that some cells that we transplant might

not be showing virus and, therefore, would be kind of less infectious risk. My point there is if you don't induce those, then your statement --

DR. HIRSCH: If you have a research basis, then you should certainly try to do that but for the FDA to

require that mitogens and cytokines be used on every single cell that you are thinking of transplanting --

DR. ONIONS: If I could just make a comment, what this discussion I think reflects is in fact our ignorance, and our ignorance is really that we don't know which of the many lines that we can identify are productive. If they all are, the likelihood is, and as Robin pointed out most of them will not be productive, we really do need to get to the stage where we can know these loci and whether these are spontaneous producers or inducible and, actually, those are the data we will eventually need to get so I am very clear, as Robin is, that that is where the research emphasis should go.

My only comment about induction, and I defer to John Coffin who is certainly expert in the area, that the experience tends to be that those that are at least inducible by 5-azacytine or RDU tend to be those viruses that also on occasion come up after prolonged --

DR. COFFIN: That is correct.

DR. ONIONS: -- passage in the cell so their

inducibility --

DR. COFFIN: That is the point I was trying to make. It really increases your efficiency of detection. You are unlikely to see anything that you wouldn't see given a long enough assay otherwise.

DR. ONIONS: Sure.

DR. COFFIN: But, for example, in mice where you have these endogenous proviruses that are very highly repressed, in cell cultures you can grow the cell cultures for quite some time before the viruses spontaneously appear, and they spontaneously appear in animals right around the time of birth but until that time the development of these animals has kept this virus -- this virus has been kept out but if you have an inducing agent they come up right away. So, it is really a matter of enhancing your sensitivity in detection.

The other aspect of induction -- there are two things that get mixed up here and the other thing is the use of cytokines as stimulating agents for cells -- I think that is actually fairly important, to get cells in cycle as much as possible in order to get your best chance of getting things going.

DR. AUCHINCLOSS: I am going to move us on to subgroup (iii) which is a relatively technical point, how long should your co-culture be for before you feel like you

have done a good job?

DR. PATTERSON: Right, the committee was asked to discuss the appropriate time periods for maintaining co-culture assays for treating more sensitive detection of porcine endogenous retrovirus. I think we saw this morning

data out to 35 days presented by Diacrin. One of the questions is how long.

DR. AUCHINCLOSS: Again, I am going to give you my perspective on this, which is I think consistent all the way through here. Since I believe that you can always get virus out if you do the assay correctly with the right induction and long enough and into human cells, this is not the issue as to whether it is a go/no go. So, learning how to do good assays and the type of virus is important but co-culturing for 52 days instead of 35 is not, to me, important.

DR. ONIONS: Can I make a very quick point? In our hands it behaves -- in those cultures where it is productive it behaves just like MELV or FELV in that we can do a nice time course and by three passages it has plateau'd; it has gone through the culture. So, we always do it routinely for five cultures and at three cultures it tends to plateau.

My only caveat to that is, and it is quite interesting, in some of the cell lines where we have only got the virus in as a provirus it appears to be

transcription silent. If you are not careful you can actually show extinction of that virus by passing the cells. For instance, I think I showed that we can get it into baboon cells, and we are confident of that but, as we pass those cells, because it doesn't replicate, you can actually

lose it because it is so weak and if you keep passing the cells, if that clone doesn't replicate as efficiently as the others, you can lose it by PCR.

DR. AUCHINCLOSS: Do you want to give any days?

DR. ONIONS: Well, I just say five passages and you have to keep the cells mitotically active.

DR. ALLAN: It seems to me that it is more important in isolating out from the patient and then the days become important because if you are trying to isolate out from the patient those days -- it may be a while before you see virus.

DR. AUCHINCLOSS: Fair enough, all right. What I took away from (b) in general was that, yes, there are some reasonable assays that a company should be able to do, and I can't phrase them for you but there are people here who probably can, and they ought to somehow be doable and affordable. That is a sort of general summary of part (b).

DR. PATTERSON: All right. In the event that infectious PoEV is identified during the screening evaluation of the porcine xenograft, please describe

biologic assays (in vitro and in vivo) most appropriate to further characterize the infectivity of porcine endogenous retrovirus present in xenotransplant products.

DR. AUCHINCLOSS: Well, obviously we are now about to come into sort of the heart of the matter. Do you want

to go directly to (i) because you have broken this question into sort of stages of discovering infectivity etc?

DR. PATTERSON: Right. The first part of this question is to discuss what sensitivity and specificity might be reasonably achievable and appropriately required -- two separate concepts -- of assays to further characterize the infectivity of porcine endogenous retrovirus present in xenotransplant products. Of course, the answer to this is dependent upon your answer to the first part of that question, what assays would be suitable to characterize the infectivity of porcine endogenous virus.

DR. AUCHINCLOSS: Let's be clear where we are. You have the pig cells co-cultures with the human cells and from the human cells got an endogenous retrovirus, and now you want to know what should be done to determine the subsequent infectivity.

DR. COFFIN: The first thing, actually, is to do a sequence analysis and see if that virus has been identified before. I think it is clear from what we have seen before that if you sequence a reasonable section of the GP70

portion of the envelope gene that you can have some confidence in identifying the virus with a previously known agent of interest with whatever is known in the literature about its infectivity. For new viruses, I mean clearly I would argue again, I think for the introduction of attempts

to get some newborn animal model infection with this virus, starting perhaps with some cell culture assays for various primate or even non-primate species. I mean, if you can see spreading infection in the mouse, if you injected a newborn mouse, I think you would be a long way toward being much better biological characterization of these viruses. So, I would argue for some studies that go toward the idea of developing some kind of animal model where you get viremia, even if transient viremia, and being able to see what the consequences are.

DR. ALLAN: I think what John is answering is research questions, and I think the question directed towards us is clinical. Is that right?

DR. AUCHINCLOSS: That is the way I see it, is this a go/no go finding and is there something you should do to determine whether it is a go/no go.

DR. LERCHE: Maybe this gets back to the question Dr. Solomon was raising and perhaps what you were getting at was at this stage to look for the ability of these activated viruses to infect primary cell lines, and what relevance

that would have or more relevance to the clinical setting. For example, is the virus not only infectious but will it transform human cells? That would certainly be -- so, perhaps that would be included here to see what the range of infectivity is in human primary cell lines. That may be

something that should be discussed or considered.

DR. COFFIN: Regarding the research versus clinical, I mean, they run together. You don't have the opportunity to take a kidney that you are going to use for transplant, if you get a virus out, to do this workup. It is just not possible. You have to have done it before in a research setting and then apply what you have to what you see. So, the two questions are really not different; they run together.

DR. AUCHINCLOSS: Well, the sponsor has done the co-culture experiment; the human cell indicator line has the virus in it; and now you tell the sponsor you can't use that tissue until you have done X to determine its human-to-human infectivity?

DR. HIRSCH: No, I don't think we should say that but, John, perhaps one of the more important things we could do is to say very strongly that the NIH, or whoever, set up systems where we can evaluate the infectivity and the pathogenicity of porcine or other endogenous retroviruses in suitable animal model systems, whether they be primates,

whether they be nude mice, suppressed mice or newborn mice, or whatever. I think that is what is critically needed in this field. If you can take a porcine, a rabbit or whatever endogenous virus and show that it causes lymphomas in some other species I would be much more concerned about these

than I am today.

DR. AUCHINCLOSS: But it is not what the sponsor needs to do when they tell the FDA that they have a tissue as their potential tissue source for clinical trials. So, it is a clinical versus a research issue.

DR. SOLOMON: Right. So, to take that guidance from the Chairman, to be perfectly practical then one of the ways we could frame this is if you have a specific protocol -- all protocols are going to be different so let's say it is pig islet cells and you want to put them into the intraportal circulation in a given protocol, which is going to be then exposed to the portal elements, which are the Kupffer cells, the hepatic epithelium and stromal elements, you should be able to provide the FDA as a go/no go, to use your terms, with some evidence for primary hepatic cell lines, and those are perfectly achievable. There are a number of different ways of growing primary cell lines in a lab. As well as leukocytes that would then infiltrate the graft that was rejected. Those would be the two elements -- I made that point earlier -- that would contact those cells

in an in vivo biological setting.

DR. AUCHINCLOSS: So you are saying every sponsor who gets a transferred human co-cultured cell now has to test for infection of human --

DR. SOLOMON: No, I said you have to take the

protocol that is being proposed. I am trying to be practical. So, I was giving you an example --

DR. AUCHINCLOSS: No, no, I understand.

DR. ONIONS: I am a little bit concerned about this conversation, or at least with one misconception already. That is, first of all if you put these viruses into primary cells, in general they won't transform as cells because to do that, as John explained, they have to sit next to an oncogene or they have to transform an oncogene. This does not occur -- well, I am not going to go into arguments about the probability of that but essentially if you put these viruses onto primary cultures in vitro you do not get transformation, with some exceptions. That is the first comment.

The second comment is that you have to define what you are trying to do here. I am still a little bit puzzled about what the aim is here. If you look at a clinical situation you are going to have a pig and it is going to be in quarantine and at some point you are going to use an organ from it. I can see that if you are going to use

hepatocytes or islet cells it is possible to test those because you can bank those cells frequently. You could test them for infectious virus and you could determine which of the viruses that we have talked about today are being released from those cells. That won't be the situation if

you move on to solid organ transplants. You are not going to have the luxury of being able to do that. The only situation would be to look at, say, peripheral blood cells and assume -- and it would be an assumption -- that the pattern of expression is the same in those cells as it is in the particular target organ, and I am not convinced of that.

I am also concerned about the idea that you should look at a representative cell like a hepatocyte. What you want to use is a cell line that is the most permissive for those viruses, and it might not be. It might be a bat lung cell or it might be, you know, a clawed toe cell. It doesn't matter. What you want is a cell line that is permissive for the range of viruses that we have been talking about today.

DR. SIEGEL: Actually, I am confused by your comment which seems inconsistent with others we have heard. You said if we use a solid organ, since we can't test that we would have to test the plasma or serum. But several other speakers said that for a strain of pigs, since it is

going to be the same why not just use the same solid organ -

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DR. ONIONS: No, that is a different argument

because, as I think we have discussed in previous meetings,

they will be sentinel animals and it is possible to test

sentinel animals that you would take out.

Again, I would slightly caution that there is a quick assumption that all these animals are the same. Actually, I think there are some interesting data from Gibian Langford where she has looked at expression of these viruses in different tissues and, in fact, even within a closely defined line of pigs there is actually a very different pattern of expression in different animals. Those have not yet been mapped to different loci but these pigs are not inbred mice; these are still outbred. Indeed, it will be very necessary for the health of these pigs to keep them to a degree outbred. We do not want completely inbred animals for all sorts of other reasons. So, I don't think it is going to be feasible to say that because a few animals in a line behave this way that every individual animal will behave that way. I don't think that is the case at all.

DR. COFFIN: Throughout our report it was very clear that we need to get the genetics of this under some control so that one knows. It is possible to identify the loci now that give rise to the viruses --

DR. ONIONS: I think that is the key end result --

DR. COFFIN: Another thing that is important to keep in mind in these discussions is that there are not -- this discussion is being approached a little bit as, well, there is a chance that every time you turn around you will

see a new virus that you haven't seen before. That is not going to happen. We may well have already seen all the infectious proviruses that there are. It is unlikely that there is a great deal more. So, the workload here of working these up is not infinite. This is not completely open-ended. It is merely a matter of getting hold of as many as possible in a research setting, figuring out what they are, what their properties are and then, as new ones come along, simply having probes and tools for telling which ones you are looking at.

DR. AUCHINCLOSS: I just want to translate for a brief second some nodding heads and shaking heads. What I thought I saw there was that it is unlikely we have yet identified all PoEV's; it is quite possible we have identified all infectious PoEV's.

DR. COFFIN: I didn't say that. I just said it is possible.

DR. WEISS: We have already identified extra sets of PoEV's that are less related to C type, more related to D type but none of the sequences so far have open reading

frames. There are certainly other endogenous retroviruses, and we have lots of them in pigs, that haven't yet been described. So, the key thing that John Coffin said is are any of them going to be infectious and, yes, it is likely that there is a small set there and we are most of the way

there. I think there will always be surprises.

DR. COFFIN: But even in outbreeding, once you have identified the loci that are involved, that are segregating, you can still use the presence or absence of loci to inform your breeding program that you have been maintaining outbred animals.

DR. ONIONS: It might be that the long-term goal is loci identification. So you fingerprint a pig and you will know what those loci relate to in terms of your patients. So, it might be that that is definitely the way to go for an intermediate, long-term goal.

DR. AUCHINCLOSS: But now let me go back to the sponsor who says he has his tissue here and, yes, I admit I have infectious endogenous retrovirus here. What assay should we require the sponsor to perform?

DR. ONIONS: I think, again, it depends on the type of tissue that we use. I think at the moment, if it would b feasible -- correct me if I am wrong -- the people who are doing work with isolated hepatocytes, and I think that is a very interesting system, and also the people who

are doing islet cell transplants -- it would seem to me feasible that they could actually look at the particular lots where you could look for expression of viruses in those systems. But I think the problem comes when we get to solid organ transplants where you can't do that, or you can rely

perhaps on at least demonstrating what is expressed in some other tissue in that pig before you get to slaughter.

DR. NOGUCHI: One of the things I think Dr. Solomon was trying to get at is as we are moving forward in this particular area, above and beyond being able to detect a virus, are there specific assays, such as using primary hepatocytes, that might allow us some comfort in saying this may be infectious, a virus that can be activated under certain conditions but when put into primary cells of a particular target organ or target embedded tissue, will that help us judge whether or not to go forward, or if you develop a sensitive assay at some point it will be positive. We all realize that that is necessary for research development to get the best possible assays, but in a practical sense how will we judge what to do with this infectious virus?

DR. AUCHINCLOSS: I think this is the point, and you have heard my point of view over and over again. There is no comfort you can take from any negative, at least in my view on this because I start with the assumption that the

infectious virus is going to be there and it is going to get into human cells one day when you have done xenotransplantation, ten years or twenty years from now. I don't know when it is going to be. It is going to happen. And just because you have done a hundred assay that say that

tissue didn't have it happen in vitro, it is still going to happen. So, all of these tests that you are requiring of a sponsor, are not in fact worthwhile, except to the extent that they demonstrate their capacity to mono patients later on. There are lots of things we will learn but for validating tissue for transplantation they are inappropriate.

DR. SIEGEL: We are asking whether we should characterize what sorts of tissues they might infect and what sorts of viruses they are, and we are saying they are irrelevant except --

DR. AUCHINCLOSS: No, I am saying you should go ahead and characterize and our experts should characterize but the sponsor, for use of that tissue, does not have to characterize.

DR. SOLOMON: I think part of the problem is the way you are putting it. You are making it that if you demonstrate a positive transmission of an endogenous PoEV, that means that the sponsor can't go on and do a clinical trial. I certainly never meant to imply that by any of my

comments and didn't say anything like that. So, that I think is creating a problem here. I mean, nobody wants to see any regulations or guidelines that are so rigid come out of these discussions, at least I certainly don't want to be associated with them, that kill xenotransplantation. I

don't think the FDA wants that. That is what we are doing here. So I think you are putting it into wrong terms.

What we are saying is are there certain tests that, by their biological relevance, appropriately blur the line between basic research and clinical practice that are relevant? I would say, as a transplant clinician, that after I have to take care of my patient who just had islet cells put in the liver I would like to know ahead of time if there is a high likelihood that PoEV is going to be transmitted to primary hepatic cells in culture. Now, that doesn't mean that I won't go ahead with the transplant.

DR. AUCHINCLOSS: Do you want to try (iii) under (c)? It is important that (ii) came before (iii) because it reflects what Dan was just saying, the fact that we might ask a sponsor, and consider it part of their responsibility in terms of safety assessments to do studies of infectivity of different tissues does not mean to imply that a specific result would influence whether they could go or not, which is what we are now asking.

DR. PATTERSON: Right. This is the major

question, the heart of question 1, in the event that infectious PoEV is identified during the screening evaluation of porcine xenografts, please discuss whether these xenografts would then be suitable for use in human clinical trials.

DR. COFFIN: Let me just say something first. Let me state that it was the consensus of the committee that such materials should not be used.

DR. AUCHINCLOSS: The focus group.

DR. COFFIN: The focus group, right. That doesn't necessarily mean it was the opinion of everybody in the group but that was the consensus in the discussion that we had ten days ago.

DR. DESROSIER: I would say in the absence of additional information on the potential infectivity of that virus for humans or experimental animals, the committee was not comfortable, in the absence of any other information, with putting in live replication competent virus into people, not knowing whether it would take or not. I think we heard this morning that it has been put in -- there have been multiple exposures of people and of monkeys in a variety of settings, and I think the answer to the question is dependent upon further analysis of those individuals.

As I stated before and I will say it again, I think it is possible that all 24 of those people who got the

brain implants could be infected with pig endogenous retrovirus but we don't know because we haven't done the right tests yet, and we need to know those results. If they are antibody positive, persistently antibody positive to the pig endogenous retrovirus and the population at large is

negative, that is going to tremendously influence our views. I think even Robin then might take a step back --

[Laughter]

-- and reevaluate these comments about slowing down a little bit. If they are negative even by that sensitive assay, then that changes the picture too. So, I think we need to know. If the information is there, and it is gatherable, but in the absence of any further information I, personally, would not be comfortable with the notion of putting live replication competent pig retrovirus into people, not knowing what was going to happen.

DR. AUCHINCLOSS: Let me be clear. Would you be comfortable putting apparently replication not competent pig tissue in based on our current knowledge? I have heard you. It was no.

DR. DESROSIER: I mean, if there is no replication competent virus, I mean it is being done in gene therapy all the time now with replication defective murine leukemia and I can live with that.

DR. AUCHINCLOSS: But would you believe any assay

that said that this was pig tissue that doesn't have replication competent virus?

DR. DESROSIER: Well, if I were to assume that it is highly unlikely that all those tissues will, so yes.

DR. KASLOW: One of the problems, it seems to me,

with the way it is phrased is that it is almost as if on one day you have no information and the next day you have all the information you need to decide when, in fact, it is going to be weeks, months before you get it. So, it really perhaps ought to be rephrased at what point you have enough information to make this decision rather than when it is identified as such. What will tip you over? At what point will you have enough information after all the studies are done to say one way or the other?

DR. DESROSIER: I am not saying that that information is. I can't answer the question. I know that based on what I have seen today on PCR analysis of blood cells and all results being negative, it is not overly convincing to me that those individuals are not infected.

DR. WEISS: PCR data of blood is of limited value really. You know, most of us in this room are infected with EVV, but if each of us today donates 10 ml of blood and we turn the buffy coat into DNA, only about 15% of those samples will show up positive TPV. You know, we have lots of viruses on board at levels of detection in the blood that

are just below that, and even have tropism for white blood cells. I think the antibody tests are going to be very important. They have lagged behind the PCR test so far because, as we have heard this morning, you know, there are tests coming along. So, one thing we can take away is to

perhaps put more effort into developing them. I know the CDC is.

DR. ONIONS: We are having a go in a rather more desultory way. We do need those tests. We can study the proteins; we can make some study peptides. We can progress, make positive reference and test those against human sera, against pig sera and do it. It will take time but we are probably talking about months, not years, in that regard. So, I think there is something positive to do there that will be useful.

DR. ALLAN: I would just comment on the fact that you could do the animal models, as we discussed before. One of the things that strikes me about this type of a setup is that you may not be looking for this but one of the things that came to mind to me was the fact that it may be that you design a standard assay to detect infectious pig endogenous retrovirus. You may be able to design your assay system such that you can detect low levels and high levels so that you may find certainly in animals that when you isolate viruses you get a notion that that particular animal may be

more likely to transmit to a human than, say, another pig or another virus. So, all viruses are not equal. Some may be more likely to induce an infection or produce a lot of virus that could be infectious.

These are questions that we can't answer yet, but

you could design assay systems to be able to begin to determine those kinds of things, depending on what your indicator cell line is. You can do that in conjunction with the animal model system so that you can take the virus that comes out that is a high level, inject that into a primate and see if that is more likely to be productive in a primate versus a virus that you inject that is at a low level in human cells or monkey cells. So, I think we can do those things.

DR. AUCHINCLOSS: I don't have a sense from this committee as to whether or not when they hit (iii)(c) here they have said is not tissue you can transplant or this is tissue that can be transplanted. Any answer to that?

DR. COFFIN: Or the middle ground where there are levels of risky procedures in tissues, which actually comes up in the next question.

MS. MEYERS: Can I address that? As a layman, it just seems this is a science fiction and how it is going to hit the home newspaper when we are talking about this should be of concern to scientists and everybody else. So,

transplanting tissue from any animal that contains a virus that is live and can replicate into a living human being just seems unethical. I can't see how anybody would do that, purposely expose them to a known disease.

DR. AUCHINCLOSS: But, Abbie, we do that all the

time in humans, with CMV for example, ones that we actually know are pathogenic as opposed to this one.

MS. MEYERS: But you know what those diseases will do.

DR. AUCHINCLOSS: No question of that.

DR. COFFIN: But would you feel better if we knew that these were dangerous viruses?

[Laughter]

It is actually a serious issue that comes up over and over again in other kind of FDA things as well. It is much easier to deal with the dangerous agents than the ones we don't know what they do.

MS. MEYERS: But shouldn't somebody be working with those viruses to see how dangerous they are before you expose the patient plus their family and maybe the people they get on the bus with, the people on the airplane with? I mean, we have no idea what these viruses can do and we shouldn't be doing it knowingly.

DR. SOLOMON: The issue that you raise is very important. What the transplant community has come together

and said is that the organ donor shortage is such that we are losing literally thousands of patients every year, thousands in the United States alone because we are not able to transplant.

So we are looking at individual risks and public

health risks, and I think that was well articulated earlier. We know that regular human-to-human organ transplantation regularly transmits viruses that create serious illnesses in our patients and sometimes illnesses that kill them, but we accept that as not a zero risk procedure because it is life-saving in the great majority.

What we are trying very hard to do is to responsibly not raise the bar too much higher for xeno. It is never going to be a zero risk procedure. I think the challenge for this group of experts and for the FDA and the public health service in general is to come to grips with these obvious issues and positively allow xenotransplantation to go forward and save thousands of lives in the future, realizing as best we can what the risks are so appropriate informed consent can go forward to my patients and to the public, and that I think is the only thing that we have to do, to be responsible.

DR. AUCHINCLOSS: Yes?

MR. BENEDI: Miss Meyers alluded to it, I am a transplant recipient; I am a liver recipient, and the risk

to the recipient from human-to-human transplant is one that is known and the recipient takes that risk. What I think is being expressed is the risk not just to that recipient of death or infection or whatever, but a risk to the general population. That is what one recipient can't be held

accountable for when they sign that consent form when they may infect a number of people in their families and outside. So, that is a whole different issue.

MS. MEYERS: That is right.

DR. AUCHINCLOSS: I completely agree that that is what distinguishes this from all other forms -- not all other forms but the standard risk-benefit calculation that an IRB evaluates. We agree with that, we really do.

Now, people in the FDA, help me out. We have not answered what you consider to be, and what I consider to be the central question in group 1. Do you want me to kind of have a show of hands here? Do you want to get some more information? I don't feel like I have satisfied you with advice from your experts, recognizing that we have very different levels of expertise here and lots of opinions.

DR. SIEGEL: Right. Let me state where I think we are and what I think I have heard relative to this question just for feedback. Obviously there is not full agreement but I think most people say that if next week somebody reports to us that they have grown this virus out of their

product and, indeed, most of the committee thinks that that is likely to happen, if not next week, sooner or later because it is going to be there, I have yet to hear that they should go ahead because I think I heard some reservations from Dr. Desrosier and from others that, in

fact, perhaps there is more information that we need on the patients who have already been treated, and we will get to that in question 2. But, as a point of information itself, what I think I am hearing is that that really makes relatively little difference; that we already knew that that is going to happen. We know that these tissues have virus. The fact that we are screening and we are going to continue to screen for virus is not based upon the fact that we think it is going to make a difference in how we act whether they recover or not because we think it is going to be there anyhow, and if they don't recover we think it is probably simply that they haven't been tweaked hard enough and, yet, we are not going to ask them to try to induce it in any case. So, we are just going to get that information. We are not going to ask them to take that virus and see what sorts of tissues it infects, but we would like maybe to find somebody to do that or sequence it --

DR. AUCHINCLOSS: If you heard all of this, you heard me, but I would be very surprised if this committee agreed with all of that.

DR. SIEGEL: When this happens, when this culture is positive, you know, Dr. Zoon and the Commissioner and other people are going to ask me, well, you know, what are you doing about that? And I am going to say, well, we consulted with the experts and they didn't seem to think

that this made much of a difference --

DR. AUCHINCLOSS: All right, you have heard my point of view now let's hear from the rest of them and see what they really think.

DR. VANDERPOOL: Do you want me to answer number (iii)(c)? It totally depends on the nature of "infectious" PoEV. I might be able to assume that that is not serious. So, proceed, by all means. But if I think, hey, this is going to form a mutant virus; it is going to be equivalent to AIDS I would say no way. So, I think it is going to be a judgment all the way. Just the fact that infectious PoEV is present doesn't mean to me that one can't proceed with human trials. It totally depends on the extent of harm and the degree to which it might be spread. And that is a judgment call and we can't guess the call.

DR. SIEGEL: I don't understand why you can't guess the call. Largely, some of you have told us not to require the companies to generate information to address that question so we are going to have to make that call on the basis of current information --

DR. COFFIN: No, no, no.

DR. SIEGEL: Well, you said it would be an interesting research question --

DR. COFFIN: No. I think everybody has said that you absolutely have to have follow-up on animals that have

received these and on humans that have received these. That I think is a complete given.

DR. SIEGEL: Right.

DR. COFFIN: That is the information that we really need.

DR. MICKELSON: I think we also mentioned that there should be a drive for eliminating these from the donor sources as well. I think one of the issues here is, again, the risk-benefit ratio. I think it is really hard. There are no data. Even though there may be a hundred or so people who have these transplants, that is not really a big group. The infectivity and whatever else might happen, those should be relatively rare events and a hundred people is not statistically robust by anyone's consideration. But to assume that the companies can test and say it is positive, no. It should be a burden placed upon sponsors, whether they are universities or companies or whatever, to the whole group working together to remove and reduce whatever they can detect that is potentially a danger. But the word is "potential." I don't understand how we can

honestly deal with a lot of this because we are not data driven here. We have in vitro tests. I don't know what it means to have these viruses, after being induced with PHA or something, being able to infect primary human cells, blood cells, in culture. I mean, there is no immune system; I

mean, even if the recipients are immunosuppressed -- I don't know what to do with this data. It is fearful but it is not a reflection, and I do not want us to deny something that is potentially helpful to people who are in dire need as well until we would have better data that this is a real issue.

DR. AUCHINCLOSS: At the beginning of the day Jay said to me we know we have asked you impossible questions and we feel bad about that, but they are, in fact, the questions that we are being asked to answer every day.

DR. MICKELSON: But there are answers here and they are all personal as to what you consider risk or benefit, but I think one of the things that we must always fall back on is exactly what is your data telling you is going on, and if there isn't any data then we have moved into the realm of value judgments as to what these experiments mean.

DR. ZOON: I think it is a little bit of the chicken and the egg syndrome because, you know, we would like data as well but the reality is the science is evolving and the question is at what stage do you let things go

forward, knowing that you have to scientifically monitor and study as you proceed, deal with informed consent with individuals that potentially may receive these life-saving therapies. I think, again, the public health balance if it were only affecting the individual would be fairly

straightforward to assess, and part of the complexity in this, and I think the committee rightly so is saying that any data that you do have or can explore with respect to those individuals already treated, their family members or whatever, to raise issues that are, are there additional data that may raise concern or not. But part of this is do you stop things while you are waiting to get that data or do you allow it to proceed? I think part of what we are trying to ask you is to help us balance that so we can move forward.

DR. AUCHINCLOSS: Here is what I would like to do. I would like to basically just run around the table and let any person who is in the mood to make a comment to this question do so, and then I think we can actually relatively quickly address (d), and then we should have a coffee break.

[Laughter]

So, I am just going to go around the table and anybody who wants to make sort of their opinion known on this central issue of question 1.

DR. PAUL: Having worked with a number of different infectious agents in swine, and also I have worked with bovine leukemia virus, if with this retrovirus there was any experimental evidence that it caused leukemia in swine I would be very concerned. I am personally more

concerned about some of the agents in swine more than the endogenous retrovirus.

DR. DESROSIER: I would just restate what I have already said --

DR. AUCHINCLOSS: Your feeling is get the antibody test up and running before you go forward. Is that fair?

DR. DESROSIER: My feeling is that I am not in favor of putting replication competent endogenous virus into people unless we have some sort of evidence of whether it is infectious for people and might potentially cause disease. I think in the absence of that information, we should seek information from animal models and the people who have already been exposed to rigorously as possible examine whether there have been infectious events.

DR. KASLOW: I think I would proceed on the current course. We do not have any evidence of human transmission. We don't have any evidence of disease as a result of this agent in humans, and it seems to me that it is appropriate to collect the information that would change that view as expeditiously as we can, and then proceed to

meet again and to think again about it, and this is going to be a continuous process.

DR. LERCHE: I agree with that last statement. It is a constant feedback process that is going to be going on. I think in the framework that we, as the focus group,

discussed this, essentially the question was posed to us would we be comfortable giving replication competent virus to human patients, and that is sort of the basis for a consensus or, I guess, a non-consensus.

I think that my own opinion is that I think that there needs to be a little stepping back and looking at where we actually are. I think we have two sources of this information. One is on animal studies, and I think the importance of this has been made very clear. The other is to fully evaluate the patients who have already received that. This morning we have seen a very good start on that. I do agree that some of the assays that need to be done have not yet been done but could be done.

So, I think my own opinion is that if this is going to proceed it should be done very cautiously and the protocols and the tissues that are being used have to be evaluated independently. All these procedures are not really the same in terms of risk and should be evaluated that way.

MR. LAWRENCE: With Deputy Commissioner Pendergast

gone, I am the only lawyer left at this table. We will talk about some of the legal stuff a little bit later. I am also a liver recipient, along with Tony. I would like for a moment to put a human face on some of this because I hear these outstanding scientists talk about accepting risk, and

I would like to suggest that perhaps the people who are accepting the risk are people like myself. So, we have a voice here, and I would like to thank the FDA for inviting us and asking us to participate in this. I resisted when Dr. Patterson called me. She called me several times to get me to agree to come here but I think this is good.

I would like to say that we have been accepting risk for a long time. In the '70s we accepted the risk that we were probably going to die because the problem then was immune response. I mean, we are talking about a porcine virus now and that is threatening but it was more threatening when the probability was that your immune system was going to kill you if we did this procedure now, and would you like to proceed? And some of us -- I say us, I mean recipients -- proceeded anyway, and science progressed and now we are in much better shape probably for that.

It seems to me we are on the cusp again of a major stride. When I was transplanted ten years ago there were 15,000 people on the waiting list waiting for organs, and today, as we sit there, there are almost 60,000. So, the

problem is getting worse. As we deliberate today 10 people will die in this country. Every 24 hours 10 people die. So, to make this short, we need to proceed. Recipients would say to you we will accept the risk. We want to be informed; we want information. We want to participate with

you here but let's go. Caution is fine but let's not stop.

DR. MICHAELS: I think a lot of the comments actually that I would make have already been voiced and I should probably just try to say that I think different procedures do have somewhat different risks and I think that we can proceed cautiously with small numbers as we go forward. I actually didn't realize how many numbers of people had already received tissue, and I believe it was Immunotron that have over 100 patients that have had some of the human tissues, and it would be nice to hear at some point how many numbers of those patients they actually have already been able to contact and get samples from to at least by the best ways possible that we have right now, while the assays are being developed, and I certainly know that a lot of effort is going into developing the other assays.

DR. HIRSCH: We have no knowledge about pathogenicity and we have no knowledge about risk, but extrapolating from other viruses and trying to get back to

Jay's question, I certainly would be more worried about a culture that was producing five logs of infectious virus than I would be about one that you had to induce with IUDR to get anything out of, but where you draw the line I haven't the vaguest idea.

DR. ALLAN: I think that in a vacuum you would like to see the animal studies done before you go forward. That may not be reasonable. Each of these procedures has different types of risks and you are looking at fetal neuronal cells; you are looking at certain types of procedures that probably have less of a risk than if you are transplanting an organ with a whole ecosystem of cell types and microbes that are associated with it. So there are differences in risk.

But I think you need to do animal model systems, and I think just looking at the animal models retrospectively is not enough. I think you have to do the hard core science. You have to blast the monkeys or whatever animal species it is with boluses of viruses, immunosuppress them and you can do that within six months. I think it needs to be done. Who is going to do it I don't know.

DR. ONIONS: I think we have two sources of data that are coming through now that are going to be very important. One is the study that is going on the patients

that are being exposed either through liver transplants, skin grafts or a variety of other procedures. I think evaluating those patients for the presence of virus, and I think also crucially for the presence of antibody -- one of the reasons we developed recombinant P30 is because I think

that is a critical part of the evaluation of these patients. I think those data are going to be very important, and I think some of the primate data is going to be useful. Unfortunately, I think not all of it is. I think we need to know more about the infectivity of these viruses for different environments and some of that data is beginning to come through.

I would like to see some of those data before I would be happy about putting in any tissue that I knew was expressing a virus that could infect human cells, whether that be pancreatic islet cells or a whole organ transplant. Having said that, once those data are on the table, I think you can begin to evaluate a little bit more sensibly what the risk factors are. I said I would like to pause at that state because I would like to reevaluate the data because, in my own view, I think it is unlikely from what we know of the virus already -- and I can only use the word unlikely -- unlikely that the virus would establish a persistent productive viremic infection in a human patient, unless immunosuppression modifies that significantly. It seems to

me that that is the danger because that is the public health danger when you get high titer infection and you probably get onward transmission.

So, I think we need to do it step by step, and to come out with the question straightforwardly, I think it

depends on the data coming through at the moment and then I think we can make a sensible decision about going on with the data that is going to be available in the next few months.

DR. VANDERPOOL: To give a bit of very recent history, the full reports released on xenotransplants recently, two of them were the Nufield Council of the U.K. and the Institute of Medicine Committee in the U.S. One of the significant differences between the Nufield Council in Britain and the U.S. was on this very issue. The Nufield Council appealed to the "principle of precaution," saying we should not proceed until we are assured that the procedures are safe. That is one way to go. The Ireland Committee is different. Our stand in the Ireland Committee is proceed with caution; proceed as long as you think the risks are controllable. So, I said a moment ago that we can't make that decision for you. What I meant is I can't make that decision for you now. I will be with you and helping you sort through that decision in the future if I am so called to help do that. But I think we should proceed as long as

the risks are manageable and controllable, and as long as they are not serious. It is interesting how it would be so nice to appeal to mathematics for comfort, and we do that all the time, but ultimately, in my judgment, we fall back on just straight word adjectives -- serious, controllable.

As long as the risks seem manageable and controllable I think we should proceed. When the red flags come up you will know it and we will know it, and that is when we take a different course in my judgment.

DR. MICKELSON: I must say I agree whole-heartedly with what you have just said. I do believe that xenotransplantation trials should go ahead cautiously but in parallel with the animal research. I think the data that we have seen and what is known now, and the types of viruses that have been recovered -- the risks seem low and they seem manageable, and placed in context with the need and the other adjectives that accompany descriptions of the diseases that could be treated by this, I do think they should proceed cautiously. I think the FDA can encourage the types of research to answer the questions that are needed so that if and when things happen they can be detected. I am not sure PoEVs are ever going to be the issue, but if that is what we are dealing with today, you know --.

DR. SIEGEL: We encourage a lot of things that are happening --

[Laughter]

-- some of the same people who, in fact, said that animal research was critical but it shouldn't be go/no go, but then subsequently said until we got the human and animal data we shouldn't be going forward.

DR. MICKELSON: Can I ask one question? When you go back to the patients who have already received transplants what do you tell them as to why you are asking for new samples?

DR. AUCHINCLOSS: Good question!

DR. SIEGEL: Per the guidelines that we put out a while back, substantial samples have been banked and archived from these patients and are being done on a regular basis. So, we are not specifically asking, in most cases -- I am sure in some of the projects you have heard about there will be a need for specifically asking for additional samples but by advice of many of the people on this panel a lot of sample collection has been going on routinely.

DR. MICKELSON: But you don't have to go back to ask for permission to test. That is what I am saying. They were collected with the understanding that they were blinded, or what?

DR. NOGUCHI: In the larger issue those samples for which specific authorization had not been, which is really the case of a lot of patients who had received it

under IND, if you are asking what do you tell them, you tell them the truth as far as we know it.

DR. AUCHINCLOSS: I am going to move on with our answers pretty quickly as a matter of fact because I think we are all going to drop before we get to number 3.

DR. WALKER: I just would like to ask a question of clarification of the people from the FDA. Am I right in thinking that the clinical hold applies to all xenotransplantation studies at this point?

DR. SIEGEL: For this specific porcine xeno-transplantation.

DR. AUCHINCLOSS: The effect of the answer is yes.

DR. SIEGEL: The hold that is pending having an assay for porcine endogenous retrovirus is for all porcine.

DR. AUCHINCLOSS: I think you have sort of stated my point of view so I am going to pass.

DR. WALKER: So essentially we are being asked whether we think the clinical hold should be taken off and t he research proceed now while data are being gathered. I think that is one way to operationalize the question that is being asked here.

DR. SIEGEL: Well, that is one of the ways. The question I thought was being asked now is when the data are gathered and the virus culture comes back positive, should

that -- if otherwise you were going to life the hold, should we go ahead and lift the hold? However, we are getting a lot of advice on a lot of different questions and I don't want to discourage you from giving it because it is needed.

DR. WALKER: Well, it is a slightly broader

question than this one that is on the screening, but I guess generally speaking I favor seeing research go forward and I think moratoria are a bad idea. In this case, I guess I would argue that another two months should be taken with the clinical hold and that during that time a risk assessment and risk control program should be developed.

DR. COFFIN: I am not of the opinion that it matters very much whether a given tissue is producing virus or not. It only takes one infection event -- if an infection is going to be initiated in a patient, it only takes one event to do that. The real issue is whether you get a spreading infection in the patient. To my mind, that is the key and that is what the research has to be directed at. And that is two things. One is that newborn animal studies should go forward as fast as possible, with negative results being fairly useless but to try to find a positive, try to find an animal in which things do happen and then one can ask focused questions. That is one thing.

Secondly, I think it is not unreasonable to consider that certain kinds of transplant studies could go

forward with the idea that they are being used to accumulate this data but that these studies should be assessed on the basis of the risk that if there is a spreading infection will transmission occur? Because it is really transmission that we are talking about. We are not talking about

individual risk to the patient despite what some of the people have said. So, I think that should take into account something we haven't talked about before, and that is what the patient population is. Clearly, some of the patient populations are going to be much more at risk for transmission, for example vertical transmission. If you excluded women who were within childbearing age you would essentially eliminate that risk from the patient population. So, one could grade up the risk by also considering what the patient population being treated is, as well as what the various types of procedures are.

DR. SOLOMON: I have already made the one comment that I don't think any longer the issue here is should we go forward in a safe way with xenotransplant trials. I think under the right circumstances we should go forward. You have to remember that there are a number of barriers after the infectious disease issue is put into context that are standing in the way of us deciding whether there is any benefit, and we can't have a risk-benefit ratio when we know nothing about benefit, and I think Dr. Hirsch made that

comment as well as others. We need to get into clinical trials in order to define the benefit, and the sooner we do that the sooner we benefit the patients and that is a major responsibility in transplantation.

So, what is the responsibility of a group like

this? I would leave you with the idea that it is to provide leadership. We need to provide leadership in where the research should be going, how the research is moved to the clinical side and, lastly, how we protect patients and public safety. With that in mind, I think it is very critical to acknowledge the important role the FDA, the NIH and the CDC has had. They have provided leadership that has moved this field forward in a practical way in the last two years in a way that I personally think is remarkable. I mean, there is data now when two years ago at this very same sort of thing we were talking about "what if?" Don't, in my opinion, give up the leadership role you have taken so far. It is going to be a very profitable industry. There are very fine scientists out there in the biotechnology world. They are doing very fine science. Don't give up. Don't take the pressure off them. Don't wave your hand and say oh, the NIH is going to support this because we all know that is baloney. They are going to try and do their part, and they are doing their part, but a tremendous amount of this work is being driven right now by the leadership you

guys have provided. Don't let up on that in my opinion.

DR. WEISS: I am also still in favor of proceeding with caution. Caution tells me that there is more information to gain in the short term from people who have already been exposed or transplanted with porcine cells. I

hear what our colleagues on the panel who are individual recipients of transplants, of allograft transplants, have said, and my mind remains focused on what is a new issue in this field of medicine, the possibility of transmission. We are going beyond the risk and benefit of the individual and we have to address the rest of the population at large, which I think is a lower risk but a potentially more devastating one, and one that we are not going to get a sensible answer from today with the blind leading the blind at the moment.

DR. AUCHINCLOSS: Abbie, do you want to make a comment?

MS. MEYERS: Well, of course I am going to get in trouble with what I say, which I always do --

[Laughter]

-- but that is probably because, you know, among the members of my organization is the Huntington's Disease Society and two of the Parkinson's groups and a lot of the diseases that are involved in this area. Besides which, for ten years my organization gave away millions of dollars

every year for cyclosporine to transplant patients. We handle 8000 patients a year. So, we certainly know the pain and the suffering and anxiety of these people, and how they are counting on this technology to move forward. However, a hemophilia patient is about 30 years old, and he was HIV

positive, and that is not really what destroyed his life; the hemophilia didn't destroy his life and the HIV didn't destroy his life. What destroyed his life is that he had given HIV to his wife before he knew it. He found out he had HIV when his wife died of HIV, and that was a public health tragedy and that is what we must avoid at all costs.

So, we must have a complete understanding of what this virus does; what its potential is. Maybe it is benign. Maybe it just gives you thick finger nails or toe nails for one week of your life -- who cares? But get the answer before you start using this technology in large numbers of people because then you are transplanting patients who are going to come to you and saying, "my wife died and I killed her and I didn't know it. " So, that is where I stand.

DR. AUCHINCLOSS: Okay.

MR. BENEDI: Being a recipient, it is a question that is really about our lives and everyone in this room obviously is here because they are concerned with saving lives, and 60,000 people, as Bill has mentioned, are

waiting; ten will die every single day. The science community needs to look at ways to save those lives. Obviously, the donor pool is not meeting the demand. So, to proceed cautiously is something that is responsible to do, but also, and this is a good start, to create a partnership

because there needs to be a partnership between the scientific community and the general public out here about this issue and about the understanding about the ramifications of this issue.

If it were up to a recipient alone to decide whether they were going to be infected or not, I think that that is an individual choice but to think that a recipient, if they were posed with a question whether they could live or die but if they lived there is a potential, just a potential that they will infect their loved ones when they are gone or in their lifetimes or others in the community, I think that is a bigger question, and I think posed to a recipient like myself, and I can't speak for Bill, if that question were posed to me before my transplant I would have probably said no. Actually, I know I would have said no.

So, I think we need to really look at it as a community to come together and to see what the risks are. Obviously we need to proceed. There are people who die but we need to proceed with caution. Thank you.

DR. AUCHINCLOSS: Well, thank you all very much --

MR. BENEDI: And if I could say one more thing, with 60,000 people waiting for organ transplants and 10 dying every day, I would like to challenge everyone in this room to sign a donor card before Christmas. Thank you.

[Laughter]

DR. AUCHINCLOSS: Thank you. It is time for a coffee break so let's just put this last question out, (d), very briefly. Does anyone wish to make a statement about the issue of whether or not, if you were to put xenografts so they are not in contact with patient cells, which I assume basically means barrier devices and isolation devices, and/or do bridge transplants specifically, alter the way you feel about anything you have heard?

DR. ONIONS: Yes, it matters because bridge transplants are so much safer. I don't know that they are but I would just guess that they are because the period of exposure is shorter. You might be the unlucky thousandth person who gets the hit straightaway but, yes, I am sure bridge transplants are safer and I am pretty sure that the kinds of barrier devices that some of the islet people are talking about, and we have heard today from groups who are using hepatocytes in various systems that potentially those are significantly safer.

DR. WEISS: The microchimerism that can occur over a short term of a fixed transplant could actually result in

long-term presence of the tissue even after the solid organ is removed.

DR. ONIONS: Well, I would be very interested because I am certainly not an expert in this area and I would be very interested later on to hear from people. My

understanding from talking to a couple of people who have done bridge transplants is that actually microchimerism does not occur in bridging of transplants. You get DNA in the circulation for the first few hours -- somebody might be able to comment on this -- but detection of microchimerism is actually almost unknown after the first few days, true microchimerism, by that I mean getting porcine cells in the circulation. But there may be somebody here who can contradict or add to that, I don't know.

DR. AUCHINCLOSS: I think I am going to suggest that we take a break. It is now 3:55 -- oh Lord, cancel my flight tonight and can we make some reservations in the hotel? Let's suggest that we come back in here and be ready to start at 4:15.

[Brief break]

Presentations of Focus Group Analysis of Question #1: Development of Programs for Patient Monitoring and Clinical Follow-up

DR. AUCHINCLOSS: We will go right ahead and move on. First a comment, when you are speaking, members of the

panel, people in the back are having difficulty hearing sometimes so please bring the microphone closer.

The bad news is that that was the easy question. I have heard from a number of members of the committee that there are flights, including trans-Atlantic flights, that

simply can't be missed and we are going to begin to lose members. What that functionally means is that we are not going to answer all of the questions that we have in front of us today but it is clear we didn't answer even the first question in a fundamental way.

The important information I think for everybody to have is that this is not the last time that this subcommittee will gather, partly because the information will change and partly because the issues are, in fact, so complicated that they deserve still additional attention.

With that introduction and the notion that there will be a time probably 45 minutes from now where we wind down and complete, let's move right into question #2 because there really are some very fundamental issues that come up in this question as well. We are going to have a presentation from our focus group on question #2. Claudia?

DR. MICKELSON: Well, just as a general summary and just as before, we will go on a question by question basis, our question in focus group #2 tried to deal mainly with development of programs for patient monitoring in

clinical follow-up issues.

Basically, the first four or five questions dealt with actual tests that might be used to monitor recipients. The questions had to do with appropriate tests, how to identify PERVs and sensitivity and specificity. Basically,

I am trying to be quick so that we can get into the questions specifically, but those types of questions. We all reiterated words that you have all heard this morning, such as co-cultivation with stimulation of the pig cells on PCR and reverse transcriptase, with the idea that we need to have some sort of quantitative estimate of the viral loads that might be present in the patient.

I think we should note too that just as we all agreed that it is necessary that CBER require sponsors of xenograft recipients to monitor for PERVs, I think most of us would agree that we would also ask that the sponsors of any of these trials be required to test the tissues and cells and, of course, you would want to test the recipients as well.

We felt that in terms of what types of tests these would change with time as the state-of-the-art shifted and things got more specific and more sensitive and those, of course, would be the tests that you would have to ask for.

The other question is at what frequency, what times would you ask to obtain clinical specimens for post-

transplantation monitoring. There is some flexibility in that, of course, and it would change depending on the transplant type. With things like bone marrow transplant you want very early time points, mid- and long-term follow-up. I think the point was also made that there would have

to be baseline pre-transplant testing and monitoring to be sure that there were true xenos., not only with PCR but with any antibodies, and we are hoping that there would be antibody tests available. That is really necessary for appropriate monitoring.

We looked at frequent intervals in terms of at least weekly, sometimes multiple times during the week, for at least the first two to three months and then follow-up every six to twelve months for a very long time frame. At least the minimum requirement would be that seen for allotransplant recipients.

As to what types of tissue would be relevant and informative in monitoring for PERVs, again it depends on the type of transplant. I think one of the points that was made was that detection in peripheral blood was going to be informative. I think one of the points that was made was that by the time you see infection in peripheral blood you probably have a reasonable infection elsewhere in the body. If you have enough infection in cells to detect it by PCR you have somewhere else a reasonable source of virus

shedding into the blood. Also, the issue would be how invasive a monitoring you want to do. Is it warranted? If these are non-accessible sites, how far do you go in terms of invasive biopsies on transplant patients who will also be immunosuppressed?

In the event of a positive test result, what strategies would be included in the diagnostic plan to identify the source of the positive signal? Now, we have heard talk of species-specific probes, but the key question there is to be sure that the positive signal arises from the human cells and not trace contaminants of pig cells. So, it looks like you want not just the full complement of species-specific probes and, so far, we are concentrating on porcine ones but I assume there would be other species that would eventually be donors. So, it would be important to also have very strong, clear human-specific probes to use as a counterpart in all of your tests. I have seen no one really mention those. I suppose those are easier to obtain, but for each species-specific one you should have a human positive.

Then, you also want to be sure that in terms of the positive test results whatever result is obtained, whether by PCR, you would also look at other host responses to see the extent of the positive result. If it is positive by PCR, are you also getting humoral or cytotoxic cell

response; is there any kind of NK cell response? Then, if you can isolate the virus from the patient, look at issues of tropism, host range and then tissues capable of being infected in the gene sequence.

If a patient were to test positive, what changes

might be made to the clinical evaluation program in terms of safety and possible public health risks? If PERV is detected in human cells, all of us agreed that at least the clinical trial should be suspended immediately until extensive analysis of the signal or the positive assay can be confirmed, and see if it can be supported by other tests.

We left those other tests vague because I think it was clear from the first discussion that a lot of the tests are still under development and, as more tests become available, we are just going to get much more information to assess exactly what is going on.

What happens in case one of the clinical participants turns positive? What do you do in terms of patient counseling? We asked that there be extensive free transplant counseling for the recipient, the expected recipient and family and close contacts to deal with issues that are usually addressed in HIV infection counseling, such as not donating blood or tissues, using your own toothbrush. The same kinds of items and issues that have been raised and

shown to be methods for HIV transmission should be pertinent in these instances.

In terms of immediate clinical care of the patient, the counseling of partners and family pre-transplant, as well as training and information for all of

the patient health care personnel and treatment, the minimum starting point would be the same types of support given for allogenic transplant recipients.

In terms of transmissibility, you would check, just to be sure that what was being shed or detected, that you hadn't developed any new types of phenotype. C-type retroviruses are not shed by respiratory methods. You rarely find them in certain type of secretion but they would all be checked and you would try to screen all contacts on an ongoing basis with PCR and serological tests, if possible.

I think one of the issues, and one of the questions that we sort of threw in is how might you change the immune suppression regimen if the patient is productively infected? What can you do? Are there possible treatments for the infection? I think one of the issues is if you do have a positive test, what are you going to do about it? Do you just sit there with it or are you going to try and do something to suppress that signal? Even if the signal goes away, it doesn't mean, honestly, that the

patient is not infected. But if the suppression can be safely reduced and possibly treatment instituted it might be advisable. But there was disagreement on that, that there would be no changes made in any kind of immune suppression regimen until there was evidence of pathogenicity or

infectivity -- again, the potential treatment with antiretrovirals.

In terms of long-term follow-up for the patient, we raised issues that these were different than allotransplants and that there would be much longer-term follow-up needed if survivability allowed that, and that it would constitute as many types of samples as we could get without being overly invasive to the patient. I think one of the things that was very hard to set up is an idea of what you would do for monitoring unless we knew what kind of tests would work. I think one of the things that came out of the discussions -- we conversed by e-mail; we actually never met altogether until today -- but I think one of the things is how do you set up a monitoring program when we are not sure how good the tests are and until they are well standardized? I am not sure what you do with the data, and then how do you use the data? What is it going to mean if a patient is positive if we don't know the outcome or have an idea of what a reasonable outcome of the infection is?

Then, if clinical trials do proceed and we

accumulate all this screening information, how will it be used? What will be important in terms of making clinical decisions to treat one of the patients and then, also, how are you going to safeguard all the information that you would get from patients?

We can go through the questions point by point.

DR. AUCHINCLOSS: Thank you very much. Before we do that, reading the handwriting on the wall, I guess, Leroy Walters has suggested that you receive the summary of the focus group #3 before we go into detail on question 2. We though that maybe we will sort of run out of energy before we got to 3 in detail.

DR. MICKELSON I think there is so much overlap that that is good.

DR. WALTERS: Our group met for about 45 minutes over lunch, and our group was actually growing over the weeks leading up to this meeting. So, this will largely be one person's reaction to the discussion of the group, and some group members may want to disown this summary.

I will give you four generalizations, three recommendations and two concluding comments. Our first generalization was that this is clinical research, not standard and accepted therapy, and it should be presented to patients as clinical research.

The second generalization is that there are many

unknowns in the current situation, and this fact too should be disclosed very clearly to the subjects.

The third generalization -- there are two very different contexts in which xenotransplantation trials are being conducted or proposed. One of them is the emergency

situation, for example, with fulminant liver failure. The other is a non-emergency situation, for example, progressive Parkinson's disease. In terms of the consent process, these two types of situations present very different scenarios and very different problems. In the emergency situation we are really talking about consent by a family member or a close friend on behalf of someone who can't give consent for him or herself.

A final generalization -- there are at least three groups of people who are potentially affected by these clinical trials. First, the subjects themselves and there can be a consent process for them if there is no emergency. Secondly, the close household contacts of the subjects and there can be a consent process for them if there is not an emergency. Third, and I don't think we have really addressed this today, the health providers who are involved in the subjects' care. Here, we think there may need to be some kind of notification procedure and, very likely, universal precaution until we know more information about actual risk.

Now, our recommendations fall into three areas. First, on the description of risks, this was a suggestion by one member of the group in particular, that there should be a standardized and regularly updated and accurate and balanced description of the major risks for each major type

of clinical trial. We even speculated about developing a web site and an 800 number to which IRBs or prospective subjects or clinicians and investigators could turn. Very likely, this web site and 800 number would have to be managed by a federal agency.

A second recommendation regarded selection criteria for subjects. In our view, inclusion criteria should be related to the clinical research and to nothing else. So, the subjects have to be willing to be monitored for many years. Their household contacts will also probably need to be willing to be monitored at regular intervals. Clearly, they have to have the capacity to understand the research protocol and to give consent to participate. But we hope that the trials will avoid selection based on social criteria. That is, they shouldn't be based on social class or gender, or ethnicity, or even a history of alcohol use early in life.

A third recommendation has to do with the consent process. Again, we go back to the web site and an 800 number. There should be a relatively standardized

description of risks that is updated regularly on the basis of the newest information. We also think that the consent forms should be simplified; should be in lay language. We observed that the two consent forms that we read were fairly complex at some points and a master's degree in immunology

would have been very helpful in understanding those forms. Again, household members may need to be involved in the consent process.

Now, two concluding comments -- there is one additional kind of subject monitoring that we think should probably be included that is a little different from the types of medical monitoring that we have just heard about. That is, gathering information on the effect of receiving a xenotransplant on one's health insurance coverage. That wouldn't occur to our colleagues from the U.K. because you couldn't easily fall out of that system, but it is at least conceivable that insurers will be a little worried about the unknowns in xenotransplantation and be reluctant to cover people who have had this kind of transplant.

A final concluding comment is that I think we need to be prepared for the moment when and if the first infected subject is detected, and have to be resolved to protect that individual's civil liberties. It will be easy to overreact and words like "isolation" and "quarantine" come readily to some people's minds. I would just say that there are many

people walking around with influenza. You have sat beside some of them in airplanes when you have flown. There are many people walking around with tuberculosis or hepatitis B or HIV infection and questions of isolation and quarantine do not come up. So, we need to be resolved not to overreact

when and if a human patient becomes infected.

DR. AUCHINCLOSS: Thank you very much. I am going to handle the individual questions slightly differently as we move through question 2, and suggest, for example, that question 2(a) which basically asks, as before, is it appropriate for CBER to require that assays be done to detect virus, I think we are going to simply suggest that the answer from this committee would be yes. Is there anybody who wants to disagree with that?

[No response]

Then, in moving to question 2(b), essentially it is a question similar to what we had with question 1, what types of assays, how sensitive, and in many respects I assume the answer is the same as what we talked about before.

So, what I want to ask the committee is what in addition do they feel are tests that should be required for patient monitoring. We have already heard one statement that we need to be looking for antibody responses. But we need to hear a little bit of amplification on this because

the antibody assay doesn't exist at this point. Can we generate one, and is it realistic or is it necessary to wait for an antibody test before any further progression of xenotransplants?

DR. ONIONS: Actually, Chairman, I think this

series of questions is actually a lot easier to answer than the previous ones. My own view is that if you are going to test -- certainly you should test patients but my own view though is those tests should be done perhaps 2 weeks, 1 month, 3 months at 3-monthly for the first year. What I would do on the first test up to the 3-month period would be to look for plasma viremia, and the way we would normally look for plasma viremia, the way, in fact, it is done commercially is 10,000 cats a year and we do 25,000 a year in my lab to look for virus antigen to detect viremia. But what we always do is confirm the positives by virus isolation. That, ideally, would be the way to do it.

However, I am concerned that the sensitivity of the tests are not good enough to do it that way at the moment, and the way I would actually do it at the moment is to actually look for virus by PCR in the plasma. You could do that by generic tests in the first place, and if it was positive I would then confirm which of the viruses of at least the four groups we now know of are present in the plasma. So, the first thing I would look for plasma

viremia.

Secondly, I certainly would look for virus in the presence of peripheral blood mononuclear cells. I think one of the questions we will have to ask is how many cells you should look at because you can look at 106 cells and you do

this in 105 aliquots. That only gives you a 96% certainty, if your assays are sensitive of course, that you are detecting it in that 105 aliquot. So, I would certainly do peripheral blood mononuclear cells. As Robin pointed out, a lot of the viruses we know about that are latent are at levels below 1 in 105; they are 1 in a million or so in circulation.

Thirdly, I certainly would have an antibody test, and the reason for that was perhaps badly described this morning. There are at least four patterns of infection we see in animals and, in fact, to understand which of the patterns of infection we are seeing in patients we must have an antibody result to actually define those. I think we are very near to having an antibody test. We are just validating one for P30. We are going through a lot of human sera as well as going through defined polyclonal sera.

So, I think those four tests over those time periods are the way I would approach it.

DR. AUCHINCLOSS: Comments from other members of the committee?

DR. KASLOW: I guess the question would be do you simultaneously or sequentially add such tests as would indicate transmissibility, other fluids at that point or do you wait until --

DR. ONIONS: I didn't bother to show it this

morning but if any of the tests were positive by any criteria, what I would then do, I would want to know if this patient is secreting virus in the saliva and possibly other fluids, yes, absolutely. I think if PBMCs are positive, we would really like to know if that is a true latent infection or is there, in fact, transcription of virus going on in those cells. That would be the second question I would want to ask.

DR. AUCHINCLOSS: Well, before you get to the positive what tissues do you screen and how invasive are you being?

DR. ONIONS: I wouldn't be invasive. The clinicians would be in a much better position to answer this question but I would have thought it is not really ethical to use invasive procedures on these patients. I don't actually see the necessity for doing that. We are concerned about a public health risk here, and the public health risk is when we get sufficient replication of virus for that to be of concern, either through blood spillage or through excretion in various body secretions. So, I don't see a

justification for invasive procedures. But there will, unfortunately, be patients coming through for postmortem to provide those tissues to do those other analyses.

DR. AUCHINCLOSS: As a clinician, I guess I started from a slightly different point of view. I can

imagine actually getting tissue with invasive assays through a number of different sources, but I came back to the same point that you just made. I am not sure that it helps me because the public health issue is the one at stake, and I am assuming that the tissues that represent a public health risk are the ones that we can easily sample. Is that a fair assumption?

DR. ONIONS: I think it is, yes.

DR. KASLOW: That may depend on how easy you think it is to sample cervical or seminal secretions I suppose. I mean, that would sort of be at the interface between easy and difficult.

DR. AUCHINCLOSS: You are right.

DR. MICHAELS: I think they can be requested --

DR. AUCHINCLOSS: Of course.

DR. MICHAELS: -- and I would also second David's comment that if patients were to die that up front in the consent process, which we will talk about in question 3, I would discuss autopsies with families if that should occur.

DR. AUCHINCLOSS: I don't know if that actually emerges in 3 but let me just make a comment. I guess it has been determined that you cannot require, and it doesn't do any good even if you did because it is not the person who gives permission for an autopsy, but you could potentially

put in a statement in the informed consent that "I have discussed with my family my desire that a postmortem occur."

DR. MICHAELS: I am not even sure that you have to make the person agree to an autopsy but I think it behooves the investigator to discuss those issues up front.

DR. AUCHINCLOSS: Okay. Under (b), I think your comments have addressed most of the specific questions. Amy and other members of the FDA, have you heard what you wanted to hear about (b), and other members of the committee, do you want to contribute further under (b) about the nature, sensitivity, timing etc. of assays on patients?

DR. MICHAELS: I have just one other quick comment. If the patient were to have febrile illnesses or unwellness that was not able to be ascribed easily to another cause, that at that time, even if it wasn't in your time point of surveillance, you would bring the patient in.

DR. AUCHINCLOSS: That sounds reasonable.

DR. SIEGEL: Let me ask for clarification on one point, which is that there has been a widespread, I think, agreement that antibody tests would be nice and should be

developed rapidly. Should we take home from that that they should be required as soon as they are developed, or I should take home the alternative, that they need to be developed, or they should be developed before we consider moving forward? We have said under 2(a) that before

somebody starts a protocol they should have a patient monitoring scheme in place, and we take that to mean that they should be looking with PCR in the blood, in the lymphocytes, whatever. Does that mean they should be looking at antibodies or they should be looking at antibodies at such point, as soon as that becomes a reasonable, controlled and validated thing to do?

DR. HIRSCH: I would think you would want to collect the sera.

DR. SIEGEL: Collect it now?

DR. HIRSCH: You know, we don't know how long it is going to take to develop a satisfactory assay.

DR. SIEGEL: I just wanted to clarify that.

DR. MICKELSON: We just felt it is very important that one be in the works somewhere.

DR. MICHAELS: Also, looking at a patient that is a year out from having had porcine tissue in them for a finite period of time, it is true that antibody testing in that person, if it is a sensitive test, might be better than PCR or PBL because you might not be having a latent organism

viremic. But looking for a primary infection, I think the time points that David Onions brought up would be very sensitive, and I would concur with the others that serum should be collected.

DR. AUCHINCLOSS: Which, I guess, Jay, indicates

that the committee is not suggesting to you that not only should the antibody test be in place but that it should be applied to all existing patients who have had porcine transplants in the past before proceeding with new trials, which is yet a further extension of what might have been suggested to you but I am not hearing that either. Is that a fair statement from the committee?

One suggestion is, yes, but don't go forward with any pig trials until you have the antibody assay and until you have used it on all those 100 or whatever patients, and this subcommittee is not making that recommendation to the FDA is what I am hearing.

Again, within this issue of testing patients there is another specific angle which is slightly different from testing tissue ahead of time, and that is how do you know whether the virus is in the pig cell or the human cell? Is there a technical answer to that? What do they need to do?

DR. ONIONS: I thought we heard a very nice presentation from Walid this morning about his approach which I think is a valid approach. I don't know whether I

am allowed to say it but I am going to say it, Ed Otto, from GTI has developed a very interesting system using the tapman quantitative PCR system, and I think it is a very elegant system and I think that also provides the tools for answering this question. It allows you to get a really

accurate ratio between the proviral DNA and the cellular DNA. So, I think there are systems to do that.

I am also going to be interested, actually, in what really is the true problem of microchimerism. I wonder if it is going to be as big a problem as we think it is. I know there are different opinions but I think it is a solvable problem.

DR. AUCHINCLOSS: Any other comments on this? Any of the experts want to add anything? So, you get the virus and you suggest that it is infective in a human cell, in a patient, and you do some things to characterize its infectivity and to further characterize the agent. I think we have indicated before that you sequence it, you look at tropism, and I think that list is already complete in your summary. Have I missed an important aspect of this because we are going to move into what it all means, sort of the heart of it?

Let's go there, which is (f), which is if the virus is in a human cell, in a human patient, what do you do? Let's go to the bottom line here, (iv), what do you do

with the clinical trial? You suggested that we all agreed that that is the stop point.

DR. MICKELSON: Yes, we all --

DR. AUCHINCLOSS: Well, I didn't agree with that and I was on that focus group. Remember, I am assuming that

that event is --

DR. MICKELSON: You didn't answer that question --

DR. AUCHINCLOSS: Oh, yes, I did. I am assuming that event is going to happen. What do other people think?

DR. MICKELSON: Well, not everybody that I e-mailed all the questions to answered me back on that one. The one answer that was given as to what you would do in case you did eventually have a patient that came in positive on a test was that the program be suspended immediately. I assume that that was done in context of until you have further analysis of what the positive signal was and if it is a true positive. He left but that was Dr. Solomon.

DR. HIRSCH: I can't remember whether I answered that question or not but I think it makes good sense to suspend the trial at least temporarily so that the FDA can gather the data. They may want to reconvene a committee and say, "well, what do we do now?" I don't think that means necessarily that the trial is permanently discontinued but that it is temporarily suspended seems to me the prudent thing to do.

DR. MICKELSON: Yes, until you figure out what it actually means in terms of the patient.

DR. COFFIN: It seems to me one should make a quantitative distinction here between localized infection and spreading infection. I think in the case where it is

clear that there is a spreading infection, viremia, appearance of virus at sites other than in the blood or at sites at other than where the transplant was, unless in the meantime the collective wisdom has been brought to bear on this problem better than we can do right now, I think that definitely calls for an assessment of what is going on and assessing what is going on.

DR. AUCHINCLOSS: Actually, that is what my answer said, no, not necessarily, but I want to hear from the experts what the features are that are positive that would lead you to say no, no, no.

DR. ONIONS: I can't cast myself in that role but I agree with John. If you get viremia, I would certainly want to hold things until we know a lot more about that situation because the viremia might increase -- if you get over viremia, I think that is five years --

DR. AUCHINCLOSS: I see a lot of nodding heads. Viremia is a bad thing.

DR. ONIONS: If you get just a few cells, maybe one in a million cells in the circulation infected, I would

still be concerned but I don't think that is the same degree of concern, particularly if you can demonstrate that is true latency --

DR. KASLOW: By viremia you mean free virus?

DR. ONIONS: Free virus, yes.

DR. ALLAN: I would go a little farther. I would think that if you had infected cells, and they are human and they are circulating the peripheral blood I think that is a red flag, and I think if you do have that you will probably also get antibody responses. If you are going to start seeing infected human cells floating in the peripheral blood you may have antibody responses, and is that a red flag? In other words, you start to see antibody responses to the viruses probably at least temporarily and maybe longer.

DR. HIRSCH: I think with the different answers you are getting it means that when you get any evidence of human infection you pause and you reevaluate. I mean, I think we are extrapolating a lot from HIV where you get viremia. With HTLV, let's remember, you really don't get a plasma viremia and it is pathogenic, albeit a human pathogen of low virulence, and it can cause serious disease and you don't get plasma viremia.

DR. ONIONS: But they are very different and I really must reemphasize again that this is not like HTLV. HTLV carries transactivated genes. Its method of

pathogenesis is quite different from these viruses. I think it is always risky dragging back to the systems we know behave in the same way. In the systems that we know of this representative group, in general, in over 90% of the cases it is the plasma viremia that is the bad news. You do find

tumors that are associated with recovering latency because transduction or insertion of oncogenes occur but generally it is when you get plasma viremia that you get into trouble and, honestly, I think HTLV-1 is a bad model in this case.

DR. HENEINE: If I could add to the criteria for positivity, a persistent ability to persistently detect viremia, antibody and all that -- it is not like a one time point when you detect the evidence of infection but if you are able to detect it over time and persistently, that is what would give you evidence of a persistent infection.

Regarding the viremia, we know very little about the biology of this virus and how it is going to behave in humans. You can argue both ways. I was about to raise Dr. Hirsch's point as well, like in HTLV-1 and 2 we don't see cell free virus in infected people. We see persistently infected PBLs. Also in fomi viruses we don't see cell free virus in the plasma, but we see infected PBLs all the time. So, there are different biologies but we are not sure how this PERV would behave in a human.

DR. ONIONS: But I think we have very good

circumstantial evidence. If you look at the same group of viruses and we look in the context where they have been put into a foreign host, into a primate host, if we take alpha tropic murine leukemia virus that has been put in by Cornetta and by Donahue, if you look at the Donahue study,

the tumors occurred in those animals developed fulminant viremia, high titer plasma viremia. Those were the animals that developed disease. In Cornetta's study and also in Donahue's study the animals that didn't develop a viremia, or they still might have had PCR evidence of virus around, they did not develop disease. So, I think actually it is confirmed. This is the likely way that this group of viruses will behave in a primate.

DR. HENEINE: This is another point, pathogenesis associated with or without viremia. But with the other viruses there is or there is no viremia irrespective of disease development in HTLV.

DR. AUCHINCLOSS: What I think I have heard as a general statement is that detection of virus in a human cell is a break point. Lots of information should be gathered, including especially viremia; that you should call the subcommittee back for another conversation with you and maybe we will be smarter then.

DR. HENEINE: Let me add again about the serology, the limitations and the usefulness and utilities of the

different tests or different assays we have, we look at this as different markers of the infection. Marker number 1 is proviral sequences and peripheral lymphocytes. Marker number 2 is antibody production. Marker number 3 is viremia, which is cell free virus in the plasma. And the

more markers we can gather for an infection, the better it is. But to say that we select only one marker, antibodies, and forge the rest is not optimal. We need to gather as much evidence as possible on all these different markers of the infection, and we will be facing a situation where we will have patients that are antibody positive, PCR positive, RT-PCR positive and RT positive, and others will have only one marker which is persistency of positivity but negative for all the rest, and we will have to figure out what is going on.

DR. KASLOW: Is it so unlikely that we would see shedding of cells in some other secretory pathway instead of or sooner than you would see it in peripheral blood, that the order in which all of that is done is kind of irrelevant and we should start with and rely on peripheral blood first?

DR. ONIONS: That is a difficult question to answer because I think the point was made this morning that maybe peripheral blood cells are not easy to infect. What we know of the pathogenesis of these viruses as exogenous viruses, not as endogenous viruses being reactivated, is

that they tend to get carried to bone marrow. The events that go on in the bone marrow are critical. So, in fact, you find a high viral load suddenly coming into the blood stream and actually the infected cells are in the bone marrow. So, in fact, you even find cells like platelets

which, of course, are non-dividing are actually releasing virus because the megakaryocyte is being infected. So, you suddenly get a burst and then you get a plasma viremia following very quickly.

So, I would still think that is the likely cell I would still go for. It is possible that you could get a sequestered pattern of infection which we see. That always comes after transient infection in the plasma and then the virus is just hidden somewhere in an epithelial surface. But if you do, your timing is correct. So, you are looking for this early phase of a transient viremia, two weeks and a month. Then I think you would pick that up.

DR. KASLOW: So we might miss it at two weeks but we are not going to miss it altogether.

DR. ONIONS: I don't think so, yes.

DR. HIRSCH: The exception to that might be the central nervous system --

DR. ONIONS: Sure.

DR. HIRSCH: -- you may never get a viremia.

DR. ONIONS: That is a very good point and that is

really quite distinct.

DR. KASLOW: The distinction there though is that that is probably not transmissible --

DR. HIRSCH: Right, but you still want to know --

DR. KASLOW: You want to know but it is not urgent

in terms of needing to make a different decision as a result of that.

DR. AUCHINCLOSS: Question number 2(f) has some other points here as well. What would you do about patient counseling? I am just going to summarize what I sort of felt was the response here. Patient counseling, where you have been counseling the patient appropriately ahead of time and so this is more patient counseling with honest information that it has occurred but we don't know what it means, and long-term follow-up and evaluation of patients really remains where it was before because we have things set in place for exactly this contingency.

The question, however, that I don't know the answer to is what do you do about treatment for this particular individual.

DR. HIRSCH: This is one point I feel very strongly about. I think you do not treat this patient. You don't know if it causes any disease at all. You know that all of the antiretroviral agents we have are terribly toxic. We don't know whether they are active against porcine

endogenous retrovirus so there is zero indication to treat these patients.

DR. ONIONS: Can I make a statement --

MS. MEYERS: I can't accept that.

DR. ONIONS: I think we need to go backwards.

First of all, I think we do need to know the effectiveness of the known antivirals on the porcine endogenous virus and John, I think, made a point at the last meeting that he treated MELV with some of the nucleoside analogs and not found them effective. But the critical point is actually is it is often so dependent so there is phosphorylation between nucleoside analogs and we need to know that. Of course, those will be operative in human cells, which are the cells we are concerned about and so we then need to know in human cells if the porcine virus inhibited by the nucleoside analogs. I think there will be some evidence that it will be actually. Sow e need to have those data. We must have those data. We would certainly not treat a patient who has just had cells that were provirus positive. If there was a viremia beginning, I have been persuaded by others that it probably is effective, and I can only defer to people who treat HIV and they would suggest that, in fact, the toxicity as I also thought. I would have to defer to other people.

DR. HIRSCH: We don't treat HTLV infections. We don't know that this virus causes any disease, and having

treated HIV patients now for 15 years, I can tell you they are toxic drugs. You don't know what your endpoint is. You can't eradicate virus anyway. So, it is something I feel very strongly about.

DR. COFFIN: Yes, I agree with that general

conclusion. There are other problems as well. Models where antivirals were used to treat murine leukemia virus infections really require pretreatment of the animal. Any treatment after infection is established in the animal -- my memory of these experiments is that it doesn't work very well. Also, only a very few agents are likely to be effective. Only a few of the nucleoside inhibitors are likely to be effective.

DR. ONIONS: John, if I remember correctly, those experiments when you actually looked at MELV in mice, the problem there is that actually mice do not phosphorylate the nucleoside analogs very efficiently.

DR. COFFIN: But you can protect a mouse against infection. That is part of the problem, but the other problem is as far as malignant consequences by the time you see it the damage may already be done. As far as transmission is concerned, that is another matter because you probably would block transmission that way if that is your concern, but then you are not treating the patient, you are treating the public and the ethical issues get very

serious then.

DR. ONIONS: This may be a question we can't resolve today and I think it probably has a lot of ethics in it as well, and I honestly can't answer it but, again, I would not treat a person who was just provirus positive but

I really, honestly, have to defer to people who know about this area more than I do. I would think there is evidence that you would treat a viremic person.

I think the other thing just to remember is that there is evidence from studies mentioned earlier of cats treated at a very early viremic phase, just going into viremia and viremia has been able to be reversed just by using passive neutralizing antibody, which is very surprising. It is very good work and it looks real. So, I think there are other strategies that if you detect infection really early, really early, you might be able to reverse infection.

DR. COFFIN: That begs the question how frequently you are willing to monitor because monthly monitoring is probably too infrequent.

DR. AUCHINCLOSS: Abbie, we have heard you gasp.

MS. MEYERS: I did because in my mind I am writing the informed consent for this project: "Please come and volunteer to go into this research program. We would love for you to be a guinea pig. But once we do this, it has a

virus in it that we know nothing about and so we want you to know that if you get sick we are not going to treat you."

DR. HIRSCH: That is not what we said, Abbie. We will treat you with the best medical knowledge we have and the best possible way we can, but we don't treat you with

unknown medications that we have no idea will work but we do know will be very toxic. We treat you the best we can with the best medical knowledge we have.

DR. KASLOW: We don't do that when we have no knowledge of the occurrence of clinical illness as a result of this infection. It would be very different if we knew that somebody was going to get sick as a result.

DR. AUCHINCLOSS: The key word is "sick." There is no presumption here of sickness; there is only the presence of the virus.

MS. MEYERS: But that is not what you said. You said that if they get sick I would not treat them. I feel very strongly that I would not treat them.

DR. HIRSCH: No, that is not what I said. I said if you found evidence of infection in the human with a porcine retrovirus I would not treat at that juncture because we don't know that it causes disease, and we don't know that the drugs work, and we do know that the drugs are toxic. If, in fact, we find that porcine retrovirus caused any disease and that they are susceptible to antiretroviral

agents I certainly would treat at that point.

DR. KASLOW: Just briefly, would the committee want to entertain a consideration or even a recommendation about whether or not, if treatment were even considered, it should be done under a protocol of some sort and not just ad

hoc?

DR. SIEGEL: It would certainly be an off-label use.

[Laughter]

DR. HIRSCH: And not necessarily compassionate.

DR. AUCHINCLOSS: The one point that I wanted to stress, I think it is really implicit in the way these trials would be set up that this is the point at which you want to redouble your monitoring of contacts, which is really the issue here, transmissibility, when now you know you have a patient who has the virus. But I guess that is sort of obvious.

Now, back to the FDA, have you heard in question 2 what you wanted to hear?

DR. SIEGEL: Having discussed question 2, there is something carried over from the earlier question I would want to get to. There was a general sentiment expressed by several panel members that having heard that there have been many individuals who have received xenotransplantation of cellular products, that it would be advisable to obtain some

amount of clinical screening, presumably the types of PCR we have been talking about in particular, as a database prior to moving on. Where the Agency is at the present time is that we have asked sponsors to develop or access appropriately sensitive assays of that form and consistent,

I think, with your consensus advice, and to the extent that they have already treated patients and have available test specimens stored, to test those specimens and report results before proceeding. We have not specifically asked or decided that a sponsor who perhaps hasn't treated anyone or has treated maybe 5 or 10 patients with a liver device, for example, we have not suggested that they would have to wait until some other sponsor who maybe has treated 100 patients with neuronal cells would have data from those 100 patients. We could ask for that, but that would obviously be a very different step in the sense that it would be entirely out of the control of that investigator to wait for results that only another investigator, who might in fact be a competitor, would have the ability to generate. So, I guess I would like some feeling, after having said that, yes, a sponsor should have a test and do the testing before proceeding, is there some need for the whole field to wait for a certain amount of information? And I don't mean to be putting that down idea. Or, if tomorrow somebody has submitted to us a really good PCR they are doing and they

have tested their cells, you know, in co-culture and they have protocol for how they are going to test the patients, is it okay to say tomorrow go ahead and do some transplants?

DR. AUCHINCLOSS: And here we are back at our science. Why are we back at our science? I will tell you

why I think as I listen to this. Question 2 turned out to be easier than I thought it would be, and the reason is that I think there is more sense of comfort as to where the stop point is on xenotransplant trials than where the sense of comfort is with where the safe start point is, and you are back to asking us where the start point is.

DR. SIEGEL: There is a difference between saying that there is no evidence for something and there is evidence that something isn't there. So, it is one thing to say, yes, it is okay as long as we don't know that it is there but there is a difference between not looking and not having looked --

DR. AUCHINCLOSS: I understand. Let me try my own personal response. We are not talking about 1000 people going out and getting pig transplants over the course of the next two months. We are talking, in fact, about very small trials and I personally view it as okay to proceed, given the small numbers, because basically I don't think we are going to really feel comfortable about this answer until we have done 1000 patients or maybe even 10,000 patients, all

the animal studies aside. We really just need to do patients and follow them extremely closely. So, I think that we should be pushing ahead under the kind of guidance, controls, supervision, careful testing, monitoring, informed consent etc. that we have been talking about all day. So,

there is one response.

DR. ONIONS: I mentioned earlier that I would like to see the data that has just been collected now. There is going to be very little in the next few months, the data on the patients who have been exposed, before going on, but I would say that after that, providing that there is reassurance from those data, that I honestly don't believe we are going to create a public health hazard from going into patients on a limited step-by-step basis. It is just almost inconceivable, I think, that you would create a new world pandemic, which is what we are all concerned about, from a limited number of trials that are closely monitored. That is not going to happen.

So, I think we just take it in this step-by-step process. Let's get the data from these people who have been exposed. If that is comforting, let's go to the next stage on a limited basis. I think it is a fairly logical progression. We may be trying to define our terms a little bit too rigidly and getting caught up in it. I think there is a fairly obvious step-by-step progression ahead of us.

MS. MEYERS: Do you think that the public is going to feel the way you do having watched TV over the last few days, worried about the Hong Kong chicken flu?

DR. ONIONS: I don't know, but the thing to remember about the Hong Kong chicken flu is the fact that I

think the biggest world pandemic that has killed more people is the 1918-1919 flu. You are not going to stop these events. What we can do here uniquely is that we can actually monitor the patients. We can actually advise the patients about their behavior. You can't control it, of course, but you can have a high degree of moral pressure on people and you can select them. I actually believe that you are unlikely to create the conditions for disaster by limited clinical trials. I think the problem, funnily enough, will emerge a little bit later on, that is, when the numbers start to go up because some of these problems may be quantitative.

MS. MEYERS: But can you promise the public that it will not start a major public health problem.

DR. ONIONS: I personally could promise no such thing.

MS. MEYERS: No.

DR. ONIONS: And never will be able to.

DR. SIEGEL: I would like to note that while you stated that this is the obvious thing to do, what you said

was very different from what Dr. Auchincloss said was a very reasonable and obvious thing to do in the sense that I believe you said we should wait a few months until we have the data from the current patients, and that sounds very reasonable although there are folks out here who have told

you that in those few months people will die from various diseases if they do not have access to treatment. So, it is a different decision from saying we will move ahead as we accumulate the data.

DR. ONIONS: The comment I should also have added is that it does depend on the process. I think, for instance, if we are using cells that are isolated cells or in some form of containment, I think those issues are very different from a whole organ transplant. My comments were actually directed at whole organ transplants. I think there are considerable differences in bridging transplants and I think there are considerable differences in isolated cell systems that are in some form of containment.

DR. AUCHINCLOSS: Is it fair to say, because I think there are two kinds of clinical trials that are probably closest to clinical application, that one involves barrier devices and the other involves central nervous system placement, that those are the two kinds of circumstances that you are perhaps most comfortable with?

DR. ONIONS: Yes, I think so.

DR. AUCHINCLOSS: Which is an important point which I wasn't personally willing to acknowledge in the beginning. I was sort of saying they are all the same and this event is going to happen, but the experts are saying, no, there are some that are safer than others. The ones

that you are looking at most practically in the near term are the safest ones.

DR. COFFIN: I would also point out again that I think attention should be paid to how one proceeds to the nature of the patient population that is being treated. I think elderly patients, like Parkinson's disease patients, are at a much lower risk for spreading virus around than are young patients from other groups, and I think that can be taken into consideration as to how one proceeds and what sorts of levels of concerns one has.

DR. AUCHINCLOSS: Other comments from the committee on this issue? They are sort of asking what they should do tomorrow -- not sort of, they are asking.

DR. SIEGEL: Those aren't the only protocols --

DR. AUCHINCLOSS: And I hesitated to make that statement because I don't know what protocols you have and I don't mean to imply those are the only ones.

DR. SIEGEL: And there are, for example, cellular therapies that are implanted intravenously or otherwise in ways -- some encapsulated and others not. So there is a

spectrum of things out there. But, you know, you have spoken to guidances and considerations that lead to higher or lower risk and one of our questions was should we take those things into account. You know, if that represents advice that is helpful.

DR. ALLAN: I think when you talk about bridging you have to be careful too. If you are talking about doing ex vivo or transgenic livers or something like that, where you can actually get seeded with lymphocytes or porcine lymphocytes or some other species lymphocytes, then that has a higher risk than a barrier filter device or even the fetal cells in the brain. It is different than if you are taking an adult organ and you can get lymphocytes from those pigs into the human body. I think that is a much higher risk. So, there are some differences.

DR. AUCHINCLOSS: Question 2, any other comments from the committee? Clarifications or questions from the FDA?

[No response]

Basically what Jay Siegel said to me was keep going until they drop --

[Laughter]

DR. SIEGEL: Until they have to leave.

DR. AUCHINCLOSS: So I am going to push on to question 3, but I do understand that if there are some

people who have things they have to do, like catch flights that they just have to catch, that some members of the committee may not be able to stay around. But his comment was as long as you have a group here that can keep on making intelligent comments or semi-intelligent comments keep them

talking to us.

DR. COFFIN: Please let us know when the comments stop being semi-intelligent!

DR. VANDERPOOL: Could I get us started on the discussion of question 3? I think the FDA should very seriously consider setting forth a set of national guidelines for whole organ xenotransplant research. I truly appreciate the appeal of the Public Health Service in section 2.5 of the guidelines that the standard procedures involving the ethical principles in the federal guidelines and the Belmont report ought to be honored. Then, in addition to those, there were explicit guidelines regarding the risk of infectious disease. Question 3, as posed, deals primarily with risk of infectious disease.

But before we focus on that, I want to make a plea for the inadequacy of present day understanding of adequate review for whole organ xenotransplants. Start with harms and benefits. The research community is very unclear as to when whole organ trials are go or not. Is this one month survival? Is this six months survival? Is this a year

survival? With what types of quality of life and so on? These are unarticulated determinations, different ones being voiced by different authors at different times.

Secondly, on questions of informed consent, I do think there are critical questions dealing with informed

consent regarding infectious disease. But I think if there is ever an example of clinical trials that will exceed the complexity of Phase I cancer research, which has been discussed and aired year after year, it is these trials. Informed consent will involve a host of things from the effect of immunosuppressive drugs to how one is supposed to educate one's close contacts, to whether or not I understand what it will mean to go through a life-long surveillance, to what I understand about infectious disease.

So, to me, these issues of harm-benefit analysis and informed consent are complex, and I don't think it is adequate to say, well, let's give the standard IRB review but have the Public Health Service guidelines in your pocket. I think more is involved than that, and I appeal to you to think about developing national guidelines. Whether they are required or not is a question for probably vigorous debate, but certainly recommended guidelines.

The third issue that is not here but I think is very important was one that Dr. Walters mentioned from our committee, and that is questions of informed consent for

close contact. I think there are a lot of arguments to say there should be informed consent for close contact because they will, in fact, be involved with many of the risks and also be involved from time to time in the recipients' process of coping with the trial, the transplant and so on.

Where informed consent should also be required of health care workers is, as our committee discovered today at noon, debatable. Is this an ordinary risk that health care workers will endure, or is this something much more?

I just want to mention one other thing, and this is where the transplant survivors, Tony and Bill, can speak with far greater eloquence than I, but I think the question of the process of informed consent needs to be very seriously considered, and I would hope that protocols would not just give an informed consent document, two unfortunately poor examples of which we have in our readings, but would also see themselves the need to describe the process of consent.

Now, that raises a final issue -- and I am just giving the grid, and that is should you also consider who else should be on an IRB besides infectious disease persons for xenotransplants? Should a psychological counselor be a required member of an IRB? Should former transplant patients be either regular consults or perhaps on the IRB?

Those are some of the questions I raise. I don't

want to derail us from dealing with our specific infectious disease questions but I want to give that grid so we will think in broader categories also.

MR. LAWRENCE: If I could add, I agree with what Dr. Vanderpool has stated. I give a talk about liability

issues in transplantation and one of the examples that I give is a comment an old law professor of mine used, that in the medical setting determined informed consent reminds him of the Holy Roman Empire which was neither holy nor Roman nor an empire. Informed consent is very often neither informed nor consent. What he stressed was that informed consent is not manifested by a signed document that says "I consent." It is a process of communication and understanding. If the consenting individual does not clearly understand what has been said to him, the fact that it was said to him is irrelevant. And that is going to be a challenge to everybody involved here, I assure you. My wife is a registered nurse and she scarcely understood what was being presented when I was in a hepatic coma and she was having to consent -- she was consenting to everything, you know, just "where do I sign?" So we will need to be very careful with the consent process here, and I can't add much more than that except that it is going to be quite important.

DR. AUCHINCLOSS: You bring up a point which is

going to be a peculiar problem in these trials, which we didn't kind of tackle head on, that you have this enormously complicated consent process that we envision, and relatives and offspring etc., and one of the treatments is for patients in hepatic coma. What are we going to do?

MR. LAWRENCE: Well, this is much easier legally than it is perhaps from the clinical standpoint. Legally it is well established what you do. The persons who are eligible to consent for the disabled person are well understood -- spouses, parents and so forth, and there is a hierarchy of those. Obviously, those continue to apply here. I am not sure that that is as troubling as it is to be sure that you have communicated with this person, or the patient himself if he is able to do that, exactly what is involved and it is going to be very challenging.

DR. AUCHINCLOSS: But you have indicated that your wife, a sophisticated, knowledgeable person -- you were in a hepatic coma -- really fundamentally didn't understand or didn't hear probably 90% of the issues involved in informed consent for liver transplantation, and now we are suggesting that she is instead going to hear not only about that but about something ten-fold more complicated that we have spent our whole life doing and not being sure we understand it ourselves.

I am suggesting the stark way of asking the

question is are such people, in fact, not candidates for xenotransplantation trials because you just simply cant get the kind of informed consent that you think is important?

MR. LAWRENCE: No, I would reject that. I understand your concern but I have to reject that because

the alternative is that you let them die because you don't want to get involved in a complex kind of a consent. I think all the law requires is that you do the best you can, but you must do the best you can. It must be a good faith effort and "good faith" is the legal operative word there. But go ahead and do it; I mean, act in good faith but do it.

DR. AUCHINCLOSS: I am not saying all of this because I believe it; I am saying this because I think it is the question the FDA wants to hear the answer to. But it does put you in the position of saying this informed consent for xenotransplantation is so important and here is what you are going to do, and you are going to have these educational meetings, and then in the end you really say but, of course, if the patient's life is at stake we can still dispense with all of that and just move ahead and do the best we can.

DR. VANDERPOOL: I think that is an excellent question because we know what proxy consent is, or at least we understand it in the clinical situations, but in terms of medical research on human subjects, do we have a handle on

what proxy consent would involve? And that is another issue to address.

MS. MEYERS: Well, the federal government just came out with regulations on emergency room types of consent where a patient is unconscious.

DR. SIEGEL: That is different. That is not pertinent. That is when the legally acceptable representative is not there and not available to consent. So, this is a different circumstance.

I would like to explore the issue of consent of the close contacts because you didn't say informing the close contacts; you said informed consent. Now, consent usually means in this setting that if you don't have consent you don't proceed. So, are you, in fact, saying, when you say you should have informed consent of the contacts, that family contacts of a prospective subject should have veto power over enrollment of that subject into a trial?

DR. VANDERPOOL: No, what we are talking about in informed consent for close contacts is informed consent about what they will be expected to do and what they may be required to do should X, Y and Z happen. It is not informed consent in a proxy type situation; it is what is my role? Surely, you know, all transplant trials will not have the close contacts playing active roles that they need to understand and consent to. See, I have a problem on the

surface, although I could be convinced otherwise, that an educational requirement for the close contacts will do because let's assume that some untoward event would happen to a close contact and the health care worker would say, well, we educated you; we told you about that. Well, you

have no indication that you talked with the person about it and that he or she actually gave consent. So, I am not saying we ought to move to that but I think we need to think seriously about why we should or should not have informed consent for close contacts.

DR. SIEGEL: And you mean consent in the sense that they would have the right to withhold it and then you wouldn't treat?

DR. VANDERPOOL: They would have a right to say I want to be involved in those ways or I won't take that role; I will not support him or her. But we can't just assume a Norman Rockwell via the family.

DR. SIEGEL: So, blood testing --

DR. VANDERPOOL: Right, figure out what you have to do to take the support roles that otherwise this person would want to play.

MR. BENEDI: Right now, in the regular transplant scenario, there are questions that are asked the family, and there are considerations by the surgeons as to what kind of support mechanism exists for a patient to continue on a

long-term basis or taking medications for life. Those decisions are being made already in the regular transplant community. I think this extends it a little bit more because it deals with that extended family person actually participating in the testing. So it is an extension of it

but it does happen, and there are programs that have decided in the past that a particular potential recipient might receive an organ because they don't have the support mechanism, and that has been done and those people have died.

DR. KASLOW: I think this is a little different though. If we are concentrating on the whole issue of infectiousness and transmissibility there is more than just the sort of passive involvement of the family and other close contacts. There is the potential risk not only to that contact with actual disease if we think it is a pathogen, and also to the spread beyond the family of this agent if it is, indeed, transmissible. So, I think it is qualitatively a little different from just more of the same numbers of people and support. And that is the issue I think we have to deal with.

MR. BENEDI: I guess my point is that there are things that are different in this situation, and to take the entire scenario of the individual, the family, those that come in close contact and to have them also be informed and

give consent to the process in these early stages, when a lot of things aren't known about it, is absolutely essential. I mean, to say that there are four family members in the house and three of them aren't going to participate is irresponsible to take that recipient and

introduce them when we don't know long-term what is going to happen to that person. Until we do know we are going to have to be a little more cautious in what that support mechanism and that family consent situation is.

DR. KASLOW: Take the example that Abbie Meyers gave, the powerful example, "the greatest guilt I feel is will I pass this infection onto my loved one." I think informed consent for the close contacts would alleviate some of that. That is, they would have been told whatever worries, infections, risks and would have said "I'm willing to take the chance" or they would not have been involved in that way.

Now, I am just thinking now from the top of my head about some of the reasons -- not entirely from the top of my head but on that point as to why informed consent might be necessary for close contacts. There might well be arguments that Tony has given from historic precedent to show that it is not needed. My point would be that we need to think this one through beyond where we have gone at the present time. The IOM committee had one line on that. We

need to think about this issue some, but I have never seen it really aired and discussed.

DR. MICHAELS: I certainly agree fully with everything that the others have been saying so far, and just wanted to go a little bit further with it. Certainly, with

allotransplantation we do look at the family structure and support systems, and even the individual's ability to comply and be able to take their immunosuppressive medication and be able to come to follow-up visits. Now, it is easier to do when you have the patient coming for evaluation as opposed to the critical point when they are in hepatic coma. But taking that type of patient who is able to come for evaluation -- I guess I hadn't thought of it in terms of the contacts actually having informed consent but that is certainly very intriguing and worth considering. I certainly feel that education of the family members is critical, and certainly just taking some examples of what has happened in the past, the patient who received the baboon bone marrow, we did go through many educational cycles with the person to let them know the unknowns, the concerns for transmission if the unknown became a real true event of transmission to himself and, therefore, could it be transmitted to a partner, then had the partner come in as well and have more education with the partner, and discussed, you know, things which I think Claudia has

already mentioned about using barriers with intimate relationships, avoiding transmission of secretions by certainly not ever donating blood. That patient, obviously, had HIV and so those things were sort of a moot point to start with. But I think that that is reasonable even for

patients who are not infected with a known virus to start with. At this point of experimental research it is reasonable for the partners and for the individual to use barriers and to say that they will not choose to get pregnant during that time or to conceive. I think this is a very experimental stage and I think that those are reasonable things to request and to discuss.

DR. NOGUCHI: If I could just also say from the FDA's point of view, having patients here, right here talking about consent and the informed process is precisely what we encourage very much. There is this other part that you need to consider from our point of view, the one thing about animal organs that is missing for human organs is that they are not going to be scarce. They are not going to be a national treasure. The whole process of being able to actually take this on will need family support and community support, but the preciousness of the actual commodity, in a way, takes away the ability of a physician surgeon to really say, well, I know this family is not going to do it so I am not going to give that person a liver. You will not have

that option. So, I think to the extent that this sounds important, it sounds even more important than the obvious infectious disease question.

DR. AUCHINCLOSS: I must say I don't quite buy into that. I think doctors in lots of ways continue to

exercise those judgments about major procedures whether the resources are scarce or not, and I suspect they will continue to do that in the future.

Let's bring this to a head here. Is there, in fact, a strong feeling in this group that informed consent of close contacts of the recipient is a reasonable requirement in xenotransplantation trials?

[Several members respond "yes."]

DR. MICHAELS: Yes, but I would be very surprised if you were to develop a fully-blown informed consent document. I certainly believe in the counseling and cycles of education where it is sort of documented what you are trying to communicate to people but I am not sure -- that is a new form of document.

DR. AUCHINCLOSS: I want to know whether I am hearing informed consent, signing the document. Is that what this group is really suggesting they think is an important part in xenotransplantation?

DR. VANDERPOOL: I have been talking a lot about it but I am not ready to move to that yet. I am asking for

deliberation of the issues to get a good readout of the situation first.

MR. LAWRENCE: I have one more comment about this. Wearing my hat now as an official of UNOS, I am continually surprised. It is a daily event in this country that people

are offered a human organ, after waiting on the transplant organ list sometimes for years, and they refuse it. They turn it down; they don't want it. Transplantation is not for everyone; xenotransplantation is not for everyone either, and there will be times when the consent process becomes so convoluted that you say this is not an appropriate therapy for you, and that is okay.

MR. BENEDI: Could I just say something? This informed consent by family members as well is going to be important in the long-run when this new science takes hold. In this information age there is a lot of information that travels throughout all kinds of different ways about each and every one of us -- our medical histories and such. I am concerned about the insurance companies even looking at someone who had lived with someone who had a xenotransplant in the future, and whether they are at risk or too high of a risk to insure. The portability insurance for us, recipients, has been an incredible challenge that we now have some laws to back us up. But to go from job to job, we couldn't do that without insurance companies closing the

door and saying that they are not going to insure us. This is going to extend that window and that concern to family members and to others that we come in contact with. It is just something to think about.

DR. WALTERS: When Dr. Siegel uses the word

"veto," that really challenges everyone's notion of autonomy and something very important that we stand for. On the other hand, this is clinical research. Clinical equipoise still exists, and these are both patients and subjects simultaneously, and I do think that we have to remember that this is qualitatively different from the context of an emergency situation with liver transplants or kidney transplants. This is still research.

MS. MEYERS: I would like to say that for the spouse or for the significant other of the patient there is a risk involved, and they really have the right to know what that risk is, and that is why they should get the informed consent document.

DR. AUCHINCLOSS: So, what I think I have heard from the committee is that there is a strong sense of lots of information to the close contact, and it is almost up to a document signed but then there is sort of a gap on the committee that they feel pretty uncomfortable with that.

DR. SIEGEL: Well, I heard Dr. Vanderpool suggest that there probably needs to be some further discussion and

exploration of the historical precedents, legal and other aspects of this.

DR. AUCHINCLOSS: That is exactly what I heard also. Again, FDA staff, tell me if I am wrong. I think we have covered most of what is in 3, but there is an issue

that I haven't seen us address yet which is whether you could take into account your judgment about the suitability of the recipient from an emotional, psychiatric, clinical view. To me, that is sort of a no-brainer. We do that all the time, as I think Antonio has already indicated. It is part of the evaluation process for liver transplantation and it would be even more so for a trial of this sort. I open it for further comment.

DR. SIEGEL: Let me clarify a specific question. In the report from one of the subgroups there was a comment about not discriminating socially, such as based on past history of substance abuse, but there are, especially as we are talking about liver support, potential patient populations who are actively involved in intravenous or alcoholic substance abuse. You could have a chronic cirrhotic who is still drinking and goes into acute alcoholic hepatitis deterioration. In particular, since those people are not generally considered suitable recipients of human organs, they are potentially a very desirable population. Nevertheless, this committee and

others experts have told us these transplants should go into people that you can count on for the rest of their lives, who will tell all of their contacts, will come in for their annual checkups and screening, give all the specimens, and should we be making the determinations whether or not there

are populations that we really can't count on to do the things necessary to protect the public health?

MR. LAWRENCE: I think we have to make a distinction between the clinical trial phase where it is very important to be able to follow these people and, should this ever evolve to where it becomes common therapy are two different issues, to me.

DR. AUCHINCLOSS: Jay, tell us about some of your other concerns within the context of question 3 that we might not have touched on.

DR. SIEGEL: Yes. One thing that we started touching on but didn't is an issue, and a very real issue. If we say that before we proceed we should have in place certain types of testing, being ready to test patients and so forth, and we have done that and you have seen the letter that was sent out, then what we will hear, and what we have heard, is, yes, but this particular patient is going to die if we don't treat him now.

Now, in many protocols we certainly have the authority and the general practice to really apply our

ability to grant single patient protocols, sometimes called compassionate use although that is not an official term. Congress has supported that in recent legislation. We will do that assuming that basically the patient is dying and signs the consent and understands the risk, obviously, you

know, has a different benefit-risk strata than just conducting general research. However, here we are balancing the potential benefit not just as a risk to the patient but a risk to the general population, which has led us to this quandary. Is the risk to the general population, which is not acceptable for certain patients, more acceptable if an individual patient is in more dire straights?

DR. AUCHINCLOSS: As we have heard, something like ten people are dying a day. I see no reason why there would ever be a reason for compassionate use for xenotrans-plantation.

DR. SIEGEL: You wee no reason why there ever would be?

DR. AUCHINCLOSS: Would ever be, no. This goes ahead because you decided it goes ahead or it doesn't go ahead but it doesn't not go ahead except for the one patient that you hear about who is in somebody's ICU. There are patients in everybody's ICU.

DR. KASLOW: We have made a public health announcement here I think, and I think we have said that the

process that we have recommended proceeds on that basis, and I don't think we are going to make an exception to that.

DR. AUCHINCLOSS: Anybody else?

DR. VANDERPOOL: Well, that is a tough one when you are saying, you know, the patient is going to die unless

we do a xenotransplant. They will probably truly die with one.

[Laughter]

But that is all the more reason why I think one needs a basic set of general guidelines, not particulars, about health benefit thresholds before xenotransplants should proceed. I mean, you can read about xenotransplants in Hungary and they do it just to see if it will work more than a few days. So, if you have some kind of a judgment about what a success entails, then I think you would have a better reason to say no to the truly desperate patient who perhaps would want to try the longest of long shots.

You know, it is interesting -- and this is a tough question because our federal guidelines and the Belmont report could have gone in a different way than they did. The federal guidelines and the Belmont report are fairly paternalistic. We don't allow you to volunteer for research unless certain harms and benefits are in balance. We could have gone the other way and said anybody who really wants to go for it can go for it, with a wild and crazy chance; that

is your prerogative. But I think we realize that this could sully all kinds of reputations and would lead to probably more consternation than success. But I think we need to stay with the spirit of the guidelines and the Belmont report that certain harms and benefits need to be in place

before the long shots can enter in.

DR. WALTERS: I just think that the clinical trial ought to proceed. There is a lot that is unknown. There is a lot of information that needs to be gained. The time and effort of the investigators is a very important and scarce resource, and in some cases the non-human organs may be a fairly scarce resource at this point.

MS. MEYERS: I think that for a compassionate program you are really talking about a drug or a biologic where you have something in a bottle that you can ship off to a doctor out in Iowa or the patient who doesn't live near a big institution, but here you have to have a surgeon; you have to have the transplant team. You know, you just can't have it done wherever. The local GP isn't going to give you a transplant.

DR. SIEGEL: But it is not a question of that. The question is largely whether while companies are, as we have asked and as I think you have supported, getting together and developing these porcine endogenous retrovirus assays to --

DR. AUCHINCLOSS: The answer is no.

DR. SIEGEL: -- so they are presumably planning to start in a month, three months, whatever, when they have the assays ready so that they can gather the data as to whether the patients are getting viremic. Should they be treating

patients who are in dire straights in the interim but not other patients --

MS. MEYERS: No.

DR. AUCHINCLOSS: Anybody want to say yes?

[No response]

Are there points in (iii)?

DR. SIEGEL: Well, did we skip over (d)(ii)?

DR. AUCHINCLOSS: I said we do this all the time in transplantation and, of course, we would do it here. We would assess the likelihood of compliance. We do it for will they take their medicines; will they cooperate with the program. So, of course, we would do it here. There, in fact, would be a higher bar that you would need to go over to be part of a xenotransplant trial. When a patient is in encephalopathy, well, that is very hard and we don't do it well. We do have lots of surrogate markers that we look at, however, and they are not perfect; they are terrible but we do the best we can. So, there is a process for dealing with this and it seems to me you would just say, yes, the process exists and we will do the best we can and it is appropriate.

DR. WALTERS: There is a history on this topic, however, that goes all the way back to 1962 and the Seattle committee selecting who would get access to dialysis when access was very scarce. One critic of the Seattle committee

and the way it smuggled criteria of social worth into the selection of patients commented that the Pacific Northwest would have been a very tough place for Henry David Thoreau to get access to renal dialysis. So, I guess just think of Henry David Thoreau and whether he would be considered a suitable candidate.

[Laughter]

DR. AUCHINCLOSS: Jay, I have the feeling that my committee is shrinking around me; that it is time to wrap up. One of the advantages of lasting longer than anybody else is that you asked me to summarize and, believe me, this summary will be very brief. But there is getting to be a smaller and smaller number to disagree with me --

[Laughter]

DR. SIEGEL: Before you summarize, if we could possibly take five or ten minutes perhaps to address the question that is before us now as it has been asked, and we need to answer, and that is (e), which takes a whole page but boils down to whether close contacts of xenograft recipients, and this is closely related to something we were

just discussing regarding their informed consent, should be counseled not to donate body fluids such as blood, and whether, in fact, they should be deferred from donation --

DR. AUCHINCLOSS: Again, I thought that was obvious. They should be counseled and deferred.

DR. SIEGEL: Counseled and deferred? Okay.

DR. AUCHINCLOSS: I assume that that was really part of this information --

DR. SIEGEL: But it is another thing to tell all the recipients that for the foreseeable future you need to tell all your intimate contacts that they can no longer donate blood, for example.

DR. AUCHINCLOSS: To put it in the overall context here, the likelihood that we are going to create a public health disaster with xenotransplantation I would take to be one in a million, or something like that. But this is the event that contact donating -- it is the transmission of that one in a million event that you are trying to prevent. I mean, this is the one thing that for sure you ought to do, keep the contacts from spreading the new pathogen that we create in that incredible unlikely event that we do.

DR. KASLOW: The more difficult question is what else should we tell them perhaps they shouldn't be doing besides that.

[Laughter]

DR. VANDERPOOL: You have to be monogamous from now on. Until we give you the green light you have to be monogamous. I think that is the epidemic that we know about has really spread, blood transfusion aside.

DR. AUCHINCLOSS: So do you have your answer?

DR. SIEGEL: Actually, in the Center for Biologics we regulate blood but, to date, we do not regulate sexual intercourse!

[Laughter]

Summary Points by the Subcommittee Chair

DR. AUCHINCLOSS: Now, under the prerogative of it is not over until you hear the Chairman singing, or something, here is the Chairman's summary.

What I think I know is that there are replication competent viruses in pig cells that are possibly, or even very likely, going to infect human cells in the course of xenotransplantation; that we have absolutely no idea what the consequences of that event will be in terms of subsequent infectivity or pathogenicity; that somehow around this table there is a sense that we ought to proceed with clinical trials of xenotransplantation; that we would like to do so with the greatest degree of comfort that we can muster. We are not quite sure how really comfortable we can every be; that the suggestion from the experts is that there are things you can do to find safer forms of clinical trials

than other less safe forms, and you have heard the specific suggestions; and that there are assays and information that still may, in fact, need to be accumulated to make us feel as safe as we are likely to ever feel before these trials proceed ahead.

Is something like that the consensus of this group?

MS. MEYERS: Very good.

DR. KASLOW: For so late in the day you are wonderfully articulate.

DR. AUCHINCLOSS: Is there any other question that the FDA wants to ask? I think I am supposed to ask for any other public comment. We are done on that?

DR. SIEGEL: I want to thank you all. I think we have a lot to digest but a lot of very useful information and advice, and we will look forward to further discussions. As Dr. Auchincloss said, we intend this, whether it be a subcommittee, committee or whatever, to be ongoing task of public consultation and we really do appreciate your help.

DR. AUCHINCLOSS: Those who have stayed to the end, thank you very much.

[Whereupon, at 5:56 p.m. the Committee adjourned]

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