FOOD AND DRUG ADMINISTRATION (FDA)
Center for Biologics Evaluation and Research (CBER)
73rd Cellular, Tissue, and Gene Therapies Advisory Committee (CTGTAC) Meeting

2-DAY OPEN PUBLIC MEETING

Web-Conference
Silver Spring, Maryland 20993

June 30, 2022

This transcript appears as received from the commercial transcribing service after inclusion of minor corrections to typographical and factual errors recommended by the DFO.

ATTENDEES

COMMITTEE MEMBERS	
Lisa Butterfield, Ph.D.	Parker Institute for Cancer Immunotherapy
Tabassum Ahsan, Ph.D.	City of Hope
Marshall Bloom, M.D.	Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institute of Health
Bernard Fox, Jr., Ph.D.	Providence Portland Medical Center
Jeannette Yen Lee, Ph.D.	University of Arkansas for Medical Sciences
Sean Morrison, Ph.D.	University of Texas Southwestern Medical Center
Joseph Wu, M.D., Ph.D.	Stanford University
TEMPORARY VOTING MEMBERS	
Hugh Auchincloss, M.D.	National Institute of Allergy and Infectious Diseases National Institutes of Health
Sridhar Basavaraju, M.D.	Centers for Disease Control and Prevention Atlanta
Paul Conway	Patient Representative
Matthew Cooper, M.D.	Georgetown University School of Medicine
Eric Crombez, M.D.	Ultragenyx Gene Therapy
Jay Fishman, M.D.	Massachusetts General Hospital
Paul Kimmel, M.D., M.AC.P., F.A.S.N.	National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health
Samantha Maragh, Ph.D.	National Institute of Standards & Technology
Kathleen O'Sullivan-Fortin, Esq.	Consumer Representative ALD CONNECT, INC.
Paul Palevsky, M.D.	Pittsburgh School of Medicine

Caroline Zeiss, BVSc, Dip ACVP & ACLAM, PhD	Yale University School of Medicine
GUEST SPEAKERS	
Kristi Helke, DVM, Ph.D., DACVP	Medical University of South Carolina
Richard Pierson, III, M.D.	Mass General Hospital
Eckard Wolf, DVM	Ludwig-Maximilians-Universität München, Germany
FDA PARTICIPANTS/SPEAKERS	
Peter Marks, M.D., Ph.D.	Food and Drug Administration
Steven Bauer, Ph.D.	Food and Drug Administration
Wilson, Bryan, M.D.	Food and Drug Administration
Judith Arcidiacono, M.S.	Food and Drug Administration
Tejashri Purohit-Sheth, M.D.	Food and Drug Administration
Elizabeth Hart, M.D.	Food and Drug Administration
Adnan Jaigirdar, M.D., FACS	Food and Drug Administration
Patricia Beaston, M.D., Ph.D.	Food and Drug Administration
Steven Oh, Ph.D.	Food and Drug Administration
Deborah Hursh, Ph.D.	Food and Drug Administration
FDA ADMINISTRATIVE STAFF	
Prabhakara Atreya, Ph.D.	Food and Drug Administration

Christina Vert, M.S.	Food and Drug Administration
Joanne Lipkind, M.S.	Food and Drug Administration
Sussan Paydar, Ph.D.	Food and Drug Administration
Michael Kawczynski	Food and Drug Administration
PUBLIC COMMENTERS	
Dr. Eliezer Katz	eGenesis
Dr. Sanjoy Dutta	JDRF International

TABLE OF CONTENTS

OPENING REMARKS: CALL TO ORDER AND WELCOME

MR. MICHAEL KAWCZYNSKI: Good morning, and welcome to FDA's 73rd meeting of the Cellular, Tissue, and Gene Therapies Advisory Committee meeting. I am Mike Kawczynski, along with my DFO Christina Vert, and today's chair which is -- Dr. Lisa Butterfield will be managing today's meetings. Please note that, again, this is Day 2 of this series. We are a live public meeting, so please note if we do run into any technical issues, just like many of you have, we may take a momentary pause just to address that, so that you the viewers do not miss any of the content.

So, with that being said, I am going to hand it off to our chair, Dr. Lisa Butterfield. Dr. Butterfield, are you ready?

DR. LISA BUTTERFIELD: Yes, I am, Michael. Thank you. All right, good morning, everyone. I'm Lisa Butterfield, welcome to Day 2 of our discussion about xenotransplantation. I'd like to welcome all the members, the temporary members, all the participants,

and the public who are viewing us remotely today.

A bit of housekeeping, I'm going to remind everyone that I will be watching for those raised hands, and that's how we'll know to call on you. We're looking forward to another very robust day of discussion on this important topic.

So, to get things started, I'd like to turn things over to our designated federal officer, Christina Vert.

ADMINISTRATIVE REMARKS, ROLL CALL, INTRODUCTION OF COMMITTEE, CONFLICT OF INTEREST STATEMENT

MS. CHRISTINA VERT: Thank you, Dr. Butterfield. Good morning, everyone. This is Christina Vert, and it is my great honor to serve as the designated federal officer, DFO, for today's 73rd Cellular, Tissue, and Gene Therapies Advisory Committee meeting. On behalf of the FDA, the Center for Biologics Evaluation and Research, and the Committee, I am happy to welcome everyone for today's virtual

meeting.

Today, the Committee will meet in open session to continue to discuss regulatory expectations for xenotransplantation products. The Committee will continue Session 2 to discuss and make recommendations on animal organ and cells for transplantation into human subjects and their associated risk.

Today's meeting and the topic were announced in the Federal Registry Notice that was published on May 31st, 2022.

I would now like to introduce and acknowledge the excellent contributions of the staff in the Division of the Scientific Advisors and Consultants, including our director, Dr. Prabha Atreya, who is my backup and co-DFO for this meeting. Other staff are Ms. Joanne Lipkind, Ms. Tonica Burke, Ms. LaShawn Marks, Dr. Sussan Paydar, and Ms. Karen Thomas. They provide excellent support for this meeting. I'd also like to thank Mike Kawczynski in facilitating the meeting today.

Also, our sincere gratitude goes to many CBER

and FDA staff working hard behind the scenes trying to ensure that today's virtual meeting will also be a successful one. Please direct any press media questions for today's meeting to FDA's office of media affairs at fdaoma@fda.hhs.gov. The transcriptionist for today's meeting is Ms. Ora Giles.

We will begin today's meeting by taking a formal roll call for the Committee members and temporary members. When it is your turn, please make sure your video camera is on and you're unmuted and state your first and last name, your organization, expertise or role. When finished, you can turn your camera off so we can proceed to the next person. Please see the member roster slides in which we'll begin with the chair, Dr. Butterfield.

DR. LISA BUTTERFIELD: Thank you, Christina. I'm Lisa Butterfield. I'm the vice president of research and development at the Parker Institute for Cancer Immunotherapy and an adjunct professor of microbiology and immunology at University of California, San Francisco. My expertise is in cancer

vaccination, immune biomarkers, and cell therapies.

MS. CHRISTINA VERT: Thank you. Dr. Ahsan.

DR. TABASSUM AHSAN: I'm Tabby Ahsan. I'm vice president of cell and gene therapy at City of Hope. I'm a bioengineer by training. I have a long history in tissue engineering, stem cell regenerative medicine, and the last few years in immunotherapy.

MS. CHRISTINA VERT: Thank you. Dr. Bloom.

DR. MARSHALL BLOOM: Hi. My name's Marshall Bloom. I'm the associate director for scientific management at the Rocky Mountain Laboratories of the National Institute of Allergy and Infectious Diseases located in western Montana -- in Hamilton, Montana -- the place where Yellowstone is being filmed. And I'm an expert in virology and viral diseases. Thanks.

MS. CHRISTINA VERT: Thank you. Dr. Fox.

DR. BERNARD FOX: Yeah. Good morning. My name is Bernard Fox. I'm the Harter Family Chair for cancer research at the Early Child's Research Institute. I'm a member and chief of the Laboratory of Molecular and Tumor Immunology, and it's at Providence

Medical Center in Portland, Oregon. My area of expertise is in cancer immunotherapy, tumor models, cancer vaccines, adoptive immunotherapy.

MS. CHRISTINA VERT: Thank you. Dr. Lee.

DR. JEANNETTE LEE: Good morning. I'm Jeannette Lee. I am a professor of biostatistics and a member of the Windsor P. Rockefeller Cancer Institute at the University of Arkansas for Medical Sciences. Thank you.

MS. CHRISTINA VERT: Thank you. Dr. Morrison.

DR. SEAN MORRISON: Good morning, everybody. I'm Sean Morrison. I direct Children's Research Institute at the University of Texas, Southwestern Medical Center in Dallas, and my expertise is in stem cells and cancer.

MS. CHRISTINA VERT: Thank you. Dr. Wu.

DR. JOSEPH WU: Good morning. I'm Joe Wu. I'm a cardiologist. I am a professor and director of Stanford Cardiovascular Institute. My expertise is in cardiac and tissue engineering, stem cells, and gene therapy.

MS. CHRISTINA VERT: Thank you. Dr. Auchincloss.

DR. HUGH AUCHINCLOSS: Good morning. My name's Hugh Auchincloss. I'm the deputy director at the National Institute of Allergy and Infectious Diseases. And my expertise is in the immune response to xenotransplants.

MS. CHRISTINA VERT: Thank you. Dr. Basavaraju.

DR. SRIDHAR BASAVARAJU: I'm Sridhar Basavaraju, director of the CDC Office of Blood, Organs, and Other Tissue Safety.

MS. CHRISTINA VERT: Thank you. Mr. Conway.

MR. PAUL CONWAY: My name is Paul Conway. I serve as the chair of policy and global affairs for the American Association of Kidney Patients. I've been a kidney patient for 42 years. I spent three years on the organ donor waiting list while I did dialysis and I've had a kidney transplant for 25 years. I work in federal policy and regulation. Thank you.

MS. CHRISTINA VERT: Thank you. Dr. Cooper.

DR. MATTHEW COOPER: Good morning, everyone. I'm Matthew Cooper. I'm a clinical transplant surgeon, Director of kidney and pancreas transplantation at the Medstar Georgetown Transplant Institute. I'm also the current president for the Organ Procurement and Transplantation Network, and the United Network for Organ Sharing.

MS. CHRISTINA VERT: Thank you. Dr. Crombez.

DR. ERIC CROMBEZ: Good morning. I'm Eric Crombez. I'm chief medical officer of Gene Therapy and Inborn Errors of Metabolism at Ultragenyx, and I'll be serving as the industry representative at today's meeting.

MS. CHRISTINA VERT: Thank you. Dr. Fishman.

DR. JAY FISHMAN: Good morning. I'm Jay Fishman. I'm professor of medicine at Harvard Medical School, director of the Transplant Infectious Disease Program at Massachusetts General Hospital, associate director of the MGH Transplant Center, and my expertise is in infections of the immunocompromised hosts and infections associated with xenotransplantation.

MS. CHRISTINA VERT: Thank you. Dr. Kimmel.

DR. PAUL KIMMEL: Hi, I'm Paul Kimmel. I'm a senior advisor at the National Institute of Diabetes, Digestive, and Kidney Diseases. I'm a clinical professor emeritus at George Washington University. My expertise is clinical nephrology.

MS. CHRISTINA VERT: Thank you. Dr. Maragh.

DR. SAMANTHA MARAGH: Good morning. I'm Samantha Maragh. I'm a human geneticist and molecular biologist at the U.S. National Institute of Standards and Technology, and there I lead the Biomarker and Genomic Sciences Group as well as the genome-editing program. And my expertise is in bioassays, particularly nucleic acid measurements and genome editing.

MS. CHRISTINA VERT: Thank you. Cathleen O'Sullivan-Fortin.

DR. KATHLEEN O'SULLIVAN-FORTIN: Hi, good morning. I'm Kathleen O'Sullivan-Fortin. I'm the consumer representative for this meeting. I'm the co-founder and general counsel of ALD CONNECT, and my

expertise is in rare disease advocacy and as a rare disease patient.

MS. CHRISTINA VERT: Thank you. Dr. Palevsky. Dr. Palevsky? Paul Palevsky? There we go.

DR. PAUL PALEVSKY: Hi, I'm Paul Palevsky. I'm professor of medicine at the University of Pittsburg. I'm chief of the Kidney Medicine Section at the VA Pittsburg Healthcare System, and deputy national program director for the VA's Kidney Medicine Program. I'm a clinical nephrologist, and I'm currently president of the National Kidney Foundation.

MS. CHRISTINA VERT: Thank you. Dr. Zeiss.

DR. CAROLINE ZEISS: Hi, I'm Caroline Zeiss. I'm a professor of comparative medicine at Yale University. I'm a laboratory animal veterinarian and an anatomic pathologist. And my research expertise is in translational animal models. Thank you.

MS. CHRISTINA VERT: Thank you. Thank you, for your introductions. I would also like to acknowledge CBER leadership, including Dr. Marks and Dr. Bryan, who may be present now or joining the

meeting later today at other times.

I will now proceed with the reading of the conflict of interest statement for the public record. Thank you.

The Food and Drug Administration, FDA, is convening virtually today June 30th, 2022, for the 73rd meeting of the Cellular, Tissue, and Gene Therapies Advisory Committee under the authority of the Federal Advisory Committee Act, FACA, of 1972. Dr. Lisa Butterfield is serving as the chair for today's meeting. The CTGAT Committee will meet in open session today to discuss the current regulatory expectations for xenotransplantation products.

The Committee will continue Session 2 to discuss and make recommendations on animal organ and cells for transplantation into human subjects and their associated risks. The topic is determined to be a particular matter of general applicability.

With exception of the industry representative member, all standing and temporary non-voting members of CTGAC are appointed as special government employees

(SGEs) or regular government employees (RGEs) from other agencies and are subject to federal conflict of interest laws and regulations.

The following information on the status of this Committee's compliance with federal ethics and conflicts of interest laws include, but are not limited to, 18 U.S.C. Section 208 is being provided to participants in today's meeting and to the public.

Related to the discussions at this meeting, all members, RGE, and SGE consultants of this Committee have been screened for potential financial conflicts of interest of their own as well as those imputed to them, including those of their spouse or minor children and, for the purposes of 18 U.S. Code Section 208, their employers. These interests may include investments, consulting, expert witness testimony, contracts and grants, cooperative research and development agreements (CRADAs), teaching, speaking, writing, patents, and royalties, and primary employment.

These may include interests that are current or under negotiation. FDA has determined that all

members of this Advisory Committee, both regular and temporary members, are in compliance with federal ethics and conflicts of interest laws.

Under 18 U.S. Code Section 208, Congress has authorized FDA to grant waivers to special government employees who have financial conflicts of interest when it is determined that the Agency's need for a special government employee's service outweighs the potential for a conflict of interest created by the financial interest involved or when the interest of a regular government employee is not so substantial as to be deemed likely to affect the integrity of the services which the government may expect from the employee.

Based on today's agenda and all financial interests reported by Committee members and consultants, no conflict of interest waivers were issued under 18 U.S. Code Section 208 in connection with this meeting.

We have the following consultants serving as temporary voting members: Dr. Hugh Auchincloss, Dr. Sridhar Basavaraju, Dr. Matthew Cooper, Dr. Jay

Fishman, Dr. Paul Kimmel, Dr. Samantha Maragh, Dr. Paul Palevsky, and Dr. Caroline Zeiss.

We have one patient representative, namely Mr. Paul Conway, serving as a temporary voting member. Ms. Kathleen O'Sullivan-Fortin is serving as the temporary consumer representative for this Committee meeting. Consumer representatives are appointed special government employees and are screened and cleared prior to their participation in the meeting. They are voting members of the Committee.

Dr. Eric Crombez of Ultragenyx Gene Therapy will serve as the alternate and temporary industry representative for today's meeting. Industry representatives are not appointed as special government employees and serve only as non-voting members of the Committee. Industry representatives act on behalf of all related industry and bring general industry perspectives to the Committee. Industry representatives on this Committee are not screened, do not participate in any closed sessions, if held, and do not have voting privileges.

The guest speakers for this meeting are the following today: Dr. Kristi Helke, Director of the Medical University of South Carolina, veterinary diagnostic laboratory; Dr. Richard Pierson III, Scientific Director, Center for Transplant Sciences, Massachusetts General Hospital; and Dr. Eckhard Wolf, Professor at the Gene Center and Department of Biochemistry at the University of Munich in Germany.

Disclosure of conflict of interest for speakers and guest speakers follows applicable federal laws, regulations, and FDA guidance. FDA encourages all meeting participants, including Open Public Hearing speakers, to advise the Committee of any financial relationships that they may have with any affected firms, its products, and if known, its direct competitors.

We would like to remind standing and temporary members that if the discussions involve any other products or firms not already on the agenda for which an FDA participant has a personal or imputed financial interest, the participant needs to inform the DFO and

exclude themselves from the discussion and their exclusion will be noted for the record.

This concludes my reading of the conflict of interest statement for the public record. At this time, I'd like to hand over the meeting to Dr. Lisa Butterfield. Thank you.

DR. LISA BUTTERFIELD: Terrific. Thanks very, much, Christina. All right, well, now we move to Question 4 over our two-day meeting and to start things off we have a presentation from Dr. Pierson from Mass General on organ rejection. Dr. Pierson, please.

INVITED SPEAKER PRESENTATION: ORGAN REJECTION

DR. RICHARD PIERSON: Good morning and thank you for the privilege of the floor. My conflicts of interest are listed at the bottom of the slide. My task today, as assigned, is to review immunosuppression as it's been studied in preclinical transplant models and to discuss also the potential for tolerance induction as a valid approach to accomplishing safe and

successful xenotransplantation.

Based on the wonderful presentations yesterday, it will be no secret to people on this presentation that the reason that we're pursuing xenotransplantation is that it offers the prospect of solving the critical shortage of suitable organs -- human organs -- for available for transplantation. Our goal is to provide healthy, genetically engineered pigs that will allow us to safely provide life-supporting treatments for patients with end-stage organ disease.

The advantage of this approach is that we can define the quality of that graft ahead of the transplant. The organ can be obtained in the absence of the deleterious consequences associated with brain death in human donors and avoiding human diseases -- human infectious diseases, in particular.

By sourcing animals from SPF, specific pathogen-free facilities, we can minimize the risk of infectious complications. This was discussed at length yesterday and will obviously be an important discussion for the Committee.

Pigs were chosen among potential donor species because of their short gestational period, rapid growth to adult human size, and multiparous characteristics. Because of these features, once we've identified pigs with suitable genetics as organ donors for humans, the supply is potentially unlimited, and their organs can then be available when needed.

The ability to know when the transplant is going to occur facilitates conditioning of the donor and the recipient so as to optimize results, potentially allowing us to reduce the immunosuppressive burden. Eventually, we hope to induce tolerance as a strategy for optimizing results with minimal long-term consequences for our recipients.

What are the risks? These were well-reviewed yesterday by Dr. Fishman, Dr. Denner, and the other speakers. That was a great conversation, and I won't dwell therefore on the contents of this slide. The other potential risk that I would put forward is the need for us to pay attention to equitable access to this potential life-saving therapy, both in the phase

where we're still conducting research to optimize outcomes, and also then, once it's successful, to be sure that both the supply can meet the demand and that financial resources and other societal constraints do not limit access to this potentially life-saving therapy.

I'd like to review some of the recent progress in xenotransplantation. My talk will, by necessity, overlaps somewhat with Dr. Wolf's designated subject, which was to review the various genetic engineering modifications that have been introduced that have been associated with such significant progress in the preclinical model, and I apologize for that overlap.

But by necessity to talk about immunosuppression and what we've learned in that context, there is an intersection with the effects of genetic modifications. And so as I say I apologize for that overlap, but I hope that I will be able to clearly articulate what we have learned about the particular characteristics of xenotransplantation with respect to the important role of co-stimulation pathway blockade

in particular.

Finally, on a heart-specific point, there's evidence that ischemia minimization is a critical feature, at least for the heart. This may be generalizable to other organs as well, but that remains to be determined, and I won't dwell forward on the infectious disease aspects, but those, of course, are quite important.

Our efforts over the past 25 years in working on the pig-to-human xenotransplant opportunity have been very much focused on understanding the mechanisms that lead to the entry of an organ xenograft when it's exposed to human blood. That starts with the preformed antibodies present in almost all humans that recognize particularly carbohydrate antigens on the surface of pig's endothelial cells. That triggers complement binding as well as cell-mediated injury mechanisms both of which are important to triggering initial injury of the graft within minutes or hours.

In addition, both as a consequence of antibody-mediated endothelial activation and

complement-triggered injury to the endothelium, clotting cascades are activated which lead to the clinical phenotype of graft infarction and thrombus within the blood vessels as well as endothelial activation and leakage of plasma and then whole blood into the tissue of the graft.

Finally, one of the earliest pathways identified in xenotransplant injury relates to the absence of self-recognition receptors with a critical role for NK cells and monocyte-macrophage populations in being activated in the absence of self-recognition signals related to HLA-E and CD47 as examples listed here.

So, over the course of the past 20 years, we have focused first on the preformed antibody and complement by either absorbing the antibody by administering complement inhibitors. What we learned in that context is that, even when we did those things, we were still seeing graft injury delayed in phenotype but still with graft injury occurring quite quickly.

And so, with the advances in genetic

engineering, fast-forward 15/20 years, we've learned to knock out, first, one and then multiple of the carbohydrate-related genes, the Gal-13 Galactosyltransferase, the CMAH knockout which relates to the new 5GC antigen and the beta 4Gal gene which relates to the SDA antigen with the Gal being the most important.

With one or more of these genes knocked out, we reduce the importance, in fact, eliminate the importance of the preformed anticarbohydrate antibodies and, as I will illustrate in the next slide, that is associated with substantial improvement in graft behavior and survival in our preclinical transplant models. There are, in many humans, still antibodies observed that cross-react with even triple knockout pig endothelial cells.

For that reason, we have, in addition to the carbohydrate knockout genes, introduced human complement-regulatory genes which particularly when expressed at high levels are quite efficient to down-regulate the complement activation cascade and confer

by themselves substantial projection. And in the context of the carbohydrate gene knockouts, substantial additional projection, not only to preformed antibody but potentially also to elicited antibody in case the immune suppression administered is insufficient or ineffective.

And so let me illustrate now the progress that's been made over the past several years and begin to come to the subject of my talk, which is the role of various immunosuppressive regimens to modulate the immune response to an organ xenograft. I'm using here primarily the example of hearts because that's where the data is most robust and easily compared across different regimens.

The dotted line, the lowest in the legend, refers to wild-type pigs, and you can see that, when we transplant a pig heart into a cynomolgus monkey or a baboon -- most commonly baboons -- and use conventional treatment, most grafts -- three-quarters -- are dead within a week or two, and that's in spite of a variety of different approaches to adds or about antipig

antibodies or to use carbohydrate-blocking molecules as well as complement inhibitors. So, the best we could do in the hands of very competent investigators never got us past one month.

With the emergence of genetic engineering to add complement regulatory proteins to pigs in the setting of either conventional treatment or anti-CD154 co-stimulation pathway blockade, between 20 percent and 35 percent of animals could be prolonged beyond one month, but attrition by two months was still quite high and long-term survivals were quite exceptional.

You can see that in some of those groups the experience was really quite extensive. You'll note the end of 90 in the complement regulatory protein with conventional treatment. The Gal knockout was a major breakthrough, and you can see that in the solid line when Gal knockout hearts were transplanted in the context of co-stimulation pathway blockade, 75 percent of the grafts spread out to two months, and a minority of grafts were able to be prolonged beyond three months and even as long as almost six months.

So, that was a substantial step forward. I would also point out that the Mayo Clinic was able, with a carbohydrate blocking molecule and human membrane cofactor protein complement regulatory molecule expression, to achieve similar results based on conventional immunosuppression in the substantial series of experiments.

So what was limiting in that model is illustrated here, primarily thrombotic microangiopathy -- formation of clots within the blood vessels to the graft as well as leak of plasma proteins and, as you can see here, red cells into the (inaudible) of the graft.

In addition, the consumptive coagulopathy was observed in the recipients quite frequently with thrombocytopenia and hemorrhagic complications away from the site of the graft. That combination of phenomena was not prevented even when we took Gal knockout organs that expressed CD46 and used the most effective -- in my view -- immunosuppressive approach to the anti-CD154 blockade combined with induction

therapy including ATG and anti-CD20, and as you can see, again, a pretty substantial series of experiments -- fourteen achieved occasional long-term survivors.

But you can see with the red horizontal lines the incidents of thrombotic microangiopathy, and consumptive coagulopathy limited the duration of the experiments even in the absence of a detectible immune response to the xenograft. That problem then led us to focus on the coagulation cascade and to introduce human transgenes, particularly thrombomodulin but also endothelial protein C receptor.

And I won't take your time today to go through the mechanistic basis for this, but our preclinical results in the lung model would suggest that those two together provide better protection than either by itself in the lung model. Whereas in the heart model, thrombomodulin seems to be quite important and effective.

That's illustrated here in the breakthrough work reported by Muhammad Mohiuddin from working at that point in the NIH when thrombomodulin was

additionally expressed in the context of the Gal knockout and CD46 and using a co-stimulation pathway-based regimen with CD40 on anti-CD40 antibody, and the only other immunosuppression given to these animals other than the CD40 blockade was mycophenolate mofetil and low-dose steroids.

They did have an induction regimen with ATG and anti-CD20, and in that context, they were able to consistently achieve graft survivals beyond 150 days.

Illustrated at the top left -- I'm sorry that this is small in size, but I'll zoom in on an important feature of this in the next slide. In the green line when immunosuppression was down titrated at about 90 days, one graft was lost at about 150 days and the other went to about 250 days. When the reduction in immunosuppression was delayed to about 400 days or 500 days, the graft continued to function for longer periods of time out to 600 days and almost a thousand days.

Zooming in, you can see the IgM response on the top and the IgG response to pig donor cells. You

can see that during treatment, consistently the immune response was controlled. Whereas when the treatment was discontinued, illustrated with the horizontal dash green line or the horizontal dashed red line, the immune response was detectible relatively shortly thereafter and that was associated with demise of the graft.

So here, this work illustrated effective control of the antipig immune response in the absence of any instances of thrombotic microangiopathy or consumptive coagulopathy. In the view of the field, this was truly a disruptive and innovative and effective contribution.

So what had we learned at that point from this work regarding the mechanisms of Gal knockout human complement factor regulatory heart injury? The modification of carbohydrate gene knockout, at least in the context of T and B cell depletion induction treatment at the time of transplant coupled with either CD40 or CD154 blocked immunosuppression, was able to efficiently prevent elicited antipig antibody and

complement-related injury.

The consumptive coagulopathy, which had previously plagued us, is efficiently controlled by the additional expression of the thrombomodulin gene at least in the context of the complement regulatory protein and, of course, effective immunosuppression. I think Eckhard will talk more about the perioperative xenograft dysfunction, but I will talk about it a little bit in the subsequent slide and come back to that.

So, what about orthotopic heart transplants? Everything I've shown you so far is just observing whether the heart is still beating in the abdomen in a non-working mode? What about life-supporting function of the heart xenograft? The state of the art in 2017 had moved very little forward from the results published in 1999 from the group in Cambridge where they used an hDAF expressing heart in a monkey and used conventional -- I believe it was actually baboon -- and conventional immunosuppression and had survival beyond one month. Ended up with a healthy animal up until the

demise of immunologic causes.

After the extensive work by Chris McGregor and then replicated by Muhammad Mohiuddin and Bruno Reichart working in Germany -- at the NIH and in Germany respectively -- consistently found that more than half of the animals transplanted with a pig heart, even in the hands of very competent sophisticated heart surgeons, were unable to get -- about 50 percent of them were unable to survive the surgical procedure. And survival of more than 14 days was rare associated with inflammation in the host and in the recipient and graft injury. In addition, graft hypertrophy was identified when Bruno did some experiments in the heterotopic heart model.

Those problems were overcome in another landmark paper published from Bruno Reichart's group in Munich that Eckhard will tell you more about in the subsequent slide.

But this work truly was a breakthrough, showing that with the same basic immunosuppressive regimen that Muhammad Mohiuddin used: perioperative

induction therapy with ATG and B cell depletion, and then either a CD40 or CD154 targeting monoclonal antibody treatment. That, with additional MMF and steroids -- tapered steroids -- the grafts were consistently protected from immune injury in the absence of detectible elicited antibody.

They did use anti-inflammatory treatments in addition, based on work from David Cooper's group showing that IL6, TNF, and IL1 were elevated around the time of transplant. And with that combination of treatments plus using erythromycin to retard graft growth, they were able to get consistent survival to 90 days or 180 days limited mainly by regulatory resistance to letting them carry the animals out further.

And they had since replicated that in a consistent series which has met the ISHLT's 2000 recommendations for six out of ten -- in fact they got six out of eight -- long-term survivors. And the only grafts that they lost in that series of eight consecutive experiments using a consistent regimen were

two that had pig CMV activation, which I think was discussed yesterday, and illustrates, again, the great importance of excluding pig CMV from the donor in both preclinically and in clinical application.

Muhammad Mohiuddin was able to replicate this as David Cooper has been able to -- not included on the slide -- with survivors up to nine months as the longest yet. And in the main, all of these groups have used co-stimulation pathway blockade, T and B cell depletion as induction therapy, chronic treatment with mycophenolate mofetil, and in some instances with mTOR inhibitor to inhibit growth of the graft. Importantly in at least two groups -- the Mohiuddin group and the Reichart group -- have found that the ischemia minimization strategy is essential to prevent primary cardiac xenograft dysfunction.

Importantly, consumptive coagulopathy and thrombotic microangiopathy, nor other evidence like chronic vasculopathy have not been observed when Gal knockout parts combined with the CD46 complement regulator and thrombomodulin. Those problems have not

been encountered -- consistently not been encountered. And finally, the graft hypertrophy seems to be inhibited with the mTOR inhibitor.

I'll turn briefly now to kidney work. You can see that, as I illustrated for the hearts, when wild-type organs -- illustrated by the dotted line -- are used, it was rare to get a subject out beyond two weeks. Not much better results with hDAF complement regulator by itself. Gal knockout did a little bit better, but only ten percent of the grafts got out past a month with conventional immunosuppression.

Importantly, when immunosuppression was switched to complement pathway regulatory blockade with CD154, 65 or 75 percent of the grafts bowed out to a month, and a minority out to nearly three months. So that, I think, is the best illustration head to head of the relative efficacy of CD154 blockade. Proteinuria was the main limiting factor seen in a lot of these experiments.

Importantly, then, in recent work that some of the groups who generated this preliminary data, upon

which this slide is based, have now extended these observations and unpublished work. But suffice it to say that it appears reproducible that, by additionally knocking out the beta 4Gal antigen or depleting CD4 T cells as opposed to both CD4 and CD8 T cells in a double transgenic animal or blocking CD28 or just with Gal knockout CD55 and CD154 blockade, that consistent long term graft survival -- in some instances out to several years -- can be accomplished with the important caveat that these results are only achieved in animals that have low preformed titers of antibody to their donor pig.

So some of those results are illustrated here from the Emory group and you can see that with CD4 depletion combined with CD154 blockade, two out of three animals had long-term graft survival beyond a year, whereas if the CD8 T cells were depleted, no such results were achieved. It is important to note that when the crossmatch was positive, survival beyond two weeks was quite unusual even with the full immunosuppressant regimen.

The MGH group has not yet published their data, but suffice it to say, that using a regimen, which has been described in the literature that is very similar to what I've just illustrated for the heart -- T cell and B cell induction treatment, mycophenolate mofetil, tapered steroids, and erythromycin -- that they have gotten multiple survivors out past 300 days and consistent survival I would say beyond 150 days. That work should be prepared for publication soon.

So, briefly at the end, I'll turn to xenotransplantation to tolerance induction. David Sachs, Megan Sykes, Kazukiko Yamada have made major contributions in this area over 20 years and have developed a tolerance induction regimen based on mixed hematopoietic chimerism. They use inbred, genetically defined swine leucocyte antigen-defined pigs which have the Gal knockout and CD55 human transgene.

They have recently done work showing that the CD47 gene added to these pigs is protective and enables improved duration of bone marrow and graft using micro-chimerism, and you'll note that the regimen that

they're using based on CD154 and tapering immunosuppression is quite similar to that that I've illustrated in for the heart for multiple groups.

And even in the lung, they've been able to get survivors out to about two weeks and the thymoglobulin -- sorry, there's a typo -- they've gotten out to almost 200 days with immunomodulation that transplants the pig's thymus under the kidney capsule as a co-graft, and that strategy appears to be very promising as a platform.

So, with the right pigs and with immunosuppression such as I've described for you here, it would appear that the prospects for both successful immunosuppression and tolerance in xenotransplantation are quite good, and it would be a regimen like this that many of us feel that we would be able to make a case for going forward to the clinic.

What I think we've demonstrated, what I hope I've convinced you of, is that the co-stimulation-based immunosuppression is effective to protect a xenograft from immune injury, at least, in the context of the Gal

knockout and complement regulatory protein-expressing pig that also expressed thrombomodulin.

We do now have examples of less complicated genetics, specifically Gal knockout with beta 4Gal knockout or Gal knockout with human complement regulatory protein expression, that at least in the context of a negative preoperative crossmatch is associated with long-term graft survival in expanding series in the hands of multiple investigators.

And importantly acute cellular rejection is rarely seen with this regimen and consumptive coagulopathy and thrombotic microangiopathy in the heart or proteinuria in the kidney are consistently not seen in the recent experiences.

Important things that we do not know are whether induction therapy is necessary for these results. Also important, we don't know -- it has not been directly studied whether calcineurin inhibitors or conventional immunosuppression could be used to substitute for co-stimulation pathway blockade in every one of these models. But where these have been

compared head-to-head, co-stimulation pathway blockade appears to be more effective than calcineurin inhibitors in particular.

And again, the necessity for MMF and mTOR in long-term outcomes has not yet been rigorously proven, although the Germans have shown that mTOR weaning out toward the end of the experiment was associated with graft hypertrophy which would imply that that is going to be necessary, at least for the heart. And I would argue the tolerance may be achievable with certain genetic modifications in that mixed hematopoietic chimerism model.

Thank you for your attention, and I'd be happy to take questions now or at the end.

Q&A SESSION

DR. LISA BUTTERFIELD: Thank you very much. That was very interesting and a lot of important progress over the last few years. So, thank you for summarizing that for everyone on the Committee. We do

have about ten minutes for questions, and so why don't we go to first Dr. Denner, then Dr. Cooper. Dr. Denner.

DR. JOACHIM DENNER: Thank you very much for this nice talk. My question concerns the problems with coagulation. Would it be possible that in the early experiments, problems with coagulation may be due to an unrecognized, undetected infection with pCMV?

DR. RICHARD PIERSON: That is possible. Jay Fishman will be able to answer this better -- more accurately than I. But even in instances, particularly in the kidney where pCMV was not demonstrated -- and I think he mentioned that data to some extent yesterday -- the grafts were lost early if pCMV was detected, but grafts were still lost later in the absence of pCMV evidence of detectible pCMV activation. And, as I say, it was mainly proteinuria in the kidney experience, but I believe that was also seen in the hearts.

So, I think that while avoiding pCMV is clearly going to be important, I don't think it accounts for all the coagulation pathway dysregulation

that was seen. And I think that Muhammad Mohiuddin's work at the NIH used pigs which were from Revivicor's facility and were demonstrated to be CMV free, specifically designed because of that concern. And the consumptive coagulopathy without human thrombomodulin was still consistently seen. So, I think that it can't be excluded. It's hard to prove a negative, but I do believe that the coagulation pathway regulation is independently important.

What triggers it remains an important question. But it is for the purposes of designing a clinical trial, knowing that if you have human thrombomodulin or similar coagulation pathway regulator in the genetics offers, in my estimation, a protective advantage at least based on what we've seen so far.

DR. JOACHIM DENNER: Okay, thank you.

DR. LISA BUTTERFIELD: Thank you. Now, Dr. Cooper, followed by Dr. Auchincloss.

DR. MATTHEW COOPER: Hey, Robin [sic]. Outstanding work as usual. Really pleased, and congratulations on your own work. I'm fascinated, you

know, as a reader of this literature and with your experience with what you've presented today.

Do you have an opinion on, kind of, what now is the sweet spot in terms of the number of knockouts and transgenes that may be necessary sort of recognizing kind of the cost that's necessary for each additional modification that we talk about? In other words, a single versus triple knockout. Again, do you have a thought about kind of where the minimums are at this point?

DR. RICHARD PIERSON: So, I think for a kidney into an unsensitized patient, you probably can get away with -- and I'll use that term advisedly -- a Gal knockout with an expression of the human complement regulatory protein. I think that simple genetics could go forward with reasonable justification.

I think you will have an advantage by additionally knocking out the beta 4Gal. Probably in humans, unlike in non-human primates, knocking out the CMAH gene will additionally give you an advantage both by greatly increasing the number of patients for whom

the crossmatch will be negative to begin with and thereby allowing a much broader swath of the population of patients who might benefit to safely receive the graft. For the hearts, the minimum genes that appears to additionally include the pig/human thrombomodulin, and I think that data is persuasive.

If you have a triple knockout, might this problem be assuaged? Might you avoid consumptive coagulopathy and thrombotic microangiopathy? Maybe, but even in the examples with triple knockouts where we can't detect elicited antibody, we still see consumptive coagulopathy. And that's not a big N yet, but I'm persuaded that you need at least those three genes.

And again, will it be an advantage to have the two additional carbohydrate genes knocked out in addition to Gal knockout? I think it will be an advantage long term. Is it necessary to go forward? Is it necessary to achieve very impressive and potentially therapeutic clinical results? It will depend on clinical trials.

It is likely that long-term results will be better with triple knockout, but that's just my inference based on what we've seen preclinically and the mechanisms as we understand. Does that answer your question?

DR. MATTHEW COOPER: Perfect. Thank you, Robin [sic].

DR. LISA BUTTERFIELD: Thank you. And now, Dr. Auchincloss, please.

DR. HUGH AUCHINCLOSS: Robin [sic], that was a spectacular presentation. Thank you very much. Sort of an extension of Dr. Cooper's question. As you piled on more genetic modifications, have you seen any cost in terms of the fitness of the animals or the organs? Is there a downside to this much genetic manipulation?

DR. RICHARD PIERSON: So, adding additional complement pathway regulators has not been adversely consequential to the best of my knowledge. There is a fundamental fitness challenge with using cloned animals and understanding the impact of adding gene edits is difficult to separate from that challenge. With

coagulation pathway regulatory molecules, it is clear that, if you are too good at doing that, there is an incidence of bleeding abnormalities in the animals. Can one get through that? Yes, at least in some circumstances.

But there is a potential adverse effect on animal health. So maybe in the ideal future, we might be able to control the thrombo-regulatory gene expression with inducible promotors or conditional expression and that might offer a safer way for us to get pigs to the size where their organs are useful for humans, turn on the gene just around the time of transplant, and if it were associated with a problem later in the recipient, being able to turn it off. But that is not I think necessary for us to -- it is possible to get healthy animals with moderate levels of thrombo-regulatory expression -- gene expression.

We have very good preclinical data with those pigs suggesting that that is enough and safe, and indeed, the animals created that have that pattern of gene expression are healthy and both in the clone form

and I understand also as in the breeding situation are able to be propagated safely. So, I think, this is a subject for other experts who are actually working with these animals on the ground, and perhaps Dr. Wolf might be able to address it in addition. Did that address your question?

DR. HUGH AUCHINCLOSS: Yes. Absolutely. Thank you.

DR. LISA BUTTERFIELD: Super. Thank you. Now final question in this session from our patient representative, Mr. Conway.

MR. PAUL CONWAY: Hey, Dr. Pierson, thank you very much. Excellent presentation. Imagine you were standing before a room with kidney patients that were on a waiting list for an organ in their families. This is a question that I asked Dr. Denner yesterday, and he was kind enough to answer it. But I'm interested in your perspective.

Knowing what you know today and the progress that has been made and the research that's out there and the caution and the details that you've laid out,

do you have an optimistic sense about what the future holds for patients and for this as a modality whereas a solution, or are you extremely guardedly optimistic?

What's your sense of it if you were just talking straight with the patients' families? Thank you.

DR. RICHARD PIERSON: I am very optimistic that good results will be easier to achieve in our human patients than they are in our baboons or our monkeys. I think the pigs have been designed for human use, and it's fortuitous that these genetic modifications, also, many of them are effective. But the barriers in the preclinical model when we try to use triple knockout pigs, it turns out that that unveils an antigen recognized by monkeys and baboons that creates a positive crossmatch where it does not in human.

So that's one example where we sort of have to take a leap of faith when we go to clinical models, and, if I were talking to you about enrolling in a xenotransplant trial, I would explain that and tell you

that my best guess is that a triple knockout with whatever additional genetic modifications is quite likely to be able to support you for a year or two or three. Quite likely. I can't promise until we try it.

And while the results in braindead humans illustrate that hyperacute rejection doesn't occur, we don't know yet whether the treatments that have worked so well in the non-human primates will translate into humans. It is quite clear that, in my mind, that experimental immunosuppression with a co-stimulation pathway-blocking antibody will be an essential part of long-term success, either as an immunosuppressive regimen or for tolerance induction.

But I think that what we've learned in the non-human primates has positioned us for success. And I think that if we can certify that a pig doesn't have cytomegalovirus and that we have strategies for managing other aspects of infectious disease or that the clinical complications that we're likely to see, as we do in all of our transplant recipients are likely to be more easily managed in the clinic.

So, I am quite optimistic that when we hit the go button for doing these clinical trials, that we will be pleasantly surprised by the outcome. I hope that that's true, and I can't know it until we try it.

MR. PAUL CONWAY: Thanks for your candor. I appreciate it.

DR. LISA BUTTERFIELD: Thank you. And a final, final, short question from Dr. Palevsky.

DR. PAUL PALEVSKY: Thank you. I was just, to put the immunosuppression in context, I was hoping you could compare the experimental immunosuppression that you believe is going to be necessary to the current immunosuppression used for allotransplantation.

DR. RICHARD PIERSON: Certainly. The calcineurin inhibitors, which are the mainstay of current clinical immunosuppression, have side effects -- - renal damage, diabetes, neuropathy -- and they require being taken several times -- twice a day. Mycophenolate causes gastrointestinal complications and other issues. Erythromycin is so difficult to tolerate, that, even though it's quite effective, it

has not achieved broad use in transplantation.

The co-stimulation pathway blockade given once a week or once every two weeks is not associated with viral activation in our non-human primate models, and so it may be that it is safer and better tolerated or allows lower dosing of conventional immunosuppression. So, I would say that our immunosuppression either -- it will be possible for us to get good protection from the graft from immune injury with less intense immunosuppression than we currently use for our patients today.

DR. PAUL PALVENSKY: Thank you.

DR. LISA BUTTERFIELD: All right. Thank you very much.

INVITED SPEAKER PRESENTATION: GENETICALLY MODIFIED PIGS FOR XENOTRANSPLANTATION

DR. LISA BUTTERFIELD: That was terrific and now we're going to move to our second and final presentation before the question discussion. And

that's from Dr. Wolf from Munich, Germany on genetically modified pigs. Dr. Wolf.

DR. ECKHARD WOLF: Yeah, good morning and many thanks for the opportunity to discuss with you source animals with intentional genomic modifications. I'm afraid I will not be able to add a lot of new information because most of the aspects have been covered in the very elegant talk by Dr. Pierson.

So, these are my disclosures, and I think there is no doubt that at the moment the pig is the most likely donor or animal for xenotransplantation because the organs are very similar to human organs in terms of anatomy and also physiologic aspects. We can breed pigs very well.

One generation pig takes only one year. They have large litters. We can breed them under designated pathogen-free conditions and ensure that they don't have a risk to distribute infections. And the most important point is that we can do genetic engineer, and in this aspect, they are superior to non-human primates where genetic engineering is not possible or very

difficult. And also, in terms of ethical acceptance, pigs are much better.

You need to do genetic engineering to overcome rejection mechanisms, also physiological incompatibilities, and eventually to reduce the risk of porcine endogenous retroviruses, and I'm sure that you remember that 20 years ago when it was discovered that porcine cells release PERV that may affect human cells. This was a very difficult situation for xenotransplantation because at this stage clearly the fears dominated over the hopes into this technology.

Well over the last 20 years, a number of genetic modifications have been made in order to produce source pigs for xenotransplantation. One group concerns the deletion of enzymes that synthesize certain sugar epitopes against which the humans have pre-formed natural antibodies or other enzymes alpha-1,3 galactosyltransferase, CMAH, B4GALNT2, and recently a second enzyme B4GALNT2-like has been discovered that also has to be knocked out in order to completely remove the SDA epitopes.

Another group of modifications are proteins that regulate the complement pathway and (audio skip) a third group concerns transgenes that need to overcome a coagulation (audio skip) regulation, namely thrombomodulin EPCR tissue affect the pathway inhibitors CD39 and CD73. Then a number of transgenes has been proposed to overcome (audio skip) rejection by T cells, T cell co-stimulation blocking molecule for the knockout of swine (audio skip) antigens for the expression of HLA-E in beta-2-microglobulin or CD47 to inhibit in K cell activity or microphage activity, respectively.

In preclinical experiments, so far mainly in mice or in combinations with other transgenes in non-human primates, anti-inflammatory proteins have been tested such as A20 human heme oxygenase or (audio skip) soluble fragment. An important modification that ought to be necessary, especially after heart transplantations (inaudible) growth hormone receptor knockout, and of course, there are modifications that are related to either the knockdown or the complete

knockout of PERV expression.

When you think about the tools that we have available for genetic engineering of pigs, the classical technique was the DNA microinjection technique, where pieces of DNA gene constructs were injected into one of the two pronuclear zygotes. This was a very random technology where one could not determine how many copies of a transgene are integrated and where they are integrated.

And nowadays most groups rely on somatic nuclear transfer because then all significant modifications can be done in cell culture. We can select cell clones that have exactly the right genetic makeup, and then you use once cloning according to the Dolly technique in order to generate the corresponding pigs to give targeted genetically modified cells.

And with the event of gene editing, especially the CRISPR/Cas9 system, it's now also possible to knock out one or even several genes by zygote injection of these systems.

Just a few comments, somatic cell nuclear

transfer, and this is a very impressive study where they have looked at the outcomes of over 200,000 nuclear transfers, and what we can really see is you see in the last column that general efficiency is very low, usually below five percent for an offspring based on the number of transferred clone embryos to recipients. And unfortunately, there is also a large number of abnormalities, and this is probably related to the effect that nuclear transfer cloning produces a very high epigenetic malleability.

This has been best investigated in terms of DNA methylation, so there's a large variety in DNA methylation levels. But recently there are also studies that histone modifications are effective, and, in some studies, there was also a phenotypic correlate. This is one example where a group grew at the placenta of cloned piglets, and, in some of those placenta, they found both pathological abnormalities, but also a dysregulation of the expression of genes that are essential for placentation.

So, I think cloning -- as a result of these

observations, cloning is a very good technique in order to derive the genetic modified founder animals but those animals that are actually used for transplantation -- as owners for transplantation, they should be produced by breeding because fortunately it has been shown that once cycle of sexual reproduction is sufficient to erase all these epigenetic abnormalities.

By coming back to the essential genetic modifications, and this has already nicely been shown by Dr. Pierson, pig cells have these carbohydrate antigens on their surface against the humans and also non-human primates in part have pre-formed natural antibodies. Antibodies find their targets, activate the complement system, which finally leads to a hyperacute rejection of the porcine cells.

This can be overcome either by knocking out these galactosyltransferases or by the expression of one or several complement pathway regulatory proteins and basically using a combination of these modifications, it's really possible to overcome the

hyperacute rejection of pigs to primate xenograft. And the removal of this antigen, of course, also eliminates the antibody-dependent cellular cytotoxicity.

When we look at the human antibody binding to normal pig cells, you can clearly see that there are already a lot of antibodies in very young children, and the antibody levels increase both IgM and also IgG. And when the three galactosyltransferases have been knocked out, basically, this binding is minimized, and therefore for humans, probably the TKO pig is the best donor animal.

However, there is a problem in the transplantation experiments in non-human primates because apparently, the knockout of CMAH increases the binding of baboon antibodies to pig cells, and therefore it may be problematic for the pig in the studies.

It has been shown by David Cooper and colleagues that this can be reduced by adding additional protective transgenes, as you can see here. But for me the question, of course, arises do you need

additional genetic modifications in order to overcome a problem that exists in the pig (inaudible) model but would not exist in humans?

This was also tested in vivo in a recent transplantation experiment of porcine kidneys into cynomolgus macaques. Here you can see that TKO pigs were used, and they were combined with different transgenes, complement regulatory proteins, but also proteins regulating macrophage activity, or the activity of natural killer cells, or PDL1, a negative co-stimulating molecule. And you can clearly see that the effect depended on the level of the expression of the transgene.

Apparently, TKO-B which was combined with a high expression level of the complement regulatory proteins work better -- at least gave in some instances better long-term results -- as when these complementary regulatory proteins were low and only the other transgenes were highly expressed.

This is another study that was already cited by Dr. Pierson, where a relatively simple genetic

background has been used only with a Gal knockout combined with the expression of human-CD55, and kidneys of these donors were transplanted into rhesus monkeys with a negative crossmatch. Also the CD4 T cells were depleted. Then consistently a relatively long survival of up to 500 days was achieved, indicating that the long-term survival of xenografts was with a relatively simple genetic background of the donor pig.

In addition to the hyperacute rejection, there are, of course, at the cellular rejection mechanisms by natural killer cells, and those can be overcome for instance by the expression of HLA-E beta-2-microglobulin in the donor pigs because they bind the inhibitory CD94/NKG2 receptor on macrophages, and this is a study from our lab that it could clearly show that the cytotoxicity is markedly reduced on pig cells that express the HLA-E and also the secretion of the proinflammatory cytokine interferon-gamma is significantly reduced.

And as mentioned already by Dr. Pierson, a strategy to overcome macrophage activation is the

expression of human CD47 that binds SIRP alpha (inaudible) receptor on human macrophages. In contrast the porcine CD47 does not interact efficiently with the SIRP alpha. But these two modifications have not been tested, at least not alone in non-human primate transplantation experiments, so, so far, their effect and their necessity for clinical studies is not clear.

And finally, we also have to overcome adaptive immune mechanisms. T cell activation that can occur directly by porcine antigen-presenting cells or indirectly by human antigen-presenting cells, a very important tool is the blockade of the CD40 ligand pathway using specific blocking antibodies or the CD86/CD80 pathway -- CD28 pathway by using synthetic proteins like CTLA4-Ig or the affinity optimized variant LEA29Y.

There are also recent studies using the expression of negative co-stimulatory molecule PD-L1, but it is not clear how big this effect actually is. And there is the idea to knock out SLAs, but this would only overcome the direct T cell activation.

In addition to rejection mechanisms -- or I should mention here that local expression of LA29Y were successful to protect islet grafts when these islet grafts were transplanted under the kidney capsule of mice.

This is a histologic infection showing expression of LEA29Y in the pancreatic islets, and, when such islets are transplanted into the diabetic mice as the human immune system, all the mice stayed normal glycemic over the whole observation period where its wild-type islet were rejected. And here you see the reaction at the transplantation site after transplantation of wild-type islets; they are barely entering the positive cells. Instead, a massive T cell infiltration in contrast after transplantation of this LEA29Y transgenic islets, a large mass of insulin-positive cells.

It's T cell infiltration, but not in the graft itself, but only in the vicinity. In the more recent study, we were able to show that this works only also in the long term. These are mice which were

transplanted seven months ago, and you still see that there is a lot of insulin-positive tissue. And these mice had a completely normal glucose tolerance, and these islets are now non-human primate studies.

On the other hand, we noticed that issue is overexpressed LEA29Y systemically in the donor pigs. It reduces the development of the immune system, and they are immunocompromised and very difficult to maintain.

Another possibility is the SLA knockout, which may also cause some immune defect in the donor pigs. To our understanding, it's not necessary if the recipients have a negative crossmatch to TKO cells. It may be useful if the recipient has a crossmatch with anti-SLA antibodies, then it might be useful to knock out or knock down SLA-I or SLA-II.

In addition to immune rejection, we have to deal with coagulation disorders, and there are some incompatibilities between the porcine and the human blood coagulation systems. One example is to pair thrombin and thrombomodulin that is on the endothelial

cells.

Peter Cowan has shown that porcine thrombomodulin is able to bind human thrombin, however, the complex is relatively weak in activating protein C. And as a consequence, we observe thrombotic microangiopathy in the transplanted organs.

And for this reason, it's necessary to encrypt the pigs with the human thrombomodulin. We did this with a construct where we place the coding sequence with the human thrombomodulin under the control of porcine thrombomodulin regulatory sequences.

And at least in the heart, it gives us a very nice expression of endothelial cells all nicely decorated with the human thrombomodulin that is biologically active and in turn prolonging the clotting time.

And when Muhammad Mohiuddin tested organs from these pigs that had a Gal knockout expressed the CD46 transgene plus the human thrombomodulin, we can see that one of these organs survived in the heterotopic abdominal transplantation model in baboons for 945

days, and it stopped only to beat when the immunosuppression was completely discontinued.

And in a later study he showed that thrombomodulin was really key to the success because we needed some transplants with hearts that had only the Gal knockout and the CD46; the survival time was markedly reduced, and there were clear morphological signs of thrombotic microangiopathy.

One question is whether EPCR, the endothelial protein C receptor, is essential -- additionally, essential to be humanized in order to prevent coagulation disorder.

This is a study also from the lab of Peter Cowan, where he clearly showed that the porcine endothelial protein C receptor works with the human thrombomodulin eventually a little bit less well than the human endothelial protein C receptor. But these differences were not significant. And at least for the heart transplantation, we believe it's not necessary to express the human endothelial protein C receptor.

Well over time, a number of techniques have

been developed to combine (inaudible) of these modifications. This is an approach by my colleague Angelika Schnieke in Munich where she simply generated large suppression constructs and injected them into cells that were randomly integrated. And using this, she was able to get a quite good expression level.

Another strategy is to do a targeted integration into a safe harbor locus, such as the ROSA26 locus, which worked for (audio skip) genes and gave also a very good expression level.

However, an even more clever approach is basically to integrate transgene expression vectors into the loci that (inaudible) to be inactivated, and this is an example from a (inaudible) where they basically integrated expression cassettes for the CD46 and CD55 into the CMH locus and four expression cassettes with thrombomodulin EPCR, CD47, and heme oxygenase-1 into the Gal locus. And this, of course, facilitates the breeding of the animals because you reduce the number of independently segregating (inaudible).

And this is so far the most expensive germline genome engineering in pigs performed by eGenesis. They performed the three knockouts and generate a large vector based on the modified piggyback vector, where they have in one expression cassettes from many transgenes. However, not all of these transgenes were expressed tastefully. You can clearly see that, for instance, the thrombomodulin shows a reduced expression, whereas the CD39 showed increased expression as compared to humans.

In spite of the new possibilities of genetic engineering of pigs, surprisingly long-term results -- consistent long-term results have been achieved with donor pigs carrying only a relatively small number of genetic modifications. For instance, here is an example from Munich where the donor pigs had only a Gal knockout CD46 transgene and the thrombomodulin transgene, and the hearts of those pigs were subjected to a special perfusion treatment with (inaudible) hyperoncotic solution that prevented hypoxic damage of the hearts before implantation, and they were then

transplanted into baboons orthotopically and consistently a survival of several months has been observed. The four experiments with the 90 days, they had to be terminated at this stage according to the experimental protocol.

In two experiments, we were allowed to run them longer, and they survived for half a year. And the main problem at this stage was that the hearts got too large for baboons. And this was already mentioned. Unfortunately, the two donors for these experiments, they were positive for porcine cytomegalovirus, which markedly reduced the survival of these hearts.

The basis for this success is a consistent transgene expression. You can see here the analysis for the CD46 and for the thrombomodulin in all the hearts that were transplanted and that we analyzed by immune histochemistry after explanation. When the experiment was terminated, we saw a very nice consistent transgene expression on the right side.

Another important message that we learned from these experiments -- and this is a very nice paper by

Dr. Denner from our consortium -- is that the donor pigs must not be infected with the porcine cytomegalovirus. It reduces the survival of the transplants. When he analyzed the viral load of different organs of the donor pig and of the recipient baboon, he found that, in the heart, the viral load was several orders of magnitude higher than anywhere else. So apparently the main infection is in the heart.

To my knowledge, it's not even clear if baboon cells or human cells can be infected with the porcine cytomegalovirus, but there is clearly a damage of the transplanted organ, and there is a systemic upregulation of proinflammatory side effects.

And the last aspect that we learned from these experiments was that we have to take care of the growth of the transplanted heart because pig hearts grow very rapidly and, at least for a baboon recipient, they get in a short time too large. We can control this for some time by treating the animals with temsirolimus, which is a erythromycin prodrug that inhibits the activation of mTOR. And for this, we can prevent the

hypertrophy of the cardiomyocytes.

This is a non-treated animal, and this is a treated animal. There you can see that the diameter of the cardiomyocytes is much smaller. However, when we discontinue the treatment with erythromycin, the heart immediately starts to grow.

So, we have to think about the strategy; how to get these donor animals smaller, since this growth phenomenon was not only observed in the heart, but also for the kidneys. This was an allotransplantation experiment performed by Kazukiko Yamada where he clearly showed that kidneys from land-raised pigs after transplantation into mini pigs, they continue to grow as they were still in the large pigs.

As a potential strategy to reduce the growth of the animals, we propose to do the knockout of the growth hormone receptor gene, and here you can clearly see that the animals are smaller, their IGF-1 levels are markedly reduced, and also the hearts are smaller. And in the meanwhile, we have this also on the background of pigs that are suitable for

xenotransplantation.

Muhammad Mohiuddin has tested growth hormone receptor-deficient hearts in orthotopic transplantation experiments to baboons, and you can clearly see there was a large effect -- significant effect on the prolongation of the life span of the animals, and also the hearts did not show any hypertrophy.

However, when we look more closely to these growth hormone receptor knockout pigs, they are not completely normal. When they are young, they display a juvenile hypoglycemia. This is absolutely the same as in patients suffering from a growth hormone receptor deficiency. We see also a major metabolic disturbance of the liver, and we see that the animals get relatively obese because the lipolytic action of growth hormone is missing, so the breeding of those animals might be relatively difficult.

Therefore, I believe although we initially proposed the growth hormone receptor knockout, and we did not see any abnormalities on the protein levels and the functional levels in the heart, I think it would be

wise to choose a genetic background that's fit for the size of human recipients.

Well, and the last topic, of course, is to activate PERV to eliminate the risk of PERV transmission. This has been done as you know by George Church. Geneticists, they were able to inactivate all PERV integrants by mutagenesis of the pol gene and were able from mutagenized cells basically to clone pigs. However, it's questionable whether this is routinely necessary because they are also some risks associated. This is only possible with some tricks, for instance, a use of a p53 inhibitor. Some of the cells or the majority of the cells that have been analyzed, they showed major chromosomal abnormalities.

There are other ways to increase the safety in terms of PERV, which is to choose PERV C3 donor animals and eventually also do appropriate tests; monitor the patients. But this is still a matter of discussion.

So, to summarize the current state, I think the use of pigs as source of cell tissues and organs for xenotransplantations offer unique opportunities

since we can genetically engineer the donor pig. Many (inaudible) modifications have been made, but only few of them have been really evaluated in non-human primates.

Genome editing is, of course, speeding the progress and the combination of genetic modifications required depends on the type of organ and tissue and especially for cellular gene and xenograft also on the transplantation side. A very important point is the cellular localization and the consistency of transgene expression for the functionality and also for potential side effects. Remarkable long-term survival has been achieved with relatively few genetic modifications of the donor pigs, and therefore xenotransplantation can be considered as a future therapeutic option.

Specifically, for the source pigs, we have to ask the question: are more genetic modifications always better? I think when we want to demonstrate the efficacy and safety of individual modification, this is definitely easier if there are only few. The same is true for the demonstration of long-term stability and

expression of each modification. The cellular localization is important.

Transgene expression is difficult to modulate once a transgene is already active in the transplant, whereas drug treatment can be dose-adjusted or even discontinued. With an increasing number of genetically modified loci, a breeding strategy becomes complicated. I think cloning is not really a reliable procedure for the routine production of organ source pigs because we have this high epigenetic variability. There may be unpredicted interactions between the various modifications, and some modifications may have unforeseen negative effects and an example is the increased antigenicity of pig organs that lack CMAH in non-human primates.

So that's it for my slides. Thank you very much for your attention and I'm happy to take questions.

Q&A SESSION

DR. LISA BUTTERFIELD: Thank you so much, Dr. Wolf. I'm going to watch for some questions. Looks like there's a lot of opportunity and a lot of complexity yet before we get where we need to get. All right, we'll start with Dr. Auchincloss, please.

DR. HUGH AUCHINCLOSS: Another beautiful presentation. Thanks so much. So, what is the sweet spot? You've emphasized that, with relatively few genetic modifications, we can do quite well. But surely relatively few is not the ideal spot. What would be the ideal spot in your mind currently?

DR. ECKARD WOLF: So I believe that for heart transplantation the knockouts of the 3 galactosyltransferases plus a solid expression of thrombomodulin on the endothelium and the solid expression of one complement regulatory protein in CD46 may be/could be sufficient for the first trials.

DR. HUGH AUCHINCLOSS: Thank you.

DR. LISA BUTTERFIELD: Thank you. Dr. Kimmel.

DR. PAUL KIMMEL: Thank you very much, Dr. Wolf, for really a tour-de-force talk. I have a

question that may be a little bit tangential. I remember 40 years ago there were data that growth hormone in rodent models was not the only mediator of hypertrophy of the kidney after uninephrectomy. Are there other mediators of hypertrophy, either for the kidney or the heart, that should be thought of besides growth hormone?

DR. ECKHARD WOLF: Well, there are other mediators, but the problem is always when we interfere with this, we still have to make sure that the animals are more or less healthy. And we can propagate them by breeding, and we looked at many other growth regulating cascades like directly the insulin-like growth factors, growth hormone itself. But the knockout of the IGF-1 is lethal before birth and the knockout of growth hormone itself is associated with breeding problems.

So, the growth hormone receptor knockout was the modification that affected the health of the donor animals minimally basically. But still (inaudible).

DR. PAUL KIMMEL: It's the same kind of question.

DR. ECKHARD WOLF: Yeah.

DR. PAUL KIMMEL: No, it's a question of the genetic sweet spot. I'm sorry I interrupted you.

DR. ECKHARD WOLF: So, but still the growth hormone receptor knockout pigs, they are not completely normal.

DR. PAUL KIMMEL: Thank you very much.

DR. LISA BUTTERFIELD: Thank you.

DR. ECKHARD WOLF: I should add we did a holistic proteome study of the heart of these animals, and we did not detect any major difference that we had the feeling could affect the function of the heart. So, the heart seems to be relatively little affected. The liver is quite affected.

DR. LISA BUTTERFIELD: Thanks for that addition. Dr. Zeiss.

DR. CAROLINE ZEISS: Hi, Dr. Wolf. I wonder what the possibilities are for conditional knockouts because if we consider things like mTOR, if you (audio skip) completely, that's also lethal, and that is pretty central to hypertrophy. Is it feasible to do

conditional knockouts and to knock this out simply in the organ before it goes into the person?

DR. ECKHARD WOLF: It is, in principle, possible we can do also induce the transgene expression, but this, of course, complicates the whole thing enormously, and I think for a routine production of donor pigs, this is not suitable. This is suitable for experimental purposes, but not for the routine provision of organs.

DR. CAROLINE ZEISS: Okay. Thank you.

DR. LISA BUTTERFIELD: Okay, Professor Fox.

DR. BERNARD FOX: I'll just add my comment that these have been two amazing presentations, and why I think it's clear to me that what you've done is really setting the stage for very successful transplantation.

One of the things that I haven't thought about and was struck by in both presentations was this concept of the aging of the organ and looking at the timeline that you've done where you've transplanted tissues that were out 900 or a thousand days and

learning about how the organ is growing and in the case of the heart. But in the case of the other organs, the aging process in the pigs, how long do you think these organs will survive in humans once they're transplanted?

If the immunobiology is appropriately handled so that rejection is not an issue, what is going to be the timeline? Is this something that over a period of 25 years, somebody might have to have multiple transplants of an organ?

DR. ECKHARD WOLF: I would say not multiple. Eventually, two. So, pigs have a life expectancy of 20 years or so, and you also have to consider that you start with a relatively young organ. Organs there is, at least in Germany, 50 percent of the organ donors are older than 50 years.

DR. BERNARD FOX: Yep.

DR. ECKHARD WOLF: So, you start with a young healthy organ.

DR. BERNARD FOX: Thank you.

DR. LISA BUTTERFIELD: All right. Thank you

again for that perspective.

COMMITTEE DISCUSSION QUESTION #4

DR. LISA BUTTERFIELD: So, I think now we'll move to discussion of our question. So, we're moving to Question 4 and have a look at that question.

Transplantation of animal cells and organs into humans is associated with hyperacute rejection, vascular injury, cell-mediated rejection, and chronic rejection. Options for controlling rejection include genetic modification of donor pigs, modulation of the immune response in the recipient. Please discuss the most promising strategies to prevent rejection of pig organs.

In our discussion, please consider the balance between potential benefits of the desired genetic modifications and/or immune response modulation and the potential for detrimental transplant outcomes. So, to get things rolling, we have two discussants this morning. The first is Dr. Cooper.

DR. MATTHEW COOPER: Dr. Butterfield, I hope you can hear me. Of course, just like technology, right at the time when I was expected to talk my computer decided it was not going to work. So can you hear me okay?

DR. LISA BUTTERFIELD: I hear you fine. And we've had a flurry of updates as well today, so it's a little touch technologically, but please carry on.

DR. MATTHEW COOPER: Yeah, I'm sorry about that, everyone. So, I have the unenviable task of actually following two of the most outstanding presentations I've heard on this topic. I'm not sure who I offended at the Agency, but it really is incredibly interesting to follow the work that's been done thus far and to consider really where we are today. I won't look to recount everything that's been shared by our speakers except to say that, again, I think we have lots of opportunities.

Particularly when we think about the question and even as we tried to ask a number of our speakers what is kind of the minimum need, and then what is the

absolutes? Those are two, I think, very sort of different questions. I think if we appreciate the fact that we do have, as opposed to allotransplantation, the advantage of time and genetic modifications. We do, I think, appreciate that we have, again, the ability to control those minimums, and then as we continue to advance the science along with, as many have suggested, the need for kind of prospective ongoing adaptive clinical trials we can continue, I think, to improve our model, and hopefully with that, our outcome.

We have really an alphabet of opportunity, so I think everyone would certainly agree, and our speakers -- as many of our questions love to get to -- would, I think, all agree that the minimum that's necessary is at least in a single Gal knockout. Although, with a triple Gal knockout -- so with a triple knockout as Dr. Locke presented during the open session, there is the ability to potentially open up opportunities for negative crossmatches for larger patient population.

I'll get to more of that in a second, but some

of the additional things are complement regulatory proteins, thrombomodulin, the ability to knock out growth hormone and PERV sounds very exciting and certainly necessary for avoidance of some of the infections that complicates this moving forward.

We even have the ability for, like we said, genetic modifications to improve the physiologic compatibility, i.e., the ability for blocking growth hormone.

What we have to recognize is as we've, again, looked at the progressive implementation of new transgenes, we don't really know in the human xenotransplant model what the addition of each of those transgenes means because we really have been unable to attest it beyond non-human primate model, and I think that's important.

Many have said throughout the course of our two days that the xenotransplant is very species-specific, and unlike the non-human primates, in xeno crossmatches, we can potentially modify that in humans with interventions that we currently use for (audio

skip) looks like, sorry I think I lost you there for a second.

MR. MICHAEL KAWCZYNSKI: No, that was me. I muted you by accident. Sorry about that, Matt. Go ahead.

DR. MATTHEW COOPER: I get that done on a regular basis, Michael. So, I'd say the interventions such as plasmapheresis, total plasma exchange and IVIG to reduce any bio levels have been demonstrated to be quite effective in reducing median channel shifts to allow for the avoidance of hyperacute rejection. And so, even in the face of a single Gal knockout, there's the opportunity to potentially allow for interventions that we currently use an allotransplantation, again, that would, again, minimize the additional cost and time that's necessary to produce that ideal transgenic and knockout pig that we continue to talk about.

What we also have to recognize and reflect upon a lot of the conversations we had yesterday that is looking at the benefit. You know, often the transplant recipient for many of the organs in which we

utilize as a gift of life, they are somewhat frail, debilitated, may have some low-level infections that individuals like Dr. Fishman are keeping at bay with antibiotic therapy. But because of the bio secure environment and pathogen-free, we really have potentially the less risk of exogenous microbes than we do have allotransplants. So, we have to look at that as a potential benefit even in the face of immunosuppression.

I think what I've heard and what I've read in terms of, again, minimums of immunosuppression that's necessary, T cell induction therapy of anti-thymocyte globulin, maintenance therapy, combination of costimulatory blockade with either CD40, anti-CD40, or anti-CD154 combination with anti-CD20 for B cell coverage, and then maintenance therapy with anti-metabolites and steroids.

It's interesting; I think Dr. Palevsky tried to get to the question about our current use of standard immunosuppression that includes calcineurin inhibitors and recognizing that that model can't even

be adequately tested in our non-human primates still sort of puts that question mark out there as to how valuable, and really, what the place is for our cornerstone immunosuppressive therapy for seeing eyes.

I think what is also important to recognize is that, again, while the effects of standard of care immunosuppression has been evaluated as best possible in xenotransplantation in non-human primate model, the specific effect of its use, mainly the effect on the xeno organ itself amongst the various transplantable organs that we talk about -- hearts and kidneys it's been a lot of times -- is still largely unknown in the human.

It's important to appreciate and exciting that groups like David Sachs and others are already well on their way to developing tolerance of mixed chimerism and utilizing thymic transplantation in TREGs, so very similar to what we're now currently doing in clinical trials and allotransplantation.

So we may be able to eventually think about that one side of the equation of our donor still

working towards perfection but yet being able to remove immunosuppression on the recipient side. And so, again, I bring it all back saying that there's tremendous opportunities. There's been science which has really demonstrated with each adaptive and increasing innovation that we've had prolonged tolerance and successful survival of these animals.

But really, we're not going to know until we eventually get to the point where we feel comfortable enough that we have those minimums, that we feel safe, which is of course the number one priority that allows us to move forward to these adaptive clinical trials that probably allow us to get closer to determining what is truly enough in addition to what other potential interventions we have that are available that we're using in the allotransplant model.

And then in addition, what's the minimum amount of immunosuppression, if at all, thinking that potentially being able to work towards tolerance, maybe allow us to successfully see this from the bench to the clinical bedside?

So, again, thank you for this opportunity.

DR. LISA BUTTERFIELD: All right. Thank you so much. We're going to move to our second discussant, Dr. Auchincloss next, and then we'll have a discussion from the Committee members after that.

DR. HUGH AUCHINCLOSS: Let me just make two points before we get to the general discussion. The first, I think, is kind of obvious now. My experience of working with the FDA in the past has been that they like to show that each component of combination therapy is an important individual component. That approach clearly is not going to work here. We can't prove the benefit of one genetic modification in xenotransplantation. I think we've heard repeatedly today that there has to be a cassette ranging from three to more genetic modifications. So, it will not be one at a time; there needs to be a combination approach.

The other point that I'd make, at least in my mind, is that in discussing the balance between modifying the donor pig and immunosuppression of the

recipient, I would strongly lean towards further modification of the donor pig rather than trying to increase immunosuppression of the recipient. To do so, I think makes xenotransplantation a second-class option for a transplant recipient if they're going to have to have more immunosuppression to make the organ survive.

So, I'd lean heavily towards further modification of the donor pig. I'd close by just turning back to Dr. Pierson and saying that you'd mentioned you think it might actually turn out that less maintenance immunosuppression will be used for pig donors, and I'm curious as to why you think that. Thank you very much.

DR. LISA BUTTERFIELD: All right. Let me see. Yeah, so there was one particular question to Dr. Pierson just now. So, I'll ask Dr. Pierson to respond, and then I'll be watching for hands for questions and discussion from the Committee members, please.

DR. RICHARD PIERSON: Thank you. Hugh, to address your question, I think it refers back to the work we published in the 1980s showing that the xeno-

immune responses were vulnerable to selective inhibition of CD4 mediated immunity, whereas allotransplantation you have to target both the CD4 and the CD8 cells to prolong allograft survival at the skin in mice. But that's now been shown for kidneys in monkeys -- pig kidneys in monkeys confirming similar work that was done by Allan Kirk and Tony Dorling in the interim.

So, I think that with more focused immunosuppression that has less deleterious long-term consequences, we're going to be able to more safely protect the xenograft. That has the specific advantage when you deplete CD8 T cells, you eliminate a lot of the antiviral immunity, and it's in that context that endogenous CMD reactivation occurs which has deleterious consequences for the host as well as potentially at least for allografts. We don't know if the xenograft will be similarly affected.

I think that co-stimulation -- I believe, based on our work for 20 years on the pathway, that co-stimulation pathway blockade will be a safer and more

effective immunosuppressive strategy as well as a bridge to tolerance. And so, for those in those two different but parallel veins, I think that co-stimulation-based immunosuppression is more likely to find its way into allotransplant in a prevalent way in the next ten years, but that's merely an opinion and that remains to be demonstrated.

One other point that I wanted to raise in reference to Dr. Cooper's very nice commentary. We have spent the last 15 years taking pig organs with progressive individual genetic modifications and studying them in a paired ex vivo lung profusion model, and in that model, we've been able to show that the advantage of individual genetic modifications on the hyperacute rejection response, which is what we have the opportunity to measure in that model.

There has also been work with limb profusion done by the German group in cooperation with the Swiss that looked at the HLA-E expression and demonstrated that that did have an effective demonstrable protective response, again, against early immune interactions when

human blood was perfused through a limb of a genetically modified pig. So, we do have human evidence that several of these individual genetic modifications do have a salutary effect, and there's other good in vitro evidence to support that as well as mechanistic basis.

So, I think it's not without evidence. It is the same time as you accurately point out, we are not going to be able to come at this and test each of these individual things by itself. But based on logical inferences from the data we have, I think for each organ people will be able to -- various investigators will be able to come up with plausible strategies and I would encourage the regulators to look at it in a flexible way as opposed to a rigid way.

And I don't know how that translates into the real world, but that's my advice. Thank you.

DR. LISA BUTTERFIELD: Thank you, and if I can ask for the specific to the CD40 pathway inhibition, is the thought there that you just need to transiently inhibit priming until tolerance is established? I'm

sure you've said.

DR. RICHARD PIERSON: So, in the mixed hematopoietic chimerism context, transient blockade for just one month is the standard approach in non-human primates and is respective 80 percent of the time, I think, in the kidney model.

I think in clinical translation the immunosuppression has been continued for longer periods of time in the MGH mix hematopoietic chimerism experience. But the conventional immunosuppression in that case has continued, I think, for six months or roughly. So, I don't think anyone knows what the minimum duration necessary, but I think it will be possible with the tolerance induction approach to only require transient immunosuppression.

In the absence of mixed hematopoietic chimerism or another tolerance induction approach, I do think that co-stimulation pathway blockade can be used safely for long-term immunosuppression, as is currently done with belatacept as an alternative to higher dose calcineurin inhibitor-based immunosuppression.

DR. LISA BUTTERFIELD: Thank you. All right, so we've had a lot of great discussion and some recommendations so far. So, I'm watching for hands from our Committee members to discuss the most promising strategies to prevent rejection and hear hyperacute rejection, vascular injury, cell-mediated rejection in pig organs. Let's hear from Dr. Bloom.

DR. MARSHALL BLOOM: Well, the one thing that I would note, which really refers back to yesterday, is one thing that definitely has to be avoided and is going to be a major problem no matter how many genes you knock out, how you deal with cell-mediated rejection and stuff, is those donor animals have to be negative for the porcine cytomegalovirus.

I mean, in the presentations from this morning, it seems to be that if that happens, it doesn't make any difference what else you do, that's going to be a showstopper for efficacy of the graft. Thanks.

DR. LISA BUTTERFIELD: Thank you. All right, other thoughts and recommendations about the most

promising strategies? Dr. Fishman?

DR. JAY FISHMAN: Thank you. It seems to me that what we've heard about are a large group of potential genetic modifications that are linked to specific immunosuppressive regimens. And so, the notion that there is a best genetic modification pig is on the genetic background of that pig, and the immunosuppression has been adapted to the modifications that are made. So, I'm not sure that I'm hearing that there is a best immunosuppressive regimen or that there's a best pool of genetic modifications or even a best pig.

I've heard that there is a set of all of the above that should be considered for each protocol, and we have found the same in human-to-human tolerance induction and the like, which is there is a bit of trial and error in it. And as Dr. Pierson pointed out, we don't know the best amount of time, for example, for tolerance induction and we use immunosuppression for a conservative period of time. But in fact, hematopoietic chimerism is not maintained for a

prolonged period of time; it's maintained for a number of weeks, and then seems to go away and the tolerizing effect is maintained.

So, there's some things we don't understand yet. So, I think that my point is that we ought to have a package of pig genetic modifications, immune suppression for each protocol, but that no one protocol should be seen as intrinsically better than any others except based on preclinical data that may be available. So, we have to analyze the experience of an individual team with an individual regimen in that setting.

Similarly, if you use certain immune-suppressive agents, you're more or less likely to stimulate infectious risk, so that there is this balance of the whole package rather than an individual strategy, I think. So, I'm not sure.

I mean, the least immunosuppression you can get away with, and Hugh Auchincloss made the point, that what we're trying to do is use as many genetic modifications as we can to minimize exogenous immune suppression because of infectious risk. That makes

intuitive sense, but it is going to be a package regardless of which one we use.

DR. LISA BUTTERFIELD: Thank you. Let's hear again from Dr. Cooper.

DR. MARSHALL COOPER: Thank you very much. I'm actually thankful that Dr. Fishman made that comment because I've come after days and days and days of preparation review and reading so many of these articles that I've come to a similar conclusion. So, I guess I would, perhaps, then bring forth a question to the Agency with that recognition -- at least maybe recognition by several -- that there is a package of genetic modifications and immunosuppression availabilities.

We think, however, to get to the next stage, which it sounds as if we're going to get to some form of clinical trials, that there is a minimum though. Is there a minimum that will allow to say that it is safe to move forward? That when a sponsor comes forward, the FDA would say, this is a minimum that we require in order for the ability for the movement into the next

phase of clinical trials. Because it seems as if, again, we can have our pie-in-the-sky perfect genetically modified pig and eventually be able to work towards tolerance, but that's not going to happen overnight as many have said.

So, what do we think is the minimum that's going to allow us to be able to go to the next stage?

DR. LISA BUTTERFIELD: I think I can put my psychic hat on and suggest the response would be they want to hear that from us. But if someone from the Agency wants to weigh in here, I'll watch for that hand. We will hear from FDA after the Open Public Hearing later today.

DR. WILSON BRYAN: Dr. Butterfield.

DR. LISA BUTTERFIELD: Yes. Thank you, Dr. Bryan.

DR. WILSON BRYAN: Your psychic powers are very good. We appreciate the concept that the package may be different in different clinical trials because there's a lot to learn. But we aren't very interested in what the minimum is and this Committee's thinking on

what that minimum should be. Dr. Hursh, you wanted to add something?

DR. DEBORAH HURSH: Just to support Dr. Bryan's position. It's not the Agency's position to tell sponsors how to create their medical products. We want you to come to us with data to support your hypothesis and see how it goes.

That being said, we think the alpha Gal knockout is probably the minimum that you need to work with, and then the rest of it, you'll provide pre-clinical data to support.

DR. LISA BUTTERFIELD: Terrific.

DR. MATTHEW COOPER: I appreciate that.

DR. LISA BUTTERFIELD: Thank you very much for adding to the conversation right then. Anything else, Dr. Cooper? Okay. Let's hear from Mr. Conway.

MR. PAUL CONWAY: Thank you very much. I'd like to, one, thank Dr. Cooper for the comments that he's raised and also for Dr. Fishman. But I wanted to highlight in particular what Dr. Auchincloss talked about, and that's from the viewpoint of patient

consumers and especially those who are taking a look at the prospect of organ failure. This therapy, I think what you want to try to avoid is having it viewed as a second-class therapy or something where patients would have to go ahead and endure a higher regimen of immunosuppression.

And I think this particular question is a good one. Right at the end of it, it asks about the balance between modifications and immunotherapy. So, on the allograft side, it's been discussed what the patient burden is in terms of diabetes, gastrointestinal issues, heart issues, and some of these other things. But I think one of the things that the Agency should keep in mind as they look at this question of the balance and this is a role for the Agency.

Sponsors will come forward, but they should be encouraged to bring forward with them patient insight data as well, and the reason why is because the burden of managing pills of trying to make certain that you're on top in terms of compliance as a transplant patient is significant that you have to make as a patient.

In my case, I've taken over 170,000 pills since my transplant -- 170,000. So I'm on it, I'm a counter, not every patient is like that. The prospect of being able to have a therapy where you could have less of a regimen that might be more predictable and might have less side effects I think is very, very important to keep in mind and be open to harvesting those insights from patients -- both recipients and those who are yet to be transplanted -- to get their voice into this conversation as the Agency moves forward and looks at it.

But I think the presentations have been fantastic, and again, I think the questions posed by the doctors are good ones in terms of tiering or segmenting different pathways of different therapies that we're considering and not having must fit all, one-size approach. Thank you.

DR. LISA BUTTERFIELD: Great. Thank you very much. So, I'm starting to hear, I think, some consensus and points agreed upon. So, if there are no more Committee comments at this time, I'll go ahead and

take a stab at some summarizing of some key points, and then we'll have a few minutes for people to add to that if they would like to.

So, in terms of the most promising strategies, I think the theme is that more -- just echoed now by Mr. Conway -- more modifications to the donor animals that could be propagated, studies, added to over adding regimens to the patients that might have a lot of side effects over time. That we heard, in fact, suggested and supported also by the Agency that in terms of genetic modifications and the data are very strong about the alpha Gal knockout, and that this might be considered the minimum. As we've seen in the guest speakers, we've seen really striking changes in the survival plots of organs with triple knockouts in these different carbohydrate molecules.

We've seen thrombomodulin complement as key pathways for genetic modifications that can have significant impact. So perhaps that minimum is closer to two, perhaps it's four genetic modifications in the donor animal that can be accomplished without donor

fitness. But also, to keep in mind that there are key pathways where the specific modulations would have to be matched to the animals and their background as well as the target organs in the clinical transplant setting to optimize. It will probably, as in all biology and certainly immunology, one size will not fit all, and that this will be based on the data package as submitted.

But those carbohydrate complement cascades and coagulopathies, those are all critical pathways and that there are going to be limitations to the non-human primate models that can be studied because there could be effects specific to those that would not be expected in humans.

And so, in all things we've talked about so far yesterday and today, it can be early, and a lot of the amazing genetic modifications were now technologically capable of making remain to be tested to the extent possible in non-human primate models, but then ultimately in patients.

And then, lastly, PERV and PCMV are also

important targets that could be genetically eliminated in the donor animals.

So, with that, let me ask Dr. Auchincloss to add to that summary, please.

DR. HUGH AUCHINCLOSS: Your summaries are really quite spectacular each time. Thank you very much.

I would simply second what you just said, I think, which is Gal knockout alone is not the minimum. I think that Gal complement regulation and coagulation modification are also a part of the minimum, but I'd be interested in our two speakers talking about what they think a minimum package might include.

DR. LISA BUTTERFIELD: All right. With that specific question to our two guest speakers for that, I'll ask Dr. Pierson and then Dr. Wolf if you would like to add a brief specific about what you consider the minimum.

DR. RICHARD PIERSON: I think for the kidney, there's a case to be made for the Gal knockout by itself in unsensitized patients. So, I personally

would be reluctant to bring that forward as a general strategy because I think the number of -- it's only a minority of patients who I would have access to this in a clinical trial even if it were successful as a clinical therapy.

I think the triple knockout there's a better case for as a minimum for the kidney, but I still -- if it were me as a patient -- would prefer that a complement regulatory protein be included in that context as a safety and as a protection in case of elicited antibody becoming a factor.

The case for thrombin regulatory molecule expression in the kidney has never been tested, to the best of my knowledge, so I don't know what we can say about that. Although, the evidence that Eckhard cited showing that when complement and coagulation pathway regulatory molecules were expressed on the kidneys, it's actually data out of MGH with the eGenesis pigs. It does look like having expression of both of those genes may offer an advantage in that context.

But in terms of it, I think a hard and fast

minimum as has been alluded to, Dr. Butterfield, in your comments and, Matt, in your comments, a hard and fast minimum is not the course that I would advise. But again, this is your job, not mine, to make a recommendation in the FDA's decision, not mine.

With respect to the heart, I think as Hugh alluded to, Gal knockout is the minimum along with complement regulator and thrombomodulin. Triple knockout would probably be better for clinical application, but it will be not possible to validate preclinically. So that is the caveat for regulator in looking at the dataset, but I think that case is very well established in the preclinical data that the triple knockout is not readily testable preclinically.

So, Hugh, I hope that answers your question at least from my perspective. I'd be very eager to hear what Eckhard thinks.

DR. LISA BUTTERFIELD: Thank you. Dr. Wolf.

DR. ECKHARD WOLF: I think I can just echo this statement. I don't have experience with kidney transplantation, but, at least for the heart

transplantation, I think it's absolutely true what Dr. Pierson said.

For me, the question is, would it be acceptable to work preclinically in baboons with a pig that has only a heterozygous CMAH knockout and later on, nevertheless, use organs that have a homozygous CMAH knockout in humans? Because there is no evidence that this CMAH knockout could have a negative effect in humans, and, simply for the preclinical testing, it disturbs the outcome of the results.

DR. LISA BUTTERFIELD: All right. Thank you. So, we have a few final words from members of the Committee. Professor Fox, Dr. Morrison, and Dr. Wu, and then we'll see where we're at.

DR. BERNARD FOX: I agree, Dr. Butterfield. Great summary, again, and I guess I was just going to comment about trying to advise the FDA. Given the data that we've heard, I kind of wonder why we don't push for more, like looking specifically at the triple knockout. And it was, again, continuing to be informative to listen to the last two presenters,

again, discuss the impact of maybe thrombomodulin and maybe that there's not enough data on the kidney.

But it seems that the inference from the preclinical data would be that it would be supportive realizing that all the preclinical data may not advise us in terms of how it's going to work in the clinic. So, it seems like at least, from our perspective, there should be at least two: the complement in addition to the alpha Gal and potentially the thrombomodulin. And I guess I'd be weighing in to support all three of those given the advantage that it has in at least one of the settings preclinically.

DR. LISA BUTTERFIELD: Thank you. Dr. Morrison, and then Dr. Wu.

DR. SEAN MORRISON: In the context of the conversation about minimal numbers of manipulations, I think it's really important to bear in mind that one of the things we heard is with a minimum set of manipulations it would be necessary to prescreen patients that have low levels of antibodies against certain antigens. So, I think it's really important

that we not pursue a strategy in which large numbers of patients wouldn't be candidates for the therapy because of preexisting antibodies.

When we're talking about minimal numbers of manipulations, it should be the minimum number of manipulations that would really maximize a fraction of the population that could potentially be treated with this approach. Thanks.

DR. LISA BUTTERFIELD: Thank you. All right, Dr. Wu, and then we'll close it out with Dr. Zeiss.

DR. JOSEPH WU: Yeah, so I have a question for Dr. Pierson with regard to the patient selection for the cardiac population. I think as you know the patient in Maryland, he was actually quite sick before he got the pig heart, and so that also makes it tougher to prove that the pig heart transplant's safe. On the other hand, if you pick your patient population that's not so sick, there are other alternatives that says these many (inaudible) that we can give to our patients.

So, I just want to get your thought on how do

you pick the right population that gives the best benefit to the patients without taking away some readily available options that's just as good, if not better?

DR. LISA BUTTERFIELD: Thank you, Dr. Wu. Question -- that was a question for Dr. Pierson.

DR. RICHARD PIERSON: Thank you, Dr. Wu. That's a great question. It's one that we've tried to address in two recent publications. One in circulation in 2020 and one that was published in *Transplantation* just last week. I would refer you to those publications for a detailed consideration that includes some treatment of kidney as well as heart. There's a sub-population of our (inaudible) heart failure patients who are either are highly sensitized to alloantigens but not to pig antigens who might benefit significantly from early access to a heart xenograft -- timely access to a heart xenograft.

Additionally, populations of patients with hypertrophic cardiomyopathies, patients with amyloidosis, for example, can get into trouble with

arrhythmias and fall off a cliff quite rapidly. And for them, an LVAD, even a small LVAD, at least the HeartMate 3 is a poor choice that doesn't feel well and doesn't give good support. And the truly micro LVADs, the Impella style VADs, are only approved for short-term use. We don't yet have a durable long-term VAD that can support patients through recovery of kidney function and onto a desensitization protocol, et cetera.

So, there are -- not to go on too long -- but I think there are populations of heart failure patients for whom a heart xenograft would be an attractive alternative either as a bridge to an allograft or as destination therapy, as a definitive treatment option. So, and as, I said, the more extensive treatment of that I think you can find published and for the Committee's reference.

DR. JOSEPH WU: Thank you.

DR. LISA BUTTERFIELD: All right. And we will close this out by hearing from Dr. Zeiss.

DR. CAROLINE ZEISS: Question for Dr. Wolf. I

wonder if the growth hormone receptor-deficient mutation may not have a use in real transplantation in children. Children with chronic kidney disease can become growth hormone receptor, also growth hormone-resistant and you can get stunted growth, and the treatment for them is recombinant growth hormone. If you would add that on top of the intrinsic capacity of the organ to grow, you could have a real problem in children, and using those particular mutants may be very valuable in children.

DR. LISA BUTTERFIELD: All Right. Dr. Wolf.

DR. ECKHARD WOLF: I'm not sure if I completely understood your question. You are asking whether growth hormone receptor-deficient organs could have an advantage for children or could be a problem for children?

DR. CAROLINE ZEISS: Could have an advantage, specifically for renal transplants in children where children with chronic kidney disease can become growth hormone-resistant, and they are treated with recombinant growth hormone and so having an organ that

is transplanted that would be resistant to responding to that.

DR. ECKHARD WOLF: Right, yeah.

DR. CAROLINE ZEISS: Because human growth hormone can bind to the pig growth hormone receptor.

DR. ECKHARD WOLF: I think for this very special case it could be beneficial. Also, even the smaller pig strains that are available, for instance, the (inaudible) pig, whose organs would fit for adult humans, they would be too large for children.

DR. CAROLINE ZEISS: Yep. Thank you.

DR. LISA BUTTERFIELD: All right, well, thank you everyone for the discussion, all the questions, and all of the perspectives. I'm sorry, do we also have a final word from Dr. Hursh?

DR. DEBORAH HURSH: Yeah, I had a scientific question for Dr. Pierson and Dr. Wolf. In regard to all the human immunomodulatory genes that have been knocked in in various of these pigs, has there been any sense that they changed the pig's ability to fight off viral infection in an unpredictable way?

DR. RICHARD PIERCE: I guess I'll start that. The short answer is no. I'm aware that the CD46 membrane cofactor protein is a receptor for -- I think it's the mumps virus or I think that's correct. To the best of my knowledge, it does not increase the susceptibility of the pig to any viruses that our pigs are exposed to. So, there's no health effect associated.

Is it possible that that gene expressed on the pig organ would have a clinical effect if our patient got mumps or measles and the kidney then, in theory, would be more susceptible to binding the virus -- being infected by the virus, whereas it might not be with pig membrane cofactor protein. That's the only potential context in which I can see the complement regulatory protein expression potentially having a deleterious effect with respect to infectious disease. Eckhard.

DR. ECKHARD WOLF: I would answer in the same way, and I think it should not be a major problem because humans express these proteins anyway, so I don't see an increased risk introducing human protein

into pig organs.

DR. DEBORAH HURSH: Yeah, I think I was more concerned about whether the pigs themselves might be more susceptible to viruses that we might not be as aware to be screening them for. I think that was more the context I was considering.

DR. RICHARD PIERSON: I think the context that I would recommend to consider that these source animals for human organ grafts are going to be -- the husbandry is going to be quite stringent, and the porcine CMB illustrates one reason why. But the regulatory Agency's been very clear that that is going to be best practice and will be required. And I completely support that.

Exposure of these pigs to human viral pathogens is preventable and should be avoided and should be -- whatever to the extent that I. Fishman tells us it's necessary to document that, that is what we ought to do. But, again, I don't want to suggest that there should be a requirement that we document, document, document all kinds of things which are highly

improbable.

If you have an animal housed derived by cesarean section and raised in specific pathogen-free environment and only coming into contact with humans who are in moon suits, I think the risk is so low that requiring documentation is probably overkill. Not necessary.

DR. CAROLINE ZEISS: Thank you both.

DR. ECKHARD WOLF: I would fully agree and also an allograft is not without infectious, risk. I think we can control the xenografts much better.

DR. LISA BUTTERFIELD: Okay. It looks like we have two more last questions that pertain directly to Question 4. Dr. Beaston.

MR. MICHAEL KAWCZYNSKI: Sorry, I had Jay first. Sorry.

DR. LISA BUTTERFIELD: Sorry. The hand had gone away. All right, Dr. Fishman, please.

DR. JAY FISHMAN: Well, just to echo what Robin [sic] Pierson said and Eckhard. The genetic modification, the only downstream effect not really

implicated for viral infection, but if complement levels are normal, they should not have increased risk for bacterial, particularly encapsulated bacterial organisms as well. So, I think it would be an easy assay to do to make sure the complement levels are normal, the immunoglobulin levels are normal in the donor animals.

But otherwise, one wouldn't necessarily anticipate an infectious risk secondary to the genetic modifications, and the easiest thing is, are the animals healthy? And I think if they're healthy, then we probably have addressed that question.

DR. LISA BUTTERFIELD: Thank you, Dr. Fishman. And finally, Dr. Beaston.

DR. PATRICIA BEASTON: Good afternoon. Thank you for these great presentations. So, I have a question about all of the manipulation. In their article, Porrier (phonetic) described altered overall structural integrity changes in the renal parenchyma and suggested that this could be related to the genetic manipulations. I was wondering how you're looking at

these animals as you're doing the manipulations and looking for the integrity of the organs over time -- because number of alterations including growth hormones.

So can you make a little comment on how these are going to be chosen and how the normal aging of pigs might be changed from these and then certainly it may not be possible that one pig could be a source for multiple transplants. Like, it could not give a heart and kidneys to patients because the manipulations are particularly developed for those organs.

So, if you could talk a little more about these choices and then how you're following up on the consequences of these choices, that would be really helpful. Thank you.

DR. RICHARD PIERSON: Eckhard, do you want to start and I'll -- I had the first go on all the other questions. Can you hear me?

MR. MICHAEL KAWCZYNSKI: He's reconnecting his audio. Hold on a second. While he's doing that, why don't you take it away first, sir, and then we'll come

back to him.

DR. RICHARD PETERSON: Sure. Sorry. So, I think that the pigs that are brought forward for heart will have sufficient genes to be used as kidney donors as well. So it may be that, while not all genes are needed for the kidney, that it will be convenient and may prove eventually to be advantageous to have additional genes. The same goes for CD47, for HLA-E, and some of the other genes that are in some of the constructs. It will take time for us to understand how consistent the expression of the various specific genes is in the individual animals that come forward for transplantation.

As Eckhard so nicely said, the epigenetic modifications present in cloned animals may have adverse effects or unpredictable effects on how particular genes are expressed and other aspects of the health of those animals. And so, his comment that, eventually, the product that gets approved for clinical use and goes into widespread clinical use should almost certainly be from bred animals in whom the breeding

process should correct those epigenetic problems that are associated with cloning.

That said, some cloned animals do not thrive and die in utero or in infancy or during their maturation phase. And some can go through crises through their maturation but then get over those problems.

And as a consequence, I would advocate that cloned animals can and perhaps must be used as the initial trial subjects, doing all we can to ensure animal health and then assessing retrospectively over or under expression of particular genes is associated with better or worse outcomes as one begins to develop a body of evidence.

Once we have a body of evidence, it'll be much easier to say that the minimum gene set for kidney and the minimum gene set for heart are the same or different, and it will become easier for us to say that additional expression of XYZ additional transgenes or XYZ additional knockouts is advantageous or not.

But I think we can't expect that that's going

to come from preclinical data at this point, and I think we are far enough along in our preclinical database that I have made the strong case that I think we're ready to start trying some of these candidate strategies. And those will depend on what pigs are available to each of these groups, and what evidence they can come forward to the FDA with showing that this is sufficiently dependable and sufficiently effective in the preclinical model as to justify confidence on the part of us and our IRB and the FDA that we're doing something sensible.

And to our patient advocate, I encourage you to hear his voice in what he would feel comfortable with going forward with. The comment by Porrier among the comments in the paper was that the tissues of that pig organ were fragile and the ultrastructural abnormalities that were described, I don't know what to make of them. I'm not a renal pathologist.

The fact that that kidney had normal function in the donor and then was still alive three days later, again, I don't know what to make of the ultrastructural

abnormalities. Is that a cloning artifact? Is that a consequence of the human transgene expression? Is it associated with the carbohydrate knockouts? Simply, we do not know, nor do we know what proportion of the pigs produced have this. It might be worth asking that question, but I don't know that I would put a lot of weight on that individual, unique observation. Eckhard?

DR. LISA BUTTERFIELD: I think that probably ties to Question 5 that we'll be coming to later today. Dr. Wolf.

DR. ECKHARD WOLF: I think in order to demonstrate the integrity, it's necessary to characterize precisely the transgene integration site, and this can now be done easily with long (inaudible) treatment sequencing and also perform functional studies on the organs. For the heart and the kidney, this can be easily done in the donor pig already.

DR. RICHARD PIERSON: By ultrasound for the heart and kidney and then just by -- I don't think you need to measure cardiac output in a healthy pig, but I

think you can measure creatine simply or B1SO and the proteinuria in the kidney.

Dr. ECKHARD WOLF: Yes.

DR. PATRICIA BEASTON: So thank you very much.

DR. RICHARD PIERSON: Did that answer your question?

DR. PATRICIA BEASTON: Yes. Thank you.

DR. LISA BUTTERFIELD: All right. So, I think a little preview of some of the things that we'll probably talk about after the break. So right now, I'd like to, again, thank everyone and we're going to move to a lunch break. The Open Public Hearing will be next. That'll be 10:00 a.m. here in San Francisco. That'll be 1:00 p.m. on the U.S. East Coast. So, thank you all. See you back then.

MR. MICHAEL KAWCZYNSKI: All right, and with that, let me switch this over to lunch. And studio again we're going to take a -- I just want to make sure -- we're going to come back at 1:00. So, we're taking a 34-minute break. So, studio, go ahead and kill our feed.

[LUNCH BREAK]

OPEN PUBLIC HEARING

MR. MICHAEL KAWCZYNSKI: Welcome back to FDA's 73rd meeting of the Cellular Tissue and Gene Therapies Advisory Committee meeting. I'm going to hand it back to our chair, Dr. Lisa Butterfield. Dr. Butterfield, take it away.

DR. LISA BUTTERFIELD: Thank you very much. All right. Welcome back and welcome to the Open Public Hearing session. Please note that both the Food and Drug Administration, FDA, and the public believe in a transparent process for information gathering and decision making. To ensure such transparency at the Open Public Hearing session of the Advisory Committee meeting, FDA believes that it's important to understand the context of an individual's presentation.

For this reason, FDA encourages you, the Open Public Hearing speaker, at the beginning of your oral

statement to advise the Committee of any financial interests relevant to this meeting, such as financial relationship with any company or group that may be affected by the topic of this meeting. Likewise, FDA encourages you at the beginning of your statement to advise the Committee if you do not have any such financial relationships.

If you choose not to address the issue of financial relationships at the beginning of your statement, it will not preclude you from speaking. So, with that, we'd like to get started with the Open Public Hearing. I'll hand this to Christina Vert, our DFO.

MS. CHRISTINA VERT: Thank you, Dr. Butterfield. What my camera's doing. Okay. I'll go ahead. Before I begin calling the registered speakers, I'd like to add the following guidance. FDA encourages participation from all public stakeholders in its decision-making processes. Every Advisory Committee meeting includes an Open Public Hearing, OPH, session, during which interested persons may present relevant

information or views.

Participants during the Open Public Hearing session are not FDA employees or members of this Advisory Committee. FDA recognizes that the speakers may present a range of viewpoints. The statements made during this Open Public Hearing session reflect the viewpoints of the individual speakers or their organizations and are not meant to indicate Agency agreement with the statements made. Now, I will go ahead and call on the first Open Public Hearing speaker, which is Dr. Eliezer Katz.

DR. ELIEZER KATZ: Thank you. Do you see my first slide?

DFO CHRISTINA VERT: Yes, we do.

DR. ELIEZER KATZ: Thank you. Thank you, everybody, and good afternoon. My name is Dr. Eliezer Katz. I am the chief medical officer of eGenesis. I'm fully employed by eGenesis and holding stock option of eGenesis. I would like to thank the Committee and the FDA for the opportunity to present some of the eGenesis perspective on this important topic that we've all

discussed here in the last two days. Next slide, please.

eGenesis is utilizing state-of-the-art gene engineering technology to produce human-compatible porcine organs for transplantation. Next slide. To bring this technology to clinal use, eGenesis, like many others, has been engaged over the last few years in extensive pre-clinical transplantation studies of porcine organs into nonhuman primates. Although a tremendous amount of data and knowledge were generated, most of us here today would agree that transplantation models of porcine organs to nonhuman primates has significant limitations.

We can also agree that first-in-human study will be critical in establishing proof-of-concept and open the door for further development of this important innovation. We can also agree that first-in-human clinical study is not aimed to provide final and definite answers. Therefore, we advocate a need for a practical and effective path to first-in-human proof-of-concept study.

Our approach utilizes F0 cloned donors produced in a specified pathogen free barrier facility for our GLP studies and our first-in-human proof-of-concept study. Next slide, please. The production of porcine donors starts with the generation of well-characterized nuclear donor cell in which the genomic edits are confirmed, the off-target affects are characterized, and screening of adventitious agent is performed.

The genetic edits include the knockout of the three sugar antigens associated with hyperacute rejection and the insertion of human (inaudible) genes at the safe harbor within the porcine genome to mitigate (inaudible), compliment system activity, and immune system activation. Next slide, please. This nuclear donor cell undergo electrofusion with oocytes from a controlled donor population to generate the embryo which then is being implanted to a controlled surrogate who gives birth to the F0 cloned donors.

These cloned donors are maintained in a clean barrier facility and are fully characterized, including

the confirmation of the genetic edit, the assessment of off-target affect, the screening for adventitious agents, and the evaluation of the donor herd. Next slide, please. Control of infectious risk from adventitious agents, including porcine endogenous retrovirus, is critical for the success of xenotransplantation as we heard in length in the last two days during our discussion here in the Committee.

PERVs have been shown to potentially infect human cells and, therefore, pose a potential risk for porcine organ transplant recipients and the larger community. To reduce this risk, we use CRISPR-Cas9 technology to inactivate the retrovirus reverse transcriptase copies in the porcine genome, eliminating viral replication and avoiding the risk of transplantation and also of transmission.

In addition, we plan to adopt practical approach to monitoring and controlling adventitious agents. To do that, we believe we need to work in collaboration with porcine and human infectious disease experts, with our colleagues in industry, and of course

this agency. Next slide, please.

In summary, eGenesis' position on the path to clinic in xenotransplantation includes the use of specified pathogen-free F0 clone porcine donor organs to be evaluated in our GLP safety studies, the use of the same organs for the first-in-human clinical study, and the reduction of infectious disease risk that will include inactivation of PERVs and the implementation of well-designed plan for the mitigation and control of adventitious agents. This approach we hope will provide for a practical path to proof-of-concept first-in-human clinical study and open the opportunity for bringing this life changing innovation to patients in need. Thank you very much for listening and for the opportunity to present for you. Thank you.

MS. CHRISTINA VERT: Thank you. Next speaker is Dr. Sanjoy Dutta.

DR. SANJOY DUTTA: Good afternoon. My name is Dr. Sanjoy Dutta. I'm the chief scientific officer with JDRF International, the leading charitable organization funding type 1 diabetes, or T1D, research.

JDRF's vision is a world without T1D, and our mission is to improve lives today and tomorrow by accelerating life-changing breakthroughs to cure, prevent, and treat T1D and its complications. JDRF does not have any financial disclosures.

The key points I will focus on today are, one, the unmet needs that exist in T1D and, two, the potential for xenotransplantation to meet these needs. In particular, porcine islet xenotransplantation presents a solution to the shortage of human islets as a potential cure for T1D. For the 1.6 million Americans with T1D, the mainstay of disease management, insulin, has been around for over 100 years, but it is not a cure.

The burden and risks of life-long T1D disease management falls almost entirely on people with T1D and their caregivers, requiring 24-hour-a-day diligence to maintain glycemic levels, prevent long- and short-term complications, and survive. While technologies to administer insulin and monitor glucose levels has improved, subcutaneous exogenous insulin replacement is

not physiologic and is insufficient to restore the body's natural ability to maintain glucose homeostasis.

For example, data from the T1D Exchange Registry in the U.S. shows us that less than one-third of people with T1D in the U.S. are consistently achieving target hemoglobin A1C levels. And on average, those with T1D have a decade-less life span than the general population. Among the leading causes of mortality for people with T1D are renal failure and heart failure.

Although human organ donors can successfully address end-organ failure, the supply of human organs is insufficient to meet the demands, and xenotransplantation could be a potential approach to address this unmet need. As evidenced by the successful phase three safety and efficacy study of cadaveric islets, led and funded by the NIH Clinical Islet Transplantation Consortium, transplantation of donor human islets could be a cure for T1D.

Results of that trial showed that islet cell transplantation can significantly improve glycemic

control, protect patients from severe hypoglycemic events, and restore counter regulatory measures while improving quality of life and, for some, provide insulin independence for up to five years or longer. However, the available supply of human donor islets is limited, and these transplants require chronic immunosuppression which further limits the use of this treatment to only a subset of those with T1D.

Therefore, JDRF is supporting a multipronged approach to support the research of curative therapies that could provide a replenishable source of cells and reduce or eliminate the need for chronic immunosuppression. This multipronged approach includes research in xenotransplantation which builds on the following. One, we know that the cell types and cellular architecture of pig islets are a very faithful model for human biology and diabetes.

Two, pigs could be a source of islets that could potentially be more abundant and could benefit from stricter quality control than is possible with human islets. And, three, there is a long history of

success with pig insulin for the treatment of this disease. Transplantation of pig islets could be a promising avenue to develop new cures for T1D. Data is available to show that neonatal and adult porcine islets are able to correct diabetes in immune-compromised mice, pigs, and nonhuman primates.

Progress in genetic modification of the source pig has allowed the generation of animals that are free of defined pathogens and also free of specific targets for immune rejection by human recipients. This offers the opportunity to improve the engraftment and survival of islets xenografts. To that end, JDRF has funded nonclinical research using gene editing of pancreatic pig islets to remove xeno antigens likely to trigger hyperacute rejections as well as research with encapsulation devices designed to provide immune protection.

First-in-human clinical studies of encapsulated pig islets have shown promising results in both early efficacy signals and safety with no zoonotic infection issue detected thus far. We encourage the

FDA and the Advisory Committee to consider all available scientific information to develop reasonable and adaptive regulatory pathways for products devised from xenogeneic sources.

We also encourage FDA and Advisory Committee to consider existing regulatory guidance from other agencies worldwide as to the extent possible globally-aligned regulatory-framed work will help research and development and speed patient access to curative therapies. This is especially important --

MS. CHRISTINA VERT: Please finish up.

DR. SANJOY DUTTA: -- for complex novel areas such as this and for diseases like T1D where the unmet needs remain significant. In summary, despite advances since the discovery of insulin over 100 years, morbidity and mortality rates as well as disease burden for those with T1D remain unacceptably high. We need cures.

We thank the Committee and the FDA for the careful consideration of not only the risks of xenotransplantation but also the potential benefits of

those awaiting organ and tissue transplants as potential cures for T1D. Thank you.

DFO CHRISTINA VERT: Thank you. Thank you. This concludes the Open Public Hearing. I thank you for your comments and presentations. I will now hand the meeting back over to Dr. Butterfield.

FDA PRESENTATION: FUNCTIONAL STUDIES OF PIG ORGANS

DR. LISA BUTTERFIELD: Great. Thank you so much. We appreciate those perspectives from the Open Public Hearing. Now, as we move to discuss our final Questions 5 and 6 for today, I'd like to welcome Dr. Beaston from OTAT and CBER for her presentation.

DR. PATRICIA BEASTON: Good afternoon. I'm Patricia Beaston, a clinical reviewer in the Office of Tissues and Advanced Therapies. Today, I will give a brief introduction for clinical considerations for functional studies of pig organs that will be used for transplantation. With improvements in surgical techniques, tools, donor recipient matching, and

immunosuppressive regimens, the success of transplantation can exceed 90 percent at one year, and 10-year survival has surpassed 50 percent.

The success of kidney transplant is greater than that for liver transplant, which is greater than that for heart transplant. Living donor transplants are more successful than cadaveric donor transplants. While these are life-saving and life-improving strides, there is a shortage of donors, living or deceased, compared to the number of patients on waiting lists. And some potential recipients have characteristics that make achieving a match near impossible.

To address the imbalance between the need for transplantation and the availability of donors, the use of organs from other species has been considered for more than a century, with tissues and cells being investigated in the more recent past. As discussed previously by Ms. Arcidiacono, there has been much interest in the considerations for donor animals, the requirements for immunosuppression, and the risks for zoonosis.

We must remember that the purpose of transplantation is to provide replacement of function for organs, tissues, or cells that are no longer able to support life or to treat serious and life-threatening conditions in patients. Therefore, it is important to consider whether the product obtained from the source animal is sufficient to approximate the physiology of the human organ, tissues, or cells that it is meant to replace.

Surgical techniques for organ transplantation, heart, lung, liver, and kidney, are well established. However, there are no data to determine the appropriate criteria for organ selection, such as the age of the source pig or the size of the organ. The clinical review starts with input provided by the Chemistry and Manufacturing Controls, CMC, and Pharmacology Toxicology, or PT, reviewers as this information forms the basis of the evaluation of the safety and mitigations contained within the composed clinical protocol.

As presented by Dr. Hursh, the CMC reviewer

determines that the organ, tissues, or cell obtained from the source animal meets the requirements for transplantation. The pharmtox reviewer considers whether the animal model is appropriate for clinical condition or disease. These considerations include but are not limited to the route of administration, which should mimic the proposed clinical routes as much as possible and include the surgical approach, delivery devices, concomitant medications, and immunosuppressive regimens that would be the same or similar as those proposed for the clinical study.

While immunosuppression regimens for allogeneic transplants are well established, immunosuppressive regimens that are appropriate for the xeno organ, tissues, or cell are not well established. The pharmtox evaluation of immunosuppressive regimens for xenotransplantation in nonhuman primates is limited because commonly-used drugs may not be as effective or well tolerated in nonhuman primates. This also limits the ability to demonstrate prolonged function in the transplanted organ.

To assess the proposed clinical studies, the clinical team considered data gathered from pre-clinical study endpoints for safety and organ function. I will introduce two of the major potential safety issues that would be considered in the review of the proposed clinical protocol. In general, if the transplanted organ, tissues, or cells cannot meet or approximate replacement of the human organ, tissues, or cells, this mismatch can pose a risk to the recipient.

Allogeneic kidney transplant has the expectation that the donor kidney will provide replacement therapy. The move to xenotransplant requires consideration of the kidney's functions and the need to explore whether the xeno kidney can provide replacement of all of these functions. And, if not, can the risks of these physiologic mismatches be mitigated?

In addition to waste removal, the kidney regulates electrolytes and is a complex endocrine organ that produces, converts, and responds to hormones. The actions of these hormones are not always conserved

across species. I will describe the few examples of these complex functions. We will start with fluid balance, blood pressure, and electrolyte balance.

Potassium phosphate wasting has been reported in pig to cynomolgus monkey bilateral nephrectomy model. And free water wasting has been reported in a nonhuman primate model and raises concerns for a potential mismatch for a response to (inaudible) present. In sodium regulations, (inaudible) excretion is influenced by several natriuretic peptides which act on the kidney until pairing (phonetic) is achieved through the renal sympathetic nervous system and the renin angiotensin aldosterone axis.

We know that porcine renin does not cleave human angiotensinogen. The Vitamin D parathyroid, or PTH, axis is critical in maintaining calcium and phosphate levels within the appropriate physiologic range. The kidney is the site of 1-alpha-hydroxylation of 25 Vitamin D to produce the active form of Vitamin D in response to PTH. PTH also promotes tubular reabsorption of calcium while inhibiting phosphate

reabsorption.

Amino acid sequence for PTH is not conserved between humans and pigs. And the response of the pig kidney to human PTH has not been described. Porcine erythropoietin is only 80 percent homologous to nonhuman primate erythropoietin and does not support nonhuman primate erythropoiesis. Similarly, porcine erythropoietin does not support human erythropoiesis. While not unique to the kidney, it should be noted that pigs and primates have a mismatch in the coagulation cascade.

This mismatch can increase the risk of thrombus formation and requires consideration during the transplant and post-transplant periods. We must also consider the pharmacokinetics and pharmacodynamics of drugs that will be used in the peri-transplant period to provide immunosuppression to manage the recipient's other medical problems or complications that may occur from the transplant procedure or immunosuppression.

There are drugs, such as SGLT2 inhibitors, for

the treatment of diabetes that act on the kidney. It is important to understand whether the xeno kidney and the human kidney had similar responses to these drugs. In addition, the xeno kidney and the human kidney may have different metabolisms of certain drugs, and this difference could result in underdosing, leading to ineffective therapy, or overdosing, leading to possible toxicity.

Such differences in metabolism would be most critical for drugs that have a narrow therapeutic range. Additionally, some drugs can be toxic to organs. It is important that the drugs used in the post-transplant period are not toxic to the transplanted xeno organ. In summary, FDA considered the potential benefit and the potential risks of all stages of clinical development.

The hope for benefits is for the transplanted organ to (inaudible) cells to provide the intended physiologic and functional replacement. However, with this benefit comes many risks, both known and unknown. Risks from the route of administration include risks

associated with implantation procedure, such as bleeding and infection, and risks associated with the site of implantation based on the organ, tissues, or cells to be transplanted.

Yesterday, Ms. Arcidiacono introduced considerations for immunosuppression regimens and infectious risk. Today, I have presented a brief discussion of considerations for physiologic mismatch in the case of the kidney xenotransplantation and considerations for clinical pharmacology. For recipient's safety, it is important to consider the requirements of the transplanted organ, tissues, or cells, in our examples the pig kidney, to provide replacement therapy.

The clinical protocols should identify the risks associated with the proposed treatment and provide a specific plan to mitigate these risks. Such a plan should consider the subject eligibility criteria, the treatment plan, safety monitoring, and management of physiologic mismatch. FDA is looking forward to the Committee's discussion of Question 5 and

6 on considerations of evaluation of pig organs that will be used for xenotransplantation to replace human organs. Thank you.

Q&A

DR. LISA BUTTERFIELD: Thank you very much, Dr. Beaston. We have time for some questions about Dr. Beaston's presentation, so I'm going to watch for hands up from the Committee members. I appreciate your highlighting a number of things that we're going to have to think about and discuss as we move into Questions 5 and 6 focusing on organ function.

All right. So I'm not seeing any questions immediately from the Committee members. Okay. We do have one from Mr. Conway. Thank you.

MR. PAUL CONWAY: Hi, doctor. Thank you very much for walking through your presentation. It was very good. I have one question for you, and I know that this has been a source of discussion at FDA and also among patient advocates. It's a pretty clear

understanding, I think, among patient advocates what the role of the science of patient insights is on the device side of FDA.

But for those patient advocates that are listening and for those patients and families that are listening that have unique insights, can you tell us what the role of those insights are in deliberations like this on the drug side of the FDA? Thank you.

DR. PATRICIA BEASTON: Well, we do really appreciate the input from patients and their caregivers. As you heard yesterday, we also have an additional consideration for public health because of the risk of (Inaudible), so we also consider those. I heard you today say that you want this to be simpler. So my goal is to make sure we have a good understanding of what is ahead of us.

So if some of these physiologic mismatches I've mentioned requires a greater burden on you, I don't know that that would be satisfactory. But it might be with testing prior to doing the transplants we may understand ahead of time which drugs may be better,

which other things that we could do to further modify that. So we do give this a lot of thought. Thank you.

MR. PAUL CONWAY: Thank you very much. I appreciate it.

INVITED SPEAKER: PIG TOXICOLOGY STUDIES

DR. LISA BUTTERFIELD: All right. Thank you both. If there are no other questions right now regarding Dr. Beaston's presentation, then I think we'll go ahead and move to our other speaker. We have in invited speaker on pig toxicology studies, Dr. Helke, from Medical University of South Carolina.

DR. KRISTI HELKE: Good afternoon. Thank you for the opportunity to talk with you. It has become obvious during these last two days why we're talking about pigs in this session. But why are we talking about toxicology in pigs? I think Dr. Beaston just highlighted why we're having this discussion now. So far, we've been talking about very relevant and specific concerns with xenotransplantation.

The talks we've heard are promising and optimistic that we are very close to xenotransplantation. I'm going to talk more about hypothetical but very real concerns that we've not yet discussed. We need to be sure that any drugs given to humans that have had a successful xenotransplant will be metabolized in a similar manner to the native organ or that we know and are prepared for any differences in metabolism so that any differences or concerns regarding metabolism can be anticipated and addressed.

Dr. Wolf was the first person today to mention the different breeds and why it may be important to consider this. Today, I'm going to discuss the different pig breeds used in research. I'm going to talk about drug metabolism, including not only some of the enzymes that are involved but also the locations and organ systems important to drug metabolism and current knowledge of such in the pig.

And one of the things we learn in vet school is that many species have breed differences, as breeds are selectively bred for specific traits or

characteristics. This is also true for pigs and leads to some of the differences we see in the drug metabolism. The Hanford breed was originally bred in 1958 and is currently used for dermal toxicity. But, with its size similar to humans, it's a good surgical model and is often selected for cardiovascular studies because the size of the heart of the adult Hanford breed is similar to humans.

The Sinclair breed was the first breed developed specifically for research. It was originally developed by the Hormel Center at the University of Minnesota in 1949. There's one lineage of this breed that actually has the melanoma that spontaneously regressed, so it is used in cancer research as well. They're currently selectively breeding this line to be even smaller and with white skin to be used in dermal toxicity studies.

The current Yucatan population used in research are descendants of only 25 animals that were imported to Colorado from Mexico in the 1960s. This breed is very easily trained and is quite docile.

Again, there's also a white hairless line for dermal toxicity studies. The Gottingen was originally bred beginning in 1969 at the University of Gottingen. They are bred from the Vietnamese potbelly pig, the Landrace, and the Minnesota minipig.

That being said, it has since been made available outside of the European Union. This is great because it's the same breed being used everywhere. But what has happened is they've developed all of these different breeding colonies. What happens with that is you end up with genetic deviation or drift from one colony to another, like you would see in mouse research. This becomes potentially relevant when looking at these drug metabolizing enzymes.

I would be remiss if I did not also mention the breeds used in Asia. There are numerous pig breeds, but I'm only going to mention these two: the micromini, which is commonly used in Japan, and the Bama, which is used China. Both of these breeds have been studied for their utility in toxicology studies. And many papers have been published examining the

amounts and activities of the drug-metabolizing enzymes in these breeds.

So if you look at the toxicity literatures, these breeds are very commonly represented. Finally, there are the agricultural breeds. There are many different agricultural breeds, but these three, the Yorkshire, Duroc, and Landrace, are the ones that are mostly commonly used in research studies. They're not typically used in toxicity studies. But, if you'll remember, as I mentioned about the minipigs, many of them have one of these agricultural breeds in their lineage.

Now, I'm going to switch gears and talk very briefly about drug entry pathways. Drugs enter the body by the mouth, by injection, or topically. After entry into the body, the drug will have contact with cells. For drugs taken orally, the drug must enter the gastrointestinal epithelium, and this can be via passive diffusion or by active transporters. In some cases, the drug is then transported intact into the blood stream, but the drug may also undergo metabolism

within the epithelial cells.

After it enters the bloodstream, the drug can then be delivered to the liver and kidney, which are both important organs of drug metabolism. Drug metabolism is composed of Phase I reactions, Phase II reactions, and finally by elimination. We'll be talking more about these later in the presentation. There are not many studies looking at transporters in the pig and comparing to those in humans.

But few of the references that are available state that the transporters do have similarity between pigs and humans, and it's about approximately a 72 percent sequence homology between the species. A couple of transporters that have been looked at in the pig are the ATP-binding cassette, or the ABC transporters, and the solute carriers, or SLCs. The ABC transporters are efflux transporters which help to move the drug out of the cell, and the pig P glycoprotein 1 or multidrug resistance 1 transporter can be inhibited or induced.

The breast cancer resistance protein, or BCRP,

is also an efflux transporter found in both pigs and humans. SLC transporters are influx transporters helping transport drugs into cells. The organic anion transporters, or OATs, and organic cation transporters, or OCT, are SLC transporters that are also found in the pigs. Although, several individual genetic variations have been found in the organic cation transporters. There is a group of scientists examining these different transporters. They're known as the International Transporter Consortium.

As we'll see later, they're still determining which transporters are present and relevant in humans. And there's really nobody looking at this in pigs. We're just basing what we look at in pigs on what we find in humans. Next, I want to go ahead and discuss the first reaction that happens after the drug enters the cell, and that is the Phase I reaction. These reactions expose functional groups of the parent compound which may result in either increased or loss of drug activity.

They result in the exposure of functional

groups for Phase II reactions. The Phase I reactions are either oxidative, reductive, hydrolytic, or dealkylating in nature. The enzymes that mediate these reactions include the cytochrome P450 enzymes which, hereafter, I will refer to as CYP enzymes or CYPs. The CYP enzymes are the enzymes in all species that are most frequently involved in drug metabolism. Other enzymes that can facilitate these reactions include the flavin monooxygenases, the monoamine oxidases, molybdenum hydroxylases, in addition to others.

For those of you that are interested, I've included the reactions catalyzed by the Cytochrome P450 families. I'm not a biochemist, but I wanted to highlight an example of a hypothetical CYP hydroxylation. After the product has been released from the active site, which you'll see at Number 6, the enzyme returns to its original state with a water molecule returning to occupy the distal port position of the iron nucleus.

Depending on the substrates in the enzymes involved, the P450 enzymes can catalyze any of a wide

variety of reactions. Because of the vast variety of reactions catalyzed by the CYPs, the activities and properties of many of the CYPs differ in many aspects. There may be overlap between isoforms, meaning that more than one isoform performs the same or similar reaction. CYPs are a family of enzymes that are functionally conserved in all mammals as we saw.

In humans, the most important Phase I biotransformation enzymes are the CYPs, and there are three primary families that are involved in the majority of all drug biotransformation. These are CYP1, CYP2, and the CYP3 families. These enzymes are found in the ER, or endoplasmic reticulum, and mitochondria of the liver, GI tract, kidney, as well as the skin and other organs. The liver is the most important organ in drug transformation in mammals, including both pigs and humans.

When looking at the content of these cytochromes in the liver -- and this is looking at nanomoles of the protein in the fraction of liver that contains the cytochromes, also known as the microsomal

fraction -- per milligram of total liver protein, we can see that there are differences among the species. In humans, there are about 0.3 nanomoles per milligram. And in the agricultural farm pigs, it's similar in that it's 0.22 to 0.46. But you'll see in the minipig that it's actually more than twice what you would find in either the human or an agricultural pig.

It looks like that's just what I've just mentioned. The study reported here found a greater concentration of the cytochromes in minipigs compared to agricultural pigs, which we need to keep in mind when we start looking at specific studies and differences between the cytochromes. We need to keep the breed that was used for the measurement in mind when we're looking at these numbers. Not only are there breed differences in levels or amounts of the cytochromes present, but there are also polymorphisms between species and within species.

There are also allelic variations leading to interindividual variations. Some individuals may carry multiple copies of certain cytochromes. With

completion of the genome sequencing of the different breeds being finalized, some pseudogenes have been found in the pig for other enzymes, which are not functional within the pig but are homologs to functional enzymes within the human.

Another source of variation in many of the published studies are not only what is measured but what assay is used or how it is measured. When discussing amounts or quantities of enzymes, many papers measure mRNA via PCR. The PCR products may be measured using qtPCR or RT-PCR. Levels of protein have been measured by Western Blot, ELISA, or mass spec, which all have very different sensitivities. And activity levels have been measured by substrate assays or using inhibition assays.

Some papers look at one, some at two, and some at all three measures. There's not a linear correlation between the RNA levels and the protein levels, nor is there always a linear correlation between protein levels and activity levels. There's also evidence for post-transcriptional regulation of

the enzyme. So a little more information on the activity level and how it's measured.

In humans, these studies have been conducted by determining whether the metabolism of a specific substrate or set of substrates happens. And this is to measure whether there is a presence or absence of a specific cytochrome enzyme. Most substrate reactions are specific for a single human cytochrome. In pigs, this is not always the case. In substrates metabolized by humans, cytochrome 2D are metabolized by the pig cytochrome 2B family.

There are other substrates that are metabolized by multiple pig cytochromes, whereas in the human it's only one cytochrome. Now I'm going to talk about the common drug metabolizing enzymes found in humans and pigs. In humans, there are 57 cytochromes which are primarily in six families. These enzymes metabolize over 90 percent of the drugs. In humans, three of these six families are most commonly involved in exogenous drug metabolism.

The remaining families are involved in

metabolism of endogenous substances. The three families important in exogenous metabolism are the CYP1, 2, and 3, as listed here. Within each family, there are several isoforms. Each enzyme is an isoform, and they are derived from different genes. I'm going to just run through some of the common isoforms.

For the cytochrome family 1, there are two common isoforms that have over 80 percent sequence similarity between humans and pigs. Depending on the reference, isoform 1A1 in both humans and pigs has been reported to both have sex differences, and it's also been reported to not have sex differences. And this is something that is consistent throughout the literature discussing these cytochromes is the lack of consistency.

No sex differences have been reported in the 1A2 isoform in pigs. That doesn't mean it doesn't happen. It just may be their methodology that was used in that paper. This family metabolizes carcinogens, including aromatic and heterocyclic amines. It metabolizes estrogens, mycotoxins, xanthenes, some

antidepressants, and analgesics. Specifically, CYP1A2 has the role of metabolism of antipsychotics, caffeine, and theophylline.

It's also been shown to be induced by drugs, including a normal dose of omeprazole, which is a common over-the-counter drug. And this induction has been shown to be consistent across species. In humans, the CYP1A family metabolizes about 20 percent of the substances tested. There have been reports of activity being sex related with higher activity in females, only in minipigs, or in males, and this is human males. And it was Caucasian males. There are also changes in the amount of CYP1 as the animal ages with decreasing levels as the animal or human ages.

The cytochrome 1B family is the predominant isoform in humans in organs outside of the liver. And this isoform has not been characterized in the minipig. Moving to the CYP2 family, here we have a menu for isoforms to discuss. On the left, I have the human CYP listed with the corresponding pig cytochrome in the next column. Then I have a column with amino acid

similarity. In the final column, I have listed any differences that have been reported in the literature. There are sex differences in some of these cytochrome families, and there are also breed differences in some of them.

The CYP2 family metabolizes nicotine, nitrosamines, aflatoxin B1. We have thus far been talking about differences between humans and pigs, but here we have information that's specifically for the 2A19 isoform. There is a difference between pig breeds, and there's a 99 percent similarity between Gottingen and conventional breeds. But that means that there's one percent that is not homologous, and that may be significant.

Female Gottingens have shown to have a 70-time higher activity level than males for this family. But when intact males are castrated, the activity in these males increases ten times, showing that androgen levels do affect CYP activity, but it's not completely related only to the androgens or sex hormones. Yucatan females have been reported to have a five-time higher activity

than males, and there have been no sex differences in activity reported in humans. Again, there are marked species, breed, as well as sex differences.

The CYP2B family metabolizes diazepam, lidocaine, cyclophosphamide, and tamoxifen. No sex differences in activity have been shown in Yucatans in this family, and levels are increased in conventional pigs relative to humans. Levels in young animals are the highest and then decrease as the animals reach adulthood. Overall, there are many inconsistencies in what is known about the CYP2B isoforms in the pig.

One of the substrates commonly used for testing activity in human cytochrome 2B family is dealkylation of 7-pentoxyresorufin. This assay was used in some of the studies examining porcine cytochromes but was not used by all groups. There are also inconsistencies in sources of the hepatocytes and thus differences in the microsomes that were used in these tests. Another variable is that the CYP2 family can be induced by phenobarbital and a few other drugs.

In humans, the CYP2C family metabolizes 22

percent of drugs, including losartan, propofol, estrogens, testosterone, and methadone. In pigs, the CYP2 isoform show cross reactivity toward many of the test substrates, not just those for human CYP2C. And it has proven difficult to extrapolate between the species for this family. In the CYP2D family, this family metabolizes antidepressants, antipsychotics, as well as beta blockers.

In humans, this family has high inter-individual variances with multiple polymorphisms or alleles. This family has not been focused on in the pig, but what has been found is that many of the human CYP2D substrates have been found to be metabolized by the pig CYP2B family. The final group in this family is the cytochrome 2E family. This family metabolizes alcohols, ketones, anesthetics, and nitrosamines. Metabolism by this family can lead to production of highly reactive toxic or carcinogenic metabolites.

I think one of the more relevant and important aspects of this family is that it can be inducible by both alcohol as well as high-fat diet. None of these

studies that have been done in pigs look at how these factors may affect levels or activity of this or any cytochrome family in the pig. This family can be induced by stress, by increased translation, and no change in transcription. In many pigs, studies have shown higher activity in females than in males.

Conversely, there have been no sex differences noted in studies of the CYP2E in any of the conventional breeds that have been examined nor have they been shown in humans. In humans, there are two important CYP3 isoforms, and in pigs there are three important isoforms. Again, both sex and breed differences have been shown in the pig for this CYP family. In humans, this family represents 30 percent of the total cytochromes in the liver.

This family metabolizes at least 27 percent of exogenous substances in the human and is involved in steroid hydroxylation and converts sex hormones as well as polycyclic, aromatic hydrocarbons, and pesticides. The CYP3 family is highly expressed in many organs in humans, and this is the primary family in humans. A

couple of highlights are that the pig also expresses 3A in several organs, although this family is not the primary one in the pig. It has been shown that transcriptional regulation is different between humans and pigs. Differences between breeds have been shown.

And, again, the diet can differentially affect the activity level of this cytochrome family in males and females. A study was done looking at the effect of chicory root in the diet, and it was shown that the presence of chicory root in the diet decreased the enzyme activity in males, whereas in females the activity was increased. To review, there are no major differences in substrates, inducers, or inhibitors, and tissue distribution between humans and pigs in CYP1A1, 1A2, and 3A.

Several studies have shown that Gottingen minipigs have higher content overall relative to three breeds of conventional pigs and two races of humans. Both content or levels of the enzyme and activities of cytochromes differ among the breeds. Significant sex differences have been shown in porcine cytochromes but

not all breeds. While sex steroids or hormones have been shown to have an effect, the sex differences are not always dependent only upon those sex hormones.

There have been several studies done by Kojima (phonetic) et al. that have looked at several cytochromes in two different breeds as well as F1 hybrids of these two breeds. The findings have shown that there may be a positive or negative correlation with administration of testosterone and some cytochromes are increased, whereas others are decreased. The takeaway is that there are significant discrepancies in the interpretation of cytochrome levels and substrate specificities. And many of these discrepancies are due to different assays and measurement techniques being used.

We've heard much about these issues in yesterday's presentations and discussions for viruses as well. These studies also show that whether a cytochrome family is inducible and the magnitude of induction differs across tissues and cell types, even when exposed to the same chemical inducer. There are

similar concerns when looking at activity. Some of the studies measure activity per milligram of microsomal protein whereas some of them look at activity per milligram of whole liver protein.

These discrepancies may account for some of the differences between the sexes if in some breeds the females have more cytochrome enzymes overall within the liver. Some of the other variables I've mentioned briefly include genetics, both breed and parental lineage, the age of the animal. For some cytochromes, very young animals may not express a specific cytochrome, whereas for other cytochromes the highest expression is in animals less than three months old.

There are sex differences as well as sex differences with age. Diet factors may be more pronounced with age. There are also epigenetic factors to consider. Circadian variation has also been reported, so the time of sampling for the study is relevant but rarely reported. Transcriptional regulation is also important but poorly studied. I've included this figure to demonstrate that organs develop

at different rates between pigs and humans.

With all of the variation I just reviewed, I believe it's imperative that we make sure that the organ that's being transplanted has matured if it's going to be placed into an adult, and I think we've covered that in some of our discussions in the last day and a half. The reason we're talking about drug metabolism at all is likely twofold. One, you want to make sure that the drug you're giving the patient can be metabolized appropriately by the xenograft.

Two, you want to make sure that the drugs are not toxic to the xenograft. There will be many cases in which drug-drug interactions also need to be considered. Another facet we need to consider is, while the drug may not be directly toxic, it may inhibit a particular cytochrome isoform that results in toxicity from another drug that would use that inhibited cytochrome. I'm going to move quickly through the Phase II conjugation pathways.

In the Phase II reactions, these reactions result in the formation of the covalent linkage between

a functional group and either glucuronic acid, sulfate, glutathione, amino acids, or acetate. This will increase the polarity of a compound to aid in excretion. In most species, glucuronidation and sulfation are most important covalent reactions in drug biotransformation. But not as much research has been done on the Phase II enzymes so far in the pig.

It is known, however, that sulfate conjugation in swine is slower than in other species and that to offset this other reactions predominate in the pig. Whereas sulfation is more predominate in humans, it turns out in the pig the pig is more efficient than the human at glucuronidation, so it will glucuronidate in place of adding a sulfate in many cases. As I just mentioned, pigs compensate by using other Phase II enzymes to metabolize, and pigs also have a high acetylating capability.

In the pig, not much is known about the UGT or its isoforms, other than the fact that it is more efficient than the human. I am going to go through the organ systems right now and just talk about what is

known in the pig. I'm just going to touch on the liver, GI, and kidney. Starting with the liver, there are numerous influx and efflux transporters. This slide represents a human hepatocyte. It's from a review in 2010, so 12 years ago. The transporters in blue are known transporters, but they were not thought to be of much importance in drug metabolism.

Then, in a review from the same group in 2018, you can see that they have added more transporters that they're aware of. Ones that they didn't think were important, now they think are, which is represented by the color change. And the point of showing this is that in eight years the study of the most important drug metabolizing organ in humans has led to advances and new knowledge, and there's funding to support studies like this.

Until there's a group of toxicologists and pathologists that can systematically examine the pig, I think we're lagging far behind in basic scientific knowledge for this species. The liver performs primary or pre-systemic extraction with the receipt of the port

of blood flow. There are both Phase I and Phase II enzymes in the liver. The porcine liver contains similar levels of glutathione transferase and UDP-glucoronosyl transferase to the human. Overall, the quantity of the isoforms are quite different between the two species within the liver.

This shows the protein levels, which is picomoles per milligram of microsomes in the pig on the left and in the human on the right. In the pig, the most abundant protein is the CYP2A19 followed by 2D25 and 2E1. In humans, the most abundant protein is CYP3A followed by 2C25, 1A1, and 2E1. So you can see that there are profound differences in the liver of the cytochromes. Moving onto the intestine. Again, just showing you that in 2010 these are the transporters that they were aware of and thought were important.

Those circled in green in this slide actually have higher levels in the pig. If they're in red, they had lower levels, and grey had similar levels. So that's just a comparison between the two species. Again, you can see there are different levels of the

transporters in the intestine. In 2018, there are more transporters that the group discovered and thought were important. In the GI tract, passive cellular diffusion is the primary mechanism of intestinal drug absorption.

Other variables to consider are that there are profound interspecies differences in the level of salivary amylase, the pH of the stomach, small, and large intestines, the rate of gastric emptying. GI transit time also differs between species, and the age of the animal again matters when discussing drug absorption and metabolism. The GI tract is the most important extrahepatic site of drug biotransformation. Most molecules pass through the enterocytes after oral administration.

In both pigs and humans, CYP3A is the most abundant bio transforming enzyme in the small intestine. Overall, pigs do have similar gut physiology to humans. Other factors to consider in the GI tract are the efflux transporters, which I discussed previously, bile salts that solubilize the lipophilic drugs, and the bile flows is similar between humans and

pigs. Here is another figure showing the cytochromes in the jejunum between the pig on the left and human on the right.

And you can see, in the jejunum at least, there is more similarities between the cytochromes. Finally, let's talk about the kidney. The kidney does have some drug metabolizing capability, and this figure should be starting to look familiar. Here it is in 2010, again in 2018. You can see that the transporter number has increased. Without doubt, whether or not the kidney contributes to metabolism, it is the most important organ for elimination of drugs and their metabolites.

Of the most commonly used therapeutics, approximately one-third will undergo elimination through the kidney. As far as metabolism, the kidney only has one-tenth of the cytochromes expression as does the liver. Although, in some cases, it's metabolic activity may surpass the liver, depending on the drug. Within the kidney, there are regional differences in regards to enzyme levels, and the

metabolism of drugs occurs primarily within the proximal tubules.

Substrates and inhibiters of renal transporters are well documented in the human, and studies looking at cytochromes in the kidney are rare. In a few studies looking at other species, it has been shown that in the rabbit the S2 and S3 segments are enriched in cytochromes levels. And in the rabbit there are sex differences in the liver, but they're not evident in the kidney. I mentioned that some cytochromes may be induced in the liver -- and this is also true in the kidney -- but there are differences.

In some cases, the same drug will induce cytochromes in both organs, or in some cases the drug is organ-CYP-inducing specific. So barbiturates would induce cytochromes in the liver but not in the kidney, whereas polycyclic hydrocarbons will induce cytochromes in both the liver and the kidney. It's going to be difficult to extrapolate findings in other species to the pig if the studies are not done in pigs.

Of note, large differences have been noted in

the renal metabolism between mice and rats, and they are more closely related than humans and pigs. There was one study in China where they attempted to cause acute kidney injury with a drug. Not only were the results of the study inconsistent between groups, they were inconsistent between individuals. There remains much to learn about the kidney reaction to drugs in the pig and renal metabolism of drugs in the pig.

In humans, the kidney expresses the 3A isoform, but levels of the cytochrome vary by race, with Africans expressing highest levels and Caucasians the lowest. This is relevant as nephrotoxicity of cyclosporin and tacrolimus, two commonly-used drugs in immunosuppression, is dependent upon the 3A5 genotype. There are similar processes and pathways between the two species, but levels of the enzyme and rate of metabolism may differ between and even within the species.

DR. LISA BUTTERFIELD: Dr. Helke, we will want to leave a few minutes for questions.

DR. KRISTI HELKE: Okay. Let me make two more

points. I'm just going to apologize to the vegans and vegetarians, but the bottom line is that most of the original work has been done in the pig examining drug metabolism in cytochromes stems from the fact that agricultural side has had an interest in making pork more palatable. Many initial studies looked at porcine cytochromes to decrease "boar taint," and breed differences emerge, as some of the studies showed.

I'm just going to skip through all of this. You guys have the slide deck for your perusal. There are holes in knowledge. Then, at the end, I have placed some value-added slides here for the Committee to consider in their deliberations. I'm not going to go through them but would recommend that the background lesions in xenotransplant models be examined systematically as it has been in these minipig breeds used in toxicology studies. They're all findings from the control animals in toxicology studies.

I'll also mention that finding the funding to do these studies is difficult. With the slides I have provided, the tissues were collected and processed as

part of a study for toxicology. But funding to do this de novo needs to be considered in order to see what sort of background pathology may be present in the populations of potential xenotransplant pigs. Thank you, and I'll end there. I'm sorry I went over.

Q&A

DR. LISA BUTTERFIELD: Thank you very much, Dr. Helke. We do have a couple minutes for questions. While I watch for hands from the Committee, I wanted to ask it seems, as you've shown, there's a lot of biochemistry in drug metabolism that's either known or anticipated to be very different between pigs and humans and more so between what could be a considerable variation from one human being to another.

Perhaps as sponsors think about the engineering that they propose in the porcine hosts for these organs, perhaps basing the strain choice in part on what's known about the metabolic changes would be valuable?

DR. KRISTI HELKE: I think so. The problem is that even between the breeds there is inconsistencies in the literature right now as it stands. If you look at one study that compares pigs to humans, then their methodology is going to be the same throughout that paper, which is great. But it's difficult to compare from one group of scientists to another because they don't necessarily use the same, like I said, methodologies.

But, yeah, there are individual differences in human as well. But I think it is something that's going to have to be considered. Like I said when I started my talk, Dr. Wolf did mention the differences in breeds and the growth rates. But I've had a hard time finding -- I see all these papers on the xenotransplant, and it says there was a genetically-modified pig used. But what I can't find is what breed was that.

DR. LISA BUTTERFIELD: Yeah. That's important. One of the things we talked about yesterday was an opportunity for some consortia efforts to help

propose standards. Do you think that there's an opportunity here in some of these biochemical and sematic-type studies?

DR. KRISTI HELKE: Oh, absolutely. I think there needs to be. You want to keep up with the science, and I understand that some of these papers were probably done in the 80s. And, yes, science has advanced. But that doesn't mean we can't redo a couple of those to see is that consistent or has this new methodology changed the outcome or our interpretation of the outcome.

DR. LISA BUTTERFIELD: I'm wondering, because the CYPs are so critical to drug metabolism and some of the drugs that are key to the clinical situations we're talking about, is there a short list of things that you would prioritize for measurements? Or would that be just very hard to think about?

DR. KRISTI HELKE: I think it's hard because you've got so many of them that overlap. It may be one CYP that does this reaction in the human. But in the pig, that reaction is metabolized by two CYPs, neither

one of which are the same as the one that's in the human.

DR. LISA BUTTERFIELD: Are these studies that can be in vitro?

DR. KRISTI HELKE: Most of them are done in vitro. They take liver samples and then isolate the microsomes. One thing I didn't get to mention is that a lot of these are isolating microsomes, which is essentially the ER. But that leaves the mitochondrial aspect out. There was a recent paper done in rats showing that you've got CYPs both in the mitochondria and in the ER.

So, if you're only looking at the microsomes, you're looking at the ER, you're leaving that whole mitochondrial component out. So maybe the better way to do it is to look at whole liver. I'm not sure. And some of the studies do look at whole liver, and maybe that's why there are differences.

DR. LISA BUTTERFIELD: All right. Great. Thank you very much. This is definitely going to factor into our discussion on Question 6. Any final

questions from other members of the Committee? Dr. Bloom.

DR. MARSHALL BLOOM: Yes, that presentation can only be described as a cornucopia of detail. I'd just be sort of curious to hear what Dr. Pierson and Dr. Wolf's reaction to all that was. You talked a lot about the kidneys, the transporters, and stuff like that. I'm curious what they're feeling about this and how much of what you talk about is something that they take into consideration or think about when they do their studies. Thanks.

DR. LISA BUTTERFIELD: Okay. I don't know if we can call on them now, if they're easy to call on, or if we should ask them to be ready to perhaps respond to that question when we have the full Committee discussion.

DR. MARSHALL BLOOM: That'll be fine. That'll be fine.

DR. LISA BUTTERFIELD: Okay. Why don't we do that. Again, I'll thank you, Dr. Helke, for that presentation. Now, we are scheduled for a short break

before we go into the long discussion of both Questions 5 and 6. So let's come back in 15 minutes. We're scheduled for 10, let's come back in 15 refreshed and all ready to weigh in on both of these questions. Thank you very much.

MR. MICHAEL KAWCZYNSKI: All right. Studio, if you can take us to break.

[BREAK]

COMMITTEE DISCUSSION OF QUESTION #5 & 6

MR. MICHAEL KAWCZYNSKI: All right. Welcome back to FDA's 73rd meeting of the Cellular Tissue, and Gene Therapies Advisory Committee meeting. That was our last break. I'm going to hand it back to our chair, Dr. Lisa Butterfield. Take it away.

DR. LISA BUTTERFIELD: All right. Thank you, very much. So, welcome back, everyone. And now we've had two presentations about our last two questions for today about xenotransplantation. So, now let's move to

discussion of Question 5. We'll have two discussants to present their views and to start the discussion ball rolling. And then we'll move to full Committee comments. And I'm looking forward to hearing from most of the members of the Committee on this.

So, Question 5 is: transplantation of pig cells and organs is intended to provide replacement for non-functioning/damaged human cells and organs. Therefore, it's important to understand the characteristics of these cells or organs in the pig to ensure they have the characteristics needed to provide replacement therapy for the human recipient before transplantation. And it is important to monitor these cells and organs to demonstrate they provide the expected functions after transplantation.

Please discuss existing data to address the following issues related to pig cells and organs intended for transplantation into humans -- so, both before and after transplant -- A, the ability of the target pig organ to support full organ function in humans, and, B, the natural aging of the target organ

in the pig relevant to expected organ function over time in humans -- so, organ function and function over time. So, our two discussants are Dr. Zeiss and Palevsky. So, Dr. Zeiss, please start us off.

DR. CAROLINE ZEISS: Thank you, Dr. Butterfield. And thank you, Dr. Beaston and Dr. Helke, for setting the stage. And all that toxicology, it certainly makes me want to live a healthier lifestyle. I wanted to address in some more detail the issue of overgrowth of the donor organ because this is not a benign phenomenon. The pathology is very significant. And it's independent of rejection associated pathology.

So, you've heard from previous speakers that the pig has a very strong intrinsic capacity for growth. Pigs are production animals. They've been bred for a long time to grow fast and very big. And that is reflected in the capacity of the organs to do the same. We see from pig-to-pig allograft experiments that this is associated with breed, and it is an intrinsic capacity.

We have also -- I also had the same experience

as Dr. Helke, that trying to find the pig breeds that are used for the creation of genetically altered pigs, it's very difficult to find this. And I'm sure that there are people here who know what these major breeds are, but they are not well reported in the literature. I do think that even if we use some of the smaller breeds, some of that potential for intrinsic growth capacity is going to be retained because the ancestral streams are still these production breeds.

When you put a pig to baboon, a kidney -- there are some reports on that -- on those xenografts, the kidneys grow very quickly. So, approximately they double their size in about three months. And that is not a benign phenomenon. It's associated with aggressive increase in creatinine. And on explantation histology there are ischemic lesions in the kidney associated with intracellular edema and fibrosis.

When it comes to hearts, you see very much the same thing, so, a very quick doubling, two to three times the size of the original size of the heart, accompanied by biventricular hypertrophy and poor

cardiac function and on histology, myocardial hypertrophy and necrosis, interstitial edema and fibrosis, as well as a microangiopathy. And these are the animals that have previously been referred to (audio skip) these die within 30 days.

So, in the same study, this is Langen (phonetic), 2018, this was overcome by taking a three-pronged approach. The first was based on the rationale that pig blood pressure is slightly lower than non-human primate blood pressure. And I think that that may be the case in some studies. However, if you look at multiple papers looking at reference values for pigs, in adult pigs they are pretty much the same as people, in the 120 over 80 range. There is some variation.

So, their first approach was to give anti-hypertensives. The second was to taper Prednisolone sooner because Prednisolone also has a trophic effect. And third, which I think turned out to be possibly the most important intervention was to use an mTOR antagonist. So, mTOR is quite central to cardiac

hypertrophy in showing rat studies -- in hypertensive rats, that the central mechanism to engaging the heart in a hypertrophic response is mTOR. And if you block that, you can block that response.

We also see hypertrophy of the heart in allograft. So, this is not restricted to xenografts. It is a complication of cardiac allografts as well. And there is evidence to suggest that extrinsic factors such as hypertension may play a role. And I think with the pig xenografts, the combination of the intrinsic capacity of the heart to grow very fast, combined with extrinsic factors such as hypertension -- which are likely to be very common comorbidities in transplanted patients, that these two could have a very strong synergistic effect.

I'd like to talk a little bit about the Baltimore patient. So, this individual was transplanted with a 10-gene edited pig heart. And this included the growth hormone receptor deficiency. So, one of our previous speakers talked about preventing this hypertrophic response in pig to baboon xenografts

by transplanting organs that had the growth hormone receptor deficiency and that that took care of the problem. And certainly, in the baboons it did.

However, in the patient in Baltimore that was transplanted with one of these growth hormone receptor deficient hearts, that did not solve the problem. So, this individual was hypertensive, and he experienced progressive biventricular hypertrophy throughout his 60-day course of survival. When the heart was examined after he had died, it had doubled in weight, and it had very similar lesions to what was seen in monkeys -- so, cardiac myocyte necrosis, edema and some evidence of humeral mediated rejection. So, there was some evidence of rejection there.

Now, the question has come up what is the role of CMV, what is the mechanism? We know it's reproducible. That having CMV in the patient decreases longevity of the transplant. However, the mechanism is not entirely defined. And I think certainly it's reasonable to assume that it engages the immune system and that it contributes to graft rejection. But there

was certainly no evidence of CMV -- classic CMV associated pathology in this heart.

So, the use of mTOR. So, in terms of the mechanisms that creates the hypertrophy, growth hormone is one. It's fairly upstream. mTOR is fairly downstream, and it connects with all kinds of upstream mediators -- upstream trophic mediators. And then it connects downstream many, many signaling pathways. And so, trying to -- I had asked a question earlier about could it conditionally knock that out. If that could be feasible, it may be one way to prevent the patient from being on mTOR inhibitor for the rest of their life.

But I think that we need to do more research to understand the mechanisms of controlling this hypertrophic response because it is not a benign response. And I think that it -- certainly in the Baltimore patient it seemed to be a very significant factor in loss of the tissue.

Dr. Beaston very, very nicely set out all of the differences in -- I'm going to switch -- leave that

topic behind and switch now to a couple comments about the kidney, about physiologic differences. I don't really have anything to add to those that Dr. Beaston listed. I will just say that with xenotransplants in baboons we have seen good GFR's, good urine output, good urine SG retention and normal serum creatinine for three months afterwards.

Pig kidneys tend to concentrate urine a little less. The urine is a little bit more dilute. There are a number of mechanisms behind that. Part of it is the anatomy. There are fewer lung nephrons. They don't respond to human ADH quite as well. They have a slightly lower albumin. And certainly, pigs -- baboons with pig kidneys can experience episodes of hypervolemia that required fluid supplementation.

Pigs have got a higher serum phosphorus that is quite significantly higher than people -- about 8.6 milligrams per decimeter compared to 3 to 4.5 in people. And that certainly, I think, could create some complications of (inaudible) phosphorus balance. But that's only in the short-term. It has not been seen in

baboons.

I want to make a couple comments on hepatic xenotransplantation. One of the major roadblocks there is that we still get profound thrombocytopenia. So, this is due to captured recipient platelets by pig Kupffer cells. In terms of islet xenotransplantation, the hitch there is that there is inconsistent efficacy. And these may be superseded at some point by human stem cell approaches.

And then lastly, I wanted to talk on the second question, the expected age and trajectory of transplant pig kidneys. So there isn't a lot of data on old pigs out there because they're food animals. We do see some data on geriatric micro-mini pigs, so, pet pigs. And they generally have the usual sort of array of not very interesting, not very pathogenic things that all of us get.

I wanted to pick out two that I thought could be relevant. The first is a kidney. There is a relatively higher proportion of interstitial fibrosis glomerulosclerosis with aging. And this occurs pretty

much across all species. However, if you combine this with potentially a hypertensive recipient, that could certainly accelerate this propensity.

And then in terms of their arterial systems, you do see some arterial thickening in the aorta, some intimal proliferation, some medial minimalization. And I will point out that pigs are fairly athero-sensitive. Many species are not. Most animals have really quite pristine blood vessels by the time they die. And that is very different from humans.

It is likely that pig blood vessels arteries will probably experience the same pathology, depending on a person's lifestyle, than ours do. So, all to say that these organs are going into people often with complicated comorbidities. And the impact of those comorbidities on the implanted organs is something that we have no data on because we simply don't have those comorbidities. So, I think that is something that -- it might be something that just needs to wait to get human data on to fully understand that.

I think the take home point that I have seen

from reading these papers is that there are quite unexpected things that happen that are quite difficult to predict from looking at pig to baboon studies. I'll finish up by saying the transgenes, these may have altered expression over time, and this may be tissue specific. And so, we could accumulate tentative rejection, coagulopathy over time. And I think with that, I will stop.

DR. LISA BUTTERFIELD: All right. Thank you very much, Dr. Zeiss. And now, our second discussant, Dr. Palevsky.

DR. PAUL PALEVSKY: So, I'm going to focus on the kidney since I'm a nephrology. And I want to thank Dr. Zeiss, Dr. Beaston, and Dr. Helke for their really setting the stage here.

When we talk about support -- having a kidney supporting human life we normally focus on the filtration aspect of kidney function -- GFR, controlling BUN and creatinine. But the kidney is a far more complex organ than just one that excretes nitrogenous waste products. And this was touched on by

Dr. Beaston in terms of issues related to fluid and blood pressure control, electrolyte balance, et cetera.

The kidney has complex transporter function, and I could find very little on data on homology between pig transporters and human transporters, which may have importance significance in terms of sensitivity to the drugs that we typically use such as diuretics, thiazides effecting the sodium chloride transporter in the distal convoluted tubule and the loop diuretics acting on the sodium potassium two chloride transporter. So, are these drugs going to function in similar fashion?

Electrolyte disturbances are frequently seen following allotransplantation. Hyperkalemia is a common problem. Phosphate wasting is a common problem. We'll have to find out what happens with the pig kidneys in individuals who've had longstanding chronic kidney disease who may have underlying severe secondary hyperparathyroidism.

What are the differences in the renin-angiotensin system in the pig compared to the human?

Erythropoietin -- there is a lack of homology and ineffectiveness of the pig erythropoietin on erythrogenesis. But is there enough homology that this is going to trigger an antibody response that could then result in resistance to erythropoietin and pure red cell aplasia from this, and will we have to deal with that as a longer-term consequence?

With regard to aging, comments have already been made about the growth of the kidney. And this poses a significant risk. You're not going to be increasing nephron number. So, as you have renal growth, you're going to have hyper filtration. How is that going to affect the development of glomerulosclerosis and early demise of the kidney due to non-immunologic injury?

So, I think that we have a tremendous number of unknowns that are going to need to be very well defined in order to move forward with clinical use of the xenotransplant. So, I think that we need a lot of research to define these issues before we can move forward. Thank you.

DR. LISA BUTTERFIELD: Great. Thank you very much. And I think to add to what our two discussants have just presented after our two presentations, we also heard a little bit yesterday on the notion that young organs are being transplanted and over time it's possible that there might need to be a second organ that needs to be transplanted. The notion of donor animal testing could be imaging before transplant, but it looks like there's a lot of depth lacking in some of the measures of function that we've been able to collect data on so far.

So, let me turn to the Committee and let's discuss these in more detail. And we'll start with Dr. Morrison.

DR. SEAN MORRISON: I've got a question about this phenomenon of organ growth. To what extent -- it sounds like there's both inflammation and edema that contributes to the increased size of the organ as well as a growth capacity in the heart and the kidney that we don't see in the human heart and kidney. So, is it known that there are stem cells in the adult pig heart

and kidney? And if so, does this growth continue throughout adult life?

DR. LISA BUTTERFIELD: All right. Thanks for that question. Let's see what we do know about that mechanism. Looking for hands of who would like to address that intrinsic organ growth. Dr. Zeiss. Thank you.

DR. CAROLINE ZEISS: So, first of all, there is very little information on these organs. There is no similar infiltrate. What we see is cardiomyocyte hypotrophy. So these are existing cardiomyocytes. They're not proliferating. They're the existing ones that are getting bigger, and then they're dying. That's what we see in monkeys; it's what we've seen in the Baltimore patient.

Pigs do keep growing quite a while after sexual maturity. So, sows will accumulate 50 to 100 pounds with every litter. The rationale behind creating the growth hormone pigs -- growth hormone receptor deficient pigs was that they would be past their growth curve to produce a heart that was of a

size for an adult human, but they would be past the growth curve. And so, that residual growth would not keep on.

The problem with minipigs is that they tend to have high curves. But we've heard that there are ways around that. So the question is do we create growth hormone receptor deficient minipigs assuming that there are other metabolic associated with -- abnormalities associated with that and then harvest those organs which are still going to have some intrinsic growth capacity?

I think at some point if you take enough measures to limit growth, you can mitigate that intrinsic capacity for growth. However, the extrinsic capacity -- extrinsic drivers like hypertension are still going to be there. So, there has to be some way to control that as well -- possibly too controlling mTOR and controlling hypertension which is obviously not always very easy.

DR. SEAN MORRISON: But (inaudible) like for the intrinsic growth capacity that it's just that the

heart grows a little bit longer than in a human but that that growth does end at some point in terms of the --

DR. CAROLINE ZEISS: Oh, yes.

DR. SEAN MORRISON: -- production of (inaudible) cells.

DR. CAROLINE ZEISS: Yes. Yeah. It will end.

DR. SEAN MORRISON: And will mTOR inhibition still help with the size of the heart once that growth capacity -- the intrinsic growth capacity is over, or is that the only thing that's targeted by mTOR inhibition?

DR. CAROLINE ZEISS: So, mTOR is a mechanism in pathologic left ventricular hypertrophy associated with hypertension.

DR. SEAN MORRISON: Thanks.

DR. CAROLINE ZEISS: So, this is a -- the enlargement in the size of the heart is a combination of intrinsic growth and pathologic hypertrophy. And it's difficult to disentangle which of those is driving this. Certainly, the intrinsic growth is a major

component. But the extrinsic amplification of this is also important.

DR. SEAN MORRISON: Is it possible to just harvest the hearts from a little bit older pigs once they've gotten past that intrinsic growth phase?

DR. CAROLINE ZEISS: Yeah. So, that was the rationale behind the growth hormone receptor deficient pigs. So, these are German Landrace. It's still a production breed. It's still pretty big. Those pigs are about 60 to 70 percent of the size. The heart is about 75 percent of the size of a regular production pig heart. So, it's still a pretty big heart.

If we shift -- again, you know, what breed is going to be optimal for this? I think that's a question that hasn't been answered yet. If we shift all of the genetic alterations to a smaller pig, then potentially we could get over that major growth curve and find a heart that has got far less intrinsic capacity to grow.

DR. SEAN MORRISON: Thank you.

DR. LISA BUTTERFIELD: All right. Thank you

both for that. So, let's see. Let's hear more discussion on question five from Committee members. Let's go next to Dr. Auchincloss and then Dr. Cooper.

DR. HUGH AUCHINCLOSS: I was simply going to go back to Marshall Bloom's question and ask our morning presenters what their reaction was to the afternoon presentations.

DR. LISA BUTTERFIELD: I'll see if we have them available. Sometimes guest presenters who are not Committee members end up moving to YouTube to continue to watch the proceedings. I'll ask for some --

DR. HUGH AUCHINCLOSS: Well, if they're not here --

DR. LISA BUTTERFIELD: Okay. All right. So, I don't think we can call on them.

DR. HUGH AUCHINCLOSS: Let me go on to my other observation or comment that I --

DR. LISA BUTTERFIELD: Thank you.

DR. HUGH AUCHINCLOSS: -- was on my mind, which was would my fellow Committee members agree that two tissues that are probably best to start with for

xenotransplantation would be heart or islets? Does that make sense? Oh, there's Robin (sic) Pearson.

DR. RICHARD PIERSON: I'm sorry. It took me a moment to get to the right screen. I apologize for putting my hand up again. I've been told I'm not supposed to do that, but I thank you for the call out. I wanted to start by -- Dr. Zeitel's [sic] points are right on. The complicating factor in the Maryland heart case -- the case of the Maryland heart recipient was complicated by the CMV activation which may have trigged inflammation in the graft that could have contributed to the diastolic dysfunction and hypertrophy independent of the mTOR -- independent of the growth hormone receptor knockout.

And so, that situation is difficult to fully interpret. The mTOR inhibitor's effect on growth in the German orthotopic heart experience -- in my estimation, it's not clear whether it's an effect to inhibit growth, to suppress elicited immunity, or both that accounts for the salutary attenuation of growth out of proportion to the physiological needs of the

recipient in that model.

And I think we won't know until we try this in human heart recipients whether -- to what extent hypertension control alone, mTOR inhibition, added to whatever immunosuppression is considered the platform or both will be necessary and sufficient to prevent pathologic remodeling, diastolic dysfunction, hypertrophy of either nongrowth hormone receptor knockout or growth hormone receptor knockout organs in the human circumstance.

Coming back to the more general question that Hugh asked about my reflections on these talks, which are very interesting and educational for me, about the many differences between pigs and humans. And we have many unknowns about pig renal physiology. There is grant funding from NIH right now that's coming to my colleague, David Cooper, at MGH, asking about some of these aspects of potentially clinically important aspects of renal function -- erythropoietin metabolism, pituitary hyperthyroid hormone metabolism and other facets related to salt retention, blood pressure

regulation, et cetera -- angiotensin pathway is of course also quite important -- that are unknowns.

The reassuring aspect to me is that when we prevent pathological elicited immunity and also at least in the heart circumstance inhibit dysregulated coagulation, those organs grow to the size of the donor pig and then -- at adult size and then seem to stop. And anecdotally, we have a heart that's nine months out after transplant. It does have the growth hormone receptor knocked out. And without blood pressure control, without any effort to modulate blood pressure, that heart has stopped growing and has not to demonstrated either diastolic dysfunction or left ventricular hypertrophy.

So, there are going -- I can cite an example where we didn't need to control blood pressure and we ended up with a pig heart in a baboon that is the right size for the pig it came from. And I think that's the message of Dr. Kawai's (phonetic) study as well. That the pig organs will grow -- will try to grow to the same size as the adult of the species from which they

come. If there is immunologic injury or physiologic damage either due to high blood pressure as Dr. Zeitel (sic) was referring to or some other pathology, then one can expect that the organ will adversely remodel in one way or another.

And so, that would -- my takeaway from those important observations and acknowledging the many unknowns is that our preclinical data would predict that a kidney and the heart are likely to be life supporting when tested in humans. And if that is not the case, we will learn that relatively early. And how far back to the drawing boards that will send us I can't predict until we see what kind of trouble we get into. But my own judgement is that the place for us to learn that is in the clinic and that I'm sufficiently optimistic, as I told our patient advocate earlier today, that I personally feel that it is reasonable to move forward in as safe a way as we can. So, thank you for the opportunity to speak.

DR. LISA BUTTERFIELD: Okay. Thank you both. Anything else for now, Dr. Auchincloss? Looks like --

DR. HUGH AUCHINCLOSS: No.

DR. LISA BUTTERFIELD: -- no.

DR. HUGH AUCHINCLOSS: Let's let some others weigh in.

DR. LISA BUTTERFIELD: Okay. Thank you. Let's move to Dr. Cooper.

DR. MATTHEW COOPER: So, thank you. So, I will let it be known, I had my hand raised before Dr. Pierson jumped on the call. And that was extremely helpful. He may have started to answer a question that I had that I'm not sure if I'm the only one thinking it. I would say our afternoon speakers gave a really intriguing, outstanding -- I think we said cornucopia of information around sort of functional mechanistic and physiologic differences between porcine and human heart and kidneys, especially.

And I wanted to challenge -- Dr. Palevsky at the end of his presentation said that we just don't know and we're going to need to be able to do more experiments to test these things. And after two days I'm sort of struck by the frequency with which pretty

much everyone who has either presented or commented has said that the only way they were going to know is to move into clinical trials. And I guess I'm uncertain, short of that model, how are we going to answer those questions?

And I'm reflecting back on the most recent FDA guidance on this that was -- I'm paraphrasing a little bit, but that was certainly rigid in its expectation that in order to move to clinical trials the expectation at that time was that there needed to be a robust non-human primate model with consistent immunosuppression that demonstrated success before the FDA would approve to move on to clinical trials.

And I'm hoping -- I'm uncertain, but I'm hoping sort of based upon a lot of this conversation that we are perhaps sort of changing that view back from 2016 because it seems as if many of us on this call, including again our experts -- and I thank them all for their presentations and being able to answer our questions -- seem to concur that we are at a point where that we feel confident that we can move forward

safely. But we are going to need -- in a very careful model answer a lot of these questions and continue in an iterative process to determine how can we make this model better.

But I just want to be certain that we are on a similar page or in a similar place, that we keep saying clinical trials are now appropriate, and I'm hoping that we can agree to that.

DR. LISA BUTTERFIELD: Thank you. Yes, we have heard some specifics around the limitations of non-human primate models and questions we cannot ask in them. All right. We have some hands. Dr. Kimmel, then Dr. Palevsky, then Dr. Fishman. Thanks.

DR. PAUL KIMMEL: Thank you. I'm actually dying to hear Dr. Palevsky's answer to Dr. Cooper. But I did want to ask -- I was hoping that Dr. Auchincloss could comment on why he thinks that kidneys should be later in the queue than hearts. I mean, there's some advantages in kidney transplantation. If they fail, patients can be treated with dialysis, but with heart transplantation it's sort of an ultimate effort. And I

think we're probably as ready to go forward with kidney transplantation studies as heart transplantation. So, could you adumbrate on that Dr. Auchincloss?

DR. LISA BUTTERFIELD: Okay. His hand is up. Why don't we have that response, and then we'll go on to Dr. Palevsky.

DR. JAY FISHMAN: I think you got the order out of sequence here. I think you're supposed to --

DR. LISA BUTTERFIELD: Yes. And then --

DR. JAY FISHMAN: -- go back to Dr. Auchincloss.

DR. LISA BUTTERFIELD: Yes. And then Palevsky and then Fishman, please.

DR. HUGH AUCHINCLOSS: Well, I'm very interested in your comments there. And you're right, of course. There is a fallback position for the kidney. I will upset my cardiac friends if I say that the heart's a pretty stupid organ and the kidney is much more complicated. And therefore, maybe we ought to stick with the organ that doesn't have such complicated functions to it. But cardiac surgeons

might disagree with that -- and islets, as I mentioned before.

I think we really have good evidence that pig insulin can be secreted and regulated physiologically. I just think that the kidney is a pretty complicated organ.

DR. PAUL KIMMEL: Well, I take that with a lot of respect. And we should never insult our cardiovascular colleagues.

DR. LISA BUTTERFIELD: Okay. I want to -- I do want to make sure we're staying focused on the functional questions that we're being asked currently in Question 5 about the data supporting organ function, regardless of which of those organs we're talking about. So, anything else on that topic, or should we move to Dr. Palevsky?

DR. PAUL PALEVSKY: Thank you, Matt. Thanks for the comments. I'm not suggesting that we need to spend years doing pig physiology research. I think that some of the questions about transporters and about the tubular physiology and the endocrine physiology can

probably be answered very rapidly knowing the pig that is going -- the pig species that's going to be used. And I think much of the data will have to be gathered in real time as we start doing in-human transplants. So, I'm not -- I wasn't suggesting that this should be a year's long barrier to proceeding with clinical trials.

DR. LISA BUTTERFIELD: All right. Thank you for addressing that. And Dr. Fishman, your hand had been up earlier. Did you want to weigh in next?

DR. JAY FISHMAN: Sure. Thank you. Just a comment, again, to try to put it into the context a little bit of allotransplantation because in humans -- I found these data, the metabolic very interesting. In humans there's a five-fold variance in CYP metabolism. And we see that and compensate for it based on drug levels. And so, we track immunosuppressive drug levels, for example. And we titrate those not based only on levels, but we titrate them to effect.

So, if they are toxic for the kidney, for example, or we do a biopsy, or if we have graft

rejection, or if we activate infection -- so that I only say that because although these metabolic functions, I think, are very important and the response to the immunosuppressant agents are going to be very important. It is a part of something that we do routinely in allotransplantation already in many ways.

And I think the only way to address that is, as Matt Cooper said, is in clinical trials. I'm not sure we're going to be answer those or predict what's going to happen. And in an individual, we can't predict what their metabolic framework's going to be either. So, the meshing of the pig metabolism and human metabolism is an experiment. And I think we're going to need clinical trials to unravel that.

DR. LISA BUTTERFIELD: Great. Thank you. We're going to move now to Dr. Wu.

DR. JOSEPH WU: So, I have a question about the long-term use of the immunosuppression in these pig heart transplants. I think as you know for most allotransplants after six months, a year, you can kind of taper off some of these heavy immunosuppressant

regimens. For these xenotransplants, is that the expectation, or you cannot do that in the sense that the xenotransplant, the immunosuppression is always going to be very heavy throughout the whole course of the organ being in the human body?

And if that's the case, what is the long-term consequence of that on the other organs that are being heavily affected by these immunosuppression? So, I just want to get the experts' thoughts on whether there is the possibility for tapering some of these medications after a while or that's not possible.

DR. LISA BUTTERFIELD: All right. Thank you, Dr. Wu. I will watch for hands of who would like to address that taper of immune suppression question. So, let's go to -- I see a hand up from Richard -- our guest -- from Dr. Pierson. Thank you.

DR. RICHARD PIERSON: At the moment, we have very little data upon which to judge this. What I can say -- there are two points I'd like to raise. One is that the co-stimulation pathway blocking immunosuppression is associated with absence of viral

reactivation, suggesting that it's less globally immunosuppressant than our conventional approach of calcineurin inhibitor, plus MMF, plus steroids as the most common regimen.

It is -- the only data that we have about tapering immunosuppression would suggest that if you turn off immunosuppression at six months that the graft will reject after that. So, the animals are not tolerant at six months. If you wait to a year and a half or two years before dialing down the intensity of the co-stimulation pathway blockade, the time to initiation of immunologic injury as measured by anti-pig antibody and subsequently by graft injury is significantly delayed with respect -- relative to earlier cessation of therapy.

And in at least one of Mohammed's experimental animals, turning down the immunosuppression at something like 300 days and keeping it there for another year was well tolerated. So, we're not going to know the answer to your question until we have substantial clinical experience. But as Dr. Fishman

just mentioned, what we currently do on our patients is to titrate therapy based on efficacy and side effects. And with -- the beauty of co-stimulation in our preclinical models at least is that you can give a lot of antibody.

And we don't know yet what the appropriate target drug level is -- circulating antibody, therapeutic antibody level is that is sufficient to suppress the immune response. But we can measure it, and we can then compare groups with different targets and learn from our patients how much is enough.

One of the concerns in xeno is that to date when we see the elicited immunity to a xenograft, graft failure almost always happens. And there's nothing that I know of that we currently do in our non-human primates that is able to abort that response. That is a concern for any clinical trialist. It is possible that the same treatments that we use in our patients who develop anti-donor antibody that -- proteosome inhibitors and intensified immunosuppression will be sufficient to reverse that immune response, an antibody

elicited immune response in patients.

We can't very well test that in our non-human primates because the complications associated with those aggressive interventions are simply not work -- you cannot manage those complications. And it's not humane for the animal subjects to be put through that kind of a regimen. On the other hand, our human patients, we can talk through the options with them and get their consent to do something experimental that might in fact rescue them. So, that's one of the ways in which a clinical trial offers us opportunities that we cannot pursue -- to learn and potentially to make significant progress in the clinical where we can't do it preclinically. Thank you.

DR. LISA BUTTERFIELD: Thank you. All right. So, I think we're moving sort of between Questions 5 and 6 at this point because this is in part sort of a holistic discussion. So, I propose that we move to discussion Question 6, have those two discussants present, and then let's have some discussion around that. And then I'll sum up and we'll check in with our

regulatory colleagues after that.

So, given that -- so, our last question, Question 6: transplanted pig organs are likely to be exposed to a variety of drugs that were not routinely used in the donor animals. Such drugs could include products to treat the patient's underlying medical conditions -- diabetes, hypertension -- as well as drugs like immunosuppressants intended to ensure the success of the transplant. And I know we've got some other folks on mic.

So, the transplanted organ may alter the pharmacodynamic and pharmacokinetic profiles of these drugs, with consequences for the medical management of the organ recipient. In addition, these drugs could be toxic to the transplanted organ. Please discuss the importance, limitations, and feasibility of studies of such drugs in the pig model prior to transplanting the pig organ into humans.

So, I know we've touched on a little of this but let's hear from our two discussants. First, Dr. Auchincloss and then Dr. Kimmel, please.

DR. HUGH AUCHINCLOSS: Well, Question Number 6 I think has been answered by Jay Fishman already. I don't think there's any predicting this -- what's going to happen to drug metabolism before we actually do the clinical transplant since we'll have one organ from a pig and another organ, say the liver, from the human recipient. So, I don't think there's any predicting.

But this is what we do all the time in transplantation is to measure drug levels, measure drug effect and adjust accordingly. In that sense, we've been asked to address a bunch of really important questions during the course of the two days. Question Number 6, I think, is the least important of the ones that we have to address. Thank you.

DR. LISA BUTTERFIELD: All right. Thank you, very much. And Dr. Kimmel.

DR. PAUL KIMMEL: All right. You know, as Dr. Palevsky said, we have to do lots of studies in pig physiology. And we shouldn't let that interfere. And this question is all about pig physiology. I'm also the last discussant, so I'm working off the work of all

the others. And maybe there will be some overlap in what I have to say. I think I'm going to end up agreeing with Dr. Auchincloss, but I'll go through this stuff that I've thought about.

And I think the goal is to have a pathogen free, if possible, porcine organ which functions at an optimal level capable of functioning for a long period. So, in effect, we'd like to know that the transplanted organ is normal and has no disease. And therefore, the evaluation of the animal donor for pathogen status and organ functional capacities dysfunction is necessary.

And Dr. Beaston's very short but comprehensive thoughtful presentation actually changed some of my ideas about what we should do. I think we also should consider whether we need to have a whole new research program before we go ahead. I think learning about the function of the porcine kidney before widespread use in transplantation in humans with ESRD will be critical.

The model used is also important. And an analogy comes to mind. The use of the oncologic models of aged and sick animals, as Ned Sharp listed, and

those with comorbidities such as hypertension and diabetes mellitus should be considered. So, perhaps the best model is the aged sick pig. Animals treated with multiple medications would also be useful in estimating how a porcine kidney will function in the complex environment of an aged host with renal disease and comorbid medical conditions treated for chronic illnesses with multiple medications.

So, it might be also useful to study porcine organs subjected to immunosuppressive therapies as suggested yesterday and as, I guess, suggested by Dr. Wu just a little while ago. The medical complications of kidney transplantation that are pertinent to porcine transplantation should also be considered. And in humans those would include short-term complications of kidney transplant including acute kidney injury, markedly reduced levels of GFR -- glomeruli filtration, and viral fungal protozoan and bacterial diseases which may complicate the short-term course.

In addition, thought should be given to how a porcine kidney would function in the long-term course

of kidney transplantation including considerations of how chronic porcine kidney graft dysfunction will manifest itself in humans over longer periods where hyperfiltration may be an important but ever-present contributor to injury. And Dr. Palevsky touched on that.

An interesting question by Dr. Beaston regarding the response to human parathyroid hormone could be studied in porcine isolated perfused kidney or isolated tubule perfusion experiments. That would be in effect repeating the physiologic studies done in kidney disease in the 1980s and 1990s. But I think much of those studies, as a couple of people have mentioned, will have to be done in humans.

A critical area of study is the treatment of serious viral infections in patients who have received transplants. How will the kidney respond and the heart respond to those treatments? And such studies should be performed in animal models, if possible. I would also argue, given the analogy of working in aged sick models that the best porcine kidneys should be studied

in nonhuman primates with those kinds of comorbidities -- aged with diabetes, with hypertension.

And of course, that's a different research question. It's a different and difficult set of experiments. And Dr. Zeiss mentioned that that might be some area to look at. But to my way of thinking, the ultimate test in kidney transplantation in humans will need to be related to the experimental care of patients with end stage kidney disease. And I'd argue this may be analogous to the early transplant studies done in the 1950s before the demonstrations of feasibility by the Herricks twin transplantation and before modern immunosuppression before and after the calcineurin inhibition era.

So, transplantation kidney disease done at the Brigham before 1955 was really quite the wild west. And there are other analogies, starting with Christiaan Barnard for heart transplantation. Translation to humans will require scrupulous attention to provision of information during the informed consent process. It'll be important also to avoid at all costs

1 therapeutic misconceptions of patients receiving
2 pioneering therapies.
3 So, I think, I agree with several of the
4 previous speakers that key clinical questions can only
5 be answered in the human transplantation model. For
6 instance, will porcine kidney transplants undergo
7 unwanted hypertrophy? How will the porcine kidney
8 interact in the human recipient and pathways related to
9 the Renin-Angiotensin-Aldosterone System, 125 hydroxy
10 vitamin D production and erythropoietin synthesis and
11 inaction, for example? And Dr. Beaston also mentioned
12 coagulation differences, which could become important.
13 We have therapeutic choices to address most of
14 these issues in patients, and I think we're going to
15 have to confront them in the human model. We'd also
16 like to know how the xenotransplant functions and be
17 cared for in the recipient if that recipient has
18 overwhelming viral infection or septic shock. So, we
19 would have to investigate the result of relatively
20 nephrotoxic drugs in that situation in patients.
21 This was touched on also earlier today. Will

genetic modifications of the porcine kidney endure, and will the genetic modifications of the porcine kidney affect other organ function in the human host that can only be tested in human beings? And I think we have to consider the role of the complement system, which has been considered in the pig, but evaluation of the complement system and interaction with the porcine transplant will be critical in assessing short and long-term human recipient kidney function.

The intensity of monitoring of the patient who recently underwent porcine heart transplantation reported in the *New England Journal* points to the unknown nature of multisystem complications in the first patients to be xenotransplanted, the need for many and perhaps unanticipated short- and long-term laboratory tests in patients and the seemingly unlimited biologic pathways which require evaluation in the first group of pioneering heroic patients.

So, I think key elements going forward will be the willingness of informed patients as participants in important medical experiments to undergo experimental

procedures having received informed consent in the most scrupulous fashion where the safety of the recipient is maximized in a relatively unknown clinical situation.

DR. LISA BUTTERFIELD: Thank you, very much, Dr. Kimmel. Let's first hear now from Mr. Conway.

MR. PAUL CONWAY: Thank you very much. And I'd like to thank Dr. Kimmel for his comments. And as always, he strikes the balance of principle and idealism and ethics. And I think that's central to this. My sense on Questions 5 and 6 is that we are now at a point at a two day meeting where we have a collection of known unknowns. And I don't say that to be funny. I actually say that to be quite accurate because it seems like we keep adding to the list of the unknowns.

But the general consensus is around those things that need to be checked. And the number of times that we have said moving to human trials is very important. I think Dr. Cooper said this. I think Dr. Fishman has said this, and Dr. Bloom and others have contributed to it. As an aside, I would say to Dr.

Auchincloss that most kidney patients have a cardiologist. And we're happy to broker between the two professions. We're used to doing that many times.

But I will say that we are at a crossroads. And I think that much of this is dependent on the idealism and the motivation of those patients who will be willing to pioneer this. I think it's very, very important, the role of FDA, in assuring safety and to make certain that things are not misstated in these early stages as we move forward in terms of what it means for patients, what patients might derive from it in terms of the benefits. But to understand that this is pioneering, and it's a new chapter in history.

But we've been here before. We've been here before with transplantation, we've been here before with dialysis, we've been here before with HIV, and we've been here before with COVID. But what has made the distinction, positive and negative, in each of those episodes has been this -- has been the inclusion of patients. And I think we're at the point now where you have a much more organized and much more vocal

kidney patient population and transplantation population around the world that are patient consumers, that want to be involved, that want to take the next step.

And we're partners in science. We're no longer the folks just on the other side of the table. We are partners in the endeavor because our lives -- we're the outcome. So, pass or fail, we have a direct stake in this. And I just want to put that on the table here because I think it's very, very important as we take a look at these questions and the answers that have been developed. And the consensus, in a sense, of the conversations, Dr. Butterfield, that you have put together so accurately that really role of the patient and the need for science to move forward is critical.

And I just want to put that our right here quite it plainly that you have patients around the world who are ready to participate. In fact, two years ago, patients began organizing the first international consortium that is patient-led for the development of artificial, implantable, wearable in the

xenotransplant. The demand for this on the consumer side is coming from the patient. And we're the ones that are behind the effort to develop an international consortium.

So, that is to give my fellow professionals inspiration and hope and for the scientists to know that patients are right next to them. In fact, we're already organizing. Thank you very much.

DR. LISA BUTTERFIELD: Thank you very much, Mr. Conway. All right. So, now we have an opportunity for the other members of the Committee to weigh in really on both Questions 5 and 6. And I'll remind you, 5, about existing data and target pig organ function to support full organ function in humans, aging of the target organ in the pig relevant to expected organ function over time in humans and then, this Question 6 about drugs, underlying conditions, immune suppressants and the importance, limitations, and feasibility of studies of these drug's intake models before transplant into humans.

So, watching for hands from the other

Committee members who would like to raise additional points for discussion on these questions. Great. Dr. Bloom, please and then Dr. Fishman.

DR. MARSHALL BLOOM: I'd just like to jump the shark and say I really appreciate Mr. Conway and Dr. Kimmel's remarks. And I don't think anyone could have summarized better than Dr. Kimmel. And I think I would certainly endorse his comments as well as Mr. Conway's. Thanks.

DR. LISA BUTTERFIELD: Terrific. Thank you, very much. Dr. Fishman.

DR. JAY FISHMAN: Yeah. You know, I've been an advocate, of course, of going into clinical trials. But there are some things that we can study and should be studied in either the primate models or in pigs themselves. And one of those is a way of enhancing safety. And I mentioned it yesterday, I think, which is to use the clinically relevant immune suppression in the pigs with level monitoring and metabolic monitoring to see if infections are elicited that we didn't attack by routine testing.

And so that it might be a way of giving us a sense -- since we have herds of animals -- then immunosuppressing selected members of those herds might be informative both about toxicities of the drugs but also about side effects relative to both metabolic and infectious side effects that might be useful for going forward into clinical trials.

DR. LISA BUTTERFIELD: Great. Thank you.

DR. JAY FISHMAN: Thanks.

DR. LISA BUTTERFIELD: So, let's hear from Professor Fox, please.

DR. BERNARD FOX: Yeah. I also really appreciated many of the reviews and most notably, I think ,Dr. Kimmel's and then Mr. Conway's comments. So, thank you. I guess my biggest concern about the current status is this whole growth of the organ once it's transplanted. I think there were many other points that were brought up by -- I think, the comment about potential immunity that Dr. Pavelsky brought up about potentially attacking erythropoietin and an autoimmune reaction that would potentially lead to

aplasia.

But I just really think the only way you're going to figure out a lot of this is going to be to do small pilot studies, those early phase one studies and do some limited number of patients to see what happens. So, I think one of the last comments that I heard from Dr. Kimmel, if I got it right, was before you started widespread studies, I would see that this is the FDA moving forward potentially with small pilot studies with these different knockouts.

And I guess from the growth side, the idea of having the growth hormone knocked out is going to be -- may become a very relevant one. But overall, I think I do agree with Dr. Kimmel's final summary. That seemed very much on target with things I've been thinking. Thank you.

DR. LISA BUTTERFIELD: Terrific. Thank you. All right. I'm not seeing other hands up. I can do a little summarizing, see where we're at and then -- so, why don't I do that after we hear from our consumer representative, Ms. O'Sullivan-Fortin. Then I'll

summarize, and then we'll have time for additional comments and checking in with the Agency about our discussion to date.

MS. KATHLEEN O'SULLIVAN-FORTIN: Thanks. I just wanted to say this afternoon has been fascinating. And more along the lines of what Mr. Conway suggested, I wonder if as we move forward with these sort of answer, tie up some of -- cross these T's, dot these I's on the things that we can move forward with scientifically and outside of transplant into humans that perhaps the FDA's mechanism for a PFDB or similar meeting might be appropriate in terms of really getting the opinions of the transplant community -- kidney, heart, et cetera, to make sure that -- not only to educate patients on where we are in the process but also to elicit their feedback and really make sure that we are -- that we understand the risk-benefit analysis that they would accept.

Because my guess is that if I was awaiting transplant and had been doing so for years, that if I heard these titans of science tell me that we're almost

at the point where we can move but it's going to -- you know, some of the burden is going to be risk to the patient that, you know, I think it would be wise to really have -- involve patients and have that two way communication as we move forward.

DR. LISA BUTTERFIELD: Great. Thank you for raising that important point for patient involvement and patient education. All right. So, let me hit some of the key notes that I have heard from our discussion this afternoon about Questions 5 and 6.

So, in terms of the ability of target pig organs to support full organ function, a lot of these things are experiments that are really to be determined. And I think this also ties -- I think it all ties together with age of the organs and of the drug metabolism and in terms of the treatments of the patients that the experiments we do are going to involve a situation of porcine organs in a human and that the porcine organ will vary as the genetic engineering of that donor animal vary in those settings -- and of the target organ that is transplanted.

So, it is highly complex. We don't have a lot of data yet. And while first half functional tests of oxygen exchange in lungs, of some of the -- some kidney functions would not then go down to the next step, of some of the more subtle enzymatic actions, that hormone secretion and ability to respond to hormones -- erythropoietin, all of these things that are the next level of complexity down that are nonetheless going to be critical for the long-term function of that organ in humans that we just do not yet have data from those studies.

So, what can we do now? There are some additional data on drug metabolism, hormone metabolism, receptors and protein interactions that could be done only in pig organs that could be done now. We can perhaps upgrade those models to include aged and sick animals that more closely model the older and some of the health issues facing the human patient recipients of those organs. Much has been done in the cancer world that you get very different answers when you look and ask questions in an older animal who's had cancer

for a while as opposed to a young animal that got cancer three days ago.

A suggestion that immune suppression could be tested in those animals to learn more about what will be -- what those organs will necessarily be exposed to after transplantation to human patients. Aged, sick non-human primates would also -- should be considered. So, there are ways to do in vitro studies now. There are ways to do model studies now. But I think the punchline that a lot of the folks around the table have brought up is that there are questions that can only be answered in transplanted organs received by human patients.

With that all being said and that being something of an unknown, the point has also been raised that in the allotransplant world and indeed even in normal drug delivery to human patients, drugs are titrated. And that's completely normal with protocols. And so, we have the ability in patients in real time to titrate these drugs according to their individual CYP levels in their livers and other organs as well as in a

transplant setting for immune suppression and the other therapeutic drugs.

So, those are some of the things that I heard around the table. So, I'm going to watch for hands from the Committee if anyone would like to add or modify anything I summarized. And then, I would also open it to Dr. Bryan or others from the Agency to see if there are other things that they would like the Committee to address to get to the heart of these questions that we haven't already touched on. All right. Dr. Beaston.

DR. PATRICIA BEASTON: Thank you for the conversation. So, I have two broad topics. So, first I want to thank Dr. Fishman because he first started well, we don't need studies because we already have paradigms for titration. But then he recognized that maybe we can learn something from doing these studies in the pigs and figure out what the dose would be and maybe some toxicities.

So, I just wanted to go back to Dr. Fishman a little bit and say do you have a short list of drugs

where you think it might be worth it to find out what the toxicity of the pig is? Especially like nephrotoxicity or cardiac toxicity where you can look in the pig and make sure that that toxicity would not necessitate figuring out a different drug that may be more appropriate because that toxicity would be the human dose that we would need to achieve the other effects that we were looking for.

DR. LISA BUTTERFIELD: Okay. And I'll ask Dr. Fishman if he can please response.

DR. JAY FISHMAN: So, I'm going to go back to your own comment which is that we may not be able to get all the organs from each animal. And the reason it's relevant, I think, is because we would say, I want to transplant organ X, a heart or kidney, from this pig and then subject them to the clinical immunosuppression at least that -- and other drugs potentially that they get routinely. But the immunosuppression would be the focus in terms of toxicity.

And we know what the toxicities of those drugs are in humans. As you pointed out, we don't

necessarily know what the toxicity of those drugs are although we've learned a lot from the preclinical studies in primates. So, we do know that a lot of these organs have been exposed to clinically relevant immune suppression. But I think it's a way of learning both about the toxicity of the drug, the metabolism of the drug by that organ, so, if you were doing, for example, liver transplantation -- and then the side effects of those drugs in terms of infectious activation.

I think that there are more data than what we might imagine because of all the numbers of laboratories that have been using different immunosuppressive regimens with different genetic types of pigs. So, those data could be collected and may exist already. But I think your question is a great one. And it's a question of assembling those data from models that exist and then perhaps doing some additional studies to be sure when you pick your immunosuppressive regimen that's matched to your genetic type, are there unanticipated side effects?

So, sure.

DR. PATRICIA BEASTON: Okay. Thank you for that. And then I wanted to follow up the interesting discussion of the pig heart size. So, one of the last comments was that the adult pig heart size was achieved in the baboon model and that everything was fine. It stopped growing. But when you look at Dr. Fox's talk, he has this very interesting slide where it shows the pig growth and then the baboon growth and the -- yeah, baboon.

And the baboon is only getting up to about 25 kilograms, where the pig is 100 kilograms where you get to sort of the best fit size for outcomes for the baboons. Well, humans are much larger than that. So, can we have a discussion -- maybe not now but as people start thinking about this, about what the criterion will be for figuring out the size of the heart that you would need for transplant?

And then the other thing I want to point out as part of this is the growth hormone knockout only goes so far because while that growth hormone knockout

may be great in the pig for preventing growth, the human recipient will have growth hormone. And that growth hormone will go the liver which will make IGF-1. And IGF-1 is another growth factor. So, do we understand enough about the organs where we are -- we're trying to transplant them and what the contribution of IGF-1 is to the ultimate size that would be obtained?

DR. LISA BUTTERFIELD: Right. I'm going to look for hands for anyone who would like to -- well, Dr. Beaston said we need perhaps more discussion than we have time for today. Is there someone who would like to weigh in on this for us now? Okay. Perhaps this is indeed something for more discussion at a later time for more specific answers to your questions, Dr. Beaston.

DR. PATRICIA BEASTON: Okay. Thank you so much.

DR. LISA BUTTERFIELD: All right. So, other topics, other comments before we -- yes, Judy.

DR. JUDITH ARCIDIACONO: Yes. If I may go

back to a question related to our discussions yesterday. And that is we'd like to know how the Committee feels about archiving and collecting samples for xenoproducts that have been exposed to well characterized animal cells. And just as a reminder, that's the lowest level of risk. So, these are cell lines that are well established, they've been tested. And so, I just wanted to get clarification or some input on what the Committee thinks as a whole about reducing the requirements for those products. Thank you.

DR. LISA BUTTERFIELD: All right. I'm going to watch for a show of hands on anyone who would like to weigh in on that lowest bar. I think from what we said yesterday -- that we talked about sort of case by case and people presenting their best data in their package. But let's first hear from Dr. Morrison and then Dr. Bloom. We can't hear you, Dr. Morrison.

DR. SEAN MORRISON: Can you hear me now?

DR. LISA BUTTERFIELD: Yes.

DR. SEAN MORRISON: Okay. Sorry. I was just

saying that I think it's very reasonable to lower the requirements when all that's happening is that the human cells are being exposed to a well characterized cell line and culture. It's a much less complex situation than actually transplanting an organ from a donor animal. And if the cell line is well characterized, I think it's a reasonable thing to do. I'll leave it there.

DR. LISA BUTTERFIELD: Thank you. And Dr. Bloom.

DR. MARSHALL BLOOM: So, I would agree with Sean. And I would note that the lack of any discussion on that topic really indicates that the -- I think indicates that the other Committee members would agree. And I think Sean said it very well. Thanks.

DR. LISA BUTTERFIELD: Great. Thank you.

DR. JUDITH ARCIDIACONO: Thank you.

DR. LISA BUTTERFIELD: And Professor Fox and then I'll have a couple last comments and we'll go to Dr. Marks. Professor Fox.

DR. BERNARD FOX: I just wanted to support

what Dr. Bloom said, right. That I also agree. I think the risk is very low. So, I didn't want him to be out on a limb. Thanks.

CLOSING REMARKS/ADJOURNMENT

DR. LISA BUTTERFIELD: All right. I appreciate the folks from the Agency asking some additional questions. And also, wanted to express my thanks for the additional comments about -- that the patients are the partners of the clinicians and researchers doing this work and that additional outreach and education would be appreciated to further garner the education and support of the patients and patient advocates. So, with that, I think we've had some terrific discussion, and I'd like to turn it over to Dr. Marks, the director of CBER.

DR. PETER MARKS: So, Dr. Butterfield, thanks very much. I really appreciate the Committee's thoughtful discussion. I wish I could have been here for all of it. I've been in and out of listening to it

over the past two days. Really appreciate the thoughtful discussion in this area. There's tremendous interest, tremendous promise, and tremendous challenges that you talked about. But really this is such an important -- such important input to get here.

And we really appreciate the incredible thoughtful information and discussion that occurred. So, thank you all so much. And really wish you a very pleasant holiday weekend. Thank you again for the time today and thanks for everyone for joining us.

DR. LISA BUTTERFIELD: Perfect. Thank you, very much, Dr. Marks. So, with that, I'd like to turn the meeting over to our DFO, Christina Vert.

MS. CHRISTINA VERT: Thank you, Dr. Butterfield.

DR. PRABHAKARA ATREYA: Christina, Dr. Wilson (sic) is going to make some comments.

MS. CHRISTINA VERT: Sure. Go ahead, Dr. Bryan.

DR. WILSON BRYAN: No. I just wanted to echo Dr. Marks, thank the Committee. It's so helpful to us.

And we really are very enthusiastic about the field of xenotransplantation and look forward to ongoing discussions in this area.

MS. CHRISTINA VERT: Thank you, Dr. Bryan. Okay. With that, with those comments, I also would like to second -- thank all the participants for today. And I will go ahead and adjourn the meeting today at 3:43 p.m. Thank you.

MR. MICHAEL KAWCZYNSKI: All right. And with that, studio, please take us -- please end the session. If you have any questions or comments, you can send them to fdaoma@fda.hhs.gov. Thank you so much.

[MEETING ADJOURNED]