DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

BIOLOGICAL RESPONSE MODIFIERS ADVISORY COMMITTEE

XENOTRANSPLANT SUBCOMMITTEE

OPEN

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C O N T E N T S

P R O C E E D I N G S

Welcome and Introduction

DR. AUCHINCLOSS: As I open this morning's meeting, let me first state that the meeting statement that was read into the record yesterday is applicable again today. What I would like to do this morning to introduce the session is to ask each of the members of the committee to introduce themselves as we go around the table, just briefly who are you and why are you here.

DR. PAUL: Prem Paul. I am Professor of Virology and Associate Dean for Research in the College of Veterinary Medicine, Iowa State University. My expertise is virology, swine viruses.

DR. COFFIN: John Coffin, Professor of Microbiology at Tufts University School of Medicine and Director of the HIV Drug Research Program for the National Cancer Institute. My expertise is in retroviruses.

DR. CONTE: John Conte. I am the Director of Heart-Lung Transplantation at Johns Hopkins Hospital. My expertise, I would suppose, is heart-lung transplantation.

MR. LAWRENCE: My name is Bill Lawrence. I am a liver recipient. I am Director of Patient Affairs at the United Network for Organ Sharing. I think I am here as penance since I am a lawyer and I don't really speak much of the language.

MR. BENEDI: I am Antonio Benedi. I am a liver recipient and Past President of Transplant Recipients International Organization.

DR. MICKELSON: I am Claudia Mickelson, the Director of Biosafety at MIT. I think I am here more as a public representative and a little bit liaison some with the NIH and Recombinant DNA Advisory Committee as well.

DR. TOENJES: My name is Ralf Toenjes, Paul Erlich Institut, Department of Medical Biotechnology in Germany. We have long years of experience with work on endogenous retroviruses.

DR. ALLAN: I am Jon Allan from the Southwest Foundation for Biomedical Research. I am a virologist. I study simian retroviruses. I guess that is why I am here.

DR. HIRSCH: I am Marty Hirsch from Mass General Hospital and Harvard Medical School. I am a virologist and infectious disease person.

DR. MICHAELS: Marian Michaels, Associate Professor of Pediatrics and Surgery at Children's Hospital, Pittsburgh, University of Pittsburgh. Transplant infections is my area.

DR. ONIONS: I am David Onions. I am Professor of Veterinary Pathology at the University of Glasgow. My primary interests are in virology.

DR. VANDERPOOL: I am Harold Vanderpool. I am a

member of the Institute for the Medical Humanities at the University of Texas Medical Branch and have a particular interest in the ethics of research with human subjects.

DR. SALOMON: Dan Salomon. I am a member of the Department of Molecular and Experimental Medicine at the Scripps Research Institute and Director of Transplantation Research. We have programs in pig-islet and tissue xenotransplantation and recently have gone forward with a porcine endogenous-retrovirus animal-model building strategy that is just in its beginning.

DR. AUCHINCLOSS: I am Hugh Auchincloss. I am a transplant surgeon at Harvard. Dan Salomon and I are also members of the Biologic Response Modifiers Advisory Committee which is the parent committee to this subcommittee.

Let me interrupt for just a second and go back to Robert Michler. We are introducing ourselves.

DR. MICHLER: I am Robert Michler. I am Professor and Chief of Cardiothoracic Surgery at the Ohio State University Medical Center.

MS. DAPOLITO: Gail Dapolito, Center for Biologics. I am the Executive Secretary for the subcommittee.

DR. WALTERS: Leroy Walters from the Kennedy Institute of Ethics at Georgetown University. I have been

associated with the recombinant DNA Advisory Committee at NIH beginning when it was dealing with biohazards and ending when it was dealing with gene therapy.

DR. LERCHE: I am Nick Lerche from the University of California at Davis. I am a virologist with expertise in non-human primate retroviruses.

MS. MEYERS: Abbey Meyers, President of the National Organization for Rare Disorders and former member of the Recombinant DNA Advisory Committee and a former member of the Biological Response Modifiers Committee.

DR. SACHS: David Sachs. I am a Professor of Surgery and Immunology at Harvard Medical School and the Director of the Transplantation Biology Research Center at the Mass General Hospital and the Department of Surgery.

DR. CHAPMAN: Louisa Chapman. I am a medical epidemiologist at CDC and the point person at CDC on xenotransplant issues.

DR. GROESCH: Mary Groesch, National Institutes of Health. I am a science policy analyst and one of our point people in xenotransplantation.

DR. NOGUCHI: I am Phil Noguchi. I am Director of the Division of Cell and Gene Therapy. My division is responsible for xenotransplantation.

DR. BLOOM: Eda Bloom, FDA, Division of Cellular and Gene Therapies. I head the Center's Xeno Action Plan

and represent FDA to the DHHS Working Group.

DR. WILSON: Carolyn Wilson. I am a member of the Division of Cellular and Gene Therapies and also have research expertise in retrovirology.

DR. MARZELLA: Louis Marzella. I am a medical reviewer in the Office of Therapeutics at CBER.

DR. WEISS: I am Karen Weiss. I am the Director of the Division of Clinical Trials in the Center for Biologics.

DR. AUCHINCLOSS: A word about the day's schedule. The presentations that are listed will take place as they are listed, with the time limits that are listed, and we will have the break which undoubtedly will be longer than 10 minutes, and then come back for the FDA perspective.

What I would suggest to you is that it is extremely unlikely that we will break for lunch prior to the completion of the group discussion. We will keep the discussion going as long as it takes to have the discussion, and then you get to eat, but that will at that point end the meeting unless I am caught by surprise, so that is what I expect will happen, that we will come back, hear the FDA perspective, and then talk until we are done.

I should mention again to all speakers, both at the table and at the podium, to please speak to the microphone, really into the microphone, so that everyone can

hear, and there is a clip-on microphone available at the podium for the speakers if they find that easier.

Karen or Jay--Jay just walked in--or Phil, would the FDA like to make a brief statement about the day's topic?

DR. WEISS: We just wanted to thank the members and the guests and to welcome everybody to the second day of the meeting. I think this should prove to be a very interesting and important discussion as we embark on I think a new era in terms of transplantation, so I look forward to all these discussions.

Open Committee Discussion Topic III

Xenotransplantation Preclinical/Clinical Issues

DR. AUCHINCLOSS: I think we will move directly to David Cooper from the Massachusetts General Hospital, who will start the day's presentations with Current Results of Experimental Xenotransplantation in the Pig-to-Primate Model.

Guest Presentations

Current Results of Experimental Xenotransplantation in the Pig-to-Primate Model

DR. COOPER: I hope you can hear me because yesterday I had great difficulty hearing most of the committee speak. Please shout out if you can't hear me.

[Slide.]

I am going to try to give you in 15 minutes a very brief overview of what has been going on in the pig-to-primate experimental model which we all think is a sort of preliminary of the preclinical model before we go into humans, so if we can get it to work in this model, we can probably get it to work in humans.

Now, as you can see, I did a review in 1991 with a colleague of mine when we were writing a chapter for a book and we found four reports in the literature on pig-to-primate transplants and xenotransplants.

When we reviewed it again in 1998, there were over 120 reports, and I think by now there is probably over 150 reports, so there has been tremendous interest in this model in this last few years, and the reason for that is that as I mentioned briefly yesterday, baboons and other old world monkeys have antibodies which are specific for a galactose sugar, which pigs have on the surface of their entire endothelium throughout their whole body, the vascular endothelium throughout the body, and it is this that is the first target for rejection by a primate including a human, and this can cause very rapid rejection.

[Slide.]

Now, there are a number of rejection barriers that we have got to overcome, and I am sorry that I know many of you will know all of this and it won't be news to you, but

there are some in the audience that I think it is important to give this background to.

First of all, these antibodies can attach to these sugars on the pig organ surface and cause hyperacute rejection, which is rejection that occurs very rapidly, usually within a few minutes, and this is caused by the activation of complement which actually does the injury, and this is an important mechanism because some of the approaches that we will see, that have been designed to try to prevent this, relate to both the antibody and to the complement aspect of that chain of reaction.

But if you get over that hyperacute rejection by various maneuvers, you come to what has been termed "delayed" xenograft rejection or acute vascular rejection, which also appears to be mediated by the antibodies, but is not mediated through the complement cascade. There is another mechanism, but antibodies are still involved.

Then, we are only just beginning to see that if you get through that, as well, you come to what we all know as acute cellular rejection, which is the same sort of rejection you get when you put a human organ into another human.

Then, finally, in humans, as time goes on, we get what we call chronic rejection, which is very poorly understood, the mechanism, and this can cause damage to the

organ, the transplant, over a number of years, for example, by about five years, a significant percentage of the organs have been lost from this rejection, and by 10 years, at least 50 percent have been lost.

We expect to see this in the pig-to-primate, so pig-to-human model, as well, probably at an earlier stage than we see it in the human-to-human model, so there are a number of barriers that we have got to overcome.

[Slide.]

Now, Randall Morris, who many of you here know, is an immunologist and surgeon at Stanford, has this saying, which I have modified slightly. He says there are three golden rules for achieving successful xenotransplantation.

[Slide.]

And unfortunately, we don't know any of them.

[Laughter.]

Now, that is not quite true. It is not quite true, and I will show you that we are beginning to get a few approaches to some of them, but he was right a few years ago.

[Slide.]

Now, if you just put a pig organ into a primate, you put a pig kidney or a pig heart into, say, a baboon or a monkey, and you give no therapy at all, or you give the standard immunosuppressive therapy that we use in human

allografting, you will find that the organ will survive from anything from five minutes up to perhaps 24 hours. The majority will be less than an hour or two. So, it is a very rapid rejection.

There are a few reports of extended survival for reasons we don't understand where, for example, a pig kidney has survived in a monkey for up to a month, but they are pretty rare and the majority will undergo this, what we call hyperacute rejection within the first 24 hours.

[Slide.]

Now, what approaches are we taking to try to overcome certainly this initial problem of hyperacute rejection, and as you will see, this has relevance to some of the later problems.

Well, we can either delete or deplete or inhibit these antibodies, so if we could get rid of the antibodies, these antigalactose antibodies, we wouldn't start off this reaction which causes the injury, or we can deplete or inhibit the complement because the antibody set the complement chain going, and if we could block the complement, inhibit it or deplete it, so there is no complement, then, obviously the injury wouldn't take place even though the antibody would bind to the surface, and that would get over hyperacute rejection, or we can try to genetically engineer the animal, the donor, and this is the

first time, remember, in transplantation that we have really had a chance to modify the donor rather than just modifying the recipient.

We can either do that by changing the antigen expression on the surface, we can get rid of its galactose and put another sugar there perhaps, say, a blood group sugar that we all have ourselves, or we can modify the donor organ to make it resistant to the human complement or the primate component, which it doesn't have much resistance to, or finally, we can try, because we know there are so many barriers, we can try to go the whole hog--which is not a pun--in one move and we can try to deplete the immune system of the recipient to such an extent that when we put the pig organ in, when the immune system recovers, it will be tolerant to this pig organ, it will recognize this pig organ is belonging to itself, and it will try to reject it, and will be tolerant.

[Slide.]

So, there are a number of approaches that people are attempting, and the first one is to deplete the antibody.

[Slide.]

Now, you heard yesterday a couple of groups, the Munich group, and one of the other groups about depleting antibody with various columns. This is a standard

plasmapheresis or plasma exchange machine, and you can see down here at our own center we have included in it a column here, an immunoabsorption column which contains this galactose sugar, synthetic sugar.

[Slide.]

When you pump the plasma through this column for two or three hours, you deplete all of the antigalactose antibody in the baboon, so the baboon no longer has any antibody against the pig, and if you do this for two or three days running, you actually get really a complete depletion of the antibody and when you then put an organ into that baboon that is depleted of antibody, it will survive at least few days or a week or so.

[Slide.]

So, this is the first approach that has been used, and using these various techniques of plasma exchange, immunoabsorption columns, and so on, you can see that we have got organ survival, will get out to about one to three weeks if you add immunosuppressive drug therapy, the standard immunosuppressive drug therapy, which suppresses the return of antibody to some extent, but not very successfully. So, we can just by removing antibody, we can get out to one to three weeks.

[Slide.]

What about if we deplete or inhibit the

complement? There are a number of drugs that will do this. Cobra venom factor depletes all your complement and soluble complement receptor 1 inhibits complement and there are a couple of other drugs that we have tried and other people have tried that will also deplete or inhibit complement, and this is pretty successful, too.

[Slide.]

This will get you, if you add immunosuppressive therapy, this will get you out to at least a week and maybe in some cases up to six weeks just by depleting the complement or inhibiting the complement and adding some basic immunosuppressive therapy.

But during this period of time, rejection is slowly occurring, this delayed vascular rejection is occurring. If you look at biopsies of these organs, you will find that they are actually beginning to undergo rejection from quite an early stage.

We have gone over the complement activated, the hyperacute rejection, but we still have antibody there which is causing problems. Now, if you combine the complement inhibition with the antibody depletion, you should technically get out a little bit further, but nobody has really done a very good trial of that to date.

[Slide.]

Now, the most successful approach--and we will

hear a lot more about this, this morning, from the two groups who are going to talk following me--is the genetically engineered donor pig, and I am only going to touch on it fairly briefly.

[Slide.]

There are two approaches I mentioned. Let's take the second one first. If we could get rid of the sugar from the surface, either by knocking the gene out that makes the sugar, or that makes the enzyme that makes the sugar, or competing with that sugar by putting in a gene for another enzyme that makes another sugar, so that we are competing, then, this might be a very good approach, but in the pig, this has not proved possible technically so far.

You can do this in mice, and it certainly does prolong survival of the mouse organ, but you can't do it in pigs. It is just technically not possible. Now, maybe cloning technology will allow us to do this, and various groups are looking into this, but at the moment this is ruled out.

So, we have to look at this complement regulatory protein expression. We have on our own organs human complement regulatory proteins that protect us from our own complement, not always, but they do most of the time.

Pigs have their own complement regulatory protein, but pigs are not protected from human complement regulatory

protein very well, and so several groups have manipulated genetically engineered pigs that express human complement regulatory protein, as well as the pig regulatory protein, and these have been really pretty successful.

[Slide.]

If we look at the results that have been published to date, we have got survival. A few of them still undergo hyperacute rejection possibly because of the expression of the human complement regulatory protein is rather low, but there have been survivals up to 99 days, three-plus months, and this is obviously very encouraging.

[Slide.]

But if we look at it in more detail--and I know Dr. Cozzi will talk about this in more detail, and I just want to briefly mention it to you--this is the most successful result to date from the Imutran group with orthotopic heart transplants.

These hearts are actually supporting the life of this baboon, so here is a pig heart supporting the life of this baboon, which is very important. You see on one occasion they got up to 39 days, and the baboon died of other problems not from rejection, which is extremely encouraging and at least does show that a pig heart will support the life of a baboon, probably also of a human if it's a big enough heart, for a prolonged period of time.

But it worries me a little bit that the median survival in this group of six was only 12 days and that as you can see, one still underwent hyperacute rejection and three underwent acute vascular antibody mediated rejection. So, despite a very successful regimen, the majority still were susceptible to rejection, so we haven't got over the rejection problem despite fairly heavy immunosuppressive therapy.

[Slide.]

If you look at their results with kidney transplants, these are monkeys now living now on a pig kidney. Neither of their own kidneys is left in situ, and you can see that they got from 9 to 71 days, and every encouraging they go to median survival of over a month, which is extremely encouraging, but again, all the animals either died or had to be euthanized, so the regimen was not 100 percent successful, and what is very worrying is that 3 of the 7 animals developed lymphoproliferative disease, which we know is a result or can be the result of fairly heavy immunosuppression, and can lead to lymphoma type conditions which could kill you if you persisted with that sort of immunosuppressive therapy.

So, despite the very considerable response, there are still problems involved in still overcoming rejection and not actually wiping out the animal with the over-

immunosuppression. So, it makes one a little worried that that approach has still got some problems.

[Slide.]

Now, I am working with David Sachs, who has been looking at tolerance induction for a number of years, and in the allograft model of monkey to monkey, he and Ben Cosimi have been very successful in that they can by manipulating the recipient at the time of the transplant, I won't go into the details of it, it requires a low dose of irradiation and some other therapy, and then putting a donor kidney in, a fully mismatched donor kidney, they can stop all immunosuppression within a month, and they have monkeys here surviving now more than five years who have never had any immunosuppression after the first month.

So, this monkey is now tolerant to this donor kidney, and we have been trying to do the same thing with regard to the pig to the baboon transplants.

[Slide.]

Now, here we have the added problem of those original antibody-mediated rejection phenomena, the hyperacute rejection and the acute vascular rejection that we mentioned, which makes it a much more difficult problem.

One of the keys to this is to get some pig bone marrow cells or pig hematopoietic cells engrafted in the baboon, which is also difficult. This is akin to the

allograft, as well, to get the donor bone marrow engrafted at the time or before you put in the kidney.

If you can get that engrafted, you almost certainly can get a kidney or heart to survive from that specific donor long term without immunosuppression.

[Slide.]

We have developed this technique of leukophoresing pigs, which will stand there quite comfortably while this is going, and we take out a large number of their mobilized white cells which are from the bone marrow originally, and we put those into the baboon.

[Slide.]

Now, if you put them into a baboon that is getting standard immunosuppression, you find that you get a huge increase in this antigalactose antibody. This is 100-fold increase. This is a log scale. It is a huge increase after you put the cells in, because the baboon is sensitized to the pig tissues or the pig cells, and you will also develop new antibodies that you didn't already have against the pig, but if you actually give one of these new costimulatory blockade molecules, such as anti-CD40 ligand monoclonal antibody, you see that when you put the cells in, although the antibody returns to the original level, you get no sensitization, and I think this is a very important finding, and I think if the groups working with the transgenic pigs

incorporate it into their regimen, they may find that they don't get this major late rejection phenomenon.

[Slide.]

But despite that improvement and despite the fact that we have now got engraftment in pig cells in baboons, we still are only getting survival of one or two weeks.

Now, one of the major reasons for this is that we are not seeing rejection, but we are seeing a coagulopathy that develops, and we are not the only people seeing it, and it always surprises me that the Imutran group in particular have not seen it.

It may be the construct of their transgenic pig protects the pig organ from this coagulopathy, and this coagulopathy is something we are looking at, at the moment, and is a significant problem.

[Slide.]

Now, Hugh also asked me just very briefly to look at a couple of points. One is if you have a patient who is highly sensitized to other humans, he has had blood transfusions or is a woman who has had pregnancy or has had a previous kidney transplant, and is highly sensitized to the point that he is probably not going to get a human organ, that you will never find a human against which he doesn't have antibodies basically, rather similar to finding that you have got antibodies against the pig, but these are

developed antibodies against other humans, so he won't get a kidney transplant.

Can that person get a pig transplant or is he going to have antibodies against the pig that will prevent that? Now, there are two groups, Cambridge and St. Louis in th USA, who have come up and say that some of these developed antibodies against other humans can also bind to pig cells.

Now, that is an important finding, and if that is the case, it may prevent some of these patients from having a pig organ transplant. It is something we need to look into very carefully.

But our own group has certainly found that if you remove the antipig antibodies from those patients, their serum is no longer injurious or cytotoxic to pig cells. Now, that doesn't mean to say that there is an incompatibility between these results because it means that from the point of view of hyperacute rejection with complement-mediated rejection, those patients appear to be safe.

They can give them a pig organ, but it may be that they develop problems later down the line because they do have some of their antibodies can still attach to pig organs, so this is a problem that still has to be looked into before we go ahead.

[Slide.]

Finally, I asked myself this question - can a patient who undergoes an allograft after rejecting a pig graft, if you put in a pig organ as a bridge, can you then say, okay, the bridge failed, but we have now got a human organ, we will put the human organ in to keep the patient alive?

Well, there is virtually no literature on this except from our own group when I was in Oklahoma, and we found that in three baboons that had rejected pig organs, we could not detect any sensitization to other baboon organs, so it looked as if the pig organ did not sensitize them to other organs of the same species, and when we transplanted baboon organs into those baboons that had received the pig organ previously, we did not see hyperacute rejection. We may have seen rejection down the line, but that may be from other factors.

So, again, there is very little work on this, so if we are going to consider xenotransplants as a bridge to transplantation, we have to get a little bit more data on whether we are sensitizing the recipient by the pig organ which will preclude him having a human organ.

[Slide.]

So, finally, this was a saying that somebody mentioned at John Coffin's Cold Spring Harbor meeting

recently, one day making a pig of yourself could have a whole new meaning, and I hope it won't be too long.

Thank you very much.

[Applause.]

DR. AUCHINCLOSS: David, thank you very much.

Would you mind staying at the podium just for a moment and we will see if there are any particular questions for David's presentation.

MS. MEYERS: There is. I am trying to understand whether your presentation said that transplantation of pig organs into baboons doesn't work for more than 30 or 60 days if you are lucky.

So, do you think that we are ready for human transplants?

DR. COOPER: Personally, I don't, not quite. I think we are getting towards there, but I think to offer somebody, say, a consistent--you can say I can consistently offer you at least a month survival, I don't think that is good enough to go into a clinical trial.

DR. AUCHINCLOSS: That is the topic for the day.

DR. COOPER: But the argument would be that it is very difficult to manage baboons in the environment they are in. They are prone to infections, and so on. You do not have any of ability to look after that you have to look after a patient in an intensive care unit or in the hospital

surroundings. So, it may be much easier to manage patients.

You can diagnose their rejection much more quickly, and so on, than it is with these baboons. These baboons generally have been prevented from having that sort of care for a number of reasons, one of which is the authorities in Britain have precluded the use of biopsies and other methods that might have helped you to diagnose rejection was happening, so there are distinct differences between this model and the human model, but I still have worried because of the fact, as I pointed out, the fact that the rejection still does occur in some of them, the fact that they are getting lymphoproliferative disease, that it seems that we have not quite got an immunosuppressive program that will take them or humans relatively long term.

MS. MEYERS: So, the pressure that we are feeling to move ahead with this, to finally see it in clinical trials is all premature, isn't it?

DR. COOPER: No, I certainly wouldn't say that I think we should hear what the other speakers have to say this morning. I am sure that is one of the reasons this committee is here, to assess whether it's premature or not. There are good arguments for going ahead, there are good arguments for perhaps waiting a bit longer.

DR. GORDON: Dr. Cooper, the examples you gave all appear to be whole organ transplants. Would you have

expected more positive results if those had been tissue transplants, for example, islets where you would not have had hyperacute rejection, it is not vascularized, so you wouldn't have had acute vascular rejection, and the mass of tissue is much smaller?

DR. COOPER: This is my main field of interest, and I am not an expert on the cell transplants. My understanding is, though, that although you don't get the hyperacute rejection because you don't have this vascular sugar--

DR. AUCHINCLOSS: I think it is a very important question because it will now enable me to try and clarify the topic for the day. We are going to be talking about solid organ transplantation today.

The FDA has approved trials at this time that are going on right now of cell transplants from pigs to humans, neurocells and others. So, today's topic is solid organ transplantation. The issue is certainly different for cellular transplants.

DR. VANDERPOOL: Dr. Cooper, very enlightening presentation. With respect to immunosuppression of the non-human primates, would you first compare the ability to do that with non-humans and human beings? I mean are the immune suppression regimens for primates up to the level of that of humans, and if they are not, do you think if you had

a better immunosuppression regimen for the non-human primates, you might get a better survival rate for these organs?

DR. COOPER: Generally, the immunosuppression, if it works in the baboon, will work in the human, but that is not always the case. There are distinct differences between the sensitivity of the baboon to certain drugs and the human, and in the human, of course, we have got so much more experience of managing it.

So, there are distinct differences, and we can't say for certain that because it doesn't work in the baboon, it is not going to work in the human or vice versa, but I do think, yes, we do need a better immunosuppressive regimen to be pretty sure it is going to work in the human.

These regimens here may work in the human, but the fact that they don't work completely successful in the non-human primate suggests to me that they may be not fully successful in the human, but there are distinct differences and one will not know what those differences are until you actually go ahead with a human trial.

DR. AUCHINCLOSS: David, thank you very much. I think we will move on to Robert Michler for the second presentation of the morning, on human xenotransplantation.

Human Xenotransplantation

DR. MICHLER: Thank you.

[Slide.]

What I would like to share with you this morning is really the notion that what we are all after is the opportunity to replace organs, and xenotransplantation truly is just one of the tools in our armamentarium for the replacement of those organs.

[Slide.]

If we look over the history of clinical xenotransplantation, and I have divided it into two eras, the precyclosporin era and postcyclosporin, I think several things are striking about what has been done since the early part of the 20th Century beginning with the variety of organs that have been transplanted including sheep and pig, but also the fact that these most successful xenotransplant in humans occurred over 35 years ago with the use of a chimpanzee organ in a human that survived for nine months.

[Slide.]

Following the introduction of cyclosporin, we see that there are several interesting cases, again, the variety of organs, two pigs, the majority of the others being baboons. Actually, this was a pig by Len Makowka using the liver, but I would like to focus your attention on the Bailey heart transplant experience, and you are all very very familiar with Suzanne Ilstad's work in AIDS therapy.

[Slide.]

Interestingly, the first human implantation of a kidney xenograft demonstrated by Keith Reemtsma shows I think several salient points that we should all try and maintain in mind when exploring human transplantation.

First, not only is the survival and the successful survival, but also the demonstration that these organs function and that if rejection occurs, they can be treated for those rejection episodes.

Keith, in his early publication on this, compared the xenograft, which he called a heterotransplant, to the allotransplant, which is called the homotransplant, and looked at variable features, from urine flow, BUN, creatinine clearance, and demonstrated almost striking similarities between the function of the two organs.

[Slide.]

At the time of death of this patient, who died from causes unrelated to the xenograft--this was a chimpanzee organ--grossly, the organ looked normal, and histopathologically, the organ looked normal, as well, demonstrating again the feasibility in that particular situation.

Naturally, non-human primates for a variety of reasons really are not to be addressed and can't be addressed in the current strategies for organ replacement therapy, but I think with the world experience summarized

here for clinical cardiac xenotransplantation, there are actually two cases I would like to focus our attention on because they lend I think two very important lessons with respect to what we are trying to discuss.

[Slide.]

The first is the case of Baby Fay, which you are all very familiar with, Leonard Bailey's experience, and the second being a case done in Poland by Drs. Czaplicki and Religa in which they implanted the pig organ into a 24-year-old man with Marfan's syndrome, who was dying of severe heart failure.

That organ was transplanted after two separate pig organs had been perfused while the patient was on the heart-lung bypass machine, essentially doing what Dr. Cooper just outlined for you, which was immunoabsorption.

This organ surprisingly survived for 24 hours, and this is actually the photomicrograph of that organ, the heart, following its explant. I appreciate the Polish investigators sending this to me.

At any rate, I think the important feature here is that this architecture for those unfamiliar with cardiac biopsies, this architecture is essentially normal. Unfortunately, what the investigators did not do is look for immunofluorescent staining for the binding of antibody and complement, so we have no understanding of really whether

there was any deposition of that.

[Slide.]

The second case. This is Baby Fay's heart transplant, and this is a right ventricular biopsy or actually a right ventricular specimen taken after the death of Baby Fay. What it shows you is striking interstitial infiltrate and hemorrhage and blood in the blood vessels of that organ.

Now, as many of you know, Baby Fay was an ABO incompatible transplant. The blood group area was crossed. Baby Fay being an infant had presumably not yet developed significant antibody to that blood group mismatch and Baby Fay survived for approximately three weeks, died on the 20th day, and this is presumed the cause of death.

The important feature here is that this is analogous to an acute vascular rejection that one might see following pig-to-human transplantation under the conditions of transgenic implantation or immunoabsorption, whatever that preclinical scenario might be to allow the organ to go out this far.

[Slide.]

With this history of what I think many could assume as failures, what is it that really xenotransplantation going? We have had no clear long-term human successes except for the 35-year historical case of

Dr. Reemtsma in a non-human primate, and I think the reasons are really very obvious to everyone.

[Slide.]

First of all, allotransplantation is extraordinarily successful, so successful that this is really the reality for every transplant that we see. There is a long line of patients awaiting, in need of transplantation, and in many centers, transplantation at one year exceeds 90 percent.

[Slide.]

The other is a point that Dr. Vanderpool and others were raising yesterday, and that is, what is the potential benefit for this therapy, and I wanted to take a moment to just outline to you the true impact of what heart failure is like is this country today and what I, as a heart surgeon, in my microcosm of my heart center have to deal with every single day with patients referred for heart failure.

First of all, there are about 400,000 new cases we see every year, 4.8 million patients, 2 million of those patients are under the age of 65. It is the most common DRG that we have go deal with. Nearly a million hospital admissions per year. The one year survival rate for patients with heart failure in the best medical therapeutic hands only approaches 50 percent, and it is the only form of

heart disease that is actually increasing in frequency.

[Slide.]

If you look at its cost, between 10 to 17 billion dollars per year, most of those expenses occur inside the hospital. These patients overwhelmingly are managed inside of the hospital, and heart failure dollars outstrip the treatment of myocardial infarction by 2 to 1.

[Slide.]

But what else has made it very exciting that keeps xenotransplantation on the table? No question there has been extraordinary advances in the development of molecular technology and the sponsors that are discussing protocols here today have really developed revolutionary technology that I think will one day benefit many millions of patients.

They have been able to humanize the donor by creating complement inhibitory proteins. They are on the verge of developing technologies that will alter the antigenic expression on the surface of pig endothelial cells, and the opportunity exists to modify the proteins that are responsible for the coagulation properties on those cells.

But if we look at the results, I think it is very important, and many of you are familiar with these results, some were presented yesterday, in the best of hands, whether it is in a heterotopic model--and just for the same of

definition, heterotopic means outside of the normal position--so a heterotopic heart transplant can be placed in the neck, it can be placed in the abdomen, it can be placed in iliac region, an orthotopic transplant means that you remove the native organ and implant the new heart in the same position, the results show that in the best circumstances, about two months outside survival has been achieved in heterotopic position, and as you just heard from Dr. Cooper, maximum survival of about a month in the orthotopic position.

[Slide.]

So, in a very elegant report, Fox and Swazey a number of years ago, in a book entitled, "The Courage to Fail: the Experimental Therapy," and I think it is a fascinating dialogue, they outlined three critical questions that investigators should address if they wish to proceed with clinical trials of any innovative therapy.

I think this is a framework in which we can begin to formulate clinical trials. First, what defines laboratory success of a sufficient magnitude to warrant introduction into the clinical arena.

Secondly, who will be the patients who we will look at for clinical trials, and thirdly, if we have defined success in these clinical trials, what would be the clinical application and who would those clinical trials be for.

[Slide.]

I think that it is very valuable to look at the experience with mechanical heart transplantation as a surrogate for some of the answers that we are trying to address today.

[Slide.]

This is Barney Clark's heart and the Jarvik total artificial heart, but I would like to focus on a device that I have a lot of personal experience with, and this is a Heart Mate left ventricular assist device.

[Slide.]

Now, what we know from mechanical heart devices is that they can form surrogates of what I like to call xenotransplants, which are biologic assist devices. We know, first of all, that if you took 100 patients with heart failure and needed to do something urgently on those patients, and you implanted a left ventricular assist device in those patients, between 20 and 25 of those patients would die before you could transplant them.

Some of those patients would die with an LVAD because they developed a stroke, an infection, or some other reason that would exclude them from transplantation, but others would die with the transplant.

Of those 75 to 80 who survive the LVAD, about 85 percent of those patients would be successfully transplanted

and be allowed to go home. So, of the 100 patients, about 60 percent of those, 60 patients would actually survive to transplantation.

So, inherent in the opportunity to include an alternative therapy, one must recognize that a significant portion of patients will die, and one has to be able to accept that and acknowledge that depending on the clinical trial one is undertaking.

The other very, very interesting point is that if you look at the mean time from implantation of the LVAD to transplantation, it is about between two and three months, 60 to 90 days, so I would submit to you, using that kind of information, before we embark on clinical trials of human xenotransplantation, I would suggest that we need to see an excess of 90 percent survival of orthotopic heart transplants for 30 days, and close to 50 percent survival beyond 60 days.

Again, I bring this up because I think it is important for us to debate those numbers and to really put some numbers on the table.

[Slide.]

Second, this is what limits transplantation, the fact that many patients are too large to get transplanted. Many patients on UNOS I wait shorter periods of time than UNOS Status II patients, and blood group has an important

impact on their survival and their likelihood to be transplanted.

[Slide.]

So, getting back to the three criteria, I think it is important to establish a survival time, but I think it is also important to address questions of rejection - are we comfortable with acute vascular rejection and the ability to diagnose it and to treat it, and what happens beyond acute vascular rejection.

[Slide.]

This is a slide depicting acute vascular rejection from Waterworth's group, Imutran group, and clearly, it is an alarming histologic picture, but as yet we have no known therapy or proven therapy or even attempted therapy that has been published in the literature.

[Slide.]

Secondly, this is a slide showing an infiltrate. This is work done in our institution on xenografts from pig-to-baboon in unmodified, untreated animals, and we have seen a significant infiltrate in animals surviving beyond hyperacute rejection, the majority of the cells being natural killer cells and macrophages.

[Slide.]

This is a therapy that we need to investigate as well, and I think there are a number of questions that we

should pose that will be important in addressing whether this is clinically applicable at the current time.

First, can cyclosporin-based immunosuppression prolong xenograft survival? I think at present our knowledge base suggests that the answer is yes. Can the xenograft heart support the circulation? Unquestionably, it can.

Is xenograft rejection reversible? As yet we do not know.

Does acute vascular rejection occur in xenografts and can it be treated? Yes, it does occur. We don't know whether it can be treated.

Does xenotransplantation jeopardize a subsequent allograft? As Dr. Cooper said, there have been only three experimental attempts at trying to demonstrate whether this is true or not.

What is the role of humoral immunity long term and what is the role of cell-mediated immunity long term?

[Slide.]

Finally, what if we look at the appropriate candidates, a destination therapy versus a bridge therapy, and in the protocols that you have seen addressed today, these questions come up.

First of all, is a destination therapy appropriate using a heterotopic heart transplant, and who will be the

patients that we use in that kind of scenario, and what would bridge therapy be like for our patients and who would we select.

[Slide.]

Well, this is what a heterotopic heart transplant looks like. I think it is important for all the sponsors to keep in mind, and I think all of us to keep in mind, as well, that this is a very uncommon operation. Only 13 were performed last year by UNOS records. With over 25,000 heart transplants being performed since the beginning, this remains a very uncommon operation, and when you take a new technology and couple it with a surgical operation that is not performed commonly, I think that the investigators must be very cautious that they not jeopardize their end results simply because they are introducing a therapeutic surgical arm that is not commonly practiced.

[Slide.]

What about a xeno bridge? Apparently, one must define whether there is reasonable supporting evidence to go on with it. It is very important to study the immune phenomenon. It is the foundation for future destination therapy. Very importantly, the public must be brought into this. Public awareness is high on this. We must ensure confidence that we can be successful in embarking on this kind of therapy.

But remember a bridge is epidemiologically inconsequential, it will not impact on the total overall strategy.

[Slide.]

If we look at appropriate therapeutic individuals, then, one has to consider not competing with a strategy that is already established, such as left ventricular assist device.

[Slide.]

Candidate selection can be an allotransplant candidate whose body size is insufficient to put an LVAD in. Death is imminent, no allodonor is available anywhere. Some of these patients might be on mechanical support devices. But for a patient in whom destination therapy might be an option, these patients will not be allotransplant candidates. These patients are likely not be left ventricular assist device candidates, and who will be those patients be? Most likely, older aged individuals or patients with multiple comormid diseases or finally retransplantation candidates.

[Slide.]

In summary, I think we always have to keep the patient as the most preeminent thing that we can consider. Never forget that the patient and the success at bringing a novel therapy to these patients must be considered, but not

to be frightened about introducing innovative therapies because it is the only way that we can address an issue that is this germane to the public health.

[Slide.]

Finally, I think it is very important for us not to be frightened by the cost of these therapies. This will be extraordinarily unimaginably expensive, and to forget that for a moment, I think is inappropriate, but at the same time, it is important to recognize that as time goes by, data will become available that demonstrates, as it has for heart and kidney transplantation, that if you look at the cost of year of life saved for heart transplantation, it is actually less expensive than a single vessel coronary bypass operation.

Finally, that the sponsors need to recognize, as well, that millions of dollars must be put into this in order to allow clinical trials to be successful, because certainly Medicare is not going to pay for it initially, the institutions are not going to burden the expense, and that the sponsors must be largely responsible for making clinical trials a reality.

Thank you.

DR. AUCHINCLOSS: Thank you very much.

Dr. Vanderpool, I believe has a question.

DR. VANDERPOOL: Dr. Michler, that was a superb

presentation. I know you are also familiar with the history of innovative transplantation.

Do you find in that history a model for beginning points and second points and third points that we could at least have in mind as we think about xenotransplant innovation? The new types of when heart transplants first started or when other transplants first started, what kind of models do we have in terms of failure and then gradual success and then greater success?

Obviously, we are not going to hit a home run the first clinical trial or two. Can you give us some historical perspective?

DR. MICHLER: Yes, I would be happy to. Actually, I included for the committee an editorial that I wrote a few years ago that includes some of the historical perspective on this.

Before the first human heart transplant was ever performed, the best survival achieved was in dogs. The control groups survived for an average of 7 days and then experimental group of Drs. Lower and Shumway survived for over 200 days. I think the mean survival was 203 days.

That was the bulk of the scientific evidence that then allowed heart transplantation to be undertaken clinically. Many of you also realize that the first heart transplant performed by Christian Barnard survived for 18

days, but the second and third transplants that survived went 18 months and 20 months. These patients returned home and did extraordinarily well.

Unfortunately, within that early time period there was an absolute explosion in the number of transplants performed worldwide with a one year survival rate of under 20 percent with 105 centers performing heart transplants within the first year of that transplant.

So, there was an astonishing attempt by investigators and clinicians all over the world who literally had no experimental experience in this, and just the desire to do something novel with results that were abysmal.

After that, there was really one medical center that persisted, and that was Stanford, and to the great credit of Norm Shumway and his team, they persisted and took this therapy and applied it and religiously made an effort to make it successful to the point where now an excess of 90 percent of patients at most centers survive one year.

DR. AUCHINCLOSS: At one point, you were considering a trial of bridge cardiac transplantation from baboon to children as a possible way of introducing xenotransplantation.

Leave aside the baboon issue, which is no longer at this point on the table, do you still consider that

population of children, poor candidates for ventricular assist, waiting for allotransplantation, to be a good population for xenotransplantation as a bridge, did you at that point do any experiments to determine what kind of effect on subsequent allotransplantation the first xeno might have?

DR. MICHLER: To address the question firstly, yes, we did give up non-human primates as a bridge and have embarked on a series of investigative efforts to try and look to see whether the pig would not in fact be a better substitute.

We feel the answer to that question is yes for a variety of reasons. First, the need is tremendous in that over 30 percent of pediatric patients die on the transplant waiting list.

Second, that the procedure is very technically facile in the pediatric population, and third, there may be an opportunity, a window of immunologic opportunity for these patients, and some of our investigative work has actually looked at the development of immunoglobulin anti-Gal antibody in the newborn baboon and the newborn human population, and we have shown that in the newborn baboon, the level of antibody is barely detectable as it is in the human, and it is not until about two months of age in the human that sufficient immunoglobulin has been developed and

it parallels about the level that is present in the adult.

So, many patients who are in need of transplantation in the pediatric population are actually infants born with severe heart failure or hyperplastic left heart syndrome, and a variety of other congenital abnormalities that we think would be good candidates for this.

The issue I have not mentioned, nor has anyone yet, but I expect Dr. Vanderpool would mention, is the issue of informed consent in the pediatric population, and Arthur Caplan has actually published quite extensively on that.

DR. AUCHINCLOSS: I do recognize that we are bringing up subjects that weren't a substantial discussion. but we will get to that in a little bit.

Martin?

DR. HIRSCH: I presume that as progress is made in xenotransplantation, progress is also being made in the mechanical assist devices.

Do you think that progress there either as a bridge or a long-term device might eventually obviate the need for xenotransplantation?

DR. MICHLER: I don't think it will obviate the need. I do think that we are in the infancy of ventricular assist development technology, and that is really why the title of my talk is replacement therapy, because as a heart

surgeon, I just want to see the patient do well and live and get on. Whether that is with the human heart transplant, a xenograft, or ventricular assist device, I suspect there will be differences in terms of how well a patient will do and what will be their ultimate outcome, but I do think that these technologies must continue to progress in parallel.

I do not believe that xenotransplantation, even if it were successful in clinical trials, and we were to see its application widespread, would ever eliminate the need for ventricular assist device implantation or vice versa.

DR. AUCHINCLOSS: Thank you very much.

I think we will move on now to the presentation from Nextran, I believe introduced by Dr. John Logan.

Clinical Applications: A Discussion

DR. LOGAN: I would like to split this morning's talk really into three parts. The first part, I will talk in a little bit of detail about some of our preclinical results. Then, we will move to potential clinical applications, and those presentations will be given by Dr. Christopher McGregor of the Mayo Clinic and Dr. Martin Levy of Baylor in Dallas, and then turn in the last part of the discussion really to what could be the potential requirements in a preclinical model in order to enter the clinical arena.

Let me first start off by saying that I believe

this is a very important forum to start the discussion on what is required preclinically in order to enter the clinic.

I think right now the data that we have does not justify an entry into the clinical arena, but I think we need to start that discussion early in order to set the goals and the framework for what we really need to achieve and what are the milestones that we need to achieve as we think about the clinical process.

[Slide.]

Let me turn then to the first part of the discussion, which really surrounds our approach and our strategy in xenotransplantation, and let me give you a little flavor of some of our results, and then discuss some of the challenges that we face in obtaining those results.

As David Cooper went into, the immunological challenges in xenotransplantation really are at least threefold today. Firstly, it is the problem of hyperacute rejection of a pig-to-primate transplant that occurs immediately after the heart or kidney is transplanted from a pig into a primate.

Then, there is a form of vascular rejection which occurs sometime around a week post-transplant, which has been named various different sources. We have called it acute vascular rejection and then presumably there is cellular rejection and chronic rejection, which are problems

yet to be overcome.

[Slide.]

If you look first at the initiative reaction of hyperacute rejection, hyperacute rejection is initiated by the binding of antibody to the antigen on the endothelial cell. In terms of hyperacute rejection, the antibody predominantly recognizes single residue, which is an alpha-gal of sugar on the endothelial cell, the binding of that antibody to the pig antigen activates the complement cascade, activation of the complement cascade results in stimulation of a prothrombotic environment, and then you see the features of hyperacute rejection, which is thrombosis, edema, and graft destruction almost immediately.

In trying to think about methodologies to overcome hyperacute rejection, the only ones that have met with success are those which attack the initiating elements either antibody and complement.

We have attempted to solve this problem by actually looking at the complement component of that and by the expression of human complement regulatory proteins on the pig endothelium.

The goal here is to provide complement regulation on the pig surface, such there are low antibody combined, complement cannot be at its effective functions.

[Slide.]

Just let me summarize some experiments we did a few years ago in which we developed a number of different lines of transgenic pigs either expressing CD59's or CD55 or CD46 alone. We transplanted these organs into baboons and applied a fairly standard immunosuppressive regimes, the cyclosporin steroid-based immunosuppressive regime with the use of cyclophosphamide in a range between 1 and 5 mg/kilo.

What we see in the transplantation of a nontransgenic kidney in this setting is hyperacute rejection, and in this set of experiments there were four nontransgenic kidneys, and they all underwent hyperacute rejection.

In the case of transgenic kidneys, we did not see hyperacute rejection, and the grafts lasted for between one and two week post-transplant. Rejection here, these are life-supporting grafts, rejection here was classified as a twice doubling in creatinine, and not death of the animals.

In the case of the heart, we essentially saw a very similar picture in that in this case we only did two control hearts, but both control hearts hyperacutely rejected, and the transgenic hearts lasted for anywhere from a few days up to two to three weeks post-transplant with actually one of the transgenic hearts undergoing a hyperacute rejection.

[Slide.]

If you look histologically at the reason that these organs overcome hyperacute rejection is because they inhibit the complement cascade. If you look at the deposition of antibody comparing nontransgenic and transgenic routes, you see antibodies deposited in both grafts.

In the case of the nontransgenic graft, we see activation of the complement cascade as indicated by deposition of C5b and MAC.

However, in the case of the transgenic animals, we block deposition of C5b and MAC. So, these organs are protected from hyperacute rejection by blocking the activation of the complement cascade.

[Slide.]

However, what we have seen with essentially all of our transgenic animals, if we apply normal levels of immunosuppression, is that we see hyperacute rejection is overcome, but all these grafts eventually succumb to a vascular rejection process, and that process starts from a few days to a week post-transplant.

In general, we see little evidence of a cellular infiltrate in the presence of immunosuppression, occasionally in the kidney, we will see some cells, but very rarely, and in the hearts, we very rarely, if ever, see any cellular infiltrate.

So, we have termed this process acute vascular rejection, and really this presented to us the major barrier at the moment to xenotransplantation.

[Slide.]

We tried to think about what could be the causative agent behind acute vascular rejection, and tried to do the follow experiment. We essentially took two sets of animals, and these experiments have been published.

We took transgenic animals under normal conditions into baboons under an immunosuppression regime of cyclosporin, cyclophosphamide, and steroids, and these grafts rejected with a few days to a week post-transplant, and this is the typical picture that we see of acute vascular rejection.

[Slide.]

However, if we went into the baboons and actively removed total immunoglobulin before transplant and the immediate days and weeks post-transplant, we could avert this course of acute vascular rejection, and this told us that immunoglobulin really was a key component in this acute vascular rejection process.

The nature of that immunoglobulin, we believe actually is targeted against alpha-gal, predominantly, if not exclusively.

[Slide.]

Really, to try and show that, we really repeated the experiment, the removal of total immunoglobulin, but this time only removed the immunoglobulin to recognize the alpha-gal in a very similar format to what Dr. Cooper described in terms of extracorporeal removal of the immunoabsorption device.

The set of controls here were transgenic animals. We performed in this case a splenectomy at D minus 6, applied immunosuppression, which was cyclosporin, cyclophosphamide, and steroids, again a loading dose of 10 mg/kilo tapered down to 1 to 5 mg/kilo.

In the case of the transgenics, as we have shown you before, these organs essentially lasted somewhere between a few days to a week post-transplant. It underwent process of acute vascular rejection.

In the case of antibody depletion, we performed exactly the same protocol in terms of immunosuppression strategy, but in this case, we actively removed just alpha-gal antibody, free transplant, and up to two weeks in the post-transplant arena.

In this particular set of experiments, we did four transplants.

[Slide.]

In these four transplants, which are heart transplants, none of the organs succumbed to rejection. We

saw no rejections defined histologically or in terms of cessation of beating of the graft.

We lost the animals at 9, 12, 34, and 39 days. The first three animals here at 9, 12, and 34 days were all lost due to complications not related to the graft of the immunosuppression. They were related to surgical complications either related to the immunopheresis or the in-dwelling catheters.

The 39-day animal was lost on infection.

[Slide.]

However, and I think this again exemplifies a point that Dr. Cooper made, which is the challenge in maintaining these animals in a healthy state when one is performing invasive technologies, however, what this pointed to us was that the strategy at least in trying to get a successful xenotransplant was to use the genetically modified, the transgenic organs expressing human complement regulatory proteins to try and develop an appropriate immunosuppressive regime, and then a therapy to control alpha-gal antibodies, which really in our case would be the specific physical removal of antibody.

What we are doing now clearly is extending those previous results, looking at different organ types, and looking at larger and longer term survival studies.

[Slide.]

However, let me now turn from the preclinical side to what potential clinical applications could exist in xenotransplantation. I just really wanted to make two critical points here.

The first point is that the goal here is to provide an additional treatment alternative for patients with end-stage organ failure.

The second point here is really a very important point and I think is a point that certainly is open to debate, and that is that the comparison and outcomes should really be with other available medical treatments, whatever they are, for the patient in end-stage organ failure, and not allotransplantation, because allotransplantation is a limited resource given to very few people.

It really is a comparison of xenotransplantation to other medical alternatives.

With that, let me now turn to the clinical applications and introduce Dr. McGregor from the Mayo Clinic.

DR. McGREGOR: Thank you, Dr. Logan. Good morning, ladies and gentlemen.

23,000 solid organ transplants are performed in the United States each year. There are, however, 65,000 people waiting for solid organ transplants. Of that 65,000 people, 4.5 thousand people will die each year, that is, 13

patients each day.

In addition, these 65,000 patients represent conservatively less than half of those patients who could benefit from organ transplantation for end-stage organ failure if there was an unlimited supply of donors.

I would therefore like to reiterate a point just made by Dr. Logan, and that is that the advent of clinical xenotransplantation will provide new, additional therapies for selected patients who would not otherwise receive an allotransplant, and reiterate therefore, the comparison of outcomes should not be with allotransplantation, which is an established conventional treatment, but with alternative methods of treatment for that specific group of patients.

[Slide.]

Let us now look at the rationale for organ selection. The most likely success will be in organs that are physiologically, metabolically, and immunologically compatible with the host.

In the spectrum of physiological and metabolic compatibility, the heart and the kidney would appear the least complex, the heart largely being a simple mechanical pump, whereas, the liver of course is a much more complex organ with the production of many complex proteins.

In the spectrum of increasing complexity of immunological compatibility, again, the heart and kidney

would appear to be at the less complex part of the spectrum, with at the present time the lung in preclinical studies being very incompatible.

Preclinical studies would emphasize these thoughts about xenograft compatibility, and would indicate that the organs of choice for initial clinical xenotransplantation trials would be the kidney or the heart.

[Slide.]

The preferred clinical indications for xenotransplantation would therefore be cardiac or renal. There are two potential cardiac applications. The first would be as a bridge to cardiac allotransplantation in patients dying waiting for an allotransplant.

The second cardiac application would be for the treatment of end-stage cardiac failure in patients who are ineligible for transplantation.

[Slide.]

Before discussing these two specific clinical indications in more detail, I would like to make an initial overall comparison for discussion between cardiac and renal application of xenotransplantation.

The comparison would be on five bases, that is, the availability of alternative treatment for the patient, the effectiveness of that alternative treatment, the outcome without xenotransplantation, the consequences of xenograft

failure, and the relative ethical bar for each application.

If we look at the heart as a bridge to transplant, one of the two proposed cardiac applications, then, apart from those patients who are VAD candidates, and clearly we are going to discuss ventricular assist devices more and more as the morning goes on, but in patients who are judged not to be VAD candidates, the outcome without xenotransplantation is death. There is no good alternative treatment.

Therefore, the relative ethical bar one would consider low. If one looks at those patients who are allotransplant ineligible, the only alternative treatment is best medical therapy. In a selected group of patients, this results in an impaired quality of life with multiple hospital admissions and an identifiable prognosis of only a few months.

One would say that this therefore had an intermediate ethical bar.

If one looks at renal transplantation, clearly dialysis is available. It is effective, but as many patients will tell you, this results in a limited quality of life. Because there is a good alternative therapy, one would think that the relative ethical bar was higher than the other applications.

However, the one advantage that renal application

would have is that there is a return to dialysis available as a consequence of xenograft failure. If the xenograft fails after heart transplantation, then, it will result in death of the patient.

[Slide.]

The use of a bridge to transplant indication would involve a transgenic pig to human cardiac xenotransplant as a bridge to cardiac allotransplantation in accepted human cardiac transplant candidates at high risk of impending death, that is within days, from irreversible cardiac failure due to lack of an available suitable human donor.

Such patients will have end-stage ischemic congestive, valvular, adult congenital or restrictive cardiomyopathy.

[Slide.]

The rationale for this application is a bridge to allotransplantation, is that there is no alternative therapy, it is potentially life-saving for that individual patient. It would therefore be an acceptable ethical choice.

The application would be of brief duration, and as one would not know when an available human donor would appear, there would be progressively longer term exploration of xenograft function, and that definitive therapy with allotransplantation would remain the endpoint and would be

available for that specific patient.

[Slide.]

This trial of cardiac xenotransplantation as a bridge to allotransplantation would be the initial entry to the clinic, and not by any manner of means, of course, as the final application.

It would answer some basic questions - will the pig heart sustain the circulation of an adult human recipient for a number of days or weeks? What are the immunologic and physiological challenges to allow patient survival, what would be the optimal immunosuppressive therapies in such patients?

[Slide.]

As I look at patient inclusion and exclusion criteria in the next few slides for the two cardiac implications, I would emphasize that these are not all encompassing lists of criteria for the sake of time, but simply highlights to give you a flavor of the patient populations that we are talking about here.

As regards to the bridge indication, patient inclusion criteria would be accepted candidates for allotransplantation, men or women in this age range, although I think that Dr. Michler makes a very good point that perhaps we should consider from birth to age 70.

These are patients who are judged clinically

unsuitable for VAD, for deteriorating from a hemodynamic point of view, who need increasing inotropic or balloon pump support, who have life-threatening arrhythmias or who are developing multiple organ failure that will result in apparently death.

Exclusion criteria would be the standard accepted published contraindications to heart transplantation and those lists are easily available to any of us.

[Slide.]

I am now going to move on to the second cardiac application of xenotransplantation, and that would be in non-allotransplant eligible patients.

Now, clearly, as you apply this technology as a bridge, you are not going to increase the number of donors, so societally, you are not making a difference. You are making a difference to that individual patient who is dying, however, as we look to the second cardiac application, that is, in patients who are ineligible for allotransplants, then, one is increasing the number of donors, and this will have a much great societal impact.

I would like to look initially at the ineligibility criteria, the inclusion and the potential exclusion criteria for such a trial.

[Slide.]

These criteria are simply to give you a flavor of

what kind of patients we are talking about, the kind of patients that people like Dr. Conte and Dr. Michler and I see every week of our lives, and they are patients who are turned down for allotransplant for a number of reasons.

They may be older patients. Many of these patients here are 65 or 70 or even 75 are extremely active and vital. Another reason patients are turned down is comorbidities that would compromise the outcome of allotransplantation, and one could list 20 of these, and I have picked some of them - diabetes with end organ disease, the presence of controlled but non-cured malignancy, the presence of systemic diseases or sustained renal impairment that would compromise the long-term outcome of an allotransplant, patients who have a very high PRA, who one knows are going to wait indefinitely, again, might be patients who would be turned down for allotransplantation because of age, because they may wait five years, and these may be ideal patients for the initial clinical application of xeno.

[Slide.]

Some of the inclusion criteria for the second cardiac application, that is the non-allotransplant eligible indication, would be men or women greater than 15 years old, they would be in chronic New York Heart Association Class III or Class IV heart failure.

They would be ineligible, as we said, for cardiac allotransplantation. They would have failed standard medical therapy and there would be a number of hemodynamic parameters, parameters such as peak consumption. I have given one arbitrary number there, and one could argue whether it should be 12 or 24.

In terms of chronic heart failure, this can be defined. It could be multiple hospital admissions within the previous four weeks. There are clear definitions that many of us have worked on over years for the application of VADs and similar circumstances.

[Slide.]

Exclusion criteria. Obviously, if patients are eligible for an allotransplant, by definition, they are going to be excluded from this trial. Factors that would result in a certain poor outcome would be exclusion criteria, such as irreversible pulmonary hypertension, severe end organ dysfunction, severe cerebral vascular or peripheral vascular disease, or active systemic infection.

[Slide.]

I will finish up by giving one potential clinical strategy for the early application of xenotransplantation. Firstly, as a bridge to allotransplantation and, of course, there would be no control group because there is no alternative.

Then, move on to xenotransplantation in non-allotransplant candidates, the controls for this trial would be best medical treatment because that is the only alternative treatment available to this group of patients.

One could then move on to prospective trials of other organs, such as the kidney, and finally, hopefully, not decades away, but within our professional lifetimes, definitive therapy for end-stage organ failure.

Thank you.

I pass the podium on to Dr. Marlin Levy from Baylor, who will talk about potential renal applications.

DR. LEVY: Good morning, ladies and gentlemen.

[Slide.]

What I would like to do in the next few minutes is perhaps explore the possibilities of a renal xenotransplant and throw out some of the questions that ought to be addressed when talking about contemplating such a trial or applying this application to patients.

[Slide.]

Certainly there is some key questions that ought to be addressed prior to initiating trials, and I would suggest that one of the most important ones is the preclinical graft survival data, some of which we have seen both today and yesterday.

Dr. Cooper alluded to the fact that perhaps

patients who are sensitized to human antigens, patients with so-called high PRA, might also be sensitized to pig antigens, and that is another barrier that would have to be overcome before we would consider a renal xenograft.

Finally, and I think quite importantly, the quality of life issues of a patient with a xenograft as it compares to a patient on dialysis would have to be addressed and explored.

[Slide.]

As my colleagues earlier this morning have already alluded to, I think that the benchmark of comparison for a renal xenotransplant trial or renal xenotransplant model really can't be an allotransplant. It has to be, in my opinion, the alternative to an allotransplant, which is waiting on dialysis for a kidney.

I would postulate that since the standard of care is allotransplant, and in applying any experimental therapy, you would probably want to apply the experimental therapy to a patient population who is unable or ineligible to receive the standard of care.

So, as I define it, the context really has to do with the waiting list for kidney transplantation, a waiting list which has some defined mortality, as I think all of you know, and a waiting list which has very definite morbidity and for many patients is quite in agony.

[Slide.]

Regrettably, this data from UNOS, which is current to February of '99, is all too familiar to us, but i think it is important to bring it out as we talk about these issues and as we try to frame the debate.

This is the number of patients on a waiting list, and the number continues to escalate, and all of us who work in the transplant field and who take care of patients understand that of the 65,000 patients who are on a waiting list, 43,000 of them are kidney transplant patients, so from a clinical need standpoint, there are certainly a large population of patients to which this could be addressed.

[Slide.]

Unfortunately, the waiting list has a defined mortality. In 1998, 2,300 people were removed from the waiting list because they died, and again, you can see the escalation in the number of patients who are dying on the waiting list.

The overall mortality from dialysis is 20 to 25 percent in this country. The overall mortality of patients who are on a kidney transplant waiting list is approximately 8 percent a year as these numbers show.

So, I would suggest to you that, in fact, a kidney transplant can be a life-saving organ for many patients. If you place a patient on the waiting list today, the chances

that they are not going to live to transplant are approximately 8 percent per year.

[Slide.]

Here is more of the obstacle and more of the problems. The waiting times for patients across the country are astronomical. The average waiting times for patients who are waiting for their first kidney--now, these are median waiting times, keep in mind that half the patients will wait longer than that--is in excess of 800 days, but there is certainly a category of patients, for example, those who have had a previous transplant, who wait far longer than that with now median waiting times of approximately 1600 days for patients who have had at least one previous transplant.

[Slide.]

If you want to address waiting times by patients with high PRAs, broken down into these three categories, there is likewise a group of patients who are waiting in excess of 1300 days who have an intermediate level of presensitized antibodies and patients who essentially will never ever get a kidney transplant, patients who are waiting in excess of six years before they can be transplanted.

[Slide.]

Again, the waiting list has defined morbidities and defined agonies for patients. There is certainly

exacerbation or new cardiovascular disease which takes place during the time that the patient is on a waiting list. Patients very commonly develop vascular access problems or risk or infections continues, both bacterial and viral.

There are some subtle, but still very significant difficulties with the waiting list in terms of lost productivity and disability to the patient, the psychological burden of dialysis, and the economic burden, both to the patient being unable to support himself or herself, and to the families, and, of course, the large economic burden to society at large.

[Slide.]

What I would suggest to you is that there is a group of patients who despite being medically suited for an allotransplant, are unlikely to ever receive one, patients who have high PRAs, patients who have had previous transplants, patients who are offered kidneys on a regular basis because they have common antigens, but who repeatedly come up with a positive cross-match and so cannot get transplanted would form an ideal population of patients in whom one would consider a renal xenotransplant.

In addition, we transplant surgeons will often give only one chance at a kidney to patients with certain diseases. We know that recurrence of certain diseases in a transplanted allograft means that the disease is going to

come back again and again, and so if, for example, in patients with focal sclerosing glomerulonephritis, which is a common indication for kidney transplantation, if these patients have recurrent disease, they are not going to be offered another organ.

Likewise, patients with Goodpasture syndrome, which is an antiglomerular basement membrane antibody, will not be offered a second kidney or a subsequent kidney if their kidney transplant fails from their original disease.

That is again another patient population in whom kidney xenotrial would be quite appropriate.

[Slide.]

One can place I think restrictions or stipulations to a xenotrial for any number of different angles. One, for instance, can say, well, we ought to reserve xenotrials for patients who have been on a waiting list a certain length of time, patients who perhaps have been on the waiting list two years, three years, five years.

You can pick a number, but it is certainly plausible to say that given that some of these patients will never, ever get transplanted, those would be good candidates for a xenotrial.

Likewise, certain patients with a degree of PRA would be good candidates for xenotrial, be it 50 percent or 70 percent. I think the number can be debated, but the

point is that that population who is unlikely to ever be transplanted, and who is sentenced to living out their days on dialysis, would be appropriate.

[Slide.]

Finally, one can also place restrictions of recipient age, and it could be an interesting debate actually. Is an elderly person more willing to take the risk of a xenotransplant because they know they are going to spend the rest of their days on hemodialysis and never be offered a kidney, or do you offer a xenotransplant kidney trial to a young person who perhaps has had a previous transplant, who has a high level of antibodies, and who is 25, 30 years old, who is facing the rest of their days on dialysis?

But those are I think questions that can be considered and can help frame the debate.

[Slide.]

I would suggest to you that potential renal xenotransplant candidates could be patients who are already on the transplant waiting list, that is to say, who are medically eligible, who have acceptable cardiovascular status, who don't have malignancy, who have psychosocial support, patients who are unlikely to receive an allograft, and to me I think ethically, that would be a very appropriate way to approach this question.

It could also include patients who have developed dialysis intolerance either because of loss of vascular access or because of debility and disease over the years.

I think a key question which we have talked about very briefly this morning is a question of informed consent. Certainly, dialysis has morbidities and mortalities, but I think Dr. Vanderpool and the other ethicists here would appreciate that dialysis does offer us a very nice safety net in which to have a very deliberate, measured discussion with patients and potential patients and their families, and give patients the time to weigh the risks and benefits of entering into a xenotrial.

So, from a renal xenotransplant standpoint, that is I think a definite ethical plus.

I will let Dr. Logan finish his presentation.

DR. LOGAN: Let me just in the last couple of slides, come back to some thoughts about preclinical requirements and just try and talk a little bit about that.

[Slide.]

Clearly, our model system that we utilize is actually the baboon, and we have used exclusively the baboon, and in here we need to look at functional graft survival in terms of in the heart, can it support the circulation, in the kidney, how well does it perform physiologically over time, as well as the immunological

questions.

[Slide.]

So, really, we are asking two issues in terms of physiology and immunology. In terms of what targets should be for graft survival, I think at the moment that is hard to say. There are a couple of challenges in these baboon models that I think individuals who work with them understand well.

As we perform procedures and protocols, and morbidity and mortality we see with baboons would not be anywhere close to the morbidity or mortality we see with humans under clinical settings. So, clearly, there are some substantial differences in trying to draw graft survival to very long periods of time in the baboon, may also be somewhat misleading in that this is a model system and there are going to be differences between the baboon and the human.

So, we picked an arbitrary time point of approximately three months and asked ourselves what would be reasonable graft survivals, and we thought a number depending on the clinical indication of perhaps somewhere around 60 percent for graft survival at the end of three months, and that could clearly go up or down depending on the clinical indications, perhaps as low as 40 percent for bridge indications, as Dr. Michler was suggesting earlier I

believe, and perhaps higher for renal applications.

But clearly the debate on these numbers I think is a good debate to start, to start thinking about what could be reasonable targets.

[Slide.]

Issues that we try and define in the preclinical protocols are organ and immunosuppressive therapies, remembering that there will be some differences between the immunosuppressive therapies that we utilize in the baboon versus perhaps the dosing that we utilize in humans.

Immunological and physiological graft survival is critical. Rejection episodes, both the detection of rejection episodes, which may be perhaps more vascular in nature in the case of xenograft and an allograft, and also methodologies to treatment.

I think it is also important to recognize that in terms of reversible steroid-resistant allograft rejection, the use of OKT3, OKT3 doesn't recognize baboon cells, so again a limitation there in the reagents that we can utilize.

And then if one does perform a bridge indication, it is very important to show that we have no significant impact on the subsequent allografts, and there have been very few studies to really address that issue.

With that, I would like to stop and thank you very

much.

DR. AUCHINCLOSS: Thank you very much.

Can I ask two questions? The problem that you are having in survival appears be antibody mediated and anti-Gal. Do you have any experience with any of the transgenic animals that might have diminished expression of gal? That is one question.

The second question is I think one that will come up to many people here, why do the results that you report look different from the results that I think we will be seeing from Imutran?

DR. LOGAN: I think those are two good points. We have derived animals with lower levels of gal. Those animals have not yet been tested preclinically in baboons, but we are moving ahead.

DR. AUCHINCLOSS: I am sorry, I am not hearing you. You have the animals and--

DR. LOGAN: And they haven't been tested yet. We should hopefully get there shortly, but they have not yet been tested.

In terms of major differences, I think between ourselves and Imutran in terms of results, there is substantial difference in terms of the immunosuppressive regimes. I think the dose levels of cyclophosphamide used initially is much lower in our studies than in Imutran's

studies. I think--correct me if I am wrong--I think they are still using relatively high levels.

DR. AUCHINCLOSS: So, the primary difference is a difference in drug therapy.

DR. LOGAN: I believe so, but it could be a difference in--

DR. AUCHINCLOSS: Do you have a reason to think there is a substantial difference between the transgenic animals that the two of you have in terms of expression of transgenes or location of expression?

DR. COZZI: My name is Emanuele Cozzi. I work for Imutran.

Yes, if you can immediately clarify, I speak immediately after you, the story regarding the immunosuppression. At Imutran, all the protocol I will show to you today except one is based on the immunosuppressive strategy which entails only four doses of cyclophosphamide, so I would like to make this clear, we are not using any more cyclophosphamide at Imutran for more than four doses, and I will show this to you in a few minutes. Thank you.

DR. LOGAN: But the four doses are quite high.

DR. AUCHINCLOSS: I will put you on the podium in just two seconds.

Are there any other particular questions?

DR. SACHS: Dr. McGregor, you mentioned that the

bridge will not increase the number of donors available, and that certainly is true, but it will increase the number of prospective recipients on the waiting list, so in essence, since you are in a situation where people are dying every day without getting a transplant, you are actually assuring that one other person won't get a transplant.

I mean that is the problem with the bridge ethically, I would say.

DR. McGREGOR: Of course, that is absolutely correct. You are just shifting the cards around. But, you know, in terms of the ethics of the application of a new technology, if you have a patient who has the potential for long-term survival, and you can save the life of that patient, just as we have done with ventricular assists over the last 15 years, then, it seems appropriate to offer that critically ill patient this option if there is a reasonable chance that it can help him or her, but absolutely, we are not going to increase the number of patients surviving, and that is always going to be the limitation of the strategy.

DR. CONTE: One comment related to that question. The number of people who could potentially be bridges with a xenograft as opposed to a mechanical or as an alternative to mechanical device, is very, very small. They would primarily be the pediatric populations where there are not currently good devices available.

There are very few in the whole spectrum of mechanical devices, whether it is a total artificial heart, a left ventricular assist device, right ventricular assist device or bilateral, there are very few additional patients, so I do not think we are going to significantly increase the numbers of patients on the waiting list.

DR. AUCHINCLOSS: Dr. Coffin.

DR. COFFIN: I had essentially the same question.

DR. McGREGOR: To respond to that, if one looks at the number of heart recipients in the last five years, who have received a VAD, in reality, as far as the clinical practice in the United States today, reported to UNOS between 1994 and 1998, only 10 to 15 percent of heart recipients are receiving VADs today.

So, I think as far as theoretically possible and what is happening in the real world, and those are the numbers currently.

MS. MEYERS: Why didn't your plan have contingency plans in it in case you find out that these patients do indeed have virus and the PERV virus or whatever?

DR. McGREGOR: Clearly, there are very many additional important issues that we have to discuss. The point I think that I would make is I don't think we are ready from our knowledge to go ahead right now.

There are issues of physiology, there are issues

of infectious diseases that have to be satisfied. Due to constraints in time, I was trying to focus for the purposes of discussion as to potential patient groups who would be suitable for xenotransplantation.

DR. WOODLE: I would like to direct this question to Marlin Levy, and would also open it afterwards to anyone else who might disagree with this point.

The issues of forcing endogenous retrovirus are resolved. I believe that there is two populations of patients with end-stage renal disease who are immediate candidates for xenotrial. Both of these populations would have to be patients that are highly sensitized with a high PRA and would have no living donors.

One of these populations of patients who have end-stage vascular access or dialysis access who are within days to weeks are going to die because of failure of access.

The other population would be patients who are demanding to be removed from dialysis because their quality of life is so poor.

Is there anyone that would disagree with that statement?

DR. LEVY: I would agree emphatically with what you are saying. You know, they are fairly small numbers and again it is difficult to make a complete list of who is available, but I guess my message to the committee is that

there are certainly patients who despite what we consider to be the excellent technology of dialysis, there are certainly patients who both suffer and who die well before they can ever get a kidney transplant, and you bring up two more examples.

DR. AUCHINCLOSS: Steve, would you agree that as sort of a rough ballpark estimate, that if you went to any busy transplant center, you would sort of find one or two patients that would fall in this kind of category?

DR. WOODLE: Probably in our program, we have maybe three or four patients a year.

DR. VANDERPOOL: The question I had, I have heard this expressed several times that one candidate would be the person who is miserable on dialysis. My only concern is that they may be jumping from the hot plate into the fire, and so unless we have a good read on quality of life for the xenotransplant, then, what the patient wants to get out of will not really be a rescue, it will be a new state of worse misery.

Could you comment on that?

DR. WOODLE: Is that fire you are talking about the fire of xenotransplant or eternal fire?

DR. VANDERPOOL: I am sorry.

Just one quotation from the Nuffield report on xenotransplants, the UK Nuffield report, it argues that we

should have a "robust concern" for quality of life issues for xenotransplant recipients, and I think that phrase is a good one to at least have in the back of our minds.

DR. WOODLE: I was talking about patients who had come forth voluntarily who normally go about, who normally would come forth and say, "I want to be removed from dialysis, I want to die."

Your point is an ethical issue, which is a serious one, which is the question of coercion, an unspoken coercion that the patient feels because now they have an option other than dying, and I think we need to be very careful in the entry criteria into trials to safeguard against that.

DR. LEVY: I just want to remind you that being miserable on dialysis for some patients, it doesn't just mean having a bad day. I mean these are patients who are physiologically devastated by this, who are hypotensive during dialysis, who feel absolutely terrible before, absolutely terrible after, people who otherwise might be very stoic individuals, very driven to work, who are completely devastated with loss of livelihood, sometimes loss of family support.

I think us transplanters here know quite well what I am talking about. Fortunately, that is not the majority of patients on dialysis, but there is many of those like that.

DR. AUCHINCLOSS: We are going to come back to this discussion following the next presentation. What I would like to do now is to move on to the Imutran presentation by Dr. Cozzi, and then we will have our break and then we will come back for, first, the FDA Perspective and then the group discussion.

Current Status of Solid Organ Pig-to-Primate Xenotransplantation

DR. COZZI: Good morning.

[Slide.]

Today, I will present to you the current status of our solid organ pig-to-primate xenotransplantation program at Imutran.

[Slide.]

Some of the aspects of my presentation have already been introduced by Dr. Cooper and Dr. Logan, therefore, I will skip over some slides.

[Slide.]

This one is just to remind you that xenograft rejection, the mechanism we have to remember that we have in addition to the cellular and chronic, possible chronic rejection phenomenon which occurs in allotransplantation, we have to deal with two additional immunological obstacles, namely, hyperacute rejection and acute vascular rejection.

[Slide.]

The approach undertaken at Imutran, as we have heard, is an approach which is aimed at interfering with the role of the activation of the complement cascade and therefore the activation and damage of the porcine endothelial cells and the onset of hyperacute rejection.

[Slide.]

We have produced transgenic pigs for the human complement regulator h-DAF. We have produced this h-DAF minigene, which has been microinjected into a porcine embryo, and we have obtained h-DAF transgenic pigs.

[Slide.]

This slide is for us extremely important. These are absolutely h-DAF pigs which grow and reproduce normally.

[Slide.]

The next step was obviously once we have obtained the transgenic pigs to show the presence of h-DAF on the endothelial cell surface where we all know the hyperacute rejection phenomenon is know to initiate.

These slides clearly show that an immunohistochemistry using an anti-h-DAF monoclonal antibody, we have a large expression of human DAF on the surface of these endothelial cells of this artery, but also on the arterial smooth muscle.

[Slide.]

Therefore, a genetic manipulation which was

successful in leading to the production of transgenic pigs which express large amounts of h-DAF exactly where we know hyperacute rejection starts.

[Slide.]

Therefore, with the availability of such animals, we initiated five years ago our preclinical pig-to-primate xenotransplantation program, which entails today the utilization of several skills which starts with a team of surgeons, immunologists, veterinarians, pathologists, and so forth.

[Slide.]

The essential three goals which we are aiming to address without preclinical studies is obviously the elucidation of the immunological mechanisms which underlie the xenograft rejection, the development of a clinically acceptable immunosuppressive regimen, and I insist clinically acceptable, and I will show to you why I insist on that point, and finally, another goal is clearly the generation of the physiological data which will be required and necessary for us to support our clinical studies.

[Slide.]

The models we have developed at Imutran are essentially four models. One is a renal model where we have the transplantation of h-DAF transgenic pig kidneys into cynomolgus monkey, cynomolgus monkeys previously

nephrectomized bilaterally.

Then, we have developed three models which are cardiac models. Two are non-life supporting, which is the heterotopic model heart into cynomolgus monkeys or into baboon, and finally, the life supporting pig-to-baboon model. I will show you to you essentially three groups of studied to give you a little bit of perception of where we are and what we are trying to achieve.

[Slide.]

As I said before, two new obstacles, two new immunological hurdle to overcome for the long-term survival of the xenograft, the first one being hyperacute rejection, what have we achieved with this genetic manipulation undertaken in our pig.

[Slide.]

It summarizes a little bit our experience at Imutran, and I have reported here almost all our transplants undertaken to date. We have done more than 350 transplants into non-human primates using either transgenic or non-transgenic control organs.

If I look at the face of our transgenic pig organs, you will see that all together we have transplanted in either of our groups or cyno, 313 xenografts, again, either the heart or a kidney, and will see that out of the 313 xenografts transplanted, only 4 underwent hyperacute

rejection, whereas, 309 xenografts were not hyperacutely rejected.

So that gives us a percentage of hyperacute rejection episodes which is lesser than 2 percent of our total experience at Imutran.

Conversely, if I look at the non-transgenic subgroup, we have here so far 37 known transgenic control organs into non-human primates. In this case, we had 22 organs which underwent hyperacute rejection, and surprisingly enough, 15 organs did not undergo hyperacute rejection, but the most important phenomenon is here, basically, less than 2 percent of hyperacute rejection episodes with our transgenic pig organs.

[Slide.]

As I said, hyperacute rejection, we consider that with the transgenic pig lines we are working with today, which is essentially the h-DAF line, although I wish to stress here that we are coming up with new lines of pigs. With our first-line h-DAF transgenic pigs, hyperacute rejection, we don't see it anymore, while we have now to attack the next hurdle to the long-term survival of our xenograft, which is acute vascular rejection.

[Slide.]

This explains to you a little bit the rationale that we have undertaken in trying to address the acute

vascular rejection, and, in general, how we are trying to obtain long-term survival of porcine xenograft into non-human primate.

We believe that the three key immunological players which we have to keep under control for the long-term survival of our xenograft are the complement cascade, the T cell compartment, and the B cell compartment.

We have data which have explored also other aspects of the immune response, but we really do feel that these are the three main immunological players to control.

The complement activation, as I said before, we have now good transgenic pigs which are able to overcome hyperacute rejection, and they are still able to control a possible role of the complement later on once hyperacute rejection has been overcome.

As far as the T cells and the B cells are concerned, I think that this part of the slide wants to convey to you essentially two points. The first one is that some of the compounds which are used to target the T cell immune response, in fact, do not just play on the T cell compartment, but also if they are chosen appropriately, this will be compound, which will also down-regulate the B cell immune response. So, that was the concept number one.

The concept number two, which is as important or maybe more important, is that if we are able to choose

appropriate compounds, it is possible to build immunosuppressive strategies with additional or even synergistic effects, and therefore with a better control of the immune response.

The compounds with which today we are more familiar with are essentially the cyclosporin A, the RAD, which is a new macrolide, cyclophosphamide, ERL, which is formulation of mycophenolate mofetil, MMF, and I would say these are the key compounds with which today we have a reasonable experience at Imutran.

[Slide.]

That is another important issue here. I allow myself to comment on what Dr. Logan just said before, because it has been a great effort for us at Imutran to work hard to come up with an immunosuppressive strategy which is realistic.

By "realistic," we mean an immunosuppressive strategy which will not kill the recipient, and the second point, extremely important for us, is to come up with the recipe, possibly an immunosuppressive strategy which is not too different from what each of us in our department use in our patients.

Therefore, the cyclophosphamide issue, I just touched on that. Cyclophosphamide was a cornerstone of our immunosuppression more than five years ago. It still is,

but as an induction treatment and as an induction treatment I meant and I mean only four doses.

Then, we do have three compounds, a triple immunosuppressive fraction which is obviously tailored specifically for the immunological compartment here and a sort of immune response we have to place in xenotransplantation.

This, let's say maintenance immunosuppression is essentially based on cyclosporin, steroids, and a so-called third agent, and I will mention this in the second--I mean not cyclophosphamide, but I mean, for instance, RAD, for instance, ERL, for instance, mycophenolate mofetil, and other compounds like this.

As an anti-rejection treatment used so far, essentially steroids occasionally, we have also used occasionally cyclophosphamide.

[Slide.]

I will show briefly to you three slides on the experience at Imutran just to give you a little bit of perception of where we are.

These are heterotopic pig hearts which were transplanted into baboons where the third agent is MMF, therefore, cyclophosphamide four doses, third agent, I mean MMF plus cyclosporin and steroids, so three compounds as we do in the clinic.

Now, the results are as follows. I will show you coincidentally the four hyperacute rejection we have had so far at Imutran. In this series, I think the key message is hyperacute rejection. Unfortunately, we have seen it, median survival 15 days, and as previously said by Dr. Cooper, our longest survivor in this series went on for 99 days.

Conversely, the median survival in our control group was 5 days, longest surviving animals 10 days, and also here, hyperacute rejection as it would be expected.

[Slide.]

This is our series of orthotopic heart xenotransplantation, and again in this case, the so-called third agent is MMF. For us, it is extremely important to stress that this is orthotopic model.

The results, as they were previously mentioned by Dr. Cooper, longest surviving animals 39 days with a median survival of 11 days.

[Slide.]

I would move now to give you a perception of our experience in the pig-to-primate renal model. We have essentially focused our attention in Cambridge in the pig-to-primate renal model, and I would say that more than 80 percent of the data generated in Cambridge are data in the renal site, so I would say more than roughly 280, 290

transplants have been generated in the kidney site, and that is where we have learned a lot of things, and that is where we have done most of our exploratory work, and maybe we will continue to do that.

Now, if I look at the results in this series, as I said, animals which were treated either with cyclophosphamide as a four-dose inductions treatment, and as a third agent, mycophenolate, RAD, ERL, or our first series, cyclophosphamide as a third agent, I would say that the key message of these slides, as we said, hyperacute rejection is not seen.

A median survival, which is comprised between 32 days and 43, 45 days, depending on the sort of third agent we have used so far in Cambridge, the longest surviving animal which went on for 78 days.

Another thing which I would like to convey and bring to your attention, as I said before, our major obstacle has been, and is, acute vascular rejection. I would say that most long surviving animals in these series are lost due to acute vascular rejection, and not due to over-immunosuppression.

While we are learning our approach in trying to improve the survival and the condition of these immunosuppressed animals, we have also made some interesting drug combinations which have allowed us to now identify a

new pattern of rejection.

So, while we used to lose our organs due to hyperacute rejection or acute vascular rejection, acute vascular rejection is still our main enemy, if you want, but we are starting to see in our grafts, mainly in our kidney, a new pattern of immunological damage, and we believe that we are altering the immunological pattern of rejection with the new compound that we are exploring.

[Slide.]

If I can in this slide, just show to you what I mean by that, is that this is a xenograft where besides some area of acute vascular rejection, we can see some areas where the damage to the xenograft is cell-mediated damage, and we have decided--this is a kidney, this is the renal tubule--and we have decided to call this cellular xenograft rejection phenomenon, which I said is a phenomenon which we see today in the presence of area of acute vascular rejection, as well, in the xenograft.

Are we witnesses a new sort of immunological rejection where maybe we have more experience, are we seeing something which is similar to what we see today in our clinical arena, I don't know yet, but certainly we are facing something new, and maybe it is something new we have maybe already seen it in our allo setting.

[Slide.]

This slide, I just want to summarize the situation. Up to 78 day survival of life supporting kidney, 90 day survival of heterotopic heart, and 39 days survival for a life supporting heart transplant.

[Slide.]

What are we doing today at Imutran, where are we focusing our attention? Obviously, we are still trying to further characterize and control AVR, to control it better, and we are also generating physiological data to support clinical studies.

[Slide.]

AVR, how are we trying to address the specific problem? We are evaluating the significance of elicited anti-pig antibodies, as other group are doing at the moment. We are, of course, pushing further our capacity to investigate the cellular infiltrate using triple immunofluorescent technology, the cellular infiltrate which we see now not just in acute vascular rejection, but also in this area of cellular xenograft rejection.

We are undertaking a big word, which is aimed at characterizing antiprimate specific monoclonal antibody, and this is an item where we are really using a lot of effort and a lot of energy, just because we work in this model.

Finally, we are testing new immunosuppressive strategies as I said before.

[Slide.]

Physiology. We are at the moment also trying to address some physiological issue in our kidney model. I will show to you some data which show excretory and osmoregulatory functions of our kidneys.

We will touch on some aspect of physiology related to erythropoiesis. We are trying to generate data on the ADH, and I will show to you some observation with respect to calcium and phosphate homeostasis in our xenografted monkeys.

[Slide.]

Now, as I said to you, we have been able to maintain cynomolgus monkeys for up to 78 days, and I think that this slide wants to convey to you an important message. I mean you and I know very well that in the follow-up of our patients we usually use the creatinine as a key marker of expression of the work in xenograft, and if we look at the creatinine in the first months in a group of eight animals, what we see is that immediately after transplant, there is a peak in the creatinine level, which usually normalizes within the first week, and then we have animals which go on for several weeks, possibly for several months, I said up to 78 days is our longest survivor, with normal creatinine.

So, the take-home message is that these animals are kept alive with a creatinine which is normal.

[Slide.]

The same thing occurs for the sodium. Normal. Again, for as long as the rejection process does not take place.

[Slide.]

I said I would have mentioned some data with respect to the erythropoietin, and these slides want to study the levels of hemoglobin over the life span up to 60 days in a group of animals.

I think that this slide has another important message brought to your attention, but if we look at the green line, these are animals which are xenografted and then not exposed to recombinant erythropoietin.

For those of you who are not familiar, erythropoietin is a hormone which is secreted by the kidney, and it is fundamental for the production of red blood cells for the presence of hemoglobin in the blood.

What we can see here is that in this group of animals, which were part of a CYP study, we see that immediately after the transplant, we have a drop in the hemoglobin, reach level as low as 4 or 5 grams of hemoglobin per deciliter, at which point we will have to sacrifice.

So, the message that this slide wants to convey to you is that the porcine kidney doesn't seem to be able to sustain the production of red blood cells, the production of

hemoglobin.

Conversely, if we treat these animals with human recombinant erythropoietin, as you can see, the initial trend, which is a drop after the first few days, is easily reverted and we have animals which survive for more than 60 days, which are hemoglobin around 12 gram, which is substantially similar with the pre-op hemoglobin.

So, we may have come across, we may--why am I saying we may--because we are deeply investigating what is going on there, and we are not sure that the phenomenon that we are witnessing here, we are not sure if this is related to a physiological incompatibility between a pig and a primate, or if this is related to an immunological phenomenon for which the porcine erythropoietin is cleared and removed.

In either case, if there is a problem with respect to the erythropoietin, the presence of recombinant human erythropoietin, which we are using every day in our clinics, is able certainly to revert and overcome this problem.

[Slide.]

Here, I would like to bring to your attention a measurement of kidney function in terms of calcium and phosphate. This is another aspect of the physiological compatibility between non-human primate and primate that we are trying to investigate very aggressively.

I showed to you before the creatinine, sodium, they substantially remain normal, within the normal value for that species for the major part of the lives of these animals until the xenograft is not rejected.

As far as calcium is concerned--and we will see phosphate in the next one--what we see here is that after the second week, there is a rise in the calcium in some of the animals. For instance, in this group, this animal, the calcium remained substantially normal, but in some of these animals, it can go up and remains like this, around 5 to 7 mEq/L with substantially plateauing out without continuing to increase with the animal, which remained substantially healthy and normal, and doesn't seem to suffer from this hypercalcemia for up to 78 days.

[Slide.]

Phosphate. We have another phenomenon in this case. It is substantially the reverse, the contrary. What we see is that after a few days--at the beginning, we have a slight increase in the phosphatemia, and then a progressive decrease, which reads very low levels around day 28 and remains low for as long as the animal remains alive.

Interestingly enough, as I said before, up to 78 days we do not have any evidence that these animals are not able to tolerate with either mild hypercalcemia or this hyperphosphatemia. On the other hand, if the problem has to

be there, we know that our colleagues, nephrologists have the necessary drugs and medications to allow our patient to normalize these parameters in case this has to be a real problem tomorrow if we had to start clinical xenotransplantation, and we had a problem like this.

[Slide.]

This is the penultimate slide. It allows me to stress again a concept which has been touched on this morning by several colleagues who have spoken before me, and that is the limitation of the preclinical model that we are forced to use today in our laboratories.

I mean although it is certainly a model which has given to us the opportunity to learn a lot, and will allow us to continue to generate a lot of data, we believe that there are several problems which are related to the use of preclinical studies, and they were mentioned earlier today.

The first point is that some diagnostic intervention of even treatment modalities are difficult to fully evaluating on human primate. The collateral ethics of some therapeutic strategies are species-specific.

Today, as I said, our aim is not to do something magic, but to do something very practical which will allow us to arrive to the clinical arena. So, what we are doing today in some respects, for some immunosuppressive regimen, is already in place in the clinical study.

We are using, for instance, RAD, which is already in this country in a Phase III clinical trial, and some of the side effects we see with animals treated with RAD are never or very rarely observed by our colleagues who are using RAD in the clinical arena.

Some of the side effects may be the reason for which we lose some of our primates. Some potentially beneficial therapeutic strategies can now be tested on appropriate animal models. I am referring, for instance, for a reagent like Compath I, which is giving great results in clinical allotransplantation, the epitome recognized by the monoclonal antibody does not exist in non-human primate, and therefore, for instance, that reagent is not an option for us to be explored in the preclinical arena.

Finally, there are limitations which are due to the absence of well-validated, primate-specific reagents. I just touched on that a few seconds ago.

[Slide.]

The last slide. Basically, our conclusion is that despite the limitation with the primate model, we have been able to show to you a prolonged life-supporting xenograft function using h-DAF transgenic pig organ and I insist a clinically applicable immunosuppression. Graft function has been demonstrated in kidney and heart, and I would like, of course, to take the opportunity to thank the large team at

Imutran, some of the colleagues are here today, would like to thank them for the great effort they have put into this program to make it successful, and I thank you very much for your attention.

DR. AUCHINCLOSS: Thank you.

On your next to the last slide, the term "collateral effects," can we keep the military terminology out and just call it complications? I think that is what we refer to them as.

A question from me. In the initial report of your cyclophosphamide series 1995, it was specifically said that none of the animals that died had evidence of rejection, that they all died of complications of the immunosuppression, so that has now shifted, isn't that correct, with the newer immunosuppressive protocol, you now see acute vascular rejection, but survival of the animal itself, is that correct? Is that fair?

DR. COZZI: That is exactly the situation. Basically, the data you are referring to was our very early experience. Today, these protocols do not exist anymore, and the side effects, which were the reason for which we were losing the animals at that time, are not the reason for which today we lose our prime, that is correct.

DR. AUCHINCLOSS: John Coffin.

DR. COFFIN: I was wondering whether you had any

evidence as to whether the variability that you see here, host-specific or donor-specific, for example, in those two animals that had the hyperacute rejection, if you go right back at those recipients with another organ, do you again see hyperacute rejection, is that a donor or a host effect?

DR. COZZI: The answer is very--it would take a lot of time. To summarize a little bit the situation it hat basically, we have gone back to try to understand and explore the reason for which we lost the xenograft, and today we have not come across the real reason for which we feel we could have predicted death.

To put it another way, we don't know if it is a donor or recipient related effect. I can tell you that two of the hyperacute rejections occurred using two litter mates.

DR. COFFIN: In the case of more later rejection, is there any evidence for an effect of genetics of the donor?

DR. COZZI: Genetics of the donor, don't know.

DR. AUCHINCLOSS: David Cooper, did you have a question?

DR. COOPER: Emanuele, that was really a wonderful presentation, and you have done some fantastic work, and I think we all congratulate you and your group immensely.

I want just to pick you up, though, on the point