FDA PUBLIC WORKSHOP

ANTIBODY-MEDIATED REJECTION IN KIDNEY TRANSPLANTATION

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PROCEEDINGS

Welcome, Topics and Goals

DR. ALBRECHT: Good morning, everyone. My name is Renata Albrecht. I'm the Division Director of the Division of Transplant and Ophthalmology Products. And on behalf of our division as well as our Office of Antimicrobial Products, headed by Dr. Edward Cox, who is present with us this morning, it is my great pleasure to welcome all of you to the Antibody-Mediated Rejection and Kidney Transplantation FDA Public Workshop over the next day and a half.

You received at the registration desk an agenda, and in that agenda, you see that we have four goals that we aim to achieve today.

One is to examine and emphasize the importance of immunosuppressive medication nonadherence in the development of DSAs as well as subsequent AMR.

The second is to discuss new developments in transplantation, their impact on patient management, such as pretransplant sensitization not manifested by DSA donor/recipient HLA epitope matching, routine posttransplant DSA monitoring.

A third goal, to discuss the natural course of acute and chronic AMR as a continuum and its temporal association with cellular rejections and changes in GFR.

And, finally, to discuss the unmet medical needs and potential clinical trial design challenges for the prevention and treatment of AMR.

Again, in your agenda, you see that over the next 2 days, we'll cover topics in five sessions.

Session 1 will be an overview, new developments, patient perspectives and diagnostic challenges in AMR.

The second will be factors contributing to antibodies in the pretransplant period in treatment options.

Third, factors contributing to antibodies in the posttransplant period.

Tomorrow, the morning will start with a session posttransplant monitoring, diagnosis, and treatment of AMR. And the final session will be clinical trial design challenges for developing new treatments as well as topics on animal models of AMR.

So as in previous workshops, this is only a day-and-a-half meeting, and therefore the scope of the meeting needs to be focused. So we will be hearing the latest scientific information on AMR, such as diagnosis, treatment, prevention, desensitization, clinical trial considerations, and, as I mentioned, animal models. The discussion of biomarkers will focus primarily on donor-specific antibodies, mainly anti-HLA DSAs.

The way each session is organized, there will be a series of formal presentations, and during that series, we'll ask you to hold your questions until all the formal presentations are concluded. Each of the sessions, after the formal presentations, will be followed by a public comment and discussion section, which will last about 45 to 60 minutes.

During that session, the moderators will first ask the audience if they have any clarifying questions about the presentations, and, subsequently, there will be a discussion of the FDA and Planning Committee-developed questions, which, again, are included in your agenda.

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During this session, we will invite the audience, along with the panel members, to interact and provide comments and perspectives on the questions that we're going to be discussing.

Now let me cover a few housekeeping issues. Dining is available downstairs. If you exit the conference room in the back and go to the right, the dining room is downstairs. Breakfast, lunch, and dinner are served. And also out of consideration for our workshop, they will have coffee available and snacks available throughout the day.

A note, credit card and debt card payments only. They do not accept cash.

There is Internet access. There is a little card with the username and password for the network that you can have available.

Okay, taxis. For those that are traveling either today or tomorrow, the request has been made that you ask at the information desk about getting taxis to take you to either other hotels or the airport.

And after I conclude my opening remarks, we're

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actually going to go around the table and ask people to introduce themselves. And what I wanted to mention is that, consistent with the FDA's Patient-Focused Drug Development Program that was authorized under PDUFA V, the Prescription Drug User Fee Act Reauthorization Number V, and, most recently, the inclusion of Patient-Focused Drug Development as a component of the 21st century act, we are very fortunate to have three patient representatives present with us today.

What I wanted to mention is we actually, the Office of Strategic Planning, reached out to and invited five patients to participate. Unfortunately, two have not been able to join us for medical reasons, which I think emphasizes the challenges that our transplant patients face.

So with that, what I would like to do is ask the panel members to introduce themselves and provide their affiliation. And I would like to start on the left with Dr. Bala.

DR. BALA: I'm Shubal Bala, FDA, CDER.

DR. WANG: Yan Wang, statistical team at FDA.

DR. IRISH: Bill Irish, statistician and

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epidemiologist with CTI.

DR. CAVAILLÉ-COLL: Marc Cavaillé-Coll, Medical Officer, FDA.

DR. KNOLL: Greg Knoll. I'm a nephrologist at the University of Ottawa.

DR. WOODLE: Steve Woodle, surgeon, University of Cincinnati.

DR. ALLOWAY: Rita Alloway, transplant pharmacist, University of Cincinnati.

DR. COLVIN: Bob Colvin, pathologist, Mass General Hospital and Harvard Medical School.

DR. HAAS: I'm Mark Haas. I'm a renal pathologist at Cedars-Sinai Medical Center in Los Angeles.

DR. VELIDEDEOGLU: Ergun Velidedeoglu, medical officer, FDA.

DR. GASTON: Bob Gaston, nephrologist, University of Alabama at Birmingham.

DR. COX: Good morning. Ed Cox, Director of the Office of Antimicrobial Products at FDA.

DR. NICKERSON: Peter Nickerson, transplant nephrologist, University of Manitoba.

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DR. MANNON: Roslyn Mannon, transplant nephrologist, University of Alabama at Birmingham.

DR. GEBEL: Howie Gebel, HLA Director, Emory University, Atlanta.

DR. WIEBE: Chris Wiebe, transplant nephrologist, University of Manitoba.

DR. DJAMALI: Arjang Djamali, transplant nephrologist, University of Wisconsin, Madison.

DR. TAMBUR: Anat Tambur, HLA Lab, Northwestern, Chicago.

DR. BELEN: Ozlem Belen, Division of Transplant Ophthalmology Products, FDA.

DR. STEGALL: Mark Stegall. I'm a transplant surgeon at Mayo Clinic.

DR. EDWARDS: Dawn Edwards, patient representative.

DR. MITTELMAN: Michael Mittelman, patient representative.

DR. LENNON: Jack Lennon, patient representative.

DR. CHALASANI: Meghana Chalasani, FDA, CDER.

DR. ALBRECHT: A couple more comments. When

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you're not speaking, if you would be so kind to turn off your mic by pressing the red button.

And, finally, on the slide, you see the link to the workshop. The information there is publicly available, and it provides all the presentations and other information.

With that, what I would like to do is turn this over to Dr. Ergun Velidedeoglu and Dr. Robert Gaston to start moderating the first session. Thank you.

Session 1: Overview, New Developments, Patients' Perspectives, and Diagnostic Challenges in Antibody-Mediated Rejection

Part I

DR. VELIDEDEOGLU: Hi. Good morning, everybody. As you might have noticed in the agenda, Session 1 is the longest session in our workshop. It consists of two parts, Part 1 and Part 2. And there will be a discussion session at the end of each part. And we will also have a short break in between the two sessions.

So the purpose of this session, as

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Dr. Albrecht mentioned, is to discuss the new developments in the field since our last FDA AMR workshop back in 2010, and also discuss somewhat controversial areas, and new developments in the field.

So one thing unique about this session is, again, as Dr. Albrecht mentioned, we have a voice of the patient session, which is the first time that we incorporated into our workshop, and we have three patient representatives. Originally, we had five patient representatives, but unfortunately two patient representatives had urgent medical conditions which precluded them from attending today. So we have three patient representatives today.

So the first talk will be given by Roslyn Mannon, from the University of Alabama. The title is "New Developments in Kidney Transplantation Since the 2010 FDA AMR Workshop, Including Nonadherence, HLA Mismatch, Banff Updates, and the New Kidney Allocation System."

New Developments in Kidney Transplantation Since the 2010 FDA AMR Workshop -- Nonadherence, HLA Mismatch, Banff Updates, Kidney Allocation

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DR. MANNON: Thank you. These are my disclosures.

I can't get the slides to advance. Could you give me the -- oh, perfect, like magic.

I was asked to provide an overview in the next 15, 20 minutes of all of these topics in the hopes of introducing the entire session. So my apologies to my colleagues that I didn't include. It doesn't mean I didn't want to, but I really did cut a lot of my 50 slides down before submitting it. And also for those colleagues that I do highlight their work, it's really a 37,000-foot overview. It's not to provide any kind of opportunity with them to not highlight their work.

I think it's important for the group to recognize the work that FDA has put into kidney transplantation over the last 5 years. Shown here is a summary of the public workshops that we have been participating in, including the 2012 meeting with the Generics Division, with the societies, both AST and ASTS.

And importantly, as has been referred to already this morning, the recent, this past fall, the

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Patient-Focused Drug Development meeting where we were actually able to hear the patient voices, learn more about patient-reported outcomes, and understand the concerns that patients have in terms of drug development.

Not on this slide have been the multiple meetings that have occurred both offline and online between the societies -- ASN, AST, ASTS -- in the hopes of developing a private-public partnership focused on transplantation.

I think one of the success stories over the last year that many of you may not be aware about is the Therapeutic Area Data Standards User Guide for Kidney Transplant, abbreviated TAUG or TAUG-KT Version 1 for short. An example of this is shown in the panel on the right. This was a compilation of terms and processes focused on therapeutic interventions to prevent rejection in transplanted kidney patients.

This was an accomplishment, and it's available online to the public, between funding from FDA, CFAST, CDISC, and C-Path, as well as the American Society of

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Nephrology's Kidney Health Initiative, and the American Society of Transplantation, and many volunteer hours.

The goal of the standard development was to accelerate clinical research and medical product development, creating and maintaining data standards, tools, and methods for conducting research in transplantation. And the very thoughtful detail here is not only available for industry, but I think quite a useful tool for many of the investigator-initiated studies performed by a number of people in this room.

Switching gears slightly, I would like to talk a little bit about some of the changes in Banff, and we'll hear more about this later this morning, but also the mental-cultural changes in transplantation and the sense that when we see allograft injury and rejection, that there are now multiple phenotypes. I recall that Banff now has acute cellular rejection consisted of T-cell-mediated rejection and acute antibody-mediated rejection. "Chronic rejection" is no longer a global term, but really separated into chronic antibody-mediated and chronic T-cell-mediated rejection, the latter of which is somewhat now undergoing some

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thorough review by the Banff working groups to define what that actually is. Many of us have seen mixed cellular and antibody-mediated rejection, particularly late posttransplant. And then the idea that there could be acute and chronic features in a rejection episode would be the antibody or cellular.

Significantly since this last meeting in 2010, there have been revised criteria for the pathology of antibody-mediated rejection, and I summarize them here. You may not be able to see them well, but you still are required to have three critical features for the diagnosis, which includes histologic evidence of tissue injury, evidence of antibody interaction with the vascular endothelium, and serologic evidence of donor-specific antibody.

Importantly, and what the arrow tries to highlight, is linear C4d staining is no longer a specific requirement. You may have, alternatively, evidence of microvascular injury, and, alternatively, you can have increased expression of gene transcripts. The idea that we would use transcriptional or gene expression data was one that has been debated for a

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very, very long time. I'll allude to this in another slide shortly. But the ENDATs were described by Banu Sis and colleagues with Phil Halloran a number of years ago.

Shown here is a panel from that paper. And while I think most of us are not currently incorporating this in our labs, and certainly not at our center, which is fairly high volume, the 2017 Banff meeting did call into question what the transcriptional features are antibody-mediated rejection and have debated whether the ENDATs really are the true signature. And I'll leave it to those individuals doing that work to comment further.

There have also been changes in the morphologic criteria for chronic AMR. Again, in Banff 2013, highlighted in red, is that the critical feature is the threshold for transplant glomerulopathy, the so-called CG lesion, and also the incorporation or the opportunity to use electron microscopy to document peritubular capillary laminations.

Why does this matter? Because in studies now, and most recently, when look at the 2017 criteria

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compared to the 2013 criteria I highlight in red on this slide, you can see that using the 2013 criteria, more of the subjects being studied in these biopsy studies fit into antibody-mediated rejection. In the De Serres study, the lack of C4d actually increased and allowed the diagnosis of AMR in their biopsy study, and ABMR was associated with worse graft outcomes.

In the Gimeno study, the main difference in inclusion of these patients, which was substantial, was the inclusion of microvascular inflammation, the g+ptc>2. Again, these were biopsy studies done typically for cause looking at the impact of ABMR on graft outcomes.

As we discussed in the previous meeting, and as the literature has accumulated, the development of de novo donor-specific antibody, meaning individuals that don't have antibody against their donor but develop it over time, has been a poor prognostic feature. There has been the creation -- and we'll be discussing this again in a little bit -- the sensitization in transplantation, assessment of risk, thankfully abbreviated as "STAR." The North American

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2017 Working Groups, led by Anat Tambur and Peter Nickerson, combine volunteer effort between the American Society of Transplantation and ASHI to look at specific aspects of both naive patients, so-called naive and so-called previously transplanted patients in all solid organs, to come up with some common recommendations in terms of monitoring and follow-up. This slide, which you cannot really read very well, indicates -- I'm sorry, I meant to go -- if you could just slide me back, not that far back.

This just is a compilation of the 25 studies on de novo DSA -- and there have been two more published since we put this together in February -- highlighting the complexity of the studies. Importantly, I think what I would like to really give you as a take-home point is that the frequency of de novo DSA really varies from study to study -- and I highlight some of the key ones here -- ranging anywhere between 2 percent in the first year up to 27 percent. This is at Colorado. This is the Manitoba group. Again, highlighting, though, that the patient population that's being studied, the measuring

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technique that's being used, the frequency of measurements, and the baseline immunosuppression, and the complexity of the patient type really determine the frequency of de novo DSA. And so having a working group to define specific follow-up patterns is critical, I think, if we're going to eventually evolve into therapeutic initiatives.

The impact of de novo DSA, as I've already alluded to, has been quite negative. This is work from Wiebe, et al., again, highlighting the half-life of a graft after the detection of de novo DSA with graft dysfunction shown in this bottom red line of about 3.3 years, significantly better than if you don't have graft dysfunction of proteinuria.

Those individuals, so-called subclinical DSA, there is a population of those individuals in follow-up. They behave very frequently worse than stable patients, but again very similar to those with allograft dysfunction from other etiologies. Again, so the notion that there is clinical dysfunction with DSA detection having worse outcomes in subclinical have also been highlighted by recent publications by Loupy

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and Orandi.

But de novo DSA, when we think of the risk factors -- typically rejection episodes, delayed graft function, for example -- I think we're going to be talking about it here, and so I want to remind everybody of the other adherence, and that is our goals for therapy conversion and minimization. We have created these protocols in order to make patients feel better, to mitigate the comorbidities associated with immunosuppression, and summarized here are a series of studies where there has either been minimization or conversion with an associated increase in risk in the development of de novo DSA. Some of this de novo DSA appeared independently of rejection, but a vast number of these studies were associated with higher rates of rejection during conversion and minimization with associated de novo DSA.

Other considerations about DSA is that not all DSA are the same. This is work by Loupy's group in Paris identifying that those DSA that bind C1q are associated with worse graft outcome, although one might really allude that this is really because of a very

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high titer antibody that binds C1q, but again bringing into question, the quantity and the quality of the donor antibody.

Another contribution to the literature this past year has been by Carmen Lefaucheur, again looking at the IgG subtypes, that not all DSA is the same. IgG3 does the worst. And I think we'll hear more later this morning about specific epitopes and also the identification and titering of antibodies, so understanding interventions and the implications of interventions.

So summarized here to the talk at this point -- and this is from one of the Wiebe papers -- again, that there is some event that occurs and that there is a period of time before clinical manifestations occur. And so I think a lot of what we'll be talking about today are to help us identify the subclinical injury in order to avert further damage, whether these can be used potentially as endpoints before getting to the critical or negative outcomes that we see here.

Switching gears to the Banff's scoring system, I want to highlight the inflammation aspects because

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there has been such a significant amount of effort focused on antibody-mediated injury. And I think this past Banff meeting in Barcelona in 2017 really highlighted the hand-in-hand association between inflammation and antibody-mediated injury.

So you may recall that a total i score was developed and incorporated into the Banff report in 2007. This means that not only do you have inflammation in non-scarred area, but a total score that includes inflammation in scarred and unscarred areas. And there is some discussion now currently underway of whether this ti score should be included with the i score as part of a new category for chronic T-cell-mediated rejection or not, and that's under debate.

Why does it matter? Well, we know from work from the DeKAF cohort that the so-called "iatr," which now has been called by Banff to be "i-IFTA," so I have to keep changing my slides around, but the presence of inflammation in scarred areas is a singularly and independently associated risk factor for patient grafts failure, independent of other features, including serum

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creatinine at the time of the biopsy and proteinuria, and that the greater strength, the more i-IFTA you have, or iatr you have, the more severe the graft outcome failure is. And so I think the community now is really recognizing that inflammation in these areas previously overlooked is important, and whether we call it i-IFTA or ti remains to be seen.

Not only does scarring in scarred areas matter, but i+IFTA -- it's very confusing, but I'm going to learn it eventually. So i+IFTA is another category. This is inflammation in non-scarred areas in the presence of fibrosis and atrophy.

here are prior older studies that have identified inflammation in sort of standard of care biopsies associated with progression of fibrosis in graft failure, more recently surveillance biopsies when you see inflammation in non-scarred areas associated with IFTA. A number of groups have identified shorter graft survivals.

And, interestingly, linking this inflammatory concept to donor antibody and outcome, a recent very nicely done surveillance biopsy study by Garcia-Carro

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identified looking at about 550 protocol biopsies. Those biopsies identified at 6 weeks with i+IFTA, meaning inflammation in non-scarred areas plus scarring was an independent risk factor for the development of de novo DSA. In this patient population that was relatively low risk and treated with non-depletional induction therapy and carefully monitored was about 9 percent per year.

I think the field is also struggling in looking -- I don't want to say struggling, I think we have a lot of competing interests right now in terms of molecular classifiers. I'm not going to be talking so much about biopsy classifiers because I'll leave that to the pathologists' presentations. But as many of you in the room know, we've been looking at markers both in peripheral blood, markers in the urine, and recently the cell-free DNA measurements. Again, the idea here is maybe to prevent us getting to the clinical demonstration of de novo DSA or the clinical demonstration of allograft injury to be able to detect and utilize these as potential biomarkers.

Moving on to therapeutic changes since the

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2010 meeting, the last approved drug on the docket has been belatacept in 2012. This medication unfortunately has not grabbed hold in the transplant community because of the higher risk of early graft rejection.

Shockingly, and not surprisingly, but I think shockingly, in the recent data, the persistent improvement in glomerular filtration rate, shown by these two upper lines, of patients on belatacept remains statistically significantly improved compared to patients which happen to be on a cyclosporine-based regimen with mycophenolate and prednisone.

And the debates are, Why is this? Is this because it's a CNI-free regimen? An important concept here highlighted in the New England Journal paper is belatacept-treated patients had lower frequency of the development of de novo DSA. This is marked and statistically significantly better than the cyclosporine-treated group. Again, this is a patient population that had a higher risk of rejection and, in fact, did not actually develop de novo DSA, again, unlinking two critical risk factors.

Why is this occurring? Whether it's a

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specific role of adherence because patients receive this as an infusional therapy every month and have less of an opportunity not to take the drug, or whether there is a specific interaction because it's a co-stimulatory blockade, there may be an interaction limiting T follicular helper cell activation of B cells when those cells become activated. And this is an area now under investigation by a number of labs.

The other area of therapeutic focus that will be likely discussed in this room is the use of complement inhibition for therapies for antibody-mediated rejection. Many of us in this room have participated in trials using eculizumab, a downstream C5 inhibitor. There are now trials undergoing for C1 esterase inhibition, a more proximal blockade.

Some of the advantages touted by this therapy are the fact that many of the co-stimulatory effects of complement activation would be mitigated and limiting injury. Not only has this been used for HLA-incompatible living donor transplants, it's been used for treatment of antibody-mediated rejection, these agents, and these agents have been targeted in studies

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for delayed graft function. I think the eculizumab trial is now undergoing analysis for the delayed graft function. So definitely therapeutic opportunities here in order to mitigate the injury associated with antibody-mediated rejection.

We will be talking today quite a bit about nonadherence. And on the panel on the left, I highlight that the association of nonadherence was strongly associated with antibody-mediated rejection, chronic antibody-mediated rejection, and mixed cellular antibody-mediated rejection in a biopsy study published by Sellares back in 2012.

And similarly, Wiebe and colleagues back in 2012 also associated the presence of nonadherence, a strong marker for the development of de novo DSA and the monitoring of their patient population.

So the presence of a nonadherence and trying to organize and focus our management strategies I think is going to be critically important.

We're back to the future. Again now we have more data about HLA mismatch, and we'll be talking about this again in probably the next speaker actually.

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Shown here is Chakkera Harini's analysis of UNOS data identifying the multiple mismatches and the hazard ratios for graft failure, showing that even with the potency of immunosuppression and our ability to get patients through the first year, that graft failure rates are higher with more HLA mismatches.

Now, I don't want to sound catty, like it's easy to match. We really do have an issue because one of the unmet needs is the lack of available transplants. And so I know that over the years we've kind of accepted the mismatch issue. But as time goes on, we are now realizing that this mismatch brings patients into a higher risk.

And so as Peter will show you -- and I keep quoting his and Chris's data -- mismatches in DR and DQ are really critical, and when you combine that to nonadherence, shown in this orange line down here and in this bottom graph, there is a significant effect on rejection-free survival, a negative effect, and a significantly negative effect on death-censored graft survival as well. So the combination of the two effects is significant.

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And, finally, the Kidney Allocation System. As if it's not enough, we've had a massive change in the allocation system, and it happened to be when I was on call, and it was the worst 2 weeks of my life, and now we only round once a week at a time and a block time.

But what happened is the notion of the algorithm that we had been using was waiting time. And so this was sort of a first come, first served basis of who got to see the transplant center first. And the goal was to have a more balanced, equitable distribution of deceased donor kidney transplants with maximal utility for those precious organs.

So from the utility perspective, we now calculate kidney donor risk indices, which are based on a number of biological and some factors out of our control in terms of developing a kidney donor profile index. And this is the one where I tell patients getting 100 is not a good thing, it's the lower scores are the better scores and the better likelihood that the graft will do well.

So now we take the 20 percent best kidneys

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that we expect to do the best after transplant and try to match them to the patients, 20 percent of the patients, with the best posttransplant survival, the EPTS score, which is based on age, dialysis duration, prior transplant, and diabetes. And so the idea is that we're maximizing the utilization of these precious organs, taking the best, and putting them into the, quote/unquote, best.

Equity has been addressed by increased national and regional sharing, priority given to those waiting for multi organs -- and that could be debated, but not here -- the role of the panel-reactive antibody. There are significant points provided to individuals who have been previously transplanted or highly sensitized. We afford zero mismatch kidneys much more so. Pediatric candidates. And we strongly give priority to individuals who were prior living kidney donors.

Most of all, the presence of listing after dialysis, if you come to the transplant center late, after you've already been initiated or you just are uncomfortable about being transplanted, you no longer

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are penalized for that, and you can still go into the system and still have that time.

There have been significant changes in KAS in terms of it, but most importantly, there has been a bolus of individuals that are highly sensitized. Some of these patients do well, and some don't, and they are clearly a high-risk patient population. There have also been impacts on transplantation in African Americans. There's been an increased rate in delayed graft function across the country which is statistically significant. There is also a lower 1-year graft survival, although this did not reach statistical significance.

Finally, related to this conference, there is the use of HIV-positive donor -- or unrelated is the use of HIV-positive organs, the use of hepatitis C treatment. Do we do it before or after? If we do it after, it allows hepatitis C-positive patients to be transplanted and get HepC kidneys. And then the whole debate about the APOL1 mutations in individuals of African American ancestry as either living donors or recipient outcomes.

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So as I always say to my -- you know, when I'm preparing my division chief annual report, I always feel disappointed because I didn't get my project ran, and my R1 may not have done well, and I sort of feel like there has been no progress, but when you look at the whole since 2010, there has been really remarkable progress made in the field. Many of those individuals are sitting here with me.

We have yet to develop a consensus on monitoring posttransplant, but I think we're close by having consensus conferences. And we really need validated biomarkers. This will obviously assist in endpoint development and facilitate the identification of new therapeutics in this unmet need in solid organ transplant.

Thank you.

(Applause.)

DR. VELIDEDEOGLU: We thank Roslyn Mannon for this excellent summary.

And before moving on to the next talk, I have a little reminder for our speakers except for the patient representatives. Next to the podium, we have a

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stand, and on top of the stand, we have a timer with three lights on it, green, orange, and red. The green light indicates that the speaker still has time. And 2 minutes before the end of the allocated time, the orange light will come on. And the red light indicates that the time has expired. But that doesn't apply to our patient representatives, which have shorter allocated time periods.

So our next talk is by Peter Nickerson, from the University of Manitoba, "A New Paradigm: HLA Epitope-Based Donor/Recipient Mismatch Assessment."

A New Paradigm: HLA Epitope-Based Donor/Recipient Mismatch Assessment

DR. NICKERSON: Thanks very much, Ergun. And I want to thank the organizers and the FDA for the opportunity to come and speak and to share some of our data and our thinking. And I'm looking forward to the next day and a half. I think it's really -- it's been 7 years since our last time we've been talking, but I think overall there has, as Ros just said, been a lot of developments.

So I'm going to talk about epitope- or

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Eplet-based donor/recipient mismatches. These are my disclosures. I will not talk in this talk about off-label.

And I don't think HLA matching is any new thing. We've known since the beginning of transplant that if you're an identical twin transplant, you don't need any drugs, and so that's the ideal, if we could ever get to that. There have been a lot of big names in the field looking at, "How do we move HLA forward?" And there have been multiple, I would say, top journal publications on the science of HLA over the last 60 years.

So why do we need to talk about it now? Well, it's really about beyond the whole molecule. So we've always talked about an HLA mismatch for a given locus as being a one whole molecule mismatch.

And Paul Terasaki, who is really one of the grandfathers of this field, I think said it well. He said, "We must now prepare for the second phase in which more sophisticated measures of HLA compatibility should be developed for more accurate prediction of outcome." And that's what this is all about. And let

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us point out that Paul said this almost 50 years ago.

And so what have we been doing for 50 years? And the answer is we've been really developing whole fields in molecular biology, and with that knowledge, we can now move forward.

So in the context of antibody-mediated rejection, which is this workshop, what I really want to talk about is, what does the antibody see on the surface of the HLA molecule? So I'm giving you a picture here in green of the cartoon of the surface of an HLA molecule. And I'm showing you in highlighted colors where the HLA antibody is actually going to bind to the surface of this molecule.

And in particular, I'm highlighting the H3 region, which is the few amino acids that are the polymorphic amino acids that the recipient is seeing on the donor HLA, and this complement determining region is determining the specificity of the antibody, and these other areas in color, the other CDRs, really stabilize that binding and lead to the affinity of the antibody binding.

And it's with this framework that Rene

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Duquesnoy developed a software package, a computational software package, where he was basically looking at, What are the polymorphic amino acids on the donor's surface for a given donor/recipient mismatch? He's identified the polymorphic amino acids that exist at the site that could be a binding for the H3 CDR, the specificity-determining target, and he has called those amino acids the "Eplet," or what we would call the functional epitope, and then there is the whole surface binding here that we would call the structural epitope. But his work is really based on this Eplet computation that is looking at the functional epitope of where the antibody could be binding.

So for a given HLA mismatch -- and I'm just using this by way of example -- to really understand this, you need to get the 4-digit or high-resolution typing. And here's a DRB1*1101 molecule of the patient, and here's a DRB1*0405 HLA molecule of the donor. And in our current language, we would say these are a 1DR mismatch.

But when we look at the Eplet level, we actually see there are 11 potential areas of amino acid

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differences between these two HLA molecules. So the degree of dissimilarity is actually quite distinct and numerous. Whereas, if we look at another DRB1 mismatch -- here's 1302 and 1119 -- again, we will call that a 1DR mismatch, but at the Eplet level, there is only one amino acid residue difference.

And the importance here is we today treat these two HLA mismatches as equivalent, but really the first one is much more different compared to the second one. And if we look at this over a whole population of donors and recipients, we can see for a conventional 1DR mismatch, you can have a whole range of Eplet mismatches, from as little as 1 or 2 up to as many as 50, almost 60, for DR, and the same is true for DQ.

So we have this broad range of different polymorphisms that exist for a given 1DR or 1DQ mismatch, and it's by getting to this level of resolution that we can start thinking about, does that give us better prediction of outcome?

So in this paper that Chris did in our group during his graduate studies, he looked at, "What are the independent predictors for forming antibodies

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against HLA DR?" And what he showed us here is that nonadherence DR Eplet mismatch load, so for every Eplet mismatch, your odds ratio goes up, and then if you have early clinical rejections preceding the antibody, that's also a risk factor.

For DQ, it's nonadherence, it's the degree of Eplet mismatching at DQ and younger age. And so this is interesting because we a lot of times think that younger individuals are just being nonadherent, and what this multivariate is saying is independent of nonadherence, a younger age is actually a risk factor, and that's probably because they have a more robust immune system.

Now, there are other ways of looking at immunogenicity, and this is the group from University of Cambridge in England where they looked at the electrostatic properties of the amino acid differences between donors and recipients. And so they used that to create an electrostatic score for the mismatches to try and see if that could predict immunogenicity better.

And then they published this paper last year,

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where they evaluated amino acid mismatches, the Eplet mismatch method of Rene Duquesnoy, and their electrostatic mismatch tool. And all three of these were basically showing the same thing in multivariate models, that this higher degree of precision of understanding donor/recipient differences gives you a better prediction of who's at risk for developing antibodies towards Class II. And this was looking at DSA development after graft loss.

So how can we use this information? Well, we can use it to get a risk prediction score, and we did it in our group using Eplet mismatch loads. What we found was that above 10 and above DR Eplet mismatches, there was an increased risk for the formation of HLA DR antibodies, as de novo antibodies, and above a threshold of 17, we had an increased risk of forming DQ antibodies.

In a similar type of study, this is a group out of the University of Toronto, Joe Kim's group, and what they looked at was using the Eplet mismatch load idea to predict in a multivariate the risk of developing transplant glomerulopathy. And you're going

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to hear a lot about transplant glomerulopathy at this meeting as one of the features that is leading to graft loss and is driven by de novo DSA formation. And, again, similar thresholds to what we saw for antibody formation, DQ above 18 and DR above 15, led to an increased risk for developing transplant glomerulopathy.

How might we apply this in a clinical setting? Well, this was a clinical trial that we did sponsored by the NIH called the CTOT-09 trial where we were trying to look at minimization for our patients, trying to get them off medications. So we enrolled living donor transplants. These are pristine patients who were given standard of care immunosuppression. And for the first 6 months, these patients did outstandingly well. They had no rejections. On a protocol biopsy, had normal histology. And at 6 months they had no DSA formation.

And we randomized these patients two to one to come off of the tacrolimus over a 3-month taper. And when we did that, what we found was that in those patients coming off tacrolimus, we had a lot of

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cellular rejections, and five of these patients developed DSA, either DSA alone or DSA in conjunction with acute cellular rejection. And in these patients, all the DSAs were against Class II DR or DQ, again highlighting the importance of Class II antibodies.

So at that point, the DSMB halted the trial. They said you're having way too many alloimmune recognition events during tacrolimus weaning. And what this taught us was that quiescence wasn't low risk to minimize. We went back retrospectively and asked, Could we have predicted who was really at high risk for developing these antibodies?

And so we went back for the DQ locus and looked at the Eplet mismatch load of these patients. And what we found was that those patients that formed the de novo DQ DSAs, all of them were above our threshold of 17, and those patients that didn't develop the DSA, only three were above that threshold.

However, one of the patients, at the end of the study, decided they wanted to say off their tacrolimus, and subsequently, they went on and developed a DSA, so now six of eight above the

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threshold had a DSA.

And one of the patients actually had an early cellular rejection, so they were restarted on their CNI, and I suspect that's partly why they didn't develop a DSA. And so that really only leaves one of our eight patients who were above our threshold who didn't develop a DSA, suggesting that this might be useful as a way of risk stratifying patients for who is at risk to developing a DSA if you're going to consider minimization trials.

And, again, a similar type of point. This was switching immunosuppression from cyclosporine over to everolimus, and they had a higher rate of de novo DSA formation. And the Paris group went and showed that those patients that were forming DQ de novo DSA upon switch, they were again having a higher Eplet mismatch load compared to those patients that didn't form an antibody, again supporting the concept that the load may be a useful way of measuring or predicting risk.

And then another paper looking at nonadherence, and this was a very interesting study out of the Minnesota group where they were using medication

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event monitoring systems, and in almost 200 patients, they found that almost a quarter of them were noting that they were dropping some of their doses in the early posttransplant period, in the first 2 months. So 22 percent were missing 7 percent or more of their doses, and that led to more late acute rejections and more premature graft loss. And this was in the 1 to 2 or the 3 to 5 year follow-up period.

And we went back and retrospectively asked the question, Could we have predicted again who was at the most risk based on the Eplet mismatch load? And here what we're showing in the orange line -- and Ros had just shown this slide -- that those patients who were both nonadherent and with a high load actually were at the increased risk for DR for late rejection or the worse graft survival, and that was true also for DQ, late rejections in DQ graft survival.

Now, what was interesting was if they were nonadherent and they had a low load, here in blue, in fact, those patients did quite well. So if you're missing your drugs, but you don't have the Eplet load to drive an immune response, this wasn't leading to

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late rejections in terms of DQ, and it wasn't leading to any diminishment in graft survival, again suggesting it's the HLA mismatch at this granular level, and understanding that degree of dissimilarity that can really be used to predict risk.

So how much we use this thinking forward? Well, today we're here in empirical medicine, and we treat everybody in transplant the same. We look at HLA mismatches, and, yes, in the allocation system, if you're a good match, you can get some points in prioritization. So a zero DR mismatch gets 2 points, that leads to maybe you getting bumped up in your allocation scheme. But we treat all the patients pretty much the same in terms of the immunosuppression we use today.

If we were to use DR or DQ Eplet mismatch load, or the electrostatic mismatch load of the University of Cambridge, and we knew that you were low for both, we could assign that priority in allocation points, we might consider these patients as individuals who might go through a minimization process in terms of their immunosuppression, and that's something that's

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imminently testable in a clinical trial. And what we could say is if you're high for either, well, probably these are the individuals we should avoid trying to minimize them.

And ultimately, what we really need to get to is not just understanding this kind of risk factor score using Eplet mismatches, but actually identify the specific epitopes that are commonly driving antibody formation, what we would call immunodominant epitopes, and if we had that, then we could really get into personalized immunosuppression, where if we knew that you had immunodominant epitopes, again we would avoid or give very low priority in our allocation scheme or we would certainly avoid minimization in the patients that have these immunodominant epitopes.

And I think this is the next 20 years of our work, is really to identify, What are these immunodominant epitopes with reliability? And in the meantime, we can maybe start to work with some of our risk stratification scoring system.

And with that, I'm going to stop and just acknowledge all the people that have contributed to

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this work, and, in particular, Chris Wiebe, who was in our group doing his graduate studies at the time when he was doing a lot of this work, and some of the work that's ongoing with Arthur and the DeKAF Consortia, and Peter Heeger and Don Hricik in our CTOT Consortia.

Thanks very much.

(Applause.)

DR. VELIDEDEOGLU: Thank you for this excellent presentation.

Now it's time for our patient representatives. And our first patient representative is Dawn Edwards.

The Voice of the Patient in Transplantation

MS. EDWARDS: Good morning. It certainly is a pleasure to be considered to be presenting this morning. It's really nice and it's really special that patients' point of view and patients' experiences get to be examined at these type of workshops. So I appreciate the FDA and all of those responsible for inviting me and bringing me out here.

I'm going to be talking about my kidney journey and how the rejection episodes affected my life.

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I began dialysis 25 years ago at the age of 23 as the result of postpartum glomerulonephritis. Three years later, after doing in-center dialysis, a doctor convinced me to try peritoneal dialysis. And I was afraid because I was told that peritoneal dialysis caused infections that kill you. So I wasn't really excited about it. But I did give it a try, and I loved it.

Ten years later, after being on a waiting list for 10 years, I did get called for a kidney transplant. Very exciting. I was ready for it. I felt good about it at the time. And we went in for the transplant. It was a great thing. The family was excited about it. And we were all very excited about having the transplant. Boy, it's great to pee and it's great to do all of those great bodily functions and everything.

So at the onset of the transplant, it was very difficult for me. I received the kidney transplant on September the 25th. I was sent home on September the 29th. And October 4th, I was back with a rejection episode. I was in a raclimune (ph) study at the time, and I don't know if that caused the rejection or not,

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but I know I didn't feel good. So we were rushed back to the hospital. The family is all upset. And, you know, we were told or we thought that once you get a kidney transplant, everything was going to be okay afterwards, you know, I was going to get the transplant, and then I would be out having pizza and beer with my friends, you know, and I was going to go back to my regular life. However, we were certainly mistaken.

I had several episodes with rejection, with plasmapheresis and IVIG and bunny rabbit stuff and horsy stuff and all kinds of different medications that they were telling me about, and I had no idea what they were all about. I just know that I was uncomfortable, I was in pain. I also developed the -- what is it? CMG. CMV. Thank you. The CMV infection as a result of my donor. So that was pretty rough on my stomach and my colon area.

The transplant was really a lot more than I expected. It was not making me happy. Actually, at some points I thought that I would have been better off staying on dialysis.

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Yes, I began to hate this new kidney because all of the attention was on the kidney, it wasn't really about me. When I came to the hospital, it was always the kidney, the kidney, and there was no Dawn involved in my transplant experience.

The biopsies were constant. And I thought that by the time they got finished snatching all of those pieces out of the kidney, there wasn't going to be any left. However, it just became a chore. The medications were very difficult.

I was able to adhere, thanks to some transplant organizations that sent us nice little medication boxes and little alarm clocks to remind us that every 12 hours you had to take that Prograf and the CellCept 4 times a day. So I had a lot of problems.

I developed colitis on several occasions. And not only that, I was having problems with my bones. I was having some body aches. The rejection episodes just kept coming. For the first 3 years, I rejected three times. And, again, plasmapheresis was just like dialysis, only you get as many blankets as you want.

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I enjoyed the freedom of dialysis finally after 3 years. Everything began to settle down and I was able to go back to work, and I was very pleased about that. It's really nice to be able to contribute to the household again.

I began working and I was traveling for my job. And one day during my travels, I stepped down off of a curb, and I felt a snap. And I knew that that just wasn't right. And this was in February of 2010. I was so nervous because the transplant center had called me that morning and told me to get home right away. And so off I went running back home to find out that I was having another rejection episode.

During that episode, I was given Thymoglobulin, and I developed an anaphylactic reaction to the Thymoglobulin. And also my hip was fractured when I stepped off that curb. After the anaphylactic reaction with the Thymoglobulin, there was nothing else that they could do, and I just went into chronic rejection, and I ended up back on dialysis.

This was 6 years. So I really expected more. I was very disappointed. I was hurt. My world was

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shattered because I had begun a job. I had started to get my life back together. At the age of 23, I didn't expect to be on dialysis in the first place. And now I thought that I had an opportunity to reestablish my life and do some of the things that I wanted to do. And this was all taken away from me. And I went into a very deep depression. I'm actually still being treated for that, but we'll talk about that another time.

And the depression was also something that I hadn't dealt with before. I actually felt like my life was over. I did not like dialysis from the first day that I did it, and I definitely didn't like going back to dialysis. However, I returned back to peritoneal dialysis. I thought that there would be more that could be done for me, but that was it. Back to dialysis I went.

So eventually I returned to peritoneal dialysis, and that only worked for a few months because of the hip fracture that was deteriorating quickly. And before that, we had a MRSA episode after going back to in-center hemodialysis. So basically I spent the whole 2010 in the hospital recovering from MRSA and

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then having a hip replacement.

And not only that, but as a result, we also discovered that I had the early stages of colon cancer and needed to have my entire colon removed. And all of this, and I wasn't even 40 yet. That was very earth-shattering for me and devastating. There was nothing that was under my control at that point. Recovering from a hip replacement, the ostomy that was completely out of control, I don't wish that on anyone, and now I've discovered that I'm not going to be able to have a reversal.

In conclusion, I'm now on home hemodialysis. And I don't like that too much either, but, you know, we have to do what we have to do to stay alive. And my outcomes are excellent. I'm very healthy, even though I'm not feeling well today. But I'm very, very healthy.

And I'm just considering -- I'm not considering having a transplant again. I have not gone back active on the transplant list. I am absolutely afraid. I can't take the chance of something more happening to me and experiencing any of what I

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experienced again. I am afraid.

And it's great to be in a room full of people who want to make positive changes for patients that have these problems with kidney transplants, plasmapheresis, IVIG, and the horsy stuff and the bunny rabbit stuff. It's good for some people, and I'm really happy for those that it works for, but I would really, really like to see something for people who have these constant rejections. I would like to have an opportunity to have the life back that I so dream and so desire.

So on that note, I thank you very much and thank you for your attention.

(Applause.)

DR. VELIDEDEOGLU: We thank Dawn Edwards for sharing her transplant experience with us.

Our next patient representative is Michael Mittelman. We are running a little bit behind. So I request our patient representatives and the following speakers to try to wrap up within their allocated times, please.

MR. MITTELMAN: Hi. Good morning. My name is

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Michael Mittelman, and I'm 35 years old. And I'm from Philadelphia, Pennsylvania. I'm a three-time transplant recipient, with the first being from deceased donors. The most recent, the third, being from a living donor, my mother. It was performed in 2001 at Johns Hopkins. And it was an ABO-incompatible transplant. She was always a perfect match for me, antigen-wise, my mother, but she never obviously had the right blood type. So I can tell you that it was a big relief when technology came along to be able to do that.

Both of my kidneys were removed when I was 5 years old. My original diagnosis at age 3 was nephrotic syndrome, which subsequently was diagnosed then as FSGS. So that has never recurred luckily. So if any of you have some wood you can knock on, that would be good. But it never recurred, so that's good. I know it recurs in a lot of patients.

During that time period before the first transplant, I did have over 20 transfusions. I did home PD, so I know I built up a number of antibodies from the transfusions that I had at the time.

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My first transplant was in 1988, like I said, from a deceased donor, and it was in January '88. It was a very poor match from the tissue typing perspective, but the surgeons and the physicians at St. Christopher's Hospital for Children wanted to get me a transplant because I was very sick. I was a patient at CHOP -- I had previously been a patient at CHOP, but they did not have a transplant program back in the early '80s, and St. Christopher's did.

So my first experience with antibody-mediated rejection was actually the same year, in 1988. I became a lot more sluggish, bloated. I was given OKT3 at the time. The AMR actually became worse in 1989, and in 1990, I received a second, and better, transplant in December 1990, so the first did not last that long of a time period.

I was also given a lot of prednisone during that time, so I obviously became a chunky little kid. But I do remember during those time periods I would gain a lot of weight. It was the protocol at the time for pediatrics; they would jack up your prednisone. I can remember the rates -- I'm sorry, the doses that I

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was on. I blame my being short on that because I was kept on that for many years.

I think during my second transplant, which was in, like I said, 1990, I did not really have any antibody-mediated rejection until about 8 years into the transplant. It was a better match for me. I was 17 years old at the time when I started experiencing the rejection again. The docs again, they jacked up my dosage of prednisone. I also had a handful of biopsies at the time period. They weren't exactly sure why I was beginning to reject the kidney at that point.

But it was during that time in 1998 at St. Christopher's Hospital for Children that I was switched over to what at the time was the new wonder drug known as CellCept, which you all know about. The AMR actually got worse, and the kidney function went almost to nothing, so it decreased drastically.

I think there is still debate I know among the physicians that used to work at St. Christopher's about whether or not we became toxic from the mycophenolate. I know a number of kids lost their transplants from being switched over from Imuran to mycophenolate. That

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was what was supposed to cause -- or stop a lot of our rejection problems. But I do know liver patients, heart patients, that all lost their organs, and I know St. Christopher's transplant program subsequently fell apart pretty shortly after.

So I did lose that kidney. Again started dialysis again in 1999 at my freshman year at University of Pennsylvania. I did dialysis as an outpatient at CHOP. They let me back into their hospital after some fighting with them since I was a student at Penn and I had been a patient and they had turned me away because they didn't have a transplant program.

But luckily my mom read an article about this new procedure going on at Johns Hopkins, the ABO-incompatible transplants. Children's Hospital advised against it. Almost every other hospital in the United States advised against it as well. I was one of the first in the United States done with the ABO-incompatible transplants. Again, you know, obviously I had to go plasmapheresis. I had IVIG post-surgery. There was also fear of my getting CMV from the kidney.

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I did get it. It was pretty bad, so I think it was meant to stop.

I have a pretty strong immune system, so every time I get sick in that instance as well, my creatinine went up. It continues to go up every time I get sick, even though I am on a fairly low dose of all the drugs that I'm on now. I'm not on prednisone anymore, thank goodness for that.

But like I said, posttransplant, I was a junior in college and in a fraternity, so I began to live a pretty normal life again, if you can have one being in a fraternity. The most difficult thing for me I think was trying to remain compliant on my medications while being a student with no regular sleeping habits. You know, it was the first time I was really away from family, and being in a fraternity and all, you are out at all hours of the night. And I did my best, obviously, to remain compliant. I have not had any issues with compliance up to this anymore.

But subsequently I've been diagnosed with Crohn's and epilepsy, and I worry about the other drugs that I take as whether or not they're going to interact

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with the drugs that I take now or if they're going to be processed by the kidneys.

And I also worry about rejection because my mother was diagnosed with ovarian cancer exactly a year after donating a kidney to me. So I wonder if I have any of that in me, and if there will be any of it recognized by my body.

So like I said, right now I don't really have any adherence issues. I did when I was younger. I didn't want to take the liquid cyclosporine, which was certainly a challenge. I did see a child psychologist. And to this day, I do have a lot of damage in my joints from long-term steroid usage.

But I would like to say that I do think that if children and teens could be educated more about the alternatives to not taking their medications, people would probably be more compliant. But I know compliance is a big challenge. I tend to see with the support groups that I work with that it works best when people have a support network behind them. I do know people that rejected their transplants because they got mad at their donor, and so they stopped taking their

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drugs.

And I do believe in digital interventions that can help. I work in the digital health field now. And hopefully I will never need another transplant, at least anytime soon.

Thanks.

(Applause.)

DR. VELIDEDEOGLU: Our next patient representative is Jack Lennon.

MR. LENNON: Good morning, all. My name is Jack Lennon, a lifelong kidney patient, born with post urethral valves, and a three-time kidney transplant recipient. And as you see, my slides today are photos of my family and I throughout my life. To give you a little bit of an insight into the life of a kidney transplant recipient, and obviously feel free to ooh and ah how cute I was when I was a baby. I'm not sure what happened in the last 29 years.

My first transplant occurred at the age of 7 back in 1995, which was from my father and lasted 15 years. My second transplant was in 2008 from my mom and only lasted 5 years due to a complicated first

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couple of years characterized by significant cellular and antibody-mediated rejection.

My most recent transplant, I actually hit my 3-year anniversary later this month, and I'm looking for wood to knock on because this one is going to last a very long time, as it's a perfectly matched kidney from my older brother. But even so, I'm running out of siblings, so family reunions become very interesting.

So obviously I've had this disease my entire life, which means my family has had to deal with this my entire life as well. And this is what happens in pediatrics. Management of the disease is not only influencing the behavior of the patient and helping the child deal and cope with being a transplant patient and being different, because kids notice when you're puffy and you get hair on your face at a young age and when you miss a lot of school, and they're too innocent not to ask why, and the kid has got to come up with an answer, and it can't be, "I'm sick," because then the kids say, "I don't want to be around him."

So that's one part. And then we have to deal with the caregivers and the parents, which is a whole

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nother ball of wax because we all know how much our mothers worry about us. So even with a family affair and them constantly, but nicely, nagging me to be compliant or adherent -- and I don't like to use those words, as they're used in manufacturing and insinuate that you can control the environment in which you are operating. And if anybody has kids, you know you can't control the environment in which you live.

So real quick interactive session. Who here takes medications for anything?

(Show of hands.)

MR. LENNON: Keep those hands up if you are perfectly adherent, you never miss a dose, you're never late, you take it with food when you're supposed to, you take it on an empty stomach like you're supposed to. Am I the only one with the hand raised anymore? And I've got to put my hand down. All right? This is the assumption, ask, and expectation of kidney transplant recipients, is that we're perfect, that we're robotic. But we're not. We're human.

So I had all the resources when I was growing up -- I had a family, I spoke English, I had good

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insurance, all of the normal barriers you would think of -- and yet I had issues with managing my care, is what I like to call it. And ultimately it resulted in me losing my first kidney transplant while I was in college.

And I've been blessed, luckily, though, to be able to receive two more transplants, as I mentioned, from my mom, but without any solid explanation, marked with significant cellular antibody-mediated, it only lasted 5 years. And I started a habit anytime I would go to the hospital to pack a bag. I didn't know what the results were going to show. I didn't know if I would have to stay in the hospital. And it's a tradition that I keep going till today. And though I'm blessed with a perfectly matched kidney, and I'm too much of a realist to think it's going to last the rest of my life, and I keep that bag packed in the back of my car just in case, and that's the scary part.

You saw my pictures today, and they're intermingled with happy family photos that you might see in your own homes, but there might have been some photos in there that for me bring back some pretty bad

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memories, and that's really the scary part. And my challenge for the folks in the room is to change the story for the next patient and have all these photos be happy family photos and that maybe I can finally unpack that bag in the back of my car.

Thanks.

(Applause.)

DR. VELIDEDEOGLU: We thank all the patient representatives for sharing their transplant experiences, their life experiences, and for their very insightful comments. And we will move on with the scientific presentations.

Our next speaker is Robert Colvin from Massachusetts General Hospital. The title of his talk is, "The Relationship Between Acute AMR and Chronic AMR? Do Acute and Chronic AMR Represent a Continuum?"

The Relationship Between Acute AMR and Chronic AMR? Do Acute and Chronic AMR Represent a Continuum?

DR. COLVIN: I would like to thank the FDA and Ergun in particular for organizing this conference. We're here to try to advance the field to address the issues that we heard so eloquently encapsulated by our

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patients here, and we're indebted to them for coming.

So my topic, the topic I was assigned, was, "Acute and Chronic AMR: A Continuum or Distinct Diseases?" And like the Yogi Berra expression, when you get to a fork in the road, sometimes you have to take it. And so my answer to the question, "A continuum or distinct diseases?" is yes.

My financial disclosures.

So antibody has multiple effects on the kidney ranging from hyperacute rejection, the first form of antibody-mediated rejection recognized; acute antibody-mediated rejection; and then a chronic form, which is by far the most prevalent form of antibody-mediated rejection. In addition, we've learned that there's a form of injury, if you will, or resistance to injury, called accommodation, where the antibody interacts with the graft, but it doesn't cause any damage, and that can be seen, for example, in ABO-incompatible grafts, but it can also be seen in other settings.

And then there's a relatively newly recognized form, which I like to call smoldering, which is entirely below the waterline. Patients do not know

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they have it, the doctors do not know they have it. The only way you know that it's going on in the kidney is by doing a biopsy, at least that's the only way we have now. And so this is characterized by a complement deposition in the small vessels of the kidney.

In the case of chronic, you get the chronic changes, fibrosis, duplication of basement membranes, et cetera. In accommodation, there is no pathology at all except the complement deposition. And in the smoldering version, you get cells in the capillaries and complement to varying degrees, but no immediate loss of graft function.

Now, are these related? Are these the same disease at different stages? And that's what I'll try to address today.

The best definition of these diseases comes from the Banff Consensus Conference, which has been going on for many years. And they're separated by their pathologic features by light microscopy and to some extent by electron microscopy.

The acute version of this disease has acute injury: microvascular inflammation, arteritis,

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inflammation of the small arteries. It can have thrombi. It can have acute tubular injury.

The chronic version has, of course, chronic pathological changes, what's called transplant glomerulopathy, which I will illustrate in a minute; duplication of the basement membranes of the small vessels of the kidney; or changes in the arteries. So that's how we distinguish them.

They have in common two things. First, they have evidence that antibody is interacting with the endothelium originally in the form of C4d primarily. Now we recognize the microvascular inflammation is an indicator of that, although it's not as specific. And we have the potential of molecular markers to detect the endothelial response.

And, finally, we would like to detect the antibodies in the circulation. These are almost always HLA antibodies, but there is a possibility that other antibodies, ABO, for example, but probably others as well, can react with the endothelium. So this is how we define it. If you have all three, that is sufficient for the diagnosis.

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Well, what does it look like? In the case of acute AMR, you have inflammation in the glomeruli, a number of leukocytes, both mononuclear and neutrophils. You have the same sorts of cells in the peritubular capillaries, mononuclear cells, macrophages, NK cells, neutrophils. You have thrombi in the capillaries of the glomerulus sometimes, congestion, and, of course, usually you have complement deposition in the peritubular capillaries, and also the glomeruli.

You can have endarteritis in this setting with polys underneath the endothelium and complement deposited on the surface of the small arteries.

Now, the chronic form, which is by far more common and probably accounts for about 60 percent of late graft dysfunction, has quite a different appearance. For one thing, there is something called transplant glomerulopathy, which has duplication of the basement membrane well seen by electron microscopy. Here you see multiple new layers of basement membrane. This is the original basement membrane, and all this has been added to it.

The endothelial cell undergoes marked changes.

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It normally is fenestrated to allow filtration through the glomerulus. This loses its specialized function and looks very activated. This cell, of course, is one of the targets of the antibody.

The capillaritis I mentioned before. The peritubular capillaries also get these laminations. These I think -- I've always thought of these as rings on a tree reflecting past individual episodes of more severe endothelial damage and repair.

This chronic disease goes through stages that last many years. This is a patient we had who had multiple protocol biopsies, started off at 3 months with a normal biopsy, no antibody, and no C4d, normal appearance by light and electron microscopy. But 11 months, there was antibody present in the circulation and complement in the peritubular capillaries, but no histologic evidence of injury. And this continued for the next biopsy, which I think was about 15 months, if I recall.

And, again, there is very little evidence, or practically no evidence, of injury by light microscopy, but you begin to see some changes by electron

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microscopy with thickening and duplication of the basement membrane.

And, finally, 5 years after transplant, the creatinine is still reasonably good, and now you finally have the changes that we would call transplant glomerulopathy, well shown by electron microscopy.

And we propose that this disease goes through stages. A slide was shown of this before, in which you begin by making antibodies. You then get some changes in the graft, C4d or capillaritis, glomerulitis, but this all occurs without any clinical evidence of disease, or for that matter, actual pathologic evidence of damage.

Then you start to get damage that you can see histologically but still is not reflected by any clinical function, clinical renal function. Finally, you get graft dysfunction, and this, of course, is when we often get the biopsies, and you can tell that this is probably far too late to really effectively intervene. And the Wiebe study showed that this whole course typically takes about 8 years and progresses over 3 years once the graft dysfunction has occurred.

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So this is a long disease.

So what are the differences and similarities between acute and chronic antibody-mediated rejection?

Acute antibody-mediated rejection is usually in presensitized patients, patients who have had exposure to blood products or pregnancies, et cetera, or a previous transplant.

Chronic is usually de novo DSA, that is, the DSA was not present at the time they were transplanted, and it is associated with episodes of T-cell-mediated rejection, which will be discussed later by Dr. Gaston.

Acute causes a rapid loss of function, measured in days, much like T-cell-mediated rejection. As I mentioned, this chronic disease is insidious, lasting months or years. And most of these cases are not associated with past episodes of acute AMR.

The antibodies could be different. The acute AMR was originally associated with Class I antibodies by Phil Halloran, but now we know from the work of many that the Class II antibodies are the principal culprit in chronic antibody-mediated rejection. Acute AMR has widespread deposition of complement typically. Chronic

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may have little or no complement deposition. Both have capillaritis. The acute tends to have neutrophils that the chronic does not.

And recently a paper has been published to try to distinguish the molecular signature of these two forms. In this case, this is the acute, this is the early presensitized DSA, in which injury repair response is the primary molecular signal. And in the de novo, the late form, you have T-cell transcripts, NK, natural killer, cell transcripts, and gamma interferon-related transcripts. So the molecular signals are somewhat different, and I would like to think this can lead us to understanding differences in pathogenesis. This is really their most important role.

Well, why are there these different effects of antibody? Let's just think in a general way why this might be. The first thing that comes to mind is the resistance and the effector strength, the resistance of the endothelium and the strength of the antibodies and the cells and the other things that mediate this damage. And you can imagine that these diseases are on

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a continuum. At the beginning, where the effector strength is maximal and there is no resistance, you get hyperacute or acute antibody-mediated rejection. With time, I think the endothelium learns how to adapt their anti-complementary molecules on the endothelium, and there are other ways of resisting the effects of antibody. And so as the resistance strength increases, the effector strength may stay the same or go down, you begin to get the slower versions of these diseases. So that's one theory. It's the balance between effector and resistance.

Another theory is the complement fixation theory. And this is nicely shown by the work of Loupy in Paris, who you've seen this slide before by Ros. In his studies, in their studies, the ability of the antibody to fix complement in vitro was correlated with a poor outcome, this red graph. And as you would expect -- let's see, I'm having trouble. I can't advance. Could you advance that for me? There. No. Yeah. Here we go.

Another study, in this case, a preexisting DSA, a study from London, showed the ones that could

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fix complement in vitro on beads, in this case, C4d, showed a much shorter graft survival. And this was primarily in the first few weeks after transplant, as you would expect. And it's nice to know that the C4d stain in the tissue correlates very strongly with the ability of the antibody to fix complement in vitro. So this can be taken as a measure of this that we see in a biopsy.

And in this meta-analysis, the presence of C4d was associated with inferior allograft survival compared with DSA or histopathology alone.

And, finally, in this theme, this study from Hopkins suggests that patients who have C4d deposited in the grafts -- and these are primarily early AMR -- have a higher rate of graft dysfunction, an earlier onset, and a higher rate of graft loss at 1 year. So these are all arguments that complement is part of the problem, but it may not be the whole story.

You can think about mechanisms, and that's what we do as pathologists a lot. And if we understand the mechanisms, that can guide us to the right kind of therapy. And we know that there are at least three

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ways that antibodies can interfere or damage the endothelium. Antibody alone in tissue culture can cause the endothelium to change, to proliferate, to secrete procoagulant factors, and this has been shown by Elaine Reed some years ago.

Complement-mediated damage is well known, and we know this through our studies of C4d and other techniques. Complement not only kills endothelium, it causes the endothelium to react and become activated.

And, finally, a relatively, I would say, less appreciated mechanism is cell-mediated injury of the endothelium via Fc receptors on the surface of either NK cells, macrophages, or neutrophils. And exactly what this does to the endothelium we have less insights on. So you would like to know in an individual patient which of these mechanisms is most important.

We know from animal studies -- and this will be discussed later by Anita Chong -- that complement-dependent mechanisms are important in acute AMR. And we know in the setting of chronic rejection in mice that NK and Fc mechanisms are very important, and complement is irrelevant to the late damage of cardiac

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allografts. So there is evidence, at least in the experimental studies, for each of these mechanisms.

Finally, I just want to mention that there is nothing unique about the kidney.

Very sensitive, very sensitive. Sorry about that. Why is that? This next one. Are you moving it, too? Okay. All right. My hands are up.

Just to make the point that there are common features in all vascular organs for antibody-mediated rejection, whether it's the kidney, the heart, the liver, or the lung. And so these principles that we are developing in kidney transplantation will probably apply in other settings.

So just to end, to summarize what I've said, acute AMR, which is also called early or type 1, is usually due to presensitization and involves both Class I and Class II antibodies. It rapidly progresses through renal failure, but it does respond to treatment typically. It may be complement-dependent or not. And I think this will be established by the drug therapy trials more than anything else.

C1q fixing antibody and C4d deposition are

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associated with more severe course and argue that complement is an important part of this.

Chronic AMR, also called late or type 2, is usually due to de novo DSA and related to Class II antigens, and as Peter Nickerson eloquently so, just a few amino acids on those Class II antigens. It's a slow pace and it has a long subclinical phase, which we need to detect better as clinicians. It progresses through these stages over many years. And this may be complement-independent and related to NK or macrophage medium mechanisms. But again, this is to be established. And if I could think of a need for a drug, it would be to affect this last mechanism, of Fc-mediated endothelial damage.

So why don't I stop there. Thank you very much.

(Applause.)

DR. VELIDEDEOGLU: We thank Dr. Colvin for his presentation.

Our next speaker is Dr. Nickerson again. And the title of his talk is, "Impact of Acute and Chronic AMR on Graft and Patient Survival -- Is Acute AMR and

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Chronic AMR Related to Memory Versus De Novo DSA the Same Process or Fundamentally Different?"

Impact of Acute and Chronic AMR on Graft and Patient Survival -- Is Acute AMR and Chronic AMR Related to Memory Versus De Novo DSA the Same Process or Fundamentally Different? HLA versus non-HLA Antibodies Causing AMR

DR. NICKERSON: Thanks very much again to the FDA for this opportunity. I'm going to echo a lot of Dr. Colvin's discussion points in my talk. Again, I will talk a little bit about off-label in this discussion.

Natural history of preformed antibodies or memory-related antibodies. And I think it depends on the context. Did you recognize that you had it or didn't you at the time of the transplant? And I think we're starting to recognize it more commonly, but in this paper, which came out of the University of Basel in Switzerland, they were doing transplants on the basis of a CDC-negative crossmatch pretransplant.

And in retrospect, they went back and tested by the more sensitive single-antigen beads whether the

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patient actually had a DSA that they had missed at the time of transplant because of the negative CDC crossmatch. And they found that there were patients that were positive for DSA by the more sensitive technique.

And when they compared the rates of ABMR in these patients compared to those that had the negative single-antigen beads, what they saw was that those patients, almost 50 percent by 100 days were having a clinical onset of ABMR compared to those patients who were negative by the single-antigen beads.

And I think because of this paper back in 2009, many groups have now moved on to using single-antigen beads routinely in their practice. But this just shows you that if you didn't know it was there, you're actually at high risk for developing a clinical ABMR.

Now, did that translate into worse outcomes? Well, yes, some of these patients, those who had a DSA and experienced an ABMR did worse compared to those patients who had a DSA and didn't experience an AMR. So clearly the combination of the antibody and an

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actual clinical event led to worse long outcomes.

And this was also reiterated in a paper subsequently in 2015 by the Paris group where again if they did a protocol biopsy at 1 year in patients and found that they had ongoing subclinical antibody-mediated rejection, these patients had a much worse outcome. And many of these patients, 80 percent almost, were these ones that had antibodies at the time of transplant that they hadn't recognized and had gone across. So, again, making it really important to know whether you have the antibody when you're doing the transplant, and then ask the question, Can I mitigate that impact?

And in this paper, a nice series of papers that came out of Mayo Clinic, and Dr. Stegall, who is here, was principal author on this group, these guys knew they had the DSA, and they asked the question, Can I overcome it with desensitization protocols? And it depended on how much antibody they had. And so as they went from weak flow crossmatches to strong flow crossmatches to cytotoxic crossmatch-positive transplants, and they put the patients through

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desensitization, they still experienced ABMR in a number of these patients, and it really showed us that the higher the titer of the antibody, the more likely you were to have an antibody-mediated rejection even when you were trying to desensitize the patients.

And a lot of interest was in, could you have predicted this based on the bead MFI? And others in this meeting will talk about the utility of that, but suffice it to say that the MFI didn't really predict who would or wouldn't have an ABMR, and this was 20 percent basically we were experiencing in ABMR.

And it didn't matter how strong your antibody was at the time of transplant, whether it was weakly positive or strongly positive, all of the patients were developing transplant glomerulopathy after the transplant in these desensitization protocols. And this really taught us a lot about -- and, again, I think what Dr. Colvin was just talking about, the smoldering nature of chronic antibody-mediated rejection.

So that's memory, not recognizing it, or even recognizing it and trying to desensitize. And then now

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the question of de novo DSA and what's the etiology and natural history? Well, in our case series, the first 315 patients, the majority of these patients had Class II antibodies; 86 percent had de novo Class II either alone or in association with Class I. And only 30 percent had a de novo Class I antibody; and only 14 percent, an isolated Class I antibody. And now we're up to 600 patients and looking at, and we see the same pattern. So dominantly Class II de novo DSA.

And throughout this whole series now of almost 600 patients, we've only had one patient with an isolated Class I de novo DSA that's resulted in graft failure out of almost 600. So really we think the emphasis should be focused on the Class II DSAs. That's where we're going to learn the most and understand how to control process.

And I apologize for the use of nonadherence. And my hand went down when the question came, do I take all my medications appropriately? And the answer is no, of course not. It's a really tough thing to do, but it becomes critical in the context of a transplant because we know that if you're adherent, the risk of

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forming an antibody is really, in our series, about 2 percent per year. But if you're having trouble complying with your regime, which it's absolutely difficult to do in life, it certainly gets in the way, you're at fourfold increased risk for developing an antibody, and that really is a course once set on is very difficult to control.

Now, once you have a de novo DSA, does that always mean that you have ABMR? And so we did biopsies at the onset of these DSAs. We were regularly screening our patients from the time of transplant. From the time of first detection, we would do a biopsy, even if the function of the graft was fine. And we found that three-quarters of our patients met the Banff criteria for ABMR, and it was largely because of peritubular capillaritis with C4d and glomerulitis.

Now, other case series, out of Vienna and out of the Mayo Clinic, have shown that when they do biopsies in these patients, they get about 50 percent ABMR detected. And so I think the range is anywhere between 50 and three-quarters we'll have ABMR when you have a de novo DSA.

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What was interesting is that we also found that 61 percent of these patients also had TCMR. So it wasn't just that they had pure ABMR, in fact, they had a mixed rejection, and while half of these were borderline, mild, TCMRs, half of them were actually Grade 1 or higher TCMRs. So these were not occurring in isolation. Only 18 percent of our biopsies at the onset of a DSA had actually pristine histology.

Transplant glomerulopathy was uncommon, and you would expect that to be the case. If the antibody is leading the transplant glomerulopathy, and this is the onset of the antibody, then you shouldn't see a lot of transplant glomerulopathy, and we didn't at that point in time. What we did see was a lot of interstitial fibrosis and tubular atrophy at the time of onset of DSA. And, again, I don't think we were very surprised by that.

When we looked at what predicted the long-term outcome in these patients on the biopsy, we found that there were two independent predictors in a multivariate model. One was transplant glomerulopathy. If you had transplant glomerulopathy, that was a very strong

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predictor that your graft was at risk for premature failure, but, again, only 13 percent had this at the onset of the antibody. Tubulitis was actually a very strong predictor of eventual graft loss, and I think it's giving us some indication of the strength of the immune response that's ongoing in these grafts.

We did see that the Banff CG score would increase by one grade per 3 years of follow-up after the onset of antibody, so this was actually something that was a strong correlation.

What was interesting was that microvascular inflammation grade, in other words, how much g+ptc you had, if you had mild g+ptc or you had more severe forms of g+ptc, that really didn't differentiate who would go on to graft loss, and I think that's partly because most patients had some degree of g+ptc, and once you have it, there is probably spottiness in the biopsy that you're doing, it doesn't really help you to predict who's going to be more accelerated in their graft loss, nor did C4d positive or negative have any prediction on who would go on to subsequent graft loss.

So not to say that they're not important, I'm

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saying that a lot of times these are telling us that there's a process underway, but the degree of that process is not predictive of the outcome.

And as already shown, if you are clinical at the onset, in other words, you already had graft dysfunction when you first had the antibody, on average, you lost your graft at about 3.3 years, but if you had stable graft function when the antibody first showed up, on average, it was taking 8.3 years to lose the graft. When you did lose the graft, there was a lot of transplant glomerulopathy and there was a lot of interstitial fibrosis and tubular atrophy. And again in multivariate models, the only thing that predicted the CG was the antibody, and what predicted the IFTA was early cellular rejection and if you had had nonadherence. Antibody did not predict IFTA. And we think that the nonadherence is really a surrogate marker of ongoing smoldering cellular rejection in the graft that's leading to IFTA.

So the model that we've derived from our de novo DSA studies is that graft loss is really the composite of IFTA and CG, that IFTA can be caused by

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multiple things. It could be drug toxicity, older donors, ischemia reperfusion injury that occurs at the time of deceased donation, and then TCMR. And we've stolen shamelessly from Dr. Colvin in using the term "smoldering" because we believe that there is a smoldering cellular rejection, and many times this is much more subclinical than clinical that's leading to IFTA.

And CG is driven by, again, ABMR, which, again, we also like the term "smoldering" and I would refer to this as predominantly subclinical rather than clinical that's leading to transplant glomerulopathy, and this is driven by de novo DSA formation.

And we'll hear later the linkages between cellular and DSA formation. And all of this is driven by HLA mismatching, and, hence, the importance of matching for HLA, and, in particular, Class II.

Under immunosuppression, whether that's because of difficulty with adhering to our regimes or us, because we're prematurely or minimizing our patients, leads to basically taking the brakes off of the immune response and allows this whole process to

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accelerate on to graft failure.

So the question of, de novo versus memory, what's the differences? Again, a nice paper that just came out of the Paris group, where they basically showed that the onset of ABMR related to preexisting was very rapid, within the first year largely, and within the first few years, for almost all the cases that they had documented.

De novo DSA was a much more slower onset of cases of antibody. They also noted that those that had preexisting DSA tended to have slightly better graft survival than those that had de novo onset of DSA.

When they looked at the pathology differences, it was actually quite interesting. The de novo DSA ABMRs had more transplant glomerulopathy, TCMR, IFTA, and proteinuria at diagnosis, and I think in part, that's because they likely had delayed recognition of the process with de novo DSA. When they looked at how many were subclinical in the de novo DSA, it was only 8.8 percent. So I think the cases that they were documenting of de novo DSA-associated ABMR were these late cases, which they weren't recognizing by

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screening, but they were recognizing by the onset of graft dysfunction. Whereas the early preexisting antibodies had a lot more subclinical, and I think that's because they were much more attuned into doing protocol biopsies in these patients anticipating the risk for early ABMR.

What was also interesting and I think also deserves emphasis is that the de novo DSA, they noted at the molecular level a lot more TCMR transcripts as compared to the preexisting DSA. So the preexisting DSAs seem to occur predominantly as an antibody phenotype whereas the de novo DSA much more commonly had this mixed phenotype with T-cell transcripts, NK, and interferon gamma transcripts.

In another paper, by Dr. Haas, who is here, and his group with Stan Jordan, they looked at again type 1, where these were really ABMRs associated with preexisting antibody or type 2, which were de novo DSA-associated antibody-mediated rejections. And what they also noted here, 72 percent of the de novo antibody-mediated rejections had a concomitant TCMR Banff 1a or borderline compared to only 27 percent in the

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preexisting DSA. They had more transplant glomerulopathy in these patients compared to the preexisting DSA.

And in terms of the activity, 70 percent were acute or active whereas in the de novo DSA-associated, 60 percent were chronic and active. So, again, similar to what you heard from Dr. Colvin's discussion, and, again, predominantly Class II in the de novo DSA-associated types.

In terms of response to therapies, this is a nice paper from Steve Woodle and his group at Cincinnati, and what they were looking at is treating ABMR, and they were basically looking at refractory ABMR, and how do we actually overcome these and how responsive are these patients to using additional agents? And in this case, they were using proteasome inhibition as a last ditch effort to try and quiet down the ABMR.

And what they found was that if this was within 6 months of the transplant, they were actually pretty good at settling things down. They had a good immune response documented by a drop in the MFI within

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14 days of treatment in three-quarters of the patients, histologic response in almost 90 percent, and improvement in graft function. But if these ABMRs were occurring after 6 months posttransplant, the response to therapy was much less dramatic, and again showing the unmet need that we have in this patient cohort.

So in summary of, "What's the difference between preexisting DSA and de novo DSA-associated ABMR?" I think it's fairly similar to the summation that Dr. Colvin gave, the HLA DSAs and preexisting are Class II, maybe a little bit more or equal to that of Class I compared to de novo, where it's dominantly Class II.

One of the things we haven't really highlighted is the level of immunosuppression in preexisting DSA. We're anticipating this. We're giving a lot of immunosuppression. We're doing induction depletion therapies and pheresis, IVIG, whereas in the de novo onset, we're really at baseline immunosuppression. So there's a real difference in the immunosuppression at the time that we're diagnosing these processes, and I think that also leads to a

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differential effect of what we're seeing on the pathology. Certainly nonadherence is pretty good in the preexisting. You don't really have nonadherence because you're under tight monitoring at that point in time, whereas it tends to be a bigger problem in the de novo DSA patients. ABMR tends to be more severe in the preexisting, less so in the de novo. And the TCMR, I think we're starting to appreciate more and more this really is a cardinal feature of the de novo DSA phenotypes, and the response to therapy is much different, it's a lot better chance to get a response with the preexisting than it is with the de novo.

So that summarizes my HLA part. Now, I was also asked to just briefly talk about non-HLA antibodies, and are they playing a role? And I'm giving you a cartoon here just to really identify what we're talking about when we talk about non-HLA. Now, in this context, I'm referring to an HLA antibody targeting HLA leading to inflammation in the graft, but this could be any kind of inflammatory process in the kidney leading to spreading of revealing epitopes inside the tissues. So we get collagen, perlecan,

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MICA, other targets, AT1R, all getting expressed in the context of inflammation leading to antigens being shed, being processed, and within the regional lymph node then getting plasma cell production of antibodies hitting these targets that are being revealed through the inflammatory process.

So there have been data supporting constructs like anti-LG3, anti-perlecan, collagen IV, AT1R, MICA, and anti-endothelial antibodies that can then come back into the graft and cause their own inflammatory processes. And the real question for a lot of us is, To what role are these non-HLA antibodies playing a role in causing an antibody-mediated like inflammatory response in the tissues?

And there's some data coming out more and more certainly supporting a role for anti-angiotensin I receptor antibodies. Preexisting, and this may be revealed from the processes that led to kidney failure in the first place and at the time of transplant then being a risk factor for acute rejection and graft loss. Anti-perlecan antibodies, again associated with vascular rejection or more chronic allograft rejection.

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And anti-collagen IV and fibronectin antibodies, leading to transplant glomerulopathy and associated with chronic allograft rejection.

The problem with a lot of these studies, though, is that they're frequently confounded by preexisting HLA DSA, and so to separate what these non-HLA antibodies are doing relative to the HLA antibodies is very difficult.

And the other problem with a lot of these studies, because a lot of them are older studies now, is that there was inadequate assessment for HLA DSA using the solid phase technology to really rule out that there wasn't an HLA antibody there that was driving the process as opposed to the non-HLA antibody.

So I think this is a field certainly ripe for investigation, but to actually attribute that these are absolutely driving processes with great frequency I think is the problem that we have today in the field.

And with that, I've already given my acknowledgements before. I'll stop. Thank you very much.

(Applause.)

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Public Comment and Discussion Part I

DR. VELIDEDEOGLU: Okay. We thank Dr. Nickerson. Now, this concludes our Part I presentations.

Now we will start the discussion session following Part I. And we are running approximately 10 to 15 minutes behind schedule. And the first approximately 10 minutes will be devoted to the questions from the audience or from the speakers specific to the presentations if they have any clarifying questions. And we will follow by our preformulated question afterwards to steer the discussion related to the presentations.

And I request all the audience members and the speakers who plan on asking questions to introduce themselves first before each question. Since this workshop is being webcast live, this is important. And so if anybody has any questions related to the presentations, Part I presentations, they can ask now.

Please, go ahead.

DR. KNOLL: Greg Knoll, from the University of Ottawa. It's a question actually for Peter from your

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first talk. Is the ability to measure those amino acid epitopes, is that something that routine clinical labs can now do, or is this something strictly being done at research centers?

DR. NICKERSON: Yeah. So certainly the software package that is used to do this is freeware, so it's downloadable from the Web, there's nothing magic about the software. What it requires is for you to do higher resolution HLA typing on the donor and the recipient, and that's really up to the labs, what they're prepared to do or not to do.

Certainly we do that already for bone marrow transplant, we do matching at a very high-resolution level. We've not really brought that into kidney transplant because we haven't really had a reason to do that until now.

I think we're going to see as the technology evolves and as the recognition of the utility of such an approach evolves, more and more labs will start doing higher resolution typing.

There has been a lot of discussion about even just using what we know about HLA frequencies to impute

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what the high-resolution typing would be. Although my colleagues would frown on that, I think that's actually a bad thing to do because using imputation, you're going to introduce a lot of error, and I think introducing error introduces noise, and if we keep doing that, we're not going to be able to get the associations we need. So I think you really do need to get to high-resolution typing.

Can we do this today? Absolutely. There is nothing preventing us from doing this today and assessing what the epitope mismatched degrees are between -- or the Eplet mismatched degrees are between donor and recipient. Anyone can do that.

DR. KNOLL: And just a follow-up then. Is there much cost to the high-res typing if you're not currently doing, the additional cost a lot?

DR. NICKERSON: Yeah, again, as the evolution of the technology occurs, a lot of times you are getting high-res or close to high-res typing, and I think that it will become very cost effective. Yes, there is some additional cost to do that, but ultimately to risk stratify your patients into whether

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you're at high or low risk, and then how you would treat them accordingly, it's a minimal investment to make.

DR. VELIDEDEOGLU: Okay. Any other questions? Okay, Anat Tambur.

DR. ROITBERG-TAMBUR: Thank you. I want to stay on the same topic and definitely echo Peter with the newer agents that we have in the market right now that will allow significantly higher resolution with minimal added cost. And just to clarify, I definitely think that that is a great way for risk stratification.

Where I see a little bit of a problem is when you look at the different papers, everyone comes up with their own different thresholds, and where I'm not sure that we can actually take it and implement it right now, as a community, as an approach, even though we definitely get way better resolution of how different the donor and the recipient are by taking that approach, is, how do we go with thresholds?

And you'll hear me talking about MFI cutoffs and my aversion to that, and I just want to caution about jumping into something using a threshold that may

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be the best for one population, and then you go to another population where they're more heavily Hispanic donors or African American donors, and you're talking about a whole different universe, and your thresholds will be different.

And thank you, Peter, for nodding for this because I think it's an important issue.

DR. VELIDEDEOGLU: Okay. The member from the audience at the microphone, please.

DR. CHONG: Hi. My name is Anita Chong. I'm from the University of Chicago. I wanted to ask a question related to the chronic as well as the acute AMR and whether there are any new technologies that are looking at whether the quality of the antibody responses are different in terms of the subclasses, the titers, the avidity, as the immune response sort of develops over time with T-cell help.

DR. VELIDEDEOGLU: Well, to whom do you want to direct your question?

DR. CHONG: Probably to Peter as well as Bob Colvin.

DR. NICKERSON: Certainly we're going to hear

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about the other technologies, the subclasses, the C1q assays from Anat and Dr. Gebel in their presentations. And, yes, I think what Anat has nicely shown is that you can start looking at titer and, how does titer have predictability?

Again, there is mixed literature out there. I think it really depends and you have to look very carefully at who is reporting what in what context. Chris Wiebe from our group is going to show some data where we had tried to look at titer or C1q.

And certainly in a univariate analysis, both of those, the higher the titer and the higher -- whether you were C1q-positive, that did correlate with graft outcome, but we also saw that that correlated with clinical phenotypes. In other words, if you had clinical rejection at the onset of your ABMR or if you were known to be having nonadherence in the mix, that seemed to associate very strongly with high titer and C1q-positive. And so those all interacted, and basically the clinical phenotype had as much prediction as any ancillary diagnostics would have had in that construct of de novo DSA.

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In terms of subclasses, I think there really needs to be a lot more work done on subclasses at this point to correlate with outcomes.

DR. VELIDEDEOGLU: Okay. Thank you for the questions. Now we are running quite a bit behind, so we will move on to the FDA questions.

And the first question for discussion is: Are early acute AMR and late acute AMR the same regardless of whether they are related to preformed or de novo DSA? Do either or both represent a continuum to chronic AMR? Discuss how.

So if anybody volunteers to make a comment or question, please you are welcome to do so.

Dr. Haas?

DR. HAAS: Yeah. I think that the late AMR, the differences between late AMR and early AMR I think are primarily due to whether this is a memory response or whether this is a de novo DSA. We do a lot of presensitized patients at our center and at Johns Hopkins, where I was previously at, and the rebound, the memory effect, can occur as late as 3 or 4 years posttransplant, where if you type the donor-specific

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antibodies, they're the same donor-specific antibodies that were present pretransplant, and these can be type 1, anti-Class I or anti-Class II, and these tend to be pure antibody-mediated rejection responses. They're not mixed rejections.

By contrast, when we're dealing with de novo donor-specific antibodies, I think as was highlighted by Peter and by Bob, these are primarily anti-Class II antibodies. They're often preceded by and occur together with cell-mediated rejection. The gene activation that occurs in these responses is different in that instead of this being a pure antibody humoral type response, this is a mixed T-cell-mediated antibody-mediated response. So I think it's not a matter of time posttransplant, but whether we're dealing with a memory response versus a de novo DSA.

DR. VELIDEDEOGLU: Okay. Thank you.

Any other comments?

DR. STEGALL: I'll comment on that.

DR. VELIDEDEOGLU: Okay. Dr. Stegall.

DR. STEGALL: I think that when you do a biopsy, they look a lot alike, but the early acute ABMR

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in a sensitized patient usually occurs if there is a crescendo rise in antibody, and you can actually usually get through that. And many of these patients actually will never develop transplant glomerulopathy long term, especially if they're Class I. So it's a different clinical situation.

One of the confusing things about this is Banff looks at antibody-mediated rejection as the same thing, which it sort of is histologically, but clinically, being a clinician, it's not even close to being the same scenario. You'll do a year later biopsy on someone who's had an early acute ABMR episode, and they won't have any peritubular capillaritis or CG, but if you do a year biopsy on anybody, and they have peritubular capillaritis, your next question, almost always it will progress at some point to CG, it just depends on how long you're looking at it.

So the question is the same. Of course, nothing in biology is exactly the same, but the histology is the same, but the clinical scenarios are very different, and I think the treatment of those two scenarios are very different.

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DR. VELIDEDEOGLU: Okay. We thank Dr. Stegall.

DR. COLVIN: Could I make one?

DR. VELIDEDEOGLU: Dr. Colvin, please, go ahead.

DR. COLVIN: I think that late acute AMR is quite unusual to be present just alone. The cases I've seen have almost always had a component of a chronic process, transplant glomerulopathy in particular. So I think the acute AMR in the late phase is just a flare-up of a chronic process rather than something different, and more related to what occurs early on.

DR. STEGALL: But the difference is that you're seeing biopsies for cause, so more than protocol biopsies. So when we see protocol biopsies, you almost always see the peritubular capillaritis and inflammation first. There's not a lot of C4d. There is commonly not transplant glomerulopathy by light microscopy. So I think it's the only time you ever biopsy that person is when they've already progressed to something.

If you do an EM, endothelial cell activation

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is there, it's like one of the first things that ever happens, it's just that you don't see that. The process of duplication of the glomerular basement membrane follows the chronic inflammation.

DR. COLVIN: Right. Can I respond, Ergun?

DR. VELIDEDEOGLU: Yes.

DR. COLVIN: Yeah, I agree with you completely, and your protocol biopsies are telling us an awfully lot about the underling pathobiology of this condition. There's a gap in the Banff classification for what I like to call smoldering. And I don't know if Mark would like to comment on this, but this is what you're seeing in your protocol biopsies, and it isn't necessarily associated with any change in renal function. And whether you call that acute or chronic, I think probably either one is not the right term, but we need another term. "Smoldering" would be one of them.

DR. HAAS: Yeah. I mean, one of the problems with the Banff is we divide antibody-mediated rejection into what's called acute active and chronic active. And acute active is a little bit of a misnomer because

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it implies that it's always acute. And if you look at the footnotes from the original, you know, the 2013, Banff, it actually states that the process may be acute or smoldering, and may be clinical or subclinical.

So at the last 2017 Banff meeting, which was held in Barcelona just a couple weeks ago, there was a move afoot to remove the word "acute" from the acute active category to reflect that this may be acute or smoldering, that it's just active and it doesn't have TG. The problem with that, of course, is that it assumes that the cases that are truly acute and the cases that are more smoldering are the same so long as they don't have transplant glomerulopathy, which is probably not the case.

So there may in fact be really three categories of antibody-mediated rejection: the true acutes, which are usually a memory response to rebound of preexisting donor-specific antibodies occur very early on posttransplant, can occur later, but are usually seen in highly sensitized patients; the more smoldering cases, which are not yet reached the stage of chronic active, but may have more in common with

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chronic active than actually the acute phase; and then the ones that are truly chronic with transplant glomerulopathy. So there may be really three forms of ABMR rather than two, as the Banff states.

DR. VELIDEDEOGLU: Okay. And I want to invite Dr. Samaniego to the microphone, please. She's been standing there.

Dr. Samaniego, you have an assigned seat at the table if you would like to.

DR. SAMANIEGO-PICOTA: Thank you. I will come to the table later. I think that everything I pretty much agree with everything that Dr. Stegall said. And we see exactly the same biology in our program, where we also do protocol biopsies. In my opinion, there are two things to look at. One is the tissue. If the patient has chronicity, that is a different type of process that is going on. Response to therapy is completely different. Outcome is completely different.

If the patient has history of noncompliance regardless of where it is happening, the prognosis is not going to be good. And even if we don't see chronicity in those patients, there may be indication,

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if not in the biopsy, that the patient has ongoing injury, proteinuria, for instance, is one of the things we tend to see in these patients.

Also, the combination of the antibodies. Are we dealing only with a Class I antibody, with a Class II antibody, or Class I and Class II? We know that Class II fare the worst. Those patients have a component of noncompliance even if you cannot detect them immediately, because patients are smart, they know how to make their numbers and their drug levels look good before they come to clinic. What happens in between, we do not know.

So it's important to have that concept because you want to select the right patients for trials. You want to select patients that will respond to therapy, where the process is early enough that you can stall it or, if possible, eliminate it.

Once antibody-mediated rejection is going on for a while, it becomes independent. It doesn't matter what you do with it. If you want to block complement, if you want to eliminate antibody, the response is so robust that you will not be able to have those patients

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to respond to anything, it's just getting ready for another transplant.

DR. VELIDEDEOGLU: Dr. Haas?

DR. HAAS: Yeah. Excuse me. With regard to treating patients with transplant glomerulopathy, is there something out there that enables us to tell which patients who have transplant glomerulopathy on the biopsy can be treated with some improved outcome, whether it be some improvement of function or at least slowing the rate of decline of function versus those patients who unfortunately are inevitably going to progress to graft loss regardless of treatment.

I think the biopsy can give us some information. Joe Kahwaji, from our group, published a small study a couple of years ago where he looked at the level of microvascular inflammation, glomerulitis and peritubular capillaritis, and found that when there was moderate to severe microvascular inflammation, that treating these patients with IVIG and rituximab did tend to stabilize the patients and that their progression was less. Whereas in patients who had no or only mild microvascular inflammation, the treatment

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had no effect on the rate of progression. But this was a very small study.

I think this is also an area where the molecular diagnostics can contribute to whether we're dealing with really an active lesion. How much endothelial activation, NK cell transcripts, are there? Measures of kidney injury transcripts, which the Halloran group found, were very, very important regardless of etiology in predicting outcomes. So there's a lot we need to learn about this, but not all patients with transplant glomerulopathy I believe have a death sentence for their graft, that some patients with TG can be treated and at least stabilized or their progression slowed.

DR. VELIDEDEOGLU: Okay. Dr. Woodle has the last word on this question. We need to move on to the next question. So we are really running quite behind.

DR. WOODLE: So, one, we agree completely with Mark in the description of these early anamnestic responses being very treatable. They're really treatable if you pick them up early. And if you wait until the antibody is so high that the graft is

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threatened to rupture, then you're looking at eculizumab or splenectomy or potentially taking the kidney out.

This is something that's been known for a long time. When we wrote the paper that was referred to, our early versus late paper with proteasome inhibitor treatment, I was at Toronto General with Carl Cardella, who many of you know, and Carl goes, "Steve, we've known this for a long time, for 20 or 30 years, that early antibody-mediated rejection is easy to treat, it does well long term."

And so this is not novel. Although there's not much in the literature, there were only two papers in the literature when we did that.

So we've looked at an endpoint that we defined just arbitrarily and picked it, and it's turned out to be fairly reliable, and that is a 50 percent reduction in the level of immunodominant DSA MFI within 14 days predicts outcome. And we'll present data at ATC. It's the strongest predictor for outcome in AMR that we've found to date, and it even works in late AMR. And it appears to us that not all late AMRs are the same, that

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the ones that do respond and show a 50 percent reduction in DSA have better survival than those that don't.

And so within that big group of late AMR that is really hard to sort out, are there different ones? They're not all the same clearly. That's one predictor that I think is out there that is starting to help us sort out some of them.

So we don't believe it's totally untreatable, it's just a very refractory form of rejection that we don't have drugs. The drugs and stuff we're using now is woefully inadequate, and we need better drugs for that.

DR. ALLOWAY: I just want to make one comment before, as Mark said, we give a death sentence to the patients that have TG. I think that a lot of people here refer to nonadherence and the impact that it has on this and how it drives it. I think that we need to be just as disciplined to give the patients and identify them a precise prescription for nonadherence to try to address those issues if there is enough kidney function there to salvage. And I think that

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what we see is the patients that are nonadherent are not going to change unless we make a definitive intervention.

And as Dr. Nickerson has referred to before, and I'll steal words from him, your first shot is your best shot. And so we need to maximize that if we can.

DR. VELIDEDEOGLU: Okay. Thank you. We only have 10 minutes for the next question. And the next question is, as you see on the screen: If acute AMR and chronic AMR is a continuum, then can we predict who will or will not progress to chronic AMR? Are acute AMR and acute mixed AMR distinct entities? What is the significance of the presence of cellular rejection component in a biopsy demonstrating AMR?

Comments, please.

Dr. Stegall?

DR. STEGALL: I'll take the second one. So when we get a biopsy, a patient comes back, and they have combined cellular and humoral rejection, so this is like the clinical approach to this. You probably treat the cellular component first, right? That's what you do. And then you also sort of make a judgment,

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what's the primary process that's -- maybe the creatinine is elevated, what's the primary thing that's driving the creatinine elevation?

If they end up with borderline cellular rejection on the biopsy, I really can't believe that that's -- and if they end up with transplant glomerulopathy is the next biopsy they get, I think that most of these patients will end up, if they get back on immunosuppression for whatever reason, maybe you decreased it because they had polyoma, they're not all nonadherent, that the primary thing that they're left with is an antibody-mediated rejection that's smoldering, it's not this acute rise. I think the nomenclature is confusing us more than actually the biological process.

So I think that it's significant obviously to have cellular rejection. And I think that if there's an association with cellular rejection and bad outcome, there's no question. What's driving the bad outcome is not known. It could just be associated with people who just didn't take any immunosuppression and that are going to end up with higher levels of antibody.

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So I don't think that you -- the cellular component of this, there are a lot of T cells in these grafts, but I'm not sure that it's garden variety T-cell rejection, and I'm not sure that it's driving the chronic process as much as people would say.

I do think that they all look like the same at the end. They all look like they get transplant glomerulopathy. And I think it is a continuum, the acute and chronic is a continuum. And I do think that hopefully the way out of this is to treat as much of the cellular rejection as possible, but you're still left with people who have peritubular capillaritis and develop CG and lose their graft to antibody over time. That's what I think.

So probably in my mind this leans more to an antibody-mediated process than not.

DR. VELIDEDEOGLU: Thank you.

Any other comments?

Dr. Haas?

DR. HAAS: I mean, from a pathology standpoint, and I'm a pathologist, the diagnosis of mixed rejection is difficult for a number of reasons.

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First, the biopsy is just a single time point, and if you have a cell-mediated component, T-cell-mediated component, and you have an antibody-mediated component, we can't tell from the biopsy which was there first and which was there second.

So we don't necessarily know if the -- although there is clearly data out there from the Manitoba group and others that cell-mediated rejection is a risk for later development of de novo DSAs, we don't know in each case if the cell-mediated rejection preceded the antibody, if the antibody preceded the cell-mediated component, or if they occurred at the same time. We don't know that. Again, we have a hard --

The other thing is that peritubular capillaritis is very hard, if not impossible, to diagnose in the context of T-cell-mediated rejection because the cells have to get there somehow into the interstitium and into the tubules, and the way they get there is through the peritubular capillaries. So peritubular capillaritis, which is an important diagnostic tool for antibody-mediated rejection is

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pretty useless in terms of cell-mediated rejection. And with late rejections, these are not infrequently C4d-negative.

So it's a diagnostic conundrum. The whole borderline category is a diagnostic conundrum. Is this really rejection? And how can we tell the borderlines that really are rejection from the borderlines that are due to another lesion?

And, again, I think we really need to go beyond pure histology here to really understand the true implication of mixed rejections. Some kind of molecular diagnostic biomarker studies are ultimately going to be necessary before we really understand that.

And then there is, finally, as Ros Mannon pointed out, the whole issue of interstitial inflammation in areas of fibrosis. Banff does not grade this as cell-mediated rejection. There's a move toward putting i-IFTA as sort of a chronic or chronic active cell-mediated rejection, but that is sort of purely a consensus at this point.

We know it's bad, and we know it occurs, and so we can call it rejection, but is this really related

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to the acute T-cell-mediated rejection that we see? Again, we don't know, and we need to go beyond histology. Histology is good as far as we can tell, but it's not the be all to end all.

DR. VELIDEDEOGLU: Dr. Woodle?

DR. WOODLE: Mark, we were bothered by this a long time ago, and so one of the things we did early on when we were looking at mixed rejections, and this was several years ago, is we asked a simple question. Forget the Banff criteria for AMR. If you just look at the predictive power of the single-antigen bead assay denoting a DSA, that alone discriminates almost as effectively as using Banff criteria.

And I don't want to make you and Bob feel that you're not necessary, but in our program, if you have a cellular rejection and you meet Banff criteria and you have a DSA and you have mixed rejection, you get treated as such.

DR. COLVIN: Could I respond? I think there is no doubt that pathologists undercall T-cell-mediated rejection in the late biopsies. Our criteria are not very good, and hopefully they will improve. The

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molecular tests pick this up very easily, and I've been impressed with how striking the T-cell signal is in some of these. And, of course, you can do it with an immunohistochemical stain for T cells as well. So I think we're going to learn a lot more about the component of the cellular aspect. And you have to, of course, remember that B cells don't make antibodies on their own, they need the T cells, and there is some evidence that local production of the antibody is occurring in the graft in some settings with helper cells, et cetera. So that's one issue.

I want to just mention, I just want to mention one other thing in the first part of this question. If acute AMR and chronic AMR is a continuum -- well, I don't think it necessarily is a continuum. We've heard that acute AMR usually responds to therapy, and chronic AMR is not usually preceded by acute AMR.

I think the salient question is when a patient comes in at year 1 or 2 and has antibodies in the circulation, how do you decide what to do with that patient? You know that patient is at risk for getting chronic antibody-mediated rejection, but how do you

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measure that risk? And you've done a DSA, of course, to get that far, but do you need to do a biopsy at that point? And how can the biopsy guide your therapy? I think that's the question, how you monitor these patients after they've been transplanted and appear with a DSA.

DR. GASTON: Bob, I was going to change it to the last question in addressing that, and that is, to me, I think all of these questions have to be addressed in the context of the immunosuppressants that we have patients on and the mechanisms by which they block alloresponses. And so I think it's very possible in a patient to use adequate dosing of the immuno-suppressants we have available to us and have them still develop antibody and AMR.

I think, however, the presence of cellular rejection always means inadequate immunosuppression, whether it's patient induced or whether it's physician induced. And so I think that's the significance of the cellular piece in it, is inadequacy of immuno-suppression.

DR. VELIDEDEOGLU: Dr. Haas.

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DR. HAAS: Okay. Well, three responses. One to Dr. Gaston. I agree 100 percent that the cell-mediated component is indicative of under-immunosuppression. And there was actually a good deal of work presented at the most recent Banff meeting, that whether i-IFTA is truly chronic active cell-mediated rejection or not, it seems to be a marker for inadequate immunosuppression, and whether by the time we detect it, it's too late to correct that or not, we really need clinical trials to determine the response of i-IFTA to correcting immunosuppression, but it clearly is associated with under-immunosuppression.

The second point, raised by Bob Colvin and others in terms of the continuum between acute and chronic antibody-mediated rejection and whether these are not a continuum, I think points out to maybe the inadequacy of the current Banff classification, which only has two forms of antibody-mediated rejection.

There's an acute form and there's a chronic form. And maybe acute and chronic may not be a continuum in all cases, but that maybe the active smoldering form, which clinically is not acute, but in

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the Banff classification is graded as acute, may be on a continuum with the chronic form.

So some acute is on a continuum with chronic, and that's the smoldering form, but other acute, which is the rebound kind of effect that's more easily treated, may not be a continuum with chronic antibody-mediated rejection.

DR. VELIDEDEOGLU: Just one question. As far as I've seen in the publications, one of the overlapping areas between antibody-mediated rejection and cellular rejection I believe might endarteritis or intimal arteritis.

DR. HAAS: And this I think also points out some of the limitations of histology because if you look at endarteritis, especially isolated endarteritis, where you see endarteritis with little or no tubulitis, this has traditionally in the Banff been called cell-mediated rejection, although there is data that has come out from the Paris group that has found that some of these cases appear to be associated with antibody, and the combination of antibody and endarteritis tends to have a worse prognosis than endarteritis alone.

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But where I think we're really dealing is that if we look at endarteritis from a molecular standpoint, and there was a very nice paper that was published I believe in the JASN from the Halloran group just a year or two ago where they looked at their TCMR classifier, molecular TCMR classifier, and molecular antibody-mediated rejection classifier in lesions with endarteritis, particularly isolated endarteritis, they found that some of their lesions were strong in the TCMR classifier and some were stronger in the antibody-mediated rejection classifier, yet histologically these lesions look the same. And the ones that were stronger in the T-cell-mediated classifier tended to be more the early isolated endarteritis, whereas later isolated endarteritis was almost always antibody-mediated rejection.

So, again, you can have the same lesion or virtually the same lesion histologically be predominantly a T-cell-mediated rejection or an antibody-mediated rejection or both, depending on the time posttransplantation that this occurs. So, again, we need to take into account more than histology, but

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also the clinical course of the patient. And hopefully the molecular classifiers will be coming online, too, because these clearly, as I'll show in my talk tomorrow, seem to add to the predictive ability of histology to predict the patient's clinical course.

DR. VELIDEDEOGLU: Thank you for all the comments. Now we have to stop here in the interest of time. And we have actually now it's down to 12 minutes. We have a 12-minute break. And we will try to reconvene sharp at 10:40 if possible, please.

DR. ALBRECHT: As people go to their break, I would like to mention to the invited speakers, including our patient representatives, we do have a speaker-ready break room for you. It's room 9224, 9225, it's outside the door to my right here. This is for the invited speakers and patient representatives.

Hi. I would just like to ask the invited speakers and patients to listen for a second. We've been told by the conference center that for the invited speakers and patient representatives, they do have an offer for a boxed lunch, four choices, smoked turkey, Caesar salad, chicken Caesar salad, sliced roast

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sirloin, or veggie, and these boxed lunches are available for $20. And if you're interested, please order them in front of the room, there's a gentleman named Devon (ph) who is sitting at a table and can take your orders. Again, this is for our invited speakers and patient representatives. People can go now.

(Break.)

Part II

DR. VELIDEDEOGLU: The time is 10:50 now, and we are starting the Part II of Session 1. And the first two talks are going to be given by Dr. Mark Stegall, from Mayo Clinic. And I believe he combined two topics into one talk. In brief, it's about the utility of protocol biopsies in the follow-up of acute AMR and tailored immunosuppression based on routine DSA monitoring.

The Utility of Protocol Biopsies in the Follow-up of Acute AMR and in the Detection of Chronic AMR

DR. STEGALL: I want to say thank you to the FDA for having this workshop. And I'm also thanking them to give my talks today. I'm not going to be here

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tomorrow, I know it's breaking everybody's heart, but actually my wife is making me go to England. Her goddaughter is getting married, and we tried to move the wedding, but we just couldn't pull it off.

(Laughter.)

DR. STEGALL: My disclosures is also the largest amount of money I've received recently is the FDA flew me to D.C. It has nice lodging. So if I say anything about the FDA, you have to take that into consideration.

The goals of the workshop have been delineated. I thought I would actually, since I'm not going to be here tomorrow, I would actually comment on this, get my comments out of the way. And the idea of nonadherence and the development of de novo DSA and antibody-mediated rejection, I would say that from the Mayo Clinic we agree, but also remember that not all patients are nonadherent who have this process.

And the nonadherent patients, I think that we've actually been able to treat their cellular rejection and get them back on immunosuppression. And in many cases, their primary problem is persistent

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ABMR, and that's kind of my comment on that.

The other goal of the workshop was to discuss new developments such as non-HLA antibody and the routine posttransplant DSA monitoring. And I think the status of this has been mentioned. A lot of our sensitized patients don't have a lot of DSA floating around, but it's very difficult to show, that we almost never find non-HLA antibodies, so we're sort of skeptical about that.

We do a lot of posttransplant DSA monitoring. And I say it would be a lot more important if there was actually effective therapy for it, but we see a lot of stuff that we can't treat, and a lot of people treat a lot of stuff because they get nervous about it. So there's that.

The other goal of the workshop was to discuss the natural course of acute ABMR continuum and its temporal association with cellular rejection and changes in GFR. And actually Mark Haas and a few people set me up because what I want to talk about is it's a major source of confusion. I think that most of it is just the terminology is poor. I think we

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understand the pathologic process better than we have.

So this is a meeting on antibody-mediated rejection, right? And it's kind of amazing, when I look back to think about this talk, a decade ago, Jim Gloor and our group, we wrote this paper on transplant glomerulopathy, and it was a big deal at the time because we were really trying to figure it out. Most people were interested in interstitial fibrosis at the time. And over here we said that originally classified as a variant of chronic allograft nephropathy of unknown etiology, TG is now recognized, yada yada yada.

Actually, we were just beginning to figure out what transplant glomerulopathy was, what the histology was, and then we figured out that there was this spectrum, that there was this peritubular capillaritis. I think Mark Haas was one of the first people to write about this, and Alexandre Loupy's group coming from Paris. And amazingly this was all done prior to DSA testing. We were really groping around in the dark about a decade ago, and I think even 3 years later when we had this meeting, we were just in that really foggy phase where we didn't have a lot of data.

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And I think what's happened is in the last decade is there's a consensus that this acute active antibody-mediated rejection, this peritubular capillaritis and glomerulitis, which, of course, is not totally specific for antibody, but if you see it in a patient who's had antibody, et cetera, that this microvascular inflammation is pretty highly correlated with whatever you want to call it terminology-wise, but it's antibody-mediated injury. And the other thing is that the demarcation between acute and chronic really is the presence of transplant glomerulopathy. It's not a clinical scenario, it's basically a biopsy finding, and that came out I think the jungle of Brazil at a Banff meeting that I did not attend, so we're still skeptical about how that actually came to occur.

So, again, this is the Banff criteria, and again the difference between acute active -- and you have to have all three features, and our patients don't tend to read this paper, so they tend to have all sorts of variants of this. So we try to give this to the patients at time of transplant so they'll come back with the right diagnosis, but they don't. And then the

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chronic active is basically transplant glomerulopathy.
But we do have a paradigm I think that is emerging in this field, and the paradigm starts like this, is that there is donor-specific antibody of some sort, and that leads to microvascular inflammation, peritubular capillaritis, and glomerulitis. Our protocol biopsies have probably shown us that that can actually precede the development of transplant glomerulopathy, but if you look closely enough, if you look electron microscopically, you can actually see ABMR ultrastructurally before you can see it by light microscopy because it's 1,000 more times sensitive. And then at some point, you get declining GFR and graft loss.
And, again, patients don't read the books. They get lots of different things in addition to this one isolated problem. They get a bad kidney or they get polyoma virus or they get a lot of other things going on. But this is the paradigm.
The other thing that's important as we talk about this at a meeting like this is sometimes we get a little sloppy in our clinical scenarios and

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terminology. And I think it's important to remind this group that there are really different clinical scenarios where you have somebody who's diagnosed with antibody-mediated rejection. There is this early acute ABMR, which a lot of people have written a lot papers around the table here. We definitely have, which is that early rise in creatinine in the first 14 days after transplant, this is almost always in presensitized patients who have high levels of DSA either going in or at some point. It's quite reversible because it tends to be due to plasmablasts that jump up the antibody levels, and sometimes the antibody levels most of the time will come back and be manageable. And, again, it's more of a pure ABMR on biopsy that you see.
This is actually kind of rare except for a few crazy programs that do desensitization. Most places will see maybe one or two of these a year. So it's hard to do a clinical trial where kidney transplant programs are seeing one or two patients a year because someone like Arjang has to be there for that weekend to get that patient enrolled.

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So much more commonly, but I think it becomes much more of a mixed bag of patients, is a couple of years to 5, 6, 7 years after transplant, somebody comes in with an elevated creatinine or for some reason they get a protocol biopsy, and that biopsy shows active ABMR, it looks a lot like early acute ABMR actually, and these patients, of course, tend to be de novo DSA patients. We also see it in our presensitized patients when they get protocol biopsies down the line. The DSA levels can be kind of all over the place.
And I say this over and over again, the fact that we're here today is the fact that there is no effective treatment for this. So you can have your ideas about how to treat it, but it's just not very commonly treated. Histology, again, it's commonly mixed ACR and ABMR. Again, nonadherence. A lot of these people show up and they haven't been taking their meds, but some of them show up and they have been taking their meds.
And the thing about this is there is a bit of a controversy about the incidents, but if it's greater than 10 percent by 5 years in tacrolimus-treated

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patients, then I think you probably should follow your patients closer because I think that this should be about that.
So you have these two different clinical scenarios. And what happens is the histology of these early acute and late active or late acute look a lot alike, and I think that that's a bit of the confusion that goes along in the terminology. And, again, the difference between late active and chronic is the presence of transplant glomerulopathy.
Okay. So that's a little bit, because I'm supposed to be talking about histology, right? And when you talk about histology, when you talk about the Banff 2013 criteria, there are these three things that are used. The first one, histologic evidence of this acute -- that's the PTCitis Gitis score. And I think that in our hands, we would say that that's very important in prognosis.
And, again, I think that the biopsies are basically a biomarker to look forward to see how the patient is going to do after they get their biopsy.
This C4d staining is actually quite variable

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especially if you do late biopsies, protocol biopsies, but it's in there. Almost all of the early ABMRs that we see in sensitized patients with C4d-positive, and it's not the case with late ones.

And then serologic evidence of donor-specific alloantibodies is actually not histology at all, it's that they would like to have something else to corroborate this. And I think that our group and the Hopkins group said back in the day, even in positive crossmatched patients, a lot of those patients will lose their antibody in the serum in a year, and I don't think that they have actually lost the antibody, it's probably in the graft.

So nothing is perfect, right? And it's not going to be. This is clinical medicine.

So microvascular inflammation has the highest correlation with graft loss. We use this combination of graft loss, or 50 percent decline in eGFR in the following 2 to 5 years. So that's something. It's not perfect, but it's something.

If you look at DSA development, DSA, by the currently FDA-approved assay, has a relatively lower

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correlation with outcome. In fact, not all people with DSA have inflammation. Non-HLA antibody possibly is out there, but is it possibly just the case where you can no longer detect a lot of serum DSA?

And then there are these other biopsy issues that I talked about, which C4d has a high correlation with outcome, Bob Gaston showed that, but it also misses a lot of patients. So if you're looking for a biomarker, you don't want to be missing a lot of patients that will progress.

And I agree that all DSA is a product of T-cell-dependent immune response, but we really may not detect ACR on biopsy in a lot of these patients. T cells, homes to sites of inflammation in ABMR. And I do throw it out there for discussion, that if you really have borderline or a small amount of T-cell-mediated rejection by itself, that generally has a pretty poor -- pretty good prognosis actually, and compared to ABMR, which has a very dismal prognosis.

So it's really a matter when you're a clinician, is, what are you going to treat? And I think that if we were designing therapy, I would try to

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get some therapy that would be treatable for ABMR.

So the talk I was supposed to give is, "The Utility of Protocol Biopsies in the Follow-up of Acute AMR and Detection of Chronic ABMR." And I think I may be on time. So the question came up, Does early acute lead to late chronic? And we get papers back reviewed that everybody knows that it does. And we thought it might. But the question really is, is it just an association? Are the same people who get early acute the same people who get late chronic, but it's not causal?

And we did a little study with eculizumab a few years ago, and then we did a follow-up paper, and we basically can summarize it saying preventing early acute clinical ABMR does not prevent chronic ABMR in patients with preexisting DSA. This is in AJT. Lynn Cornell put a bunch of this together.

And just to run through it quick, we had eculizumab, 30 patients, control group, it was a historical control group. They had a fair amount of antibody. Their total antibody at the time before we started pheresis was about 10,000 MFI. So I think

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these patients were truly sensitized.

They got eculizumab for the first month, all of them. If the B flow crossmatch was less than 200, we stopped it, and if it was greater than 200, we continued it because at the time we didn't know anything about what we should be doing, we just wanted to get them off this really expensive drug and also possibly not have the risk of infection.

So it turns out that when we did this, we had biopsy-proven acute clinical rejections, in the paper, which is an increase in creatinine over .3, so this is clinical, we call this clinical. You had to have a biopsy. The first 3 months, the control group had 40 percent rejection, and eculizumab had 6.7 percent rejection.

And there have been other studies that do, but the problem is they didn't have a high enough rejection rate in the control group to show a difference, but I think those of us who have used eculizumab are fairly impressed with what it can do in these patients. And, again, eculizumab was given for a minimum of 1 month and continued if the antibody levels were persistent.

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And what happened was we prevented antibody-mediated rejection, which was actually a good thing. These patients became much easier to take of. Long-term graft survival was not changed in these patients. And as we looked at it, the problem is, is that we didn't prevent this smoldering antibody-mediated rejection, if you want to call it that.

And so if you look at the peritubular capillaritis, moderate to severe in these patients, control is in that sort of crimson color, and the blue is the eculizumab patients. And so they still have this smoldering -- they never had that early acute rejection, but they do have the smoldering.

Transplant glomerulopathy was actually higher in the control group, but it was not prevented in the eculizumab group. So you can avoid this early catastrophic event and still get long-term injury.

And the C4d is another thing. I throw this in here. Why we're not in love with C4d is because, again, it seemed to be a lot lower than the other histologic lesions.

So this is Figure 7 of this paper that Lynn

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Cornell wrote, is a great figure in my mind that I like, and nobody else likes, but it's pretty common nobody likes my ideas. So what it was is we looked at the control patients, and if you had at 6 months a B flow crossmatch that was less than 200 -- let's see if I can show this.

So let's look at this. So you had a B flow crossmatch in the control groups less than 200. A fair number of these patients got transplant glomerulopathy by 1 year. So they had low levels of antibody at 6 months. They should have done well, right? But they went ahead and got transplant glomerulopathy.

When you looked at the eculizumab-treated patients, you still got transplant glomerulopathy in the people at high levels of DSA because these patients are pretty wound up and are going to have transplant glomerulopathy eventually. But even if you got a short course of this, you actually didn't get transplant glomerulopathy, which was counterintuitive because we thought those patients maybe should have never gotten eculizumab, they never had rejection, and therefore they didn't the drug. But it could be that the low

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levels of DSA needed complement activation in order to cause the inflammation and the graft damage to get transplant glomerulopathy, where if you had high amounts of antibody, it didn't matter whether you had complement blockage or you had complement, the kidney was going to get damaged.

So I think that lessons learned from eculizumab -- and it was asked from me, "What can you learn from protocol biopsies?" And I think that you can learn a lot from protocol biopsies. I think that you can learn that preventing early clinical ABMR does not prevent chronic ABMR. And, again, these are subclinical cases almost all the time.

You learn that complement blockade may prevent injury in patients with low levels of DSA, but high levels of DSA patients are not as complement-dependent. So I think that you can learn a lot. I think that give you some sort of signpost of where to go with some of this research in small numbers of patients, because you're never going to get a big prospective randomized trial to teach you all of this, at least not at the beginning.

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And I think that, more importantly, protocol biopsies help us delineate progression of chronic injury in many different facets of transplant. They can actually provide some indication of who to treat. And now it's a question about, Do we have the drugs to treat patients?

So the other question is, Discuss the natural course of acute/chronic AMR continuum and its temporal association with cellular rejection and changes in GFR. And I think that there is an emerging paradigm, a different group of patients, not this early, on the left side of that graph, but the ones on the right side of that figure that I made, that late after transplant, many patients present with a combination of ACR and ABMR on biopsy, and this is a real clinical entity. And ACR may be the primary cause of the acute rise in creatinine in these patients. Also, they just could be dehydrated or their Prograf level got high, and you call that a biopsy for cause; half the time that's it.

But I think what's happened is if at some point ABMR becomes the primary cause of late graft loss in this setting, in a nonadherent patient, they're

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having an acute cellular immune response against the graft, which you actually might be able to treat, but what you're left with at the end of the day is a patient who has persistently high DSA, just like you do in that positive crossmatch patient 14 or 16 days after kidney transplant, and that leads to that paradigm of PTCitis, CG, and graft loss.

So the mechanism of DSA development. I do believe it's T-cell dependent. Its nonadherence actually definitely must play a role, but I think that it may persist after the treatment or resolution of cellular responses. I think Matt Everly has a paper that he did from East Carolina that the biggest risk factor in that patient population was polyoma virus, and that was the reduction of immunosuppression to treat polyoma.

So there are multiple pathways to having the immune system become activated. I think we've just become so accustomed to being able to block cellular rejection that we forget that it actually has evolved over hundreds of millions of years.

Planned reduction immunosuppression, such as

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polyoma virus, cancer, minimization/tolerance protocols are also another way you get there.

And I think that at Mayo Clinic we actually have people who come back for their appointments and take their medicines, a unique group of patients, I guess, but we'll take any of those that you would like to refer to us. And some of those people come in with antibodies to their graft. And I actually believe them that they are taking their medicines, I don't think I'm trying to undercut them. So nature finds a way.

And I think the other thing is treating acute cellular rejection does not prevent late graft loss from antibody-mediated rejection. I think we've got to figure that out.

So what you're left with, you're left with patients with DSA, and other problems are taken care of. And so maybe now we can go to work. Maybe there will be a lot of other people in this room will work on adherence and a lot of other things. I'm a surgeon, I usually don't get to go to those kind of clinics, which is good. And what we end up with is this, a patient who already has DSA, and they're on their way to maybe

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getting this paradigm of chronic injury due to antibody.

So let's talk about de novo DSA. I'll go over this real quick. The incidence varies with the patient population studies, somewhere at 13 to 22 percent. I think that some of these older publications included a lot of patients on cyclosporine. They also included a lot of patients who were never tested at the time of transplant for single-antigen beads and DSA because it was a different era. So I think overall we're transplanting patients with less antibody, probably a cleaner population today.

And I think that we've gone through this all, but the last one is DSA-positive patients who do not develop ABMR on biopsy. I think more and more it's getting to be they do pretty well in the short term, but if you get a person with de novo DSA and you do a biopsy -- and the way that we handle these patients, our standard of care is to monitor antibody yearly, and if a person has antibody, a new antibody, in the circulation, we'll bring them in and do a biopsy. And if the biopsy shows this, we get nervous.

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And the paradigm here I think is that 50 percent of patients with DSA will develop ABMR within about a year of developing it. It's definitely more common with all the things that's been talked about this morning, but none of those assays are FDA approved, so we're not really doing a lot of those.

But I think the last one is that if you're DSA-positive and ABMR-negative, it doesn't mean you're never going to get in trouble, it just means in the short term you're going to do pretty well. And I think that from a clinical trials perspective, if you treat everyone with de novo DSA, you won't have an endpoint that you can really measure outcomes out there very well.

So Carrie Schinstock, who is Jim Gloor's replacement, came and has put together our de novo DSA data, and it uses protocol biopsies, which I guess is one of the reasons I'm here. And so this paper in AJT is 967 patients that are in 2007 to 2014. So the reason this era was chosen is because they're all tacrolimus patients and they all had single-antigen bead testing throughout their transplant, so they had

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it at the time of transplant. So this is kind of state of the art.

We actually only had 54 patients in a mean follow-up of 4.2 years, and they also got surveillance biopsies and included everything else. So 54 patients who had de novo DSA. So a pretty low incidence. And if you look at it, it's about, I think conservatively, about 2 percent per year developed de novo DSA today. That's still 10 percent incidence at 10 years. And I do think that, is de novo DSA lower in tacrolimus-treated patients? And also, but it also may be a function of our DSA testing. Again, low levels of DSA, people with memory, finally get up and have a response.

So it's not good to have de novo DSA, everybody knows that. Even in compliant patients, they do less well. And if you do surveillance biopsies 1 year after the detection of de novo DSA, 50 percent of those patients had acute active ABMR and a normal creatinine, but what happened is 37 had already, Bob, had cABMR, had some transplant glomerulopathy. Usually it's pretty mild at this stage, but it nevertheless does exist.

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And I think that this is the slide -- this is the figure from the table. I would say that every paper has one table that's the entire paper, and this is that from this paper, and it tells you basically is 2-year outcomes after de novo DSA detection. So this is possibly a timeframe when we could do a clinical trial, is you could detect de novo DSA and follow the patients for a couple years. And 34.5 percent of the patients who had de novo DSA who actually had antibody-mediated rejection on their biopsy at either the time of detection or within a year, 34.5 percent of those had graft failure or 50 percent decline in GFR by 2 years. So now you're getting to numbers that might actually have an endpoint that you could follow. Now, not everybody had graft loss. Only 20 percent had graft loss.

Over here, there were no graft losses in the people who had de novo DSA and no AMR, but actually some of them probably did develop something over time because 18 percent of those had a decline in GFR over time. The people with no DSA, there are still people who have a decline in GFR over a 2-year period from

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other etiologies.

So I'm sort of saying that we're going to catch most of the patients that are going to do poorly if we do de novo DSA testing and then do a biopsy once they have de novo DSA. Make sense? So this is kind of getting toward, "How do we get there?"

Tailored Immunosuppression Based on Routine DSA Monitoring (both in sensitized and nonsensitized patients)

DR. STEGALL: So then people ask -- the next talk I was supposed to give is "Tailored Immunosuppression Based on Routine DSA monitoring." And the answer is nothing works. So the treatment of ABMR, again, no proven therapy exists. Why else would we be here? If there was therapy, we would be here for some other reason.

So what we do at Mayo Clinic primarily is we optimize tacrolimus and MMF. We only use IVIG or plasma exchange if there is acute graft dysfunction. And some might treat it if it occurs early after transplant or if the biopsy shows a little chronic injury.

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I think the desire to treat in this area is because that you know the kidney is not going to do well long term, and yet there really isn't a lot that's that effective. I think we all kind of know that plasma exchange, IVIG, and all the rest is probably not doing a lot to these grafts. So, again, did I say no proven effective therapy? There's no proven effective therapy.

And there's more than one study. And people kind of have a nickel's worth of effects sometimes in this area. Really, patients don't need a nickel's worth of effect, right, guys? You need your kidney to work a lot longer than that. You need it to kind of go away. And so we need better therapy.

So the goals of the workshop were to discuss unmet medical needs and trial design. So I have 5 minutes and 22 seconds to talk about this, about what a clinical trial would look like. And I think the problem in this, again, gets back to this thorny issue about these patients are a mixed bag, and which patients aren't. Fifty percent is caused by nonadherence, some have these other problems. You have

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this.

So how do you get patients in a study? And we suggest that you have to get all those other problems cleared up before you can start the study, but that's okay, I think we can do a lot to get patients to the point where we can look at them.

And I think a conservative estimate, many studies in transplant overestimate the patient, the incidence of the problem, and then they end up with no enrollment, which is not good. And so we think 2 percent per year is probably good, 10 percent incidence at 5 years.

And I'm asking the FDA at some point if they would help us out with this combined endpoint of graft loss and a 50 percent decline in eGFR as our clinical endpoint for this study.

So surrogate endpoints, I'm advocating the histologic changes of cABMR are a good surrogate marker -- or ABMR, excuse me -- for allograft loss because they precede allograft loss by years. And they're pretty specific. Obviously there are other things that go on. And, alternatively, we could just use DSA

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alone. And I think that what we're trying to do ultimately is prevent graft loss decline in GFR.

And so chronic irreversible changes that need to be considered, this is another thing. If a biopsy has a lot of chronic changes, at Mayo, we're a lot less likely to treat. I wouldn't put those patients in clinical trials. Retransplant is probably a better option.

And I think that Peter and his group have looked at this before. And the 5-year timeframe for DSA is probably pretty real. Eventually these patients with de novo DSA will lose their grafts, but 5 years is a long time for follow-up. So I think that we can probably use other clinical endpoints.

I would talk about the surrogate being resolution of DSA versus resolution of antibody-mediated rejection on biopsy. If you use DSA's inclusion criteria, you're going to have to pick some MFI that's reasonable, yes/no. If you use 1,000, and then you have 6 months, treat, and recheck the DSA, and then the endpoint would have to be, as a surrogate endpoint, the resolution of the DSA, right? I don't

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think we're going to be talking about in the next couple of years that it's going to be a 50 percent reduction in MFI. That's not validated.

So the incidence of graft loss with MFI at 1,000 at 2 years is 18 percent. That's not a really huge endpoint. And C1q might be better, but, again, it's not yet approved. So if you look at the numbers for a DSA trial, if you look even for 50 percent of the patients who have a complete resolution of their DSA, just to study one drug, you would need 116 patients to just study one drug for the surrogate endpoint, and probably hundreds of patients to study the 2-year endpoint, and that's not feasible, we're not going to find those patients.

The other study that might get done -- so the other thing I say, too, is DSA can resolve without treatment, it kind of goes away in these patients, and the rate of graft loss is low. So intervention trial number 2 that I would like to talk about is patients with de novo DSA that get a biopsy, following a little bit what Carrie Schinstock showed, and if they have antibody-mediated rejection on the biopsy, would go in

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the trial; if not, you just follow and rebiopsy. And then the numbers start looking a lot better.

And, again, I think that the power calculations for these, I would like to suggest that we might use complete resolution of antibody-mediated rejection or complete histologic response as the endpoint. If you look at that, you only need 28 patients to show efficacy with one drug and a clinical study showing a clinical endpoint, you would have 90 percent chance showing a Phase 3 clinical trial with just 128 patients in each group. So, again, these numbers are a little more feasible.

And I was going to go through adaptive trial design, but I think that's the next phase that we should be looking at in this area where we basically have parameters for changing the trial. Small numbers of patients. And I think again we showed that as few as eight patients can be used to decide if a therapy is ineffective.

And another thing is it enhances the efficacy that a single ongoing control group, where you can have multiple experimental groups, and therefore the vast

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majority of patients can be assigned to an experimental group.

And minimizes the number of patients receiving ineffective treatments and limits unnecessary treatment risks. I think the FDA has gone on record saying they like that aspect of it.

And it's cheaper. Drug companies don't have to have people coming all the time wanting to do a Phase 3 prospective randomized clinical trial in this area, and I think that that's not the way to go.

And I'm frozen. There you go.

So remember this, you have 14 patients in each arm. You actually can do a study where you have one control arm and three treatment arms, and if they're all ineffective, you only have to have 32 patients. If they're all ineffective and you want to use combination therapy, if one of the combos is working, you can actually do the whole study with 74 patients, the whole Phase 2 clinical trial with 74 patients. So we're kind of thinking that that might be a smart way to do these kind of studies.

So in summarizing, there are different

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clinical scenarios that antibody-mediated rejection occurs. There is this early acute, late acute active, and I think that we have to keep those in mind when we're talking terminology.

I would also say that the first ones are really hard to enroll. And we can talk about those in a lot of different studies, but if you're looking at all comers, that's going to be a hard study to do. I think that the chronic injury is a much more pressing need for transplant patients overall in this with respect to ABMR.

This paradigm actually I think has some merit, and I think the protocol biopsies are showing that that is something that has emerged from those papers from 2007 to get to this point. I think that Jim Gloor and I would have argued about this paradigm a lot back in those days, but I think it really is emerging.

I think the biopsies are very important in this field. And people talk a lot about genomics, and we do genomics, but I think biopsies are really important because you can rule out other causes of graft injury. We have a lot of interest in other

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causes of graft injury.

You can assess the amount of chronic injury, whether or not it's really worth putting this patient, who's had polyoma and everything else, through another round of therapy because maybe that's not the way to go.

And in my mind, it's just a biomarker. That's what a biopsy does. And it might assess response to treatment, which I think is unknown, but it's something that we need to try to approach.

So I think, most importantly, is if your biopsy is normal, your chance of graft loss is low. So we probably shouldn't be treating a lot of people with normal biopsies or near normal biopsies. I don't think you're going to have a very good endpoint for clinical trials, and you probably aren't going to do a lot for the patient.

So our conclusion is developing therapy for antibody-mediated rejection is a major unmet need in kidney transplantation. Validated surrogate markers are needed. I think histology is a very good one. I think clinical trials are feasible. And it's best to

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employ adaptive trial design.

Thank you.

(Applause.)

DR. VELIDEDEOGLU: Our next speaker is Howard Gebel, from Emory University. And the title of his talk is, "Scientific Aspects: A General Overview of the Currently Used Antibody Measurement Methods, Issues of Standardization, Validation."

Scientific Aspects: A General Overview of the Currently Used Antibody Measurement Methods, Issues of Standardization, Validation

DR. GEBEL: Well, thank you to the organizers for the opportunity to present here. I have no financial relationships related to this presentation.

It's been about 50 years since Paul Terasaki published his seminal paper that showed an overwhelming association between positive crossmatches and hyperacute allograft rejection. For the next 30 to 40 years, the assay that was used to detect those antibodies was shown in this slide. And it's a cytotoxicity crossmatch.

I'm showing you this slide specifically

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because half the cells are alive and half the cells are dead. This is what we had to deal with. And what does that mean? You can use the right kidney, not the left kidney? Or that half the cells fix complement and the other half don't? Of course, the answer is no. We were just obligated to use this test because nothing better existed.

And we had numerous problems that we knew right from the beginning. The sensitivity wasn't optimal. We had false negative results. Specificity was also not optimal with false positives. We got to compose our own panels. Cells needed to be viable till the end of the study. And typically we were restricted to identifying Class I antibodies. And as we've heard throughout this morning, Class II antibodies are very relevant.

So over the ensuing time, there has been a dramatic evolution of the types of tests that became available. And on the upper right side, the test that has become the most used one today is a solid phase assay that takes microparticles and coats them with HLA antigens. And in this situation, we don't have to

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worry about other cell membrane-bound antigens that are attached to a cell. We're looking exclusively at HLA targets.

And here are two types of assays that are utilized. On the left side is an assay that's a screening assay, and it's typically simply a yes or a no. There's a little extra data in there. That bell-shaped curve on the left side in purple indicates what a negative reaction looks like. There are no antibodies whatsoever. To the right of that, in pink, are reactions from a patient who had antibodies, and in this case, we say they reacted with 99 percent of the beads. It tells us that there is a positive reaction; it doesn't tell us what's positive. That's on the right side of the slide.

And here we're looking at a suspension array. And as you look along the X axis, each one of those numbers refers to a different bead, and each bead is coated with a different HLA antigen. And as you go from bottom to top, you are going up a scale that's numerical from zero to about 25,000. And in general, the stronger the antibody, the stronger, we think, is

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the role of that antibody in a clinical situation.

Now, what we thought is we could begin using these assays to identify the pathogenicity of these antibodies. And, in fact, we've seen this slide multiple times already from Mark Stegall. And on the right side, we see that we had some ability, we thought, to quantify what the antibodies where, and that once we got even over no DSA at all, between 5,000 to 10,000 and greater than 10,000, there was a likelihood of antibody-mediated rejection. And one began to think that we could compare these assays from laboratory to laboratory. But it's not as simple as we thought.

So here is some data from a publication that Elaine Reed led a couple of years ago. There are numerous people who are on this publication. It involved seven different HLA laboratories. And what I want to show you highlighted in blue is what we looked like before we attempted any standardization. We all tested, as it turned out, sera that we each had in our possession, the same exact sera, and we were all asked to just perform the assay. And what you see is while

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we identified the same HLA antibodies in every case, the coefficient of variation was 62 percent, and that's not particularly impressive.

There are a lot of reasons why laboratories don't get identical results when testing the same samples. We use different vendors for the source of the beads. The antigen source on the beads is different, it could be native or recombinant. The expression of the antigen can differ from bead to bead, whether it's confirmationally correct, how much is there. I'm going to be talking about this in a while, interfering factors that can bother us in terms of interpreting a result. The reagents that we use aren't standardized. There is certainly tech-to-tech variation that will impact the outcome of the result. And, finally, the protocols that are used from laboratory to laboratory aren't truly standardized. And so the assay conditions, even things such as ambient temperature, can begin to affect our outcomes.

So how did we do, once we did standardize everything that we could, we used the same reagents, we used the same protocol, the same technologists did the

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assay in each of these seven different laboratories -- on the left side and right side are looking at single-antigen beads Class I and Class II with the standardized protocol. And you see under those circumstances that we got to a point where our CV was 20 percent; still not particularly impressive, it wouldn't pass a chemistry test, but much, much better than we had.

Now, the fact is we could do this, we can standardize, but we don't. Right now, each laboratory is still using their own protocols, and we're not using standardized reagents across the board.

Now, one of the things that we have to consider is the other things that can impact our test results, and in particular, so-called interfering factors. And in its simplest form, interfering factors are going to interfere with our ability to detect antibodies.

Here is one example of an interfering factor. So shown on this slide is we have a matrix that's going to be your bead, and we have antigen on the bead, and in yellow is the patient's antibody binding to the

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bead. And here the interfering factor is complement.

Complement fixes to the bead. Complement and the molecules that are deposited are big and they block the ability of that blue antibody to bind to the immunoglobulin that's attached to the bead. We can't see it, and it looks like there is no antibody there whatsoever.

If we do something to remove the complement, break up the complex so that instead of it binding to the bead itself, the red, green, and blue molecules are now dissipated, the antibody has the ability to bind to the bead. And in these circumstances, we've eliminated the interfering factor.

Here is just an example of how these interfering factors can present. And if you look in the middle of this complex slide, you see that there are lines that have gone from low to high, and what that means is as those interfering factors were removed, the ability to detect the antibodies are present. So something that had zero MFI, once it was treated to remove the interfering factor, came up to 20,000. So you totally missed an antibody if you

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didn't get rid of the interfering factor.

These are examples of the different things that can be used to remove interfering factors, such as EDTA, which will chelate the calcium, a necessary component of complement activation. We can heat and activate the complement. You can add dithiothreitol. You can heat and activate it, as I said. There are a number of different ways. There is no standardization and no mandate to do this from laboratory to laboratory.

So we've seen this slide multiple times, too. It's interesting that complement can block the ability to detect antibody, and at the same time, it has been reported that complement-fixing antibodies are the ones that we have to worry about the most.

So here we are just looking, comparing DSA to no DSA, and when you look at the DSA that fixes complement versus the DSA that doesn't fix complement, there's a huge difference. And so the interpretation is that it's due to the complement-fixing ability of these antibodies to cause the rejection.

If you look at little deeper into the

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supplement of this paper, there is some data I want to point out. And, in particular, the red line and the blue line indicate individuals who lost their graft, and the brown line and the yellow line are patients who did not lose their graft.

When you take a look more carefully, you see of the ones that did not lose their graft, 90 percent of the patients were made up of individuals who had MFI values of less than 6,000. And when you look at the ones who did lose their graft, 70 percent were greater than 6,000. And as I'll be showing you in a minute, greater than 6,000 could mean greater than 6 million. So it's not necessarily the complement-fixing ability, it's the level of MFI values.

Here is some data to back that up. This was done by Tom Ellis at the University of Wisconsin. And on the left side, we find out what happens when you take complement-fixing antibodies and dilute them. If you dilute them to a lower MFI value, they no longer fix complement. Alternatively, on the right side, if you take those same antibodies that did not fix complement and concentrate them, you can find that you

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can elevate their MFI values. Once you do that, they fix complement.

Other things that are appearing in the literature are focusing on subclasses of immunoglobulin. And here there is a recent study that came out of Carmen Lefaucheur's center where they were breaking down the different subclasses of immunoglobulin and associated them with either no ABMR, acute ABMR, or subclinical ABMR. And on the right side, you're seeing the subclasses that are associated with the rejection or lack of it.

However, when you go a little bit deeper into what these slides are actually showing, these overlapping Venn diagrams will show you unequivocally that the likelihood of finding any subclass by itself is pretty remote. Everything is contaminated for the most part with other subclasses.

And another important component of this is when you look at these data, there were a total of 125 patients that were studied. Twenty-one of them did not have a positive subclass at all, they couldn't find one. Now, there are only four subclasses of

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immunoglobulin, so this is something that did not work. And so my question is, What are these? You have several assay concerns under these conditions: that the reagents that you're using aren't necessarily appropriate, that they're not sensitive enough. And from personal experience, I can tell you that these reagents are very, very cross-reactive. And while I believe there might be something here, I think it's premature to use this in a fashion that we can rely on 100 percent of the time.

Recently, I published a personal viewpoint on, "The Road to HLA Antibody Evaluation: Do Not Rely on MFI." And what you need to remember is that beads were never meant to be quantitative, they weren't approved to be quantitative. Semi-quantitative, yes; 3,000 is less than 4,000. But it is not quantitative because what you have is an MFI value that reflects a given bead's fluorescence, but it's not compared to a standard.

So here's an example of a reaction where less than 1,000 is not considered a positive response. And I and some of my HLA colleagues can look at these and

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say even though all these antibodies were less 1,000, there's something about them that makes us believe there's a real antibody. Using the terminology that everybody else is using, they share an epitope that is common.

If we look at another assay, even though it wasn't positive by this criteria, this is our screening assay, under these conditions, the screening assay was clearly positive, and using another flow-based assay, flow cytometric-based assay, everything over the vertical line is positive, you see several beads that do show up as being positive. They're all part of that reactive group, they're all part of the group that expresses an antibody I'm concerned about.

What we think is happening when you have a limited amount of antibody and a lot of target, like you see on the right side, these are the single-antigen beads, you are not looking at one bead, you're looking at hundreds of beads at the same time, you're diluting that antibody over a large surface area, and you don't get a strong enough signal on any one bead. On the left side is a positive reaction just using the

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screening beads, where is less target.

Finally, what about the MFI value itself? Here, again from Tom Ellis, what we see is MFI values that are virtually identical, about 14,000 in each case. But we see when we begin to dilute them, there's a big difference between the two subjects. The one on top winds up staying at 14,000 no matter how much we dilute it. The one on the bottom serially goes away. Dr. Tambur I'm sure is going to be going over this in much more detail in her presentation.

So I believe that we have a test that is better than anything we've had in the past, but it's not necessarily at the point where it's perfect. I do believe that we can use this information, but we have to know what its limitations are. We have a long way to still go to make it more standardized. And we all know the beginning of the Charles Dickens novel, A Tale of Two Cities, the best of times, the worst of times. Well, it was the best of tests, it was the worst of tests. And my apologizes to Dickens, I'll stop there.

Thanks.

(Applause.)

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DR. VELIDEDEOGLU: Our next speaker is Anat Tambur, from Northwestern University. And the title is, "Consideration of Quantitative Use of HLA Antibody Assays and a Summary of the 2017 AST/ASHI Antibodies in Transplantation Consensus Conference."

Consideration of Quantitative Use of HLA Antibody Assays and a Summary of the 2017 AST/ASHI Antibodies in Transplantation Consensus Conference

DR. ROITBERG-TAMBUR: Thank you. And being Howie's student, I think it is very appropriate that I am going to be speaking after him. And Howie called me a few weeks ago, and I was like, "Our talk is going to be very redundant. What do you think?" And, yeah, there will be some redundancy, but I told Howie I'm not concerned about this at all because I think there are some points that we really need to make sure that the message goes across to the clinician and how they are using this.

So I hope there will be some new things here and that I can strengthen some of the message.

These are my disclosures here.

And my topic was specifically to talk about

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trying to quantify the assays. And I was trying to think about, when will be the times that we need to quantify the assay? And I know a lot of centers, all they want to know is, "Do I have an antibody or not? Can I go ahead with this transplant?" But I think there are a lot of times that we do need to quantify the antibodies.

So part of it -- and I apologize, it doesn't project very well -- is really for the pretransplant testing period. And this is something that we might want to use immediately to make a decision whether we go into transplantation.

But I think when we are talking about measuring antibodies, it's really important to try to get a sense of how much antibody we have, because it's not a yea or a nay, or a black and white, we're talking about a very significant gray scale, is to look at a patient and say, "Are we going to be successful in treating those antibodies?" where we're talking about desensitization and then how to monitor their response to desensitization. What are we talking about? A diagnostic aid for biopsy or a clinical presentation

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posttransplantation when we're thinking about, do we have an antibody-mediated rejection or not? And when we're treating it, to monitor those responses.

And a lot of people are talking about the strength of antibody as a predictor for long-term outcome. And let me tell you, coming from Chicago, this is going to be even more difficult from trying to predict the weather in Chicago, not just for 5 years down the road, but sometimes also for tomorrow. Only 2 weeks ago we had a 70 percent day followed by a 50 -- sorry -- 70 degrees day followed by a 50 degrees day followed by a 30 degrees day.

The antibody, the transplant, everything surrounding it is a very active process with a lot of moving parts to it. And I think we will be amiss if we're trying to look at a snapshot and try to make predictions with this. But I do think antibodies can help us as a monitoring tool in conjunction to a lot of other things.

So what is realistic to expect from the assay? And I know Howie talked about the assay. And I want to very quickly kind of go through this. We have the

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beads, we have the patient's serum, we have the detection antibody, eventually we're getting MFI results.

And the reason that I wanted to schematically show this is because we have one detection antibody binding to one HLA-specific antibody, and the expectation as a result of this, even though this is not an assay that was released to be quantitative, is that it will be quantitative, right? We have a one-to-one relationship. So why are we not using MFI as a tool that can give us antibody strength? And I think the vast majority of us realize that this is not a reliable tool for antibody strength.

So I want to talk about, why is it not working as we would have expected? And Howie talked about reagents issues, and I'm not going to repeat this at all.

I think we have to appreciate the manufacturing issues. And I'm not trying to provide any excuses to the manufacturers, they know, they've heard from me many, many times, but what we need to appreciate is that the reagents that are going into

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those assays, those are not DNA probes that were synthesized in a laboratory. The ability to manufacture those reagents is really, really difficult. We have to have 100 different analytes for a Class I, 100 different analytes for a Class II. We want to extend the panels. And all the HLA community comes and talks about having more and more of them, but generating them is not an easy thing.

So, again, I would like to get a way better assay, but I think we need to appreciate this part as well.

On top of this, we are talking about a very small market. We have about 200 laboratories in the United States, maybe 1,000 around the world. We're talking about huge expenses. So the question is, How can we make that assay to work better for us with those limitations?

Another limitation -- and Howie touched upon this a little bit -- is this particular slide. I have a little different spin to show you about this. We're talking about assays that are performing in very, very small volumes, and they are multiplexed. And I think

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we all thought this is a wonderful thing, but like a lot of other things, this is a two-edged sword. And taking you back to your days in medical school, if you need to pick 5 microliters, and you look at the outside of your pipette, there will be a film with some liquid on it, and if it's 1 microliter compared to the 5, there you go, here's the 20 percent variability or the CV that we received in the assay despite the fact that we were trying to standardize it.

And Peter and Howie and myself and the others, in this assay, we spent probably more time trying to standardize the assay than actually executing the assay. We still couldn't get there.

And I think this is a very important thing to appreciate because if what we're trying to get is a particular MFI value, and we're going to have 20 percent variability, that there is no way to get around this. And we tried with automation. We never published that part of the study. I think many of us had tried this internally. There is just no way to get anything better than this because of the design of the assay. And if we appreciate this, I think it will help

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us to step away from MFI as a particular number on its own.

But really where I want to spend more time today are serum-specific issues that we need to be aware of.

So Howie mentioned inhibition, and I want to show a slide about this. I want to talk about issues of saturation of the assay. I want to mention the shared epitope phenomenon. I'm not going to talk much about this because I don't think we have a solution for this right now.

And most of you know that I've been using titration studies for a long time. We started using this pretty much when I arrived to Northwestern, so we're talking about at least 12, 13 years ago, and we've been using this clinically for many, many years.

And the thing is that the antibody assay, like a lot of other immunological assays, mostly agglutination assays, have been using titration studies since its creation. This is how we're referring to antibodies to blood groups, to antibodies to other antigens, to antibodies to response to vaccinations.

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We don't have an MFI to put our hats on.

But all of us have done this going through med schools. You dilute the serum and you figure out when the response stops becoming positive, and you know what is the strength of the antibody. So why not apply this to HLA antibodies? And this is what we've been doing at Northwestern.

This is one slide to talk about, inhibitory factors. And what you're seeing here are on the left, the MFI values. And you see result of one serum sample, one patient, one assay, 10 different beads. Okay?

Let me see if I can point here. On the very left, you see the responses that we get in the regular assay. This is what I call the neat assay. Okay? You run the assay following the manufacturer's recommendations, and this is what you get, and you see that that patient has some antibodies that are fairly strong and some antibodies that are actually negative.

And what we did and what we're doing when we need to get the strength of the antibody is we dilute the serum sample. Unknowingly at that time, we

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actually dilute also the inhibitory factors. So now we're removing inhibition.

And we can start seeing that some of the beads will dilute, as we expect them, right? The MFI values will go down, and you seeing doubling dilutions coming up here. Some of them will actually increase in MFI values.

And I do want to point here that there are actually two different patterns of dilutions. So this is one patient, one serum sample, one assay. Some beads will be affected by inhibition, and some will not. And I think it's an important point to make because people sometimes kind of jump over this when they look at an overall statistical data instead of looking at an individual patient.

And again, the fact that the different antibodies can be affected by the inhibition differently I think is also very important. So at the end of the day, what we see is this patient is really very highly sensitized.

We can remove inhibition by other means, not just by titrations, and Howie showed multiple ways to

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do this. In this particular study, we used EDTA. We used a C1q assay as a comparison. If we're talking about EDTA, in this particular case, the inhibition was not removed from all the beads, especially those that had the strongest inhibition there. So knowing what protocol you're using for the EDTA is important, but even when you remove some of the inhibition, it really doesn't tell you how strong the antibody is. It tells you there is an inhibition, there is something that is masking the response, but how strong that is I think is definitely not revealed by using EDTA.

When you're doing the C1q assay -- and I've kind of numbered the beads here so you can see the correlation -- you're actually getting them fairly neatly organized by the strength of the antibody, and this is mostly I think because there is a step of dilution when you're running the C1q assay, there is a step of heat inactivation.

So if you want to know as a quick and dirty thing, "Do I have a lot of antibodies, yes or no?" and you run the C1q assay, I think you're getting a good response, and that's a way to remove inhibition. And I

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will return to inhibition a little bit later. I think this is something that needs to be a must as we're moving forward, and this is something that we need to keep very close to our minds when we're interpreting the assays, that we not remove the inhibition.

And now we're saying there is no correlation between the level of the antibody and what we're seeing clinically because if you don't remove the antibody and you think you have no -- sorry, you do not remove the inhibition and you think you have no antibodies while in reality you have a lot of antibodies, I think that can change the way you interpret things.

Something else that I think goes currently underappreciated a lot is the fact that we saturate the beads. There is a limited amount of antigens that is attached to the beads. This is a paper that is now in press in Transplantation.

And what we've done here, you can see the separation to the different loci and the number of beads that we were looking at, at each and every one of those loci. And what I'm providing here is the median MFI value of each and every one of the groups of beads

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that ended up to have a particular titer. So the titers are going up here. And as you look at the low titer antibodies, you can see that there is an increase in the median MFI. This is really what we would expect.

But you reach a point where you reach a plateau, and if you take, I don't know, a value of 19,000 MFI, you can find antibodies with a titer of 512 and antibodies in a titer of all the way up to 65,000, which I don't know what else is in those patients' serum when they have so much antibodies.

And by the way, I think, Steve, going into your comment before, if you have a 50 percent reduction of antibody, you must be somewhere here in order to see it because otherwise, you are beyond saturation.

And I know Steve had seen this before, but I wanted to mention it here because really what we're talking about, the strength of the antibody, and you get to a point where you just don't see the difference in the amount of antibodies. So saying we have responses some of the times and don't have responses some of the times, and I'll show you some examples, I

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think it's really dependent more on how much antibody you have there.

So this I believe is underappreciated. We have the same data for the C1q assay going to a little bit higher titer because of the nature of the assay. And this is still coming from that same paper.

And what I've done is I took the median MFIs and I've plotted them here because a lot of my colleagues will tell me, "We can get with MFI everything that you're saying with titers. We really don't need to do this," which is true when you're talking statistically, at the median of large groups, and it's true to a certain point, and I think everything that falls beyond 10- or 15,000 MFI, statistically speaking, correlates pretty nicely to the titer. But when you go beyond that, you're losing that correlation.

So, again, for statistics, this is wonderful, but if you have a patient -- so this is the raw data from which we derived this information. And let's say we have a patient with an MFI of 15,000 on the B locus, how strong that antibody is, is really important to see

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with the MFI.

So if you want to desensitize this patient, if you're trying to treat AMR for this patient, you really don't know whether your titer falls here and your patient is likely to respond to treatment, or your titer falls here, and you can treat that patient endlessly and make him even more sick, but the level of antibodies won't go below what should be clinically significant. So I think this is really something that is adding a lot of information to the way we can treat patients.

I'm going to very briefly talk about this. Howard mentioned this before. I just use a very cartoon form to this. But this is another reason why I think we should not rely on MFI as a number.

And I want to talk about the shared epitope phenomenon. So let's say we have five beads and we have antibodies that recognize the bead. We come with our secondary antibody. And now we have an MFI of 5,000 for the blue bead. No MFIs associated with the other ones, right? Very, very simple.

What would happen if we have an antibody that

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recognize a target that is shared by all of them? And we recognize that shared thing all the way from the days of CREG, right? This is simply a CREG antibody that recognize something on all those beads. What would that do to the MFI? Right?

So it's very difficult to take an MFI as it's being spit out of the computer and just assume this is good enough to do a clinical study. It really needs to go through a more rigorous analysis. And right now we don't have a good solution for this. We can run different approaches. Howie had shown an example, which I thought was great to do this, but at least we can get a better sense to say the MFI is not indicative of the antibody strength. We cannot give you a different MFI, but we can tell you there is an antibody there that we are not seeing.

Just to share some examples, I actually showed this in the previous FDA workshop. I want to repeat this, as we've been using titers to make clinical decisions. So this is a study of patients that were undergoing desensitization using rituximab and then cycles of plasmapheresis, low-dose IVIG.

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So this is one patient, and we were monitoring all the antibodies before and after treatment, and we've seen results like this from multiple papers over time. If we use the C1q matrix to look at those results, we see a trend down at everything, but different gradations of reduction. But if we convert to a titer metrics, we see a very unified response of the antibodies. And what I compare to this is to the delta, the difference, between the pre and the post, and you can see that the delta titer is very neat, very narrow.

And this is the complete study. And again you can see that the delta titer runs within the 20 percent CV of the assay pretty much. So you can measure. You can quantify the antibodies pretty well by converting them instead of an MFI metric to a titer metric.

And let me just walk you through a patient. This is actually in collaboration with Johns Hopkins. And we are looking at three different metrics of measuring the antibodies and looking at four different serum samples with the color codes, the pretreatment, the posttreatment immediately pretransplant. This

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patient received additional treatment immediately posttransplant. And this is a 3- to 6-month follow-up of all the patients that we've been looking at.

And what I want to show you here is two groups of beads that have the same MFI. But if you look closer, this group of beads, or antibodies, responded very nicely to treatment, maybe not sufficient, but responded very nicely to treatment, versus this one that did not.

If we look at the titer metrics, it's clear from the get-go that this group of antibodies will respond to treatment, and the same goes with the C1q, those that responded versus those that did not, and you can see that that correlates very nicely with titers. So, again, this is something that can help you predict this.

And I think this is the last slide of data that I want to show here. This was accepted as an abstract for ATC again in collaboration with Johns Hopkins. And what we've done here, we blindly took patients that were treated -- and this is going all the way back to 2001 -- they were treated based on the

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center's standard of care. So they didn't have the information as they determined how many cycles of plasmapheresis they want to go, and those are listed separately for Class I and Class II on the X axis.

And on the Y axis, you see the log2 or the delta reduction in the titer. The size of the circle represent the amount of data points that we had for each and every one of the individual data that you are seeing there.

And when I look at the data, I think there is a very nice linear correlation of delta reduction with increased number of cycles up to a certain point. And I remember I talked with Bob about this, and I was like, well, I knew this all the time, right? I knew that there are some patients that have so much antibodies that if I need to use that extra cycles of plasmapheresis and IVIG, it's probably not going to be that great, and many of those patients eventually had rebounds of those antibodies.

But I think by using this approach, you can determine up front that patients that have -- depending on how much antibody you want to remove, up to

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something that can be removed by 6, 7 cycles of plasmapheresis, those can be removed. If your antibody is stronger than this, if you need to use more cycles, don't even attempt because it will not go there at all.

So what do I think should be the remediation of everything that we have presented right now? I think we need to adjust our expectations from the assay. I think the assay is good. I think we're trying to force it to give us something that I don't think it can. I definitely -- and I've been saying this for a long time -- do not use strict MFI as a cutoff.

We need to put our thinking caps together and find something that the community feels comfortable with. But a strict MFI is not the route to go. We need to use additional tools to assess presence and strength of antibody. And I'll talk about STAR in a minute. We definitely need to make sure that we're removing inhibition.

And just as food for thought, patients' serum samples are very different than -- transplant patients, I should clarify this, that patients that receive

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transfusions, that multiparous woman, they have so many other things in their serum samples that affect how those assays work.

And I just want to throw there, I know it's not going to be an easy thing to do, but I would want to throw, there is some collaboration between the -- I don't know if the FDA regulatory bodies and the vendors, that allow the vendors access to transplant patients' serum samples so they can QC and improve their assays better, and I think that can really help us with this.

So very, very quickly I want to take you through the STAR. Ros had mentioned this, the STAR workgroup that we had. I just want to acknowledge a lot of the people that were a part of this. This is not the full group. We had about 40 people that were a part of this. I definitely want to acknowledge very much Peter Nickerson, who helped me drive that meeting.

We have several goals going into this, and really I think we put a lot of focus trying to start thinking differently about patients that are immunologically, alloimmunologically, naive versus

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those that have a potential memory. And I know this is an area that can have a lot of discussions, but we were trying to separate it to those that have sensitization against allo and nothing else to this.

We had several guiding principles that were listed here, and we really were trying to be very strict to state-of-the-art clinical diagnostics and trying to provide grade of evidence as we were moving forward.

Our goal was to come up with four deliverables. The first is the technical primer, and that we tried to finalize before going into the meeting, so this is where I will show you a little bit more information.

We had a section, and Howie Gebel and Frans Claas were the ones leading this, that were really trying to narrow down the definitions of what is immunologically versus naive patients. And Howie will talk about this later, so I'm not going to mention this at all. And we had two major groups that were trying to come up with clinical applications and recommendations.

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And, again, I'm not going to read all of this, you can see that information. The memory group was divided into the four different organs, so we had heart, lung, kidney, and liver groups. We did not separate the pancreas outside of this. And then the immunologically naive -- and Ros mentioned there isn't a lot of data on this -- was separated for thoracic and abdominal moving forward.

The groups are finalizing their recommendation as a result of that meeting, but I think we had a very fruitful day, a lot of discussions. Many of the people in the room were actually part of this. We had invited to the room together with us representatives from the FDA, from NIH, and from UNOS, because I think they're significant shareholders in the discussions, and I think we had very good discussions with them thinking about this.

So what I can present to you today is really the recommendation for testing. I think this is really critical, and I really want you to remember this as you're rereading papers that were published 5, 10, 15 years ago when we didn't know a lot of the things that

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we know today because the conclusions might be different. But the important thing is that we really need to try to get comprehensive typing of the donor and the recipient.

And as I commented earlier, we are having now new reagents in the field that would allow not next generation sequencing, but fairly high resolution, at least at the level that we have the reagents to test for antibodies, for donor and recipient that can be done in a few hours and not a huge added cost. And I think we really need to adopt this because we can learn a lot of things on multiple levels definitely going into the Eplet route. Molecular methods is something that was adopted in the United States a long time ago. We just kept this here.

And really for antibody assessment, we need to look at all the different loci. And I listed here very specifically the DQ alpha/beta together, the DP alpha/beta together. I think this will be an important angle to look at antibodies the way they are expressed on the cell surface and not the way they are expressed on the molecular level.

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And I think the most important thing is we will recommend that inhibition must be removed. We're not going to recommend which methods need to be used to do this, but it needs to be removed, and the labs have to be able to prove that they are able to remove the inhibitions, so at least we won't miss those patients that we think they don't have antibodies and actually have much stronger antibodies.

And then there should be some mechanism in place to detect the phenomenon of potential epitope sharing because I think this is another place where we're underappreciating the strength of the antibodies.

So I think with this I'll end. Thank you.

(Applause.)

Public Comment and Discussion Part II

DR. VELIDEDEOGLU: Thank you for the excellent presentations. Now we just completed the Part II, the scientific presentations, and Part II of Session 1. And we will move on to the Public Comment and Discussion session. If anybody has any specific questions about the presentations, please ask your question.

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Dr. Haas?

DR. HAAS: I had a question for Mark actually. There was something in the eculizumab study that I guess maybe it's my naiveté, but the fact that the eculizumab seemed to prevent development of TG in patients who have low titer antibodies. Now, the low titer antibodies I guess, as I understand it, are the ones that are most likely to be C1q-negative in terms of the C1q binding. So one might expect that complement inhibition might have the least effect with these antibodies, yet it seemed to have the most effect. Am I missing something or are we dealing with the fact of the imperfections in the assays?

DR. STEGALL: Yes, you're missing something.

(Laughter.)

DR. HAAS: Yeah.

DR. STEGALL: So I think that these antibody levels are higher than most. These are sensitized patient, positive crossmatch patients, so these weren't low level of antibodies. I think it's small numbers of patients, so it's just a signal that you kind of think, well, maybe there is something to complement inhibition

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in chronic injury, was the kernel of the hypothesis that we're working on.

And the way I kind of look at it is that the final end result we see clinically is the tip of the iceberg, right? A lot of things are working underneath that, moving that clinical endpoint forward. And a lot of it is -- our interpretation of that is undercut by all the limitations of histology, the assay, and everything else, right?

But there is at least hypothesis that there are certain antibodies that -- that you have a certain amount of antibody that truly is going to injure the graft and cause transplant glomerulopathy without any other -- with complement, right? Direct activation or -- it's large enough that it causes proximal C3 activation because it's only C5 you're blocking, but that the immune system, the way it works, is that it evolved complement for a reason, it probably augments. So if you have lower levels -- lower affinity antibodies, they may need C5 activation to move downstream.

IG3 activates complement, it binds complement,

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no question. So if you try to say that IG3 is a C1q-negative antibody, I think the rest of the immunology community is going to say you don't know what you're talking about kind of thing. And so the idea is the C1q assay is an assay that it's a little bit arbitrary, right? It's just an assay. It has some sort of correlation with outcome, and mostly it has to do with level of antibody, right? If you make enough antibody, you'll be IG3-positive. So the C1q piece is a tool, but it's not a biological phenomenon that immunologists talk about. Does that make sense?

DR. HAAS: I think actually you could look at your question a little differently and kind of turn it around and suggest that maybe those low-level antibodies wouldn't have ever developed TG, and that the eculizumab is really maybe not doing anything with the low-level antibodies, but it may be preventing AMR with the really strong antibodies.

And I think your control group suggested that the antibodies that were below 200 with or without eculizumab didn't seem to correlate with chronic rejection.

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DR. STEGALL: No, I mean, I've kind of got to stop presenting that slide to tell you the truth. It just talks more discussion than it is. But I think that it -- the control group that had low level -- that had a B flow crossmatch less than 200 at 6 months, right? I don't know if that's low-level antibodies or not -- had transplant glomerulopathy, they developed transplant glomerulopathy, and the same sort of range of patients who had 1 month of eculizumab at a year didn't have transplant glomerulopathy.

So it's just an observation. I throw it in there more as starting discussion that antibody-mediated injury is a spectrum, right? And there's a reason why some people with DSA don't develop peritubular capillaritis or at least to a significant degree.

And I think every study shows the more antibody you have, the more injury you have, right? And if you can get around the acute part, the more chronic injury you have, the more antibody you have. And I think that's just the biology of the system. And I'm sure there is something to do with the kidney's

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ability to accommodate the antibody, too.
But I think the eculizumab -- just say complement is probably important to chronic injury, too, we just don't measure it very carefully.
DR. VELIDEDEOGLU: Okay. Any other specific questions related to the presentations, clarifying questions?
DR. WOODLE: So I had a couple of questions for Howie Gebel if I could.
DR. VELIDEDEOGLU: Oh, okay.
DR. WOODLE: Howie, if you take just single-antigen bead strength, antibody strength, and use that as predictor, can it replace C1q or can it be as good as C1q?
DR. GEBEL: I think I'll just answer it a different way, which is at my center we don't do the C1q assay. So we believe in the MFI value as the cutoff. So in our center, that's how we operate, yes.
DR. WOODLE: And my next question pertains to the isotype-specific assays. One of the issues with -- as you know, IgG3 has been advocated to some degree, but in our center, we don't see IgG3 in early antibody-

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mediated rejection, and we see it in about half of the late rejections. And so it's actually only in a small -- it's only in a minority of the population, and that's problem number 1.
Problem number 2 is that -- I think you pointed this out very well with the Venn diagrams, it's never in the context of where there is just an IgG3, it's in the context of other antibodies. Problem number 2.
And problem number 3 I'd like you to comment on, which I don't think you did, was that when you assay for IgG1, 2, 3, and 4, you use a different secondary antibody. So it's really the sum of the isotypes and the amount that you have in each isotype. How do you get over that problem and getting at a summary when most patients actually have multiple isotypes?
DR. GEBEL: There's no argument with me at all, Steve, because I agree with what you've said. I think the reliability of the assays as they currently stand is quite questionable because of the cross-reactivity of your secondary reagents.

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As I alluded to, when we were trying to develop this assay ourselves several years ago, we took commercial reagents, we coated Luminex beads with IgG1, 2 3, or 4. We came back with supposedly monoclonal antibodies specific for IgG1, 2, 3, or 4. And there was a ton of cross-reactivity. And so it's not that I don't believe that there might be nuggets of good information in there, but I think it deserves much more attention to reliably create the secondary reagents and then determine the integrity of the assay itself.
DR. VELIDEDEOGLU: Anat Tambur.
DR. ROITBERG-TAMBUR: So just to add to, first of all, the comment about C1q versus strength of antibody. I think we showed very, very clearly not -- Tom Ellis definitely had shown it by either concentrating the antibody or diluting the antibody, that there is a correlation, but I think we've shown very clearly that there is an actual titer cutoff, if you will. Very strong correlation from you get to a titer of 1 to 16 or 1 to 32, you suddenly get to cC1q binding.
So it's very strongly correlated with a titer

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of antibody, and I think quite a lot of centers right now, instead of running all the titration studies, will run a dilution, whatever they pick as a dilution, as a measure to say whether they have a strong antibody or not, and I think it removes inhibition and it gives you a lot of information.
For the subtype, the IgG subtype question, I totally agree with Howie. I don't think that we have very good reagents, to talk about that point and how we had indicated several other things. But I still want to remind everyone, those subtypes is a dynamic process, and the snapshots that we take today are not necessarily going to tell us where that patient will be in a month or 2 months and 3 months. So we need to take that part into consideration as well.
DR. WOODLE: Yeah, I think that's a good point. We have data that we'll present at ATC that indicate that the amount -- the quality or the quantity of IgG3 isotypes goes down when you treat with proteasome inhibitor therapy. So that subclass goes down, as does the FcR binding capacity of the antibodies. And so what you start with may be totally

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different than what you have at the end after you finish treatment.

DR. VELIDEDEOGLU: Ros Mannon, please.

DR. MANNON: Yeah. So my question is tangential to that and for Mark. In this hypothetical trial, since we're talking about DSAs, how do you envision it if you've got people with multiple? And, granted, usually in my personal experience, the early acutes have more multiples than the late, but the late can have multiples. So how would you deal with that? And that's going to come into number 3. Do we do this MFI some or what?

DR. STEGALL: So I think that's the reason that I think that we're in this era today -- right? -- looking at DSA as a screening tool. And it doesn't matter, one, two, ten, it doesn't matter. That's not what's going to determine whether I think that we should try to then develop therapy for patients at that juncture.

So we allow the tissue-typers to say this person has an antibody. It's probably real. You can do dilutions and do whatever you want. And then we'll

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biopsy the patient. That's what we want to do. And then we're going to use the biopsy for the -- the biopsy then becomes the entry into the clinical trial.

You get around all of these ASHI kind of meetings, arguments, and that's fine with me. I know that it's important to do quantification and all the rest, and someday we'll probably get there, at least for certain things, but today -- and also really what matters is the histology anyway.

So I'm not worried about quantification, that's not what we're -- the trial I put up -- I put up trial number 1 primarily to condemn trial number 1 obviously.

DR. MANNON: But you mentioned one potential surrogate endpoint was dropping DSA and understand the effect and --

DR. STEGALL: Correct. Again, that was mentioned to basically --

DR. MANNON: Yeah. So that's my question, is, Do we say were going to take the highest? And do we really know it's the -- you know, I mean, and I don't want to be contentious because I want us to be

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agreeable.

DR. STEGALL: That trial cannot be done. In my opinion, that trial cannot be done. You can't use DSA as an entry criteria -- as a surrogate endpoint for a clinical trial today. We crossed that bridge a few years ago, and that's the reason that we're not proposing that.

I only threw that out just purely to point to the limitations because people want to use DSA for a clinical trial, I guess, and I think that the assay is not quantitative, it's unrealistic to think you're going to be able to measure a 50 percent decline in MFI, just for all those -- there are going to be 1,000 reasons. And also for another reason, because you don't want to treat all those people. They're not all going to do poorly for a lot of different reasons.

So you use it as a screening tool. You screen people, you can't biopsy people necessarily every year. You use it as a screening tool. And I think a lot of the peripheral assays that are being used, a lot of the tools used for acute cellular rejection will be screening tools to tell you who to biopsy. And then

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there is going to be a different -- indication biopsy is not going to be for elevated serum creatinine, it's going to be for some sort of other test that turned positive, whether it's a peripheral blood assay for T-cell activation or serum.

So I wouldn't worry about -- the quantification is not the issue, the issue really is who you biopsy. And the thing about that, even with a C statistic of like .9, you're still going to biopsy a lot of people who don't have the disease, but you're not going to biopsy everybody.

DR. VELIDEDEOGLU: Peter Nickerson?

DR. NICKERSON: Yeah. So a couple comments just to come back to Steve's original question, and it sort of echoes Anat's question, point, about titer. So when Chris actually looked at EDTA-treated serum, so we removed inhibiting factors, there was a very tight correlation between the MFI and the C1q positivity, and I think that this goes to titer.

But I thought one of the most important things that Chris had in the paper was that if you were C1q-negative with the antibody, and you did the biopsy, 40

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percent of those biopsies were C4d-positive. In other words, in the biopsy, you were getting complement activation despite the fact that your in vitro test was C1q-negative.

And what that tells me is that -- and a lot of people have tried to use the language that the in vitro test tells you whether you have a complement-fixing antibody or not, and that is not true. In vivo, you can very well have a complement-fixing antibody, which is physiologically the important question, that tests negative in vitro. And I think we have to get this language and concept clarified in our thinking.

DR. VELIDEDEOGLU: Robert Montgomery.

DR. MONTGOMERY: So I disagree with Mark. When I was at Hopkins, we found that actually there was a very tight correlation between semi-quantitative report of DSA and what we would find on the biopsy, to the point that after 5 or 6 years of doing that, we would just treat on the basis of the tissue typing data.

And I think that, you know, if you go to a barber, you're going to get a haircut, right? At the

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Mayo Clinic, they biopsy, right? I think that it's a very good idea to have some redundancy in terms of being able to determine the level, the quality, of information that you're getting, so I think biopsying is a great thing, you should do it a lot, I totally agree with it, but when you're getting really high quality data, I think you can believe what you get from the tissue typing lab.

The other thing I would just say is that this whole C1q thing to me is very problematic, it's become sort of like religion, there are believers and non-believers, and I think it's taking us off in kind of a weird direction that is distracting, and it really isn't, in my opinion, something that is going to change the field in an important way.

DR. STEGALL: Can I? I agree with Bob. I think that you didn't understand what I was proposing. In the early posttransplant period, yeah, the antibody levels are very helpful, there's no question, but what I'm looking at about the biopsy is the de novo DSA posttransplant 5 years out patient, and in that situation, the levels aren't very high at all, and

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that's the group of patients I'm talking about.

So, yeah, posttransplant, obviously early posttransplant the levels are very high and correlate very well with the histology, but in 5 years, when the MFI is 2,000, and it's a de novo DSA, then the histology and the DSA do not correlate, and that's the reason I think at that point you need to do the biopsy.

So it's a different patient population. And, again, that's the reason I made those slides, to try to tell you that it's not -- yeah, histology and DSA do correlate, but it just depends on the time posttransplant -- right? -- and the setting. That's all I was saying.

DR. WOODLE: So to try to mediate this --

(Laughter.)

DR. STEGALL: No, it's just a clarification of what I'm trying to -- actually, I never said what he said.

DR. WOODLE: No, I understand. Bob is right, you've got to have an HLA lab that pays attention to the precision of their measurements. So we do ours robotically, and our CV percent is 10 percent, it's

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actually less than 10 percent. And so when we lower the MFI from, say, 7,000 to 5,500, I believe that, I believe that we're on the way down. And when we see progressive reductions, we're even more confident in that. And so I agree with you.

In terms of Mark's point about early AMR, absolutely. In terms of late AMR, we do have some data that's starting to emerge that's indicating that if you reduce the MFI in late AMR, it can impact graft survival. That data is early. Clearly, other centers are going to need to show that.

The one thing that we haven't done in our program is we haven't given quite the attention to dilutional analysis, like Anat recommends, in AMR, late AMR, as we have in desensitization. In our prospective trial, our iterative trial, of bortezomib-based desensitization, we used the exact techniques that Anat illuminated, and without that, we would not have had a quantitative trial. It's not easy to do, and you have to put a lot of time and effort into it. But I hope that clarifies where we stand.

DR. VELIDEDEOGLU: Okay. In the interest of

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time, I plan on moving to the FDA questions, but I tend to shorten them and revise them a little bit because we won't have time to cover everything that we planned on initially discussing.

So actually, we got answers to most of our questions during the presentations. Regarding the first question, "Discuss the utility of surveillance biopsies and single antigen beads and DSA, routine DSA, monitoring," what we would like to hear from the speakers and the other attendees is that we realize that there are different practices among different centers with regards to routine DSA monitoring and the utilization of protocol biopsies.

So if anybody wants to comment on -- and we also realize that the landscape and the practices at different centers are rapidly changing, especially with regards to routine DSA monitoring. So if anybody has a different point of view or wants to talk about their center's practices in terms of DSA monitoring and protocol biopsies, we would like to hear that.

Dr. Nickerson?

ATTENDEE: I think Mark wanted to.

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DR. HAAS: Oh. I guess with regard to protocol biopsies, maybe we're doing protocol biopsies in the wrong patients. And we do protocol biopsies, a lot of centers do protocol biopsies, at 3 months, 6 months. And I think Bob's point is very good. When we're talking about the very early, sort of acute AMRs, these are highly correlated with rebounds of DSA, and it may be acceptable to, especially if a patient has a rebound of DSA in a highly sensitized patient, to just treat that patient to reduce that DSA.

However, when we're talking about protocol biopsies in 1 year farther out, the histologic lesions look very different. These are rarely pure ABMRs. They're frequently mixed rejections. The molecular data tells us that there's a strong correlation between the histology and whether these are a mixed rejection versus a pure ABMR, depending on what the interstitial inflammation looks like.

And so in terms of guiding treatment, a year or 2 years out, there still might not be a whole lot of graft dysfunction going on, but it may pick up a smoldering ABMR at a point before you have overt

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transplant glomerulopathy and treat it, and also to design therapies to whether you want to treat pure ABMR or whether you need to give something to treat the T-cell-mediated component as well.

So I guess I would say that maybe later protocol biopsies might be of more value than early protocol biopsies, where you can usually tell what's going on just from the DSA assays.

DR. STEGALL: So I can say what -- so I'm the protocol biopsy guy. We never did protocol biopsies necessarily with the idea that we're going to manage any individual patient. We started our protocol biopsy study in '98 because we wanted to learn the natural history of what happened to a kidney after we transplanted it, and out of that, we thought we would learn enough then to be able to design clinical trials and improve the graft survival of the entire population, and any one patient who participated therefore would contribute to that knowledge.

So I don't think you have to do protocol biopsies to manage patients at all. I think that we've outlined what we think. If you have DSA, we would call

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that indication biopsy at that point.

So I think the utility of protocol biopsies is outlying the biology of the disease, and I think that over the past 10 years we've actually started to do that. And so don't misquote me to say that I think everybody should do protocol biopsies. That's not the case.

DR. VELIDEDEOGLU: Please go ahead, Dr. Haas.

DR. HAAS: Okay. Just to respond briefly to that, so if you detect a DSA 1 year posttransplant, you'll do an indication biopsy, and that indication biopsy may show AMR, it may show mixed rejection, it may show nothing. If that patient -- say the protocol biopsy shows nothing or shows just T-cell-mediated rejection, given that this patient has a DSA, would you do a series or one or more protocol biopsies, say, at 2 years or at 3 years in that patient to see if there has been a change in the histologic status?

DR. STEGALL: We would not do any different biopsy regimen than we currently do. So we do a 4-month, a 12-month, a 24-month, and now we do 4, 7, and 10 years. That's what we would do, but we wouldn't --

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the idea is that if we are doing a clinical trial, then we would actually have a better idea about -- you know, we have a reason for doing -- I think there's a reason to do a follow-up biopsy if you're doing treatment to assess efficacy.

DR. VELIDEDEOGLU: Dr. Nickerson?

DR. NICKERSON: Yeah, so in our program, we do a surveillance biopsy at 6 months, although we've debated 6 versus 12, and I don't think it's really any different. The reason we do it is to look for adequacy of immunosuppression, mainly around cellular, and I think there's a lot of data that comes out at 6 or 12 months that if you have ongoing cellular inflammation in that graft, you actually have a high risk of premature graft loss. So we look at it in that sense for trying to define adequacy of immunosuppression. And then we don't do any other surveillance biopsies.

We do, do single-antigen bead screening, and we do that routinely starting at -- we do early, a few months, but 6 months on, we're doing it semiannual to then annual at 2 years on with the idea that if we find an antibody, we're going to do a biopsy to look for

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whether there is any ABMR, because I also agree with Mark, that you can have a de novo DSA, and there is no pathology, I'm not going to go aggressively in that patient per se.

If we are initiating treatment, we will do a follow-on biopsy to see whether we've seen a change or a progression. Ideally, that would be with a clinical trial, but that's where we all want to have a clinical trial. But the whole concern, and I think more and more people are doing surveillance with single-antigen bead measurements as a screening assay. The problem is getting reimbursed for it and the cost of doing it, and some programs have had real trouble getting that within their programs. So we've managed it in our program, but that's not necessarily true across all programs.

DR. ALLOWAY: Rita. I am afraid that perfection is the enemy of good as we continue to talk about DSA, and I think if you compare and contrast it to what we do with tacrolimus assay monitoring, we do not look at a single, or we should not look at a single, tacrolimus level in isolation, we should look at them as a continuum, just as we look at DSA.

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Number two, when we improve our ability to measure the DSAs and get the most quantitative MFI and remove as many variables as we can, the coefficient of variation is actually low or lower than especially what you're getting with our immunoassays for tacrolimus these days. So I think that we're sending the wrong message to the FDA in terms of what we maybe can or cannot do with DSA in a clinical trial.

Now, I know that we're limited by the label of the DSA and all of those different kinds of things, but we are trying to use DSA and put more restrictions on that than even we do on a tacrolimus assay today. So I just think we need to compare and contrast when we're thinking about those.

Right now, we don't know what the exact range or level for tacrolimus is, but yet we use it every day and we monitor it. We don't know what the exact number of MFI is going to be, but we know that if we drop it, we have an improvement.

DR. VELIDEDEOGLU: I'm sorry. I feel like I should intervene at this point. We are into our lunch hour, but I want to give the last word to Michael

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Mittelman. He has been raising his hand. And some of the issues that we have been discussing, the routine DSA monitoring, also overlap, and there's another trial design related question, which also falls under that subject of the upcoming sessions, which could be discussed further. So in the interest of time, I would give the last word to Michael Mittelman.

MR. MITTELMAN: First, this is awesome. This is a real privilege to kind of watch you guys debate all of this. And you may get to this later, I'm trying to figure out the agenda, but I would sort of urge you guys to pursue other activities and research protocols that do not involve biopsies. They are the worst. And protocol biopsies give me nightmares.

I'm glad I'm not at a center and being treated at a center that does protocol biopsies because, I'm sorry, but I would walk away as a patient and go somewhere else. So I just want to say that I urge you guys to pursue other methods of detection because they are terrible and horribly invasive. And that's all I wanted to say. Thanks.

(Applause.)

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DR. VELIDEDEOGLU: Okay. We thank Michael for his comment. And this concludes the discussion session for Part II of Session 1. Now we have the lunch break until 1:30, and we plan on reconvening and starting at 1:30.

(Lunch.)

Session 2: Factors Contributing to Antibodies in the Pretransplant Period and Treatment Options

DR. CAVAILLÉ-COLL: Good afternoon, everyone. I am Marc Cavaillé-Coll, from the FDA, and with my colleague Milagros Samaniego-Picota, who will be moderating this session. Session 2 is on factors contributing to the antibodies in the pretransplant period and what treatment options there are.

Our first speaker is Dr. Arjang Djamali, from the University of Wisconsin, who will be talking about the highly sensitized transplant candidate and give us an overview. Thank you.

Highly Sensitized Transplant Candidate -- An Overview

DR. DJAMALI: Thank you. And thank you to the FDA for the invitation. It's a pleasure to be here and

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to give you the first talk after lunch. So please try to keep your eyes open. We will talk about the highly sensitized patient. That's the topic.

Would you please advance it for me? Just this one. Okay. Thank you.

This is the disclosure. And I will be talking about unapproved investigational use of products in this presentation.

This is the outline of the talk. Background, a few slides on that. The clinical studies that have been conducted for the highly sensitized transplant candidate, some of the outcomes related to those, the limitations of those studies, and then future directions.

So this is to set the problem. There is an accumulation of the highly sensitized transplant candidate on the wait list. You see here in the red bar graph the definition of patients that are sensitized with a PRA of higher than 80 percent to 98 percent from 2005 to 2015 based on the recent data from 2017, annual data report. And the very highly sensitized have also increased so that the overall

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number of patients approximately right now is around 14,000 on the wait list. And importantly, there has been a modest improvement of transplant rates, overall transplant rates, in highly sensitized patients despite the Kidney Allocation System.

In fact, as you see here, while the very highly sensitized are being transplanted at the higher rates, those with a PRA between 80 and 98 percent are declining in their transplant rates. So the problem persists despite some successes.

And this is the problem. This is the immunoglobulin, the IgG. And in our scenario, patients get sensitized due to one of these three mechanisms: blood transfusion, pregnancy, and/or previous organ transplantation.

Some of the clinical studies that I'm going to approach now are the most representative ones. I apologize if I don't represent, I don't talk about all of the clinical studies, but I selected the ones that I thought were most important.

The first one and the only randomized clinical trial in desensitization was published about 17 years

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ago by the Cedars-Sinai group, and the investigators looked for the first time at the role of high-dose IVIG in desensitization. This was a randomized controlled trial. Patients received 2 g/kg of IVIG per month times 4 compared to placebo. The first impact was to see a decline in PRA. The second was that there was a significantly higher transplant rate in those patients that received IVIG.

A couple of additional observations are important here. One is that the effect of IVIG was temporary or transient by about 6 months. Second is that the starting PRA was less than 80 percent, so these were not highly sensitized. And third is that the time at which this gap was closed was the time approximately at which the two transplant curves started to split. So maybe there's an impact of IVIG later on, but at least it's important to note that the desensitization effect on cPRA was relatively transient.

You have seen this or another source of figures on the role of IVIG in desensitization, but from our perspective, the two important components are

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the properties, the immunomodulative properties of the F(ab) segment and the FC segment. For desensitization, the primary important part here is neutralization of antibodies and cytokines. Then the other ones are related to the Fc segment.

And more specifically, downregulation of plasma cells, modulation of dendritic cells or downregulation of their activity, inhibition of activation of additional immune cells, upregulation of Tregs, and for endothelial cells is saturating the Fc receptor.

So this is an important role for IVIG that you will see throughout either at the low dose or at high dose has been utilized, and we can discuss that in more detail later on.

The same group then added rituximab to IVIG. Now, these are patients that got IVIG here and here, so about 30 days apart, and in between they got rituximab at 1 gram. Twenty patients were enrolled in this study, and out of these 20, 16 got transplants, 10 of them live donor and 6 of them deceased donor transplants. Please note that their PRA declined also

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significantly. Nevertheless, it was again less than 80 percent. And also the T-cell flow crossmatch declined quite significantly, but the acceptance cutoff for the T-cell crossmatch was at 250 at the time of transplant, and at that time, already half of the patients were in that acceptable range. So the combination of rituximab and high-dose IVIG was effective to some extent.

The role of rituximab, I have summarized it with this study, single study, from Hopkins. And I think that it's important to remember that rituximab, as you know, is an anti-CD20 agent that reduces B cells in general, but not memory or plasma cells. So its impact is primarily on rebound.

And the intensity of the HLA after receiving rituximab is lower compared to the control group. And these are graphs that are depicting the same impact, no rituximab versus rituximab, whether it's a DSA HLA or non-DSA HLA.

Having looked at the impact of rituximab and high-dose IVIG in sensitized patients, but not very highly sensitized patients, it's important to look at the other negative studies that were conducted by at

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least three other groups and published by three other groups either at Montefiore in New York, this is in Indiana University, and this is the Hopkins group. In aggregate, these groups were unable, as we were at the University of Wisconsin, Madison, to reduce any PRA in patients that had a significant -- had a PRA of 80 percent or higher. So there is this limitation that is important to note.

Now, what about live donor transplantation? And this is the work of Bob Montgomery and his team at Hopkins published sequentially in the New England Journal, and in this category of patients, it is feasible and the investigators have been successful to reduce the PRA mean of 82 percent to the point to transplant these patients through sequential plasma exchange and low-dose IVIG therapy combined with tacrolimus, mycophenolate, induction with Thymoglobulin or IL-2 blockade, and then additional plasma exchange sessions.

This has to do with the intensity of the MFI or the intensity of the crossmatch, and the number of sessions increased as the sensitization increased. But

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obviously this is very cumbersome, and you need to have a live donor available because you can't continuously plasma exchange patients.

I'll come to another very interesting study that was conducted in Cincinnati by Steve Woodle and his team. This was a combination of proteasome inhibition with B-cell inhibition and plasmapheresis. They hypothesized that B-cell inhibition alone is not enough and you need to combine that with plasma cell inhibition.

So in an intent-to-treat iterative study, they included 52 patients with a cPRA with an unacceptable antigen MFI level of 1,500 of 91 percent. They enrolled these patients in various combinations of bortezomib, rituximab, and plasma exchange. Thirty eight patients, or 73 percent, completed this study. Nineteen of them were transplanted, which is a reasonable rate of 37 percent.

Importantly to me is that they had about a quarter of patients that responded by a decline in their PRA defined as 1,500 MFI. And when you look at PRA, you see that a good group of them had a PRA of 90

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percent or higher.

Additional studies have been published over the past couple years looking at different elements of the B-cell development and maturation, specifically this one that was done at Indiana University looked at anti-BAFF therapy with tabalumab, and they looked at the impact on BAFF levels, that was positive, so they had an effect, but unfortunately the effect again on cPRA was quite minimal, and you see that a majority of the patients were very highly sensitized.

Mark talked about this. This is a different representation of a study by the Mayo Clinic to inhibit C5 complements. And I will just summarize this again by showing that there was clear success in transplanting these patients with low clinical rejection rates, but at 2 years, there was not a significant difference in eculizumab versus control groups.

Finally, another relatively recent study that was published from a Cedars-Sinai group using an IL-6 receptor antagonist, tocilizumab, combined with high-dose IVIG in 10 patients who were unresponsive to IVIG

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and rituximab, and these patients received tocilizumab plus IVIG pre- and posttransplant if they got transplanted.

It ended up that half of them got a kidney transplant, the other half didn't, and the overall complication rates, severe adverse effects, were 40 percent. So some success at least in declining DSA in patients who were transplanted and in transplanting them, but the clinical safety was maybe not as great as we would like it to be.

So in summary, all of these studies, I have depicted them for you here. This is the first author, these are the number of patients. This is the cPRA to start with. This is the regimen. This is the impact on PRA. What you see was kind of minimal overall. So the transplant rates, which are hard to determine whether this was an effect of the treatment or it was just a random effect of transplantation.

And here I am also reporting the four trials that are in clinicaltrials.gov. One of them has stopped because the BAFF inhibition was not successful. So quite modest impact on therapy. And even when we

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are successful in transplanting these patients, as you know, if we don't bring down their DSA or their antibody levels at the time of transplant to reasonable levels, then long-term graft and patient outcomes are not very good.

So why are we having some of these limitations? One of the first things could be that we are missing some of the non-HLA antigens. And over the past 15 years, there have been a number of studies looking at this potential of AT1 receptor antibodies or other endothelial cell antigens that could be considered.

Another option is -- or another explanation is that, as we discussed this morning, this B-cell response is not just purely a B-cell response. It starts with T cells. Even in the germinal center, there are T cells, and they continue to activate the B cells in the presence of antigen. We don't have the right treatment strategy to eliminate or inhibit plasma cells or memory B cells, and what we really need definitely is a multipronged approach or a combination therapy that would affect B cell maturation, B cell

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development, and plasma cell activation as well.

I am going to spend just a few seconds on this slide because this is an important point from my perspective, and that is the targeting of bone marrow plasma cells and their survival niche.

Plasma cells, once they are mature, they go primarily to the marrow. Some of them stay in the lymph nodes, but the marrow is their primary place to home. It's important to know why they home there and what is it that keeps them alive because they are long-lived, and nevertheless, as soon as you take them out of the bone marrow, what happens is that they die. And, finally, it's important to understand what signaling molecules are there to make this survival happen.

So it turns out that there are a number of potential cells that could constitute this niche, but stromal cells as well as eosinophils are important components of this niche.

And if you wanted to consider the whole process of desensitization, you may have to consider targeting upstream as well as downstream molecules or

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cells to B cells so that we can be effective, yet being safe.

So the future directions I would say primarily at this stage, we have the kidney paired donation as the numbers are increasing. We have the combination of desensitization and KPD, and a lot of institutions, including us, are doing a lot of this.

And, finally, I think that we can conclude that there is limited success, nevertheless, some success, but also a transient impact on antibody and PRAs.

We still don't know about the pathogenesis of sensitization as well as we would like it. We don't know why some patients don't get sensitized. We don't know why some patients get highly sensitized, depending on the genomics or cellular pathways behind it.

And, finally, defining the best combination therapies that target the plasma cell niche as well could be an important approach for desensitization.

And regarding the endpoints that we need to target, what should be these endpoints? Should we focus on cPRA and define the antibody strength for the

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unacceptable antigen? Should it be transplantation? Should it be the immunodominant antibody? Should it be non-HLA antibodies? Or a combination of all of these?

On this note, thank you very much for your attention.

(Applause.)

DR. SAMANIEGO-PICOTA: The next speaker is Dr. Howie Gebel, who this morning already spoke to us, Emory University. "Recognized and Unrecognized Sensitization: Assessment of the Pretransplant Immunologic Memory and Its Importance."

Recognized and Unrecognized Sensitization: Assessment of the Pretransplant Immunologic Memory and Its Importance (with reference to the 2017 AST/ASHI Antibodies in Transplantation Consensus Conference)

DR. GEBEL: Thank you. I already disclosed this, that I have nothing to disclose.

Earlier this morning, I alluded to the data that are shown in this slide. This was a paper that was published by Paul Terasaki in 1969 that did crossmatches between recipient sera and donor cells. And without going through the data, the outcome of this

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study was just remarkable, and one can see that there was an overwhelming association of positive crossmatches with what turned out to be hyperacute graft rejection. It was the reason that HLA labs become operational 24/7 365. Whenever Paul Terasaki was in the audience, I would always look at him and tell him that he's either blessed or cursed for making that discovery in terms of keeping us busy 24/7.

Paul passed away last year. He spent his entire career devoted to the humoral theory of transplant rejection, and we wouldn't be here without all of the contributions that he made.

So I already showed this slide this morning. I won't spent any time on it other than to focus on the fact that we're using these Luminex beads, a solid phase assay, to detect HLA antibodies. And I showed you a version of this slide earlier. And all the red bars, no matter what lab you're in, there is a cutoff. It doesn't matter what the number is, but there is a cutoff above which those are considered unacceptable antigens. And for deceased donors, if you put those into the UNOS database, you are not going to get

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offered any donors that have any of the corresponding antigens.

To the right of the red bars what you have are a lot of blanks, you have nothing showing up, no red bars. Those are antigens that did not react with your patient's sera, so those are considered acceptable antigens.

Over the past few hours, we've heard people talking about sensitized patients, we've heard talking about desensitization of these patients. I think it's time to put some definitions to these terms.

So here is an antibody profile of three potential candidates. And I'm having you look at Class I and Class II. The first thing you should see is that there are no red bars. So all three of these patients have no Class I or no Class II antibodies, neither. Now, the question I'm going to ask you is, are they unsensitized?

At the beginning of my career, when I first came into the lab, if a person presented with a zero PRA, panel reactive antibody, activity, we were told they were unsensitized. That's really not necessarily

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true, so let's get into the details, and as you all know, that's where the devil is.

So the first patient in my study was a non-transfused male. So this is one patient that you can potentially consider unsensitized. I'll get to it in a minute as to why even this person might not be unsensitized.

The second candidate is a multiparous female. She's got three children. Now, she clearly has been exposed to the antigen, so there are mismatched paternal antigens. But the question still remains, Is she unsensitized or sensitized? And I like a term that Steve Woodle came up with, which is "quasi-sensitized." So we quasi know the answer to this.

The third patient is a previous allograft recipient, and I don't think there is anybody in the room who would deny that this person has been sensitized, but there is still this question that they all present with no antibodies. And that's the test that we use. We use antibody detection as our surrogate for whether somebody has been sensitized.

And I think what we need to remember is that

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indeed it's a surrogate. It's not the endpoint. The antibodies are a surrogate for the cells that produce them. And clearly it's plasma cells that produce them, but plasma cells come from B cells, and B cells won't make the antibody unless they've been helped by T cells.

So it's time to move on because as far as I'm concerned, we have one test, like the antibody detection. Much like this horse hospital, if you take a look, there is only one solution to every time that patient presents. Let's do an antibody test. Well, I think we can do better. We at least have to recognize that we can do better.

For the next set of slides, I want to thank my colleague Mandy Ford, who is an immunologist over at Emory. She gave me these slides and then helped tutor me through this so I could explain them correctly.

So alloreactive memory at the level of the T cell doesn't just have to come from HLA antigen exposure, it can come from pathogen exposure. This is a paper that one of our HLA colleagues, Frans Claas published several years ago, and taking clones of T

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cells that are specific for different viruses -- Epstein virus, CMV, or flu -- one can put them into a gamma interferon production assay and stimulate them with uninfected allogeneic cells, which I'm showing you here.

And you can see that there was interferon production in these cells after stimulation with just A2. So somehow the A2 is being recognized by these cells that are specific for a virus. So the virus itself might be one of the factors involved in generating what looks like alloimmunity.

And what you can see is, in this particular slide, on the top, if you have, in an animal model, if you have you low donor-reactive memory T cells, the likelihood is that you're going to be able to induce tolerance, but if you have a lot of these cells that have donor-reactive memory, you wind up not being able to tolerize this individual or these mice.

And in a human study and some work out of Rob Fairchild's laboratory, one can take a look at again a gamma interferon production assay. And what you're looking at on the left side is rejection episodes in

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patients who made a lot, we'll say a lot of spots, greater than 25 of the -- excuse me -- 50 percent of the patients made more than 25 spots compared to four out of 23 with less than 25 spots. The ones who had lots of spots had more episodes of rejection, and the ones with fewer spots had better GFR rates.

So all of this just goes on to show you that we have the ability potentially to quantify our cells, at least T cells, that are involved in alloimmunity. But we look at peripheral blood.

And as you can see here, the T cells that are involved in a number of different functions reside all over the body. They reside in non-lymphoid tissues and peripheral tissues like the lung and liver and spleen. And what we're doing is focusing only on the peripheral blood, and we have to recognize that cells that reside elsewhere can contribute. If we don't look for them, we're not going to be able to find them.

The same thing can be said for B-cell differentiation pathways. We look for the production of antibody, but just looking at this slide, one can see that there are a number of other cells that either

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can make antibody themselves or become antibody-producing cells. They don't all reside in the peripheral blood. And it's another limitation of what we can do with the information that we generate.

So we're not the first to come up with this idea that we need to look for something better than antibody. And, in fact, Sebastian Heidt and Frans Claas came up with this notion recently, published it in Transplantation, and we need more than serum antibody screening.

And actually, I can go back to Bob Montgomery and his colleagues at Hopkins when they first began looking at circulating B cells that were specific for antibody production to certain HLA antigens. They did tetramer-specific testing to look at the circulating number of cells that had the ability to produce an anti-HLA antibody.

And they found what I think is a pretty remarkably high number in some patients, up to 4 percent of the antibodies, in fact, in this slide, up to 6 percent, of the circulating B cells had apparently the ability to make a particular HLA antibody. And

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when they went into the next phase of the study to look at B cells in patients who are about to be transplanted or were transplanted, they looked at the frequency of these cells, and found that somebody who had a previous transplant had a stronger number, a higher number, of these B cells that had the ability to make a particular antibody.

So this is an assay that gives you a quick peek at the ability of the cells that have the potential to make antibodies. This doesn't prove that they make antibodies.

And so Claas and his colleagues again went one step further with these data and developed an assay, an ELISPOT assay, to look for HLA-specific B cells where they actually quantified the number of cells per million that would make specific HLA antibodies.

And in this particular slide, you see that the cells that they took from different patients, these two patients had the ability to make antibodies to HLA-A2, but they didn't make antibodies to HLA-A1 or HLA-A11. But these were from cells that at the time the patients were not necessarily making any antibodies there were

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appearing in the serum, but they were able to detect them in this assay using gamma interferon binding or, in this case, spots as a detection assay.

They continued along these lines. And the purpose of this slide is to take a look among the people -- let's look at the top two individuals -- who were both immunized in the past with HLA-A2. And you see that one of them had 99 spots compared to a second one, who had zero spots. So one could take this to the next level and at least imagine that the one with 99 spots had more likelihood to produce A2 antibody upon reexposure to A2 compared to the second patient.

The question, of course, is, How many spots do you need to see before that patient is recognized as it's a risk factor for that patient to be transplanted with an A2 donor a second or third time? I don't know the answer to that, and that's not been identified yet.

So, again, here, this is more of the same, looking at data in patients who have been exposed to antigens in the past. The key element of this particular slide, if you look at the third set of bars on the left graph -- excuse me, the fourth set of bars

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on the left graph, we see that in the serum, the patient had no circulating antibody to DRB1*10.

However, in this culture of media that I'll be getting to in a moment and how they did this, the cells that were grown in the supernatant from this culture media actually produced an antibody that was not seen in the serum. That meant they had the capacity to do it. It's giving you information of exposure to an antigen that you would have relegated as not being an antigen of concern.

So I didn't even get the chance to read this paper. This is now in press, and it's an early view of a paper that was just accepted as I sent my slides in that Frans and his colleagues have got more data that has published the ability of using this particular assay, which involves culturing peripheral blood B cells from your recipients in the context of C40 ligand and cytokine supernatants and a number cells, cell lines, that express certain HLA antigens. It's tedious, but it is a reproducible assay in his hands.

But it's not as easy as it looks. All right? As again you start with peripheral blood. You have to

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isolate the cells. You enrich the cells, deplete the T cells, culture the cells for 7 days. You can see where I'm going with this. It's not very easy to accommodate in a clinical laboratory. Once you've done that, you have to use those cells in number 4 as a stimulator, add IL-2, IL-10, IL-21, T-cell receptor ligand. And then you collect, freeze, and store all of these reagents and look at them for the production of spots or antibody testing.

It's very labor intensive. It's going to demand extensive QC, proficiency testing of these particular components in order to know that you're doing the right thing. You have to maintain the cell cultures. This goes on and on. I don't see us doing this as a routine until we have the ability to do robotics, but I see this as an immediate need in the clinical setting.

So one of the other factors that we also have to consider is once we take these B cells out and look at them in vitro, what have we now done to the system? Recently, there has been a great deal of attention on follicular helper T cells. And follicular helper T

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cells have the ability to regulate antibody production. The way they might do it is by acting on other T cells that help B cells become antibody producers, or the follicular regulatory cells work directly on B cells and prevent them from becoming antibody-producing cells. So the question becomes once you start to remove them from the physiological environment and put them into an artificial environment, what are you doing?

So the summary from my point of view is that there is only one test right now that we have for all issues related to antibodies, and that is our solid phase multiplex assay. It's not truly quantifiable. It's not uniform. And it really is the tip of the iceberg. We have all of these different things to consider when dealing with the sensitized patient, and it's critical that we pay attention to these details.

So to conclude, the current tools are better than anything we've had before, but they still remain rudimentary. The antibodies -- I said it once, I'm going to say it again -- they're surrogates for sensitization and memory. They tell you one part of

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the story, not the entire story.

Risk assessment by antibody alone is, at its very best, incomplete, and at times it can actually be misleading.

We need to transition to cellular assays for additional and perhaps better information to help us treat our patients. And the current testing for T and B cell memory is still very early in the stages of development. It's not quantifiable. It's labor intensive. The clinical applicability, barring my reading of that paper I showed you, is still speculative. And as far as I'm concerned, moving forward, it's definitely going to require some form of automation. We're going to have to vet all of these different assays to make sure that they meet our standards, and then we have to test for the clinical utility.

Thank you.

(Applause.)

DR. CAVAILLÉ-COLL: Thank you, Dr. Gebel.

Our next speaker is Dr. Robert Gaston, from the University of Alabama, who is going to address,

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"Prevention of Sensitization: Blood Transfusions, Nonadherence During the Previous Transplant, and the Management of the Failed Graft."

Dr. Gaston.

Prevention of Sensitization: Blood Transfusions, Nonadherence During the Previous Transplant, and the Management of the Failed Graft

DR. GASTON: Again, thanks to the organizers for the opportunity to attend and participate in this outstanding meeting.

Those are my disclosures. I apologize for changing the aesthetics to green. Maybe not.

And I didn't have any conversation with Howie relative to the talk beforehand, but he really did a good job of setting the stage for what I want to say in this. And that is to go back to really our basic understanding of antibody responses, and they're basically an appropriate immunologic response to foreign antigen. They derive from basically four sources: heterologous immunity, which he presented, and I use that term very broadly, but the idea that sensitization can come from sources other than what we

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usually think, which is human antigen exposure.

For the patient undergoing initial transplantation, the primary sources of sensitization are pregnancy and blood transfusion. Those remain important in the patient with a failing transplant, but their importance is far superseded by exposure related to a previous transplant, and that's going to be the focus of what I talk about here.

The response can be directed at both MHC and non-MHC antigens, but you've heard that addressed by people far more competent than I. And so as we look at this, we're basically talking about the specificity related to exposure to MHC antigens Class I and Class II.

This is a very nice study from Cambridge that looks at the relationship between matching and sensitization or DSA production. This is looking at the serologic mismatching and presensitization. The definition of sensitization is an MFI greater than 2,000. And this is 131 patients who had failed grafts.

What you see in the panel on the left is the Class I antigens, Class II on the right. You can see

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that largely the patients are unsensitized pretransplant. At the time the grafts fail, there is greater sensitization, with the red being greater than 85 percent basically PRAs in this. And then during their time on the list, after they've lost a graft, there may be a time at which the antibody response has become even greater.

And you can see here for really all categories, both Class I and Class II, perhaps the Class II response is a bit more intense, but it does correlate with the degree of mismatching in the first graft that was present. So that by the time at some point in the time that the patient is on the waiting list, the majority of the patients do show sensitization or development of DSAs to the previous donor that pose problems when thinking about retransplantation.

So what we do know about all this? We know that the development of DSAs is attenuated by immunosuppression. That really comes from two sources, as Dr. Nickerson has already showed, with CTOT-09, one place this comes from, that basically withdrawal of

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tacrolimus, even in patients who were pristine, resulted in alloresponses that resulted in discontinuation of the study. You can see this in numerous other minimization studies. This is a CNI minimization study in which patients randomized to be treated with mTOR inhibitor everolimus were substantially more likely to undergo development of DSA than patients who remained on calcineurin inhibitor, and as you can see, there were consequences of that in terms of risk of AMR.

So not only do we see that in minimization studies, but we see that in what I really think is ultimate minimization, and that's related to adherence. A lot of the specificity about adherence I will defer to Rita Alloway's talk later in the day, but clearly adherence plays a major role in this.

And you can see this work from Chris Wiebe and the Winnipeg group, that on this axis this formation of DSAs among the nonadherent patients, roughly 72 percent by 12 years had developed DSA versus significantly less DSA development in the patients who were adherent.

And there are consequences of the DSAs. These

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are the histologic consequences with both IFTA and transplant glomerulopathy. You can see whether the injury was subclinical on protocol biopsies or clinical. The clinical expression was much more likely in patients who were nonadherent than in patients that were adherent, but, again, the risk of DSA associated strongly with graft failure, particularly over years and years, and you've already heard that time course today.

So when you're thinking about the impact of these events in retransplantation, as you've also heard already, that not all DSA exerts adverse impact in retransplantation. It's dependent on the characteristics of DSA. It's very rudimentary, but things are progressing in terms of defining in relation to class and subclass and so on. It also depends very much on just the gross specificities of the DSA.

One clinical impact is that it's hard for these patients to receive another transplant. And that's in this study from Toronto, as sort of exemplified in this graph, and that is that patients who were highly sensitized before the second

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transplant, basically in order to be transplanted, required a very closely matched graft. So I stuck this slide in basically to say that the first consequence of sensitization in the patient with the failed transplant on the waiting list is it's going to be really hard for them to be retransplanted, and it will be from a very limited well-matched pool for those patients.

The other characteristics that may play significance, it appears that the antibodies that may be most detrimental in retransplantation are Class II, Class II rather than Class I. This comes from several studies.

This is an analysis of USRDS data from the Toronto group just recently published. What you see here is all-cause graft failure, and then just death-censored graft failure or immunologic graft loss here. And so they fixed at no repeat mismatches as a risk of 1 patients who had only Class I mismatch, as it exerted very little impact on outcome of the subsequent transplant, but Class II exerted a much stronger impact in terms of risk of both death-censored and all-cause graft failure.

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You can see, though, that if you really then tease it out by patients who are nonsensitized versus patients who are sensitized, then the effect of Class II really comes out much stronger in the patients who are sensitized in terms of risk of all-cause and death-censored graft failure with a second graft. So, again, emphasizing the importance of Class II relative to Class I in influencing outcome in the second transplant.

You can see a little bit different approach in this data from a French study, that it's a fairly small number of patients, 34 patients, and they looked in these patients -- they defined the patients most at risk of adverse outcomes with the retransplant as those who had persistent DSA that persisted after the transplant, and then they contrasted that with patients that had transient DSA.

You can see there is not a lot of difference in terms of transfusion and pregnancy. The patients who had persistent DSA were much more likely to have been sensitized by the previous transplant or to have had more than one sensitizing event. The adverse

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outcomes were most associated with Class II and/or both Class I and Class II DSA.

Immunodominance, regardless of how meaningful or meaningless these numbers are, they seem to come out from time to time whether you looked at immunodominant DSA or some of DSA, you see the same sort of effect. They're more likely to be persistent and therefore injurious, and then to be much more broadly reactive associated with a persistent antibody.

They then used these data to look at sensitivity and specificity. And actually there's an error in this table because this should be sensitivity and specificity across, and as they raise the MFI, the 1,500, 3,500, and 5,500, you see that it becomes much less sensitive as a predictor of DSA -- excuse me -- of persistence in retransplantation, but much more specific with the higher MFI, so the correlation. And then you see in the ROC curve whether you look at immunodominant or some of DSAs, you see the predictive value of changing this relative to the standard.

So beyond the characteristics of the DSA, I think a great deal, as also Dr. Gebel said, depends on

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the milieu that exists in the recipient in terms of preexisting memory, and not to belabor this, this is a 20-year old article from Peter Heeger and the group, and these are basically looking at preexisting T cell, memory T cell, response in interferon gamma ELISPOT testing, and these are for two different donor recipient pairs who were equally matched, at least matching as it was termed in those days, and you can see that the T cell reactivity was very different, even with the same degree of matching that was there. So the degree of memory that's present in the patient is very important in determining the relevance of the antibody that's detected in the assays.

Another way of looking at this -- and this group has been doing it for some time, as a marker of underlying inflammatory or immunologic reactivity, is looking at soluble CD30. And here where the patient does not have this underlying evidence of immune activation, if you will, you can see no real effect of the DSA on subsequent outcome, whereas if they're positive, if the inflammatory milieu is a bit more aggressive, then DSA becomes a much more detrimental

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influence.
So, finally, how do we manage the patient with a failed allograft? And the two questions are: Should they remain on immunosuppression or should they undergo transplant nephrectomy? And if you look at that, you have to first, in terms of the immunosuppression question, consider -- and this is in a very gross fashion, the drugs that we have available to maintain immunosuppression in these patients. And what you see here are drugs with basically fairly pure T cell reactivity. Obviously there is some broadness as you move across the spectrum, and this is as close as we come to purely B-cell reactive stuff.
So that when you talk about maintenance of immunosuppression, you're basically talking about drugs over on this side of the equation that are predominantly T-cell focused drugs in terms of maintaining immunosuppression. But as has not been said here as eloquently as Kathryn Wood says most things, and this slide is borrowed from her, is that you don't have to have specific anti-B-cell therapy. Antihumoral therapy is not essential if the T cells are

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kept under control. The problem is that we don't keep the T cells under control.
So this is a study again from Cambridge looking at patients, again this same cohort of 131 patients with failed allografts who were sensitized. They look at the effect of maintaining the patient on no immunosuppression, fix that risk as 1, of developing significant DSA. Patients who were maintained on steroids alone, it had no benefit. Patients who remained on CNIs and steroids, there was a substantial reduction in univariate analysis here and risk of developing DSA. There was also a relationship to time since graft failure. And no statistical relationship at all of previous blood transfusions or pregnancy, again emphasizing the primacy of the transplant.
The study that really has impacted significantly approach to transplant nephrectomy was this one published in 2010. The investigators were from the Brigham, but this was USRDS data. And it basically showed that undergoing transplant nephrectomy reduced overall mortality and enhanced the rate of retransplantation, and they really did not look at

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specific sensitizing events.
This is the raw reduction in mortality from 36 to 32 percent, but when they adjusted for other variables, it was about a 30 percent risk reduction of mortality. And this basically has been used as strong evidence that transplant nephrectomy is beneficial in the patient with a failed graft.
You have some other data. This is recently published data from Berlin. This looks at patients, Group A, or patients who underwent nephrectomy and withdrawal of immunosuppression at the same time. Group B, patients who underwent nephrectomy but remained on immunosuppression. Group C is patients who underwent withdrawal of immunosuppression, but retained their grafts in place. And you can see that all three approaches were associated with increased sensitization, perhaps a difference there with Class II and the patients who remained on immunosuppression but had the graft removed.
If you go back to the Toronto series that was published as well, they looked at the effect on retransplantation of nephrectomy again, and again this

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is in the context of repeat mismatches, all-cause graft loss, and death-censored graft failure, and they showed that nephrectomy was actually associated, and this was not an endpoint in that previous study, they showed that nephrectomy was associated with an increased risk of all-cause graft failure and of death-censored graft failure versus patients with no nephrectomy. But, again, that seemed to be exacerbated, that risk was exacerbated in the patients who had Class II mismatches, again sort of emphasizing the primacy of Class II DSA in this scenario.
So to summarize this, the development of DSA is attenuated by immunosuppression. Not all DSA exerts adverse impact in retransplantation. And in management of the patient with a failed allograft, I have no firm answers to this. I think in terms of continuing immunosuppression, our practice has become to continue it, particularly if the graft is in place and if the patient is a candidate for retransplantation. If the patient undergoes allograft nephrectomy and is a candidate for retransplantation, many times we'll continue immunosuppression as well.

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I think that nowadays we've really gravitated to the approach to transplant nephrectomy, that only if it's clinically indicated, there may be some benefit of leaving the allograft in place, although it could, as I think is hinted at in the study by Ayus, et al., that the adverse effects associated with sort of a chronic inflammation milieu may have some negative impact there as well.

Thank you.

(Applause.)

DR. SAMANIEGO-PICOTA: The next speaker is Dr. Robert Montgomery, Director of the Langone Transplant Institute at NYU.

Welcome, Bob.

New Developments in Desensitization Protocols. Is There a Standard of Care?

DR. MONTGOMERY: Thank you. And good afternoon.

These are my disclosures. And specifically, I am going to be mentioning quite a few off-label drugs and focusing on the three that I listed here.

So it's pretty well established that patients

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who have an antibody-mediated rejection do poorly in comparison to control groups. And certainly when a patient is sensitized and being desensitized, they are at significantly higher risk of developing antibody-mediated rejection than other transplant patients. And so the results aren't as good for patients who have been desensitized.

But I think it's important to mention that we should be comparing apples to apples. So when we're looking at the outcomes of desensitization protocols, we need to compare those patients to options that are actually available to them. Okay? So if you're a patient who has a cPRA of 100 percent, receiving a compatible kidney has not been a realistic option, and that should not be our reference group. In other words, we shouldn't be comparing unsensitized patients to sensitized patients in terms of outcomes.

And this slide has already been shown, but basically the point I want to make here is that when you look at the other alternatives, which would either be remaining on dialysis or staying on the transplant list, there's a very significant survival benefit to

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undergoing desensitization.

So this is a cohort of patients drawn from 22 transplant centers in the U.S. And at the time that each of these patients were transplanted, they were matched with five patients who were either on the waiting list or on dialysis, and with sort of an intent-to-treat type of methodology, we watched to see what happened to the patients.

So when we first did our single-center study, we looked at this group of patients -- so these are patients who are on the transplant list who are eligible for a transplant -- and looked to see what happened to those patients, and you can see that there was a significant improvement when the patients were desensitized and transplanted versus staying on the list waiting for a compatible organ.

But when we actually drilled down to look at -- is that me or is that -- maybe it's the way the pointer is. But when we actually looked at this group of patients, only 16 percent of the patients received a transplant during that period of time. So, in fact, for 84 percent of your patients, the option wasn't

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between waiting for a compatible organ versus desensitization, it was staying on dialysis versus desensitization and transplantation. And so that's what this bottom line shows. And this has been reproducible in our single center and in this cohort of patients from 22 centers. There's about a doubling of patient survival at 8 years for patients who undergo desensitization.

Now, this slide shows the results of a study that was done at our institution many years ago. And what we did was patients who were desensitized, we looked to see whether their immunodominant antibodies were either eliminated or persisted after desensitization a month after plasmapheresis was stopped.

And what you can see is there is a difference between Class I and Class II in terms of whether the antibodies could be eliminated or persisted. So Class I antibodies were eliminated at a much higher rate than DQ antibodies and DR antibodies. And this may explain why patients who have Class II antibodies do more poorly because patients who are sensitized, after they

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undergo desensitization, are more likely to retain Class II antibodies than Class I.

And what we do know -- and many centers have demonstrated this now -- is that when you desensitize a patient, the strength of immunodominant antibody, DSA, going into the desensitization, determines to some degree the fate of the patient after desensitization. So patients who have higher level antibody, greater strength, at a CDC cytotoxic level do more poorly than patients who have antibody that's only detectable by Luminex.

And this is probably part of the explanation for why that is. So the way this is -- I'm afraid to use the pointer now -- but the way this is set up is that -- so these are patients who are desensitized who had a negative flow crossmatch but had detectable antibody by Luminex. These are patients who had a positive flow in a negative CDC. And these are patients who had a positive CDC. And this is roughly correlated with MFIs down at the bottom. And what you see is that the rate of rejection is about twice as much for patients who have stronger antibody.

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Now, one thing that we showed in our original single-center paper is that even patients who have a positive CDC crossmatch going into their desensitization, we know they don't do as well, but they still do better than the alternative, which is either waiting for a compatible organ or undergoing dialysis during that period.

So a number of years ago, we came up with this concept. Again, we were trying to figure out a way to have patients who showed up with a live donor better served than to just desensitize them to their live donor. And so this concept of combining paired exchange and desensitization so that you have a pool of potential donors for a patient, and you match that patient to a donor that will give them the highest likelihood of a good outcome, which is the lowest level of DSA.

So in this three-way swap that we did a number of years ago, you can see that pairs 1, 2, and 3 all had CDC crossmatches with titers greater than 1024 to their original donor, their loved one, but by swapping the kidneys around, we were able to get two of them to

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a negative CDC crossmatch, and one of them to a positive crossmatch with a titer of 4, and then we desensitized that patient to that paired donor.

Now, there has been kind of a game-changer that's happened recently, and I think we should talk about the impact that the new allocation system may have on desensitization.

So this shows you the old system. The red line demonstrates that at a cPRA of 80 percent, greater than 80 percent, patients were given 4 points for their priority scoring. Now it's a graduated scale, and as you get closer to 100 percent, the curve gets exponential. And for patients who are at the very high end of that, they get a tremendous amount of benefit on the allocation scoring.

And this is the effect that that new system has had at 2 years. So for patients who have cPRAs of 99 to 100 percent, the total number of transplants that were done during that period, if you look at this very highly sensitized group of patients, they used to contribute about 2 to 3 percent to the total. Right after KAS was implemented, 17.7 percent of the

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transplants that were done in the first few months were in patients who had cPRAs of 99 to 100 percent. You saw this bolus effect. Now it's settled down to about 10 percent.

And if you look at the data from our institution, it's pretty interesting. So the current waiting list at Hopkins has about 1,300 patients on it. And there are about 164 patients who have cPRAs of 98 to 100 percent. Since the new KAS system, the number of patients that were transplanted with deceased donor organs that had a cPRA of greater than 98 percent were 66, and 64 out of the 66 had cPRAs of 100 percent.

So this new system is really only benefiting patients who have cPRAs of 100 percent. And when an organ with an unusual genotype comes out, you'll see a whole bunch of 100 percent patients listed, and you never get down to less than that.

And what we saw during the same period is the number of patients that we desensitized with live donors decreased.

Now, so let's talk about why this has really changed things. If you consider let's say I have a

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cPRA of greater than 80 percent, and that's the blue line, in this room, I could find somebody who I would not have any antibody against. Okay? So then why -- the red line on this graph is the likelihood of finding a match in a paired donation pool. So the likelihood for that same patient, me, of finding a match is less than 10 percent, even if you show me 300 different potential donors, right?

So why is there this big gap between the two? The reason is that common antigens are common in the population. Common antigens share epitopes with less common antigens. And so all the highly sensitized patients are looking for the same rare genotypes, and it's the competition that makes the transplant rate so low. But if you increase the pool, like we've done with the KAS system, you can increase the number of patients who find that rare genotype.

But the important thing to say is that that is going to change because we're just going to shift the patients. So there are patients with 100 percent cPRA who are relatively easy to match compared to other patients with 100 percent cPRA. So this is a spectrum.

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And then there are patients at the other end of the spectrum that you'll never find a kidney for because they require the rarest genotype. So we're going to shift those down so eventually we will enrich our population of highly sensitized patients for patients who are unlikely to find a match, and that curve is going to continue to come down. It won't come down to 3 percent, but it will come down. So the point is there is going to be a need to desensitize some of these patients.

So what's the standard of care? Well, there are basically two, and these are accepted by KDOQI, they're accepted by insurance companies, as being a standard of care. There is plasmapheresis and low-dose IVIG, which has been shown earlier, in which you desensitize the patient by doing every other day plasmapheresis, give 100 mg/kg of IVIG after each treatment, get the patient to a reasonable level of antibody, do the transplant, and then continue your plasmapheresis afterwards until you get to a negative flow crossmatch.

So the question has been, What is the role of

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rituximab? And this was mentioned earlier. Rituximab does not seem to be effective to treat patients who have antibody-mediated rejection -- and this was recently shown in a French study -- but it does seem to be fairly effective at preventing an anamnestic type of response.

So Howie showed some of the data from two of the papers, but this is another paper where we use the tetramers to look at B-cell frequencies. So these were patients who had donor-specific B cells but were not making donor-specific antibody. Okay? So they had these cells, memory cells, primarily, that were primed, but weren't making antibody.

And then we looked at what happened in the two groups, one that -- so on the left side of the screen, yes and no, is whether they made donor-specific antibody to those specificities after the transplant, and then red is whether they had received rituximab or not. So patients who did make antibody did not receive rituximab, patients who did make antibody did.

So there may be some protective effect of rituximab. And I think is shown well in the other

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standard of care therapy, which is a combination of IVIG and rituximab.

And as also mentioned earlier, this combination seems to be a lot more effective than IVIG alone. And this is data from Cedars showing that, again, this special soup of IVIG and rituximab produces better outcomes than IVIG alone.

Now, one kind of encouraging thing is that there is a lot of interest now in therapeutics in this space. And so I've listed here sort of the standard of care therapies and then different drugs that are being used, are being either used or tested, as add-ons to standard of care.

So I'm going to focus really on one of these because other ones have already been mentioned. So I'm going to skip over the eculizumab and talk about this new drug called IdeS.

So IdeS is an enzyme that's produced by Strep pyogenes, and it's kind of an evil enzyme in that it cleaves human IgG, and it's released in sort of a halo around the bacterium and prevents binding of human IgG antibody to the bacterium. And so it basically

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inhibits all the Fc-mediated activities.
It doesn't affect other antibody classes. It's species-specific, just human and rabbit. Figure that one out. And it cleaves and produces an F(ab')2 fragment and an Fc fragment, and this happens very rapidly. And the important thing to say is it happens across the entire space in the body.
So the way plasmapheresis works is that you remove the IgG from the vascular space, and then it has to reequilibrate because it doesn't do anything to the IgG that's in the interstitium, and that's why it's a very inefficient way to remove antibody, and that's why you wait 2 days in between treatments, so that reequilibration can happen.
This drugs knocks out all the IgG in the body, and it does it within 4 hours.
So here's an example. This is a highly sensitized patient. So in blue you see all the various antibody specificities and the strength when the patient was given a placebo, and then in red after they received IdeS. The same thing Class II antibody. So very dramatic effect.

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However, there is trouble in paradise, and the trouble is that the IgG rebounds, and it rebounds within about 14 days. And you can't give more than two doses because humans will make an anti-IdeS antibody, and the immune system reacts very strongly to this enzyme for evolutionary reasons.
So the study that we're currently doing is we're taking patients who are very unlikely to receive a transplant, and the FDA was very clear that if we were going to do this study without a control group, the patients had to be unlikely to receive a transplant and otherwise could not receive that organ because of a positive crossmatch.
So what we do is bring a patient in who has a positive cytotoxic or flow crossmatch. We give them a dose of IdeS. Two hours later, we recheck the crossmatch. If it's turned negative, we move to transplant. If it's still positive, we give a second dose of IdeS. We do the transplant. We give Solu-Medrol for 4 days because the half-life of the drug is about 4 or 5 days, and remember that it will cleave all of the induction drugs except for Atgam.

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And the other thing to mention, too, is that it doesn't only cleave IgG, but also B-cell receptors. So the B-cell receptors are all removed from the surface of the B cells. We use Campath after it's safe to do that in terms of the drug. And then we immunomodulate with IVIG and anti-CD20.
So I'm just going to give you an example of one patient that we did last week. And this was a 45-year-old who was on dialysis for 20 years and had a cPRA of 100 percent. So what we did is we eliminated all the unacceptable HLA antibodies with an MFI less than 20 percent from her profile. She still had a cPRA of 100 percent, so that's how sensitized she was.
We received an offer, 100 percent PRA offer, and this is the flow crossmatch both at the time the patient came in and then 2 hours after IdeS, so it had reduced the crossmatch significantly, but not eliminated, it was still a positive crossmatch. And the CDC crossmatch was positive at a titer of 8, so a very strong antibody.
And you can see here that this is the pre-IdeS, and these are the MFIs. So there was a A2

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antibody -- sorry, an A1 antibody at a titer of 24,000, and several Class I antibodies at very high titer. And then this is 2 hours after IdeS. So 24,000, went down to 10,000, and you can see the other antibodies. And then 48 hours, further decrease.
And this is 5 days pretty much down to a negative result. So she just got her Campath yesterday. We're starting her high-dose IVIG today. But, again, this is a pretty remarkable response to this new drug.
I can't tell you whether this is going to be effective or not, but what I can say is that reproducibly it's lowering the donor-specific antibody very dramatically after the patients receive the drug.
So there are lots of people who are involved in this work, and they're listed here.
Thank you for your attention.
(Applause.)
DR. CAVAILLÉ-COLL: Thank you, Dr. Montgomery.
Public Comment and Discussion
DR. CAVAILLÉ-COLL: We are now going to proceed into our public comment and discussion session.

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Before we go to the questions from the FDA, I would like to first go around and see if there are questions or clarifying questions for our speakers right now.

Dr. Knoll?

DR. KNOLL: I have a question for Howie. Is he still over there? Yeah. I just want to go back to that example of the male, unsensitized male, never transfused or transplanted with an antibody that's presumably from a pathogen, as we've been discussing.

So are those antibodies, do they have the same pathogenic potential as the same antibody with the same MFI, for example, from a previous transplant?

DR. GEBEL: That's a great question that I ask myself every day. And I don't know the answer to that. We treat them as if they do, but the way that they were generated is clearly different, and our readout is the fact that it has the same binding ability, but that doesn't mean that they're going to have the same pathogenic potential, but I don't know how to go about proving that it would or wouldn't.

DR. KNOLL: So there is no series of, for example, transplants that have occurred across

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antibodies that were presumed to be formed in this way where there was in fact a documented bad outcome?

DR. GEBEL: Not to my knowledge.

DR. SAMANIEGO-PICOTA: Just as an anecdote or as an experience, in a sensitized patient you have desensitized and have recurring urinary tract infections, develop osteomyelitis, those patients all experience a spike in their DSA. Many of them, it's not uncommon to see the patient who's compliant, had a bad infection 2 or 3 weeks before, presents with antibody-mediated rejection.

So in particular, patients with osteomyelitis are particularly difficult to treat because we know that infection in the bone, osteomyelitis, is a B cell mitogen. That's why all those patients used to develop amyloidosis in the good old days, stimulates the plasma cell.

So we're actually aggressive on those patients. If we don't think they're going to get better, we try to amputate those patients as soon as possible because at the end, it's going to be like the deli, right? The little salami stick. They're going

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to get amputated at the end, but by then, they have very bad rejection and are also losing their allograft. So I do believe they are equally, or not all the cases, but at least in my experience they are.

DR. CAVAILLÉ-COLL: Okay. Could I have the questions for the Session 2, the public discussion, please? Very well. Okay.

The first question we have is sort of a rhetorical one, but we'll see what the strength of the evidence is. How important is it to identify transplant candidates who have donor HLA-specific quiescent memory B cells, but do not have DSA? And should their induction or immunosuppression regimens be different?

Anybody want to attack that question?

DR. WOODLE: So, Marc, I think what we need is we actually need clinical correlation for these memory B-cell assays that if the assay is positive, what is the actual degree of risk that you have?

So even if you identify a patient that has increased risk, then the question is, What are you going to do posttransplant? And I can tell you what

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we're doing now in the absence of those.

We monitor intensively patients who are at high risk for memory responses, that is, a marked DSA response within the first 7 to 10 days posttransplant. And when we see -- and if their antibodies are negative, we will see epitope clustering and marching of those antibodies towards the 1,500 MFI cutoff, and we can often see that for 2 or 3 days before the antibodies ever exceed 1,500. We will treat those responses before they hit 4,000 MFI. And in the 18 months that we've been doing this, we have not seen a clinically overt AMR. We prevent any elevation in creatinine, and we intervene very early.

So our answer in our program is the intensive monitoring for epitope clustering in patients that are antibody-negative that you don't need a predictive marker, you're going to intervene anyway. I'd be interested to hear what Howie Gebel and others think about that approach.

DR. GEBEL: I think we just need more data, Steve. I mean, it's a good start. And the question becomes there has to be an initiation point. You're

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doing it.
DR. WOODLE: Yeah. So we think the answer is actually intervention and intensive monitoring. And so we presented this data the year before last at the ATC, and we'll be submitting the manuscript very soon.
DR. ROITBERG-TAMBUR: So I can speak for Northwestern a little bit. And when we are crossing historic antibodies, we usually add Rituxan just for good measure as we're doing this, but we definitely intensely monitor those patients, and the minute we see a spike in the antibodies, then treat it.
Those respond very well to treatment, and the antibody usually is gone, and you continue monitoring those patients, and it's not coming back. If you don't treat them right away -- and you've shown this in several publications -- the horses are out of the barn, it's very difficult to then stop the response.
DR. WOODLE: Yeah, that's exactly what we think. We think actually the earlier you catch an anamnestic memory response, the better it responds to therapy.
DR. CAVAILLÉ-COLL: Thank you. I think you're

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talking about interventions after transplantation. Is there anybody who wants to speak about any testing or any results before the transplant that could be used to identify these patients who may have quiescent memory B cells?
DR. MONTGOMERY: Well, the tetramers are very effective. The problem is that there is a very limited number of specificities that we have tetramers to. So that's the downside of that.
Anat?
DR. ROITBERG-TAMBUR: Javeed Ansari, transplant nephrologist from Northwestern, is actually using the One Lambda single-antigen beads in a B-cell assay, I think it's 12, 13, whatever number of colors on a flow cytometry, so it can actually qualify at what stage of development those B cells are. And he has some interesting data, and I'm part of those studies, but I do see significant limitation of those studies.
What you can see is the whatever, 40 cc's, 100 cc's of blood that you're testing, and you don't really know what else is going to be there, those are rare events. So if you get a positive response, you know

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that you have a B cell that might have the ability to produce those antibodies. If you don't get a positive response, it doesn't mean that you don't have those cells. And I think that's going to be problematic with all the cell-based assays, not to mention those that are at different niches, et cetera, et cetera. But I think there will be tools that at least can give you a positive predictive value, not a negative predictive value.
DR. MONTGOMERY: One approach that we've adopted with our desensitization is that patients who have repeat mismatches, we just treat those patients with rituximab in addition to plasmapheresis and IVIG.
DR. SAMANIEGO-PICOTA: Yeah, obviously, rituximab will be the drug that will be more effective, at least what we have right now, in elimination of memory B cells. However, something that I learned recently coming from the Pittsburgh group is that many of the transitional B cells, although a very small population in peripheral blood, have a high expression of C20. And transitional B cells are essential for the development of B-cell tolerance at the level of the

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spleen.
So the question would be we can still use the rituximab as an induction or as a -- we used to do in Hopkins a week or two before the transplant, because in that case, by the time the transitional cells are moving from the bone marrow to the periphery to the spleen, anti-CD20 hopefully will be out of the way, and that subset will not be depleted.
The question with rituximab continues to be, When is the right timing to give the drug? At what period of the transplantation history is the best time? And only by doing these peripheral studies and using different combination of drugs, we're going to be able to learn what is really happening in these patients.
DR. GEBEL: So, Millie, also in regards to when is the right time to give Rituxan, I think an alternative question is, When might be the wrong time to give it? I went to a CIAT (ph) meeting recently, and I think it was somebody from Anil Chandraker's group who tried to use Rituxan in patients to prevent AMR and compared it to placebo, and actually the patients with the placebo had less rejection, as if you

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were getting rid of a regulatory B cell.
DR. SAMANIEGO-PICOTA: Yeah. These products of rituximab is very well known. Clatworthy described it in the New England Journal several years ago. Those patients treated with rituximab actually had more T-cell-mediated rejection. But then the Scandinavians and the Japanese that may give it at a different timing, those patients have less incidence of cellular rejection even when they do not have a major impact in incidence or lack thereof antibody-mediated rejection. So we really do not know the right timing.
And there is a very old paper now from Francis Larned (ph) about the use of rituximab and what has been learned with rituximab from the lupus and the autoimmune diseases trials in which many of these patients actually did better, not because of any effect that rituximab would have in antibody production, but in modification of T-cell responses. The timing is when we're going to have it. When we have the B regulatory cells or the B effector cells, I can leave that to Anita Chong to talk a little bit more in detail tomorrow, but the timing of giving the drug seems to be

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important.
DR. CAVAILLÉ-COLL: Dr. Haas?
DR. HAAS: Yeah, I actually had a question for Bob, and this actually is not one of these two, but concerns sort of the combining of paired exchange and desensitization.
Do you specifically pair exchange away from DR mismatches or Class II sensitivity, Class II DSAs, even at low titer? So if you were faced with a patient who had a low titer anti-Class II against their donor versus a high titer, even a positive cytotoxic anti-Class I, would you pair exchange away from those and go ahead and try and desensitize against the high-titer Class I given that the Class II is more likely to persist and cause TG?
DR. MONTGOMERY: That's a great question. I would say that our selection of a donor is based primarily on the strength of the antibody rather than the class of the antibody, but it is noted when we're reviewing whether this is a good candidate for that patient, whether it's all Class II antibody, and we try to avoid that, but it's not formalized in our

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algorithm. Basically what we do is we decide what level of antibody we're willing to desensitize to, and then we drop out all the unacceptables that have antibody at that strength and below.
So I think your point is very good. That probably should be part of the decision-making process.
DR. SAMANIEGO-PICOTA: Bob, I have a question for you about your IdeS protocol. Why Campath and why CD2?
DR. MONTGOMERY: Why?
DR. SAMANIEGO-PICOTA: Campath.
DR. MONTGOMERY: And what was the second one?
DR. SAMANIEGO-PICOTA: In the CD2.
DR. MONTGOMERY: Oh, right, right. Okay. Well, you know, it was primarily driven by, you know, when you're doing a study, a multicenter trial, you have to compromise. And Stan's group was very committed to alemtuzumab, and the other alternative would have been to start Atgam earlier because Atgam is not cleaved. But, anyway, we compromised, and we decided on this.
I think the important thing about the protocol

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is that you're immunomodulating the patient's immune system in a quiescent state. So when we do these transplants where there is significant amount of donor-specific antibody, you get the innate response from the transplant itself, from transplant injury. And then you get antibody injury, you get a tremendous amount of endothelial disruption, and then this thing sort of spirals out of control.
When we go in with no antibody and we're able to maintain that for a period of days to weeks, I think that this approach, at least philosophically, seems to make more sense.
Stan and I, both of our fathers were World War II pilots, and Stan's dad used to always say it's easier to repair an airplane on the ground.
(Laughter.)
DR. MONTGOMERY: And so I think to some extent that's what we're doing with this protocol, we're intervening at a time when there's not a tremendous amount of inflammation, and we'll see what happens. But this is the most exciting drug, this is the most exciting thing I've seen in this field in the last 15

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years. So we're hopeful.
Bob?
DR. COLVIN: Bob, what happens to the F(ab')2s? Do they stay in the circulation? They might be very good blocking reagents for that very same patient later on.
DR. MONTGOMERY: Yeah. I mean, it's -- so there's that thought, and then there's also the idea that you still will get binding of the fragment of antibody to the endothelium, it can't activate complement, but it may be able to induce endothelial apoptosis or injury -- a la Elaine Reed's work -- and so it may not completely eliminate inflammation, but your thought is an interesting one as well, yeah.
DR. SAMANIEGO-PICOTA: Steve Woodle?
DR. WOODLE: So, Bob, we're following that work kind of closely. And there are a couple of things that came to mind when we first looked at it. One is, as you mentioned, the lattice formation that a F(ab')2 can form with a Class I or Class II complexes on the endothelium.
The other one is when you look in the papers

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that have been published, and you look at the western blots representing the degraded protein fragments, it looks as if there are actually fragments that are smaller than light chains, suggesting that maybe the enzyme doesn't just stop cleaving at the points that you pointed out, but that it can further cleave the light chains and heavy chains.
And I wonder, if you cleave several grams of protein suddenly in a patient, will you not create a situation similar to that seen in myeloma nephropathy, where you have tremendous amounts of protein that hit the kidney that cannot be cleared at once? Indeed, there is some literature suggesting that you get a significant proteinuria within hours after administration of IdeS. So the question I have is, How are you looking at that and what does your protocol include as far vis-à-vis the FDA in terms of looking at injury to graft resulting from this massive protein degradation?
DR. MONTGOMERY: So I haven't seen those data that suggest that there is ongoing cleavage because this enzyme is very specific for the hinge region. And

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if you look at the westerns that I've seen anyway, you just see two bands. You see F(ab')2 and an Fc, but you may have seen data that I haven't seen.
So the FDA was very worried about this as well, and so we're doing 24-hour creatinine clearance with protein measurements for the first 9 days after the transplant, which it's not as bad as a biopsy, but it's still very difficult to accomplish. But that's what we're doing.
DR. CAVAILLÉ-COLL: Dr. Haas?
DR. WOODLE: Yeah, one other question, Bob. How long do you think the IdeS molecule is enzymatically active after single-dose administration?
DR. MONTGOMERY: So it's about 3 to 4 days.
DR. WOODLE: And that's why you delay administration of any IVIG or any monoclonal or polyclonal antibody, therapeutic antibody, for that many days.
DR. MONTGOMERY: Yes. Right.
DR. CAVAILLÉ-COLL: Okay. Dr. Haas, please.
DR. HAAS: Steve, in answer to your question, I have looked at some biopsies of patients who have

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received the IdeS, and I haven't noticed anything that resembles like a myeloma cast nephropathy. I haven't specifically looked at protein reabsorption droplets in the tubules, but the light chains, you know, they specifically combine with the Tamm-Horsfall protein, and certain pHs and interactions of the light chains with the Tamm-Horsfall proteins are important. And there are certain light chains that are tubulopathic and there are certain light chains that are not tubulopathic, and it has to do with the property of the light chain.
So just having a lot of proteinuria per se due to cleavage of the immunoglobulin wouldn't necessarily in itself produce a cast nephropathy, although you would have to have sort of a tubulopathic light chain to really do it. And maybe it's important to first study the light chains of the DSAs that you're trying to cleave to make sure that they wouldn't qualify as a tubulopathic light chain. But I haven't seen it in any of the biopsies.
DR. WOODLE: Yeah, I just wanted to correct what I had said about the westerns. The actual data is

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from IdeS cleavage of IgG in human blood from one of the IdeS articles. And there is actually further degradation of the heavy chain, but not the light chain. I misspoke.

Mark, I think that's reassuring data, but I think that -- do you think urinary pH plays a role?

DR. HAAS: Yeah, I do. Certainly it does in cast nephropathy, that patients whose urinary pH plays a role, concentration plays a role, dehydration certainly plays a role. We see cast nephropathy very often in people who are dehydrated. So maintaining strong hydration during IdeS therapy would, if there's any kind of a risk, would decrease that risk.

DR. WOODLE: Are we getting too far off the path and going on for too long? Because there are other questions we could go on with.

DR. SAMANIEGO-PICOTA: Any other questions?

DR. WOODLE: So you could alkalinize the urine is another thing you could do?

DR. HAAS: I don't know. I would have to actually look that up. But hydration and urine pH --

DR. WOODLE: Renata, I just wanted to bring up

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one additional point, and Bob got to this, but there has actually been what I think is an important advance in looking at the transplantability of patients with a cPRA of 100. And this is a paper by Marcelo Pando Rigal. It's been published in Human Immunology just within the last months. It may actually be in press. It's actually early, early view.

But what he did was he calculated -- so at UNOS, with a calculated PRA, once you hit 99.5 percent, you're automatically rounded up to 100. And what he has done is taken what he calls a decimal PRA calculator where he actually calculates the PRA up to six digits rather than the traditional two, with cPRA, but actually four that's available with the UNOS CPR calculator on the Internet. And so what it can do is it can calculate chances of being transplanted up to 1 in 1 million.

And if you look within the 100 percent -- so one of the things he did in the paper is he looked within the cPRA 100 percent population in UNOS and looked at -- I can't remember exactly how many patients it was, I think it was between 1,000 and 2,000. Most

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of them are clustered near 99.5 percent, which is about a 1-in-200 chance of actually finding a match, but there are others that extend out to 1 in 1 million.

So within that population of a cPRA of 100 percent are patients who are very transplantable under the old allocation, very much more transplantable under the new KAS, but there is still a population, very importantly, who are virtually untransplantable.

And so what he then did was took a number called "number of patients required to match." We have been applying that number for the past couple of years in the context of our IND-approved carfilzomib protocol as an endpoint for desensitization. We actually think it is the endpoint that the agency should view now as potentially the preferred endpoint for desensitization, that is, the reduction in the number of donors required to match with desensitization. It is sensitive, it is powerful, and it far exceeds the old cPRA data.

ATTENDEE: Hear, hear.

DR. ALBRECHT: So just to follow up, has that been corroborated by others or do you --

DR. WOODLE: So this is really new.

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DR. ALBRECHT: Right.

DR. WOODLE: It's really new --

DR. GEBEL: It's not that new, Steve. Last year I published a paper with the SRTR where we modeled 2010 data, and basically out of the 5,000 people who were in the 100 percent category, if you allocated all the organs that were transplanted to that group starting 100 percent and then it went down to 99, 98, what you found -- and you would allow every organ to be offered to every patient.

So as a group, it turned out that the 100 percenters had an average of three offers per individual. However, if you broke those down, there were 3,700 people out of the 5,000 who were actually able to get a median of six offers, an average of 17, but there were about 1,300 patients who didn't get a single offer. And if we look at those patients, those patients were all over 99.9. And more recent data from Nicole Turgeon and UNOS, the Kidney Committee, has shown that it's 99.95 which is the cutoff.

So I believe Marcelo's data, no question about it, but it's not unexpected.

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DR. WOODLE: Yeah, so 99.95 is 1 in 2,000, which when you look at the number of donors that are out there per year in the United States, and break it down by blood group, that's an offer per patient, actually one offer or less per patient. And so --

DR. GEBEL: It's 99.95, not 99.5.

DR. WOODLE: Oh, I'm sorry, I'm sorry.

DR. GEBEL: 99.95.

DR. WOODLE: So it's 5 in 10,000.

DR. GEBEL: Yeah.

DR. WOODLE: Okay. So, but the point is that these are actually -- not only that, but these are kidneys that are shipped, and they usually have several hours of cold time, and you don't know about the quality of them. And so if you really need a high-quality kidney with short cold time, these patients are still at desperate risk to find a matchable donor.

I mean, the point is, is that there are better ways than cPRA that are out there. They're emerging. They aren't currently existent. But calculating the change in the number of donors required to match before you desensitize and after you desensitize is actually

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the direction which we think the primary endpoint of desensitization is going to move. It's not there yet, but it's moving.

DR. ALBRECHT: Thank you. So we look forward to hearing more on that topic.

DR. SAMANIEGO-PICOTA: So, Bob and Steve, if there is anything new in desensitization treatments, would you say, in addition to IdeS?

DR. WOODLE: I think Bob's data with IdeS is great. I mean, he and Stan are really forging a pathway to that agent, and I think that it's something we've really got to watch.

I would mention that in the proteasome inhibitor work, that there's a lot going on. I'm going to talk a little bit about it tomorrow. I specifically wanted to mention Stuart Knechtle's recent primate work published in JASN that indicates that there's a strong proliferative response that's associated with proteasome inhibitors that may explain the rebound.

I don't know, Stuart, if you want to talk a little bit about that.

DR. KNECHTLE: Sure. I'll be talking about it

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tomorrow, of course, although it is the last talk of the day tomorrow, so --

(Laughter.)

DR. KNECHTLE: We've just shown mechanistically in a non-human primate model that proteasome inhibitors actually activate the lymph node germinal center. So BAFF levels are increased, IL-6 goes up, and so we are proposing that dual targeting is a better strategy if you want to have a durable effect of reducing DSA with proteasome inhibitors or any other means of targeting of plasma cells. So that's turned out to be a useful model.

Thanks, Steve.

DR. MONTGOMERY: I would say the anti-IL-6 receptor blocking antibodies are interesting. The C1 INH, the C1q inhibitor, because it's blocking the pathway to a much more proximal level before the anphylatoxins are being produced, which are not blocked by C5 inhibitors. And there's a lot of work going on there right now.

And I think probably what we're going to end up with at the end of the day is some combination of

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the standard of care therapy with these add-on therapies that will produce better results.

And I think also trying to identify the patients that are going to most benefit from desensitization versus those who are likely to be matched with the KAS system is going to be a really important contribution.

So I think -- I mean, I have to say I'm more optimistic that we're getting a better handle on this now than I was 5 years ago, where we just seemed to be doing the same thing over and over again. And there were some centers, referral centers, that had a lot of experience, that that experience was difficult to translate and to proliferate to other centers, and a lot of it was kind of anecdotal, but those centers had pretty good results.

I think now we're starting to develop some tools that are going to be able to be used by a wider audience because they're more effective.

DR. SAMANIEGO-PICOTA: Dr. Djamali?

DR. DJAMALI: I have a question for you guys. How do you handle a slight decline in PRA from 99

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percent to, let's say, 97 or 96, and then losing their priority with the Kidney Allocation System?

ATTENDEE: Don't let them lose priority.

ATTENDEE: Right.

DR. MONTGOMERY: You can always find some low-level antibodies that you might otherwise have eliminated. And there are groups, I think Emory, you know -- certainly I know UCSF, they list all the unacceptables regardless of the strength, and they're still getting a lot of organs, so that it depends on your comfort level at your institution. If you want to list all of the unacceptables, you're less likely to have your patients match, but you're probably more likely to have a better outcome. And the places that are really comfortable with desensitization would probably be a little more supporting and allow flow-level antibodies, and we'll see how that turns out.

But clearly what we know is that if your patient is not at 100 percent, they are very, very unlikely to get an offer. Now, that may shift as we -- the bolus effect, you know, if you think about it, we're going to transplant all those 100 percenters who

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are easier to transplant, and then we're going to be left with this group of patients that are 1,000 percent cPRA. And we probably will start to shift down then to patients who are at the 99, 98, 97 percent, because this is competition. That's all it is. You run the 100 percent list on a rare genotype that everybody is looking for, and you get like 70, 100 percenters who match, and they're listed by wait time and everything else.

It's never going to get down to that 99 percent patient. But that's going to thin out those -- those 100 percent patients are going to thin out, and so then I think it's going to be less important. But right now, having your patient at 100 percent by changing the MFIs that you count as unacceptables is the key.

DR. WOODLE: Yeah. So if you have no living donor, you game the system. Okay? And the idea is to get the patient --

DR. MONTGOMERY: I don't think that's gaming the system.

DR. WOODLE: Well, I think it's gaming the

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system when you -- you want your patient at 99.5, transplantable at 99.5 percent, because that gives them national priority, a cPRA of 100, and gives them a 1-in-200 chance of finding a donor. Okay?

So ideally, even if you remove an antigen, you can declare it nonacceptable and still have it count towards the PRA. So if you overshoot with desensitization, you take somebody that's got a decimal PRA that gives them a chance of 1 in 10,000, let's say you overshoot and they get to 98 percent, I would have unacceptables in there where that patient would be at 99.5, and that's what's going to get them transplanted.

And that's gaming the system to some degree, but it's actually improving their chances of being transplanted, and at the same time, keeping national priority.

DR. MONTGOMERY: It's really -- you know, it's establishing your threshold for positivity, and there are no regulations about that right now.

DR. WOODLE: Exactly.

DR. MONTGOMERY: So when I came to NYU there were like 10 patients with cPRAs over 90 percent, and

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our list was like 350 patients. I was like, how is this possible? This is crazy. And so then I discovered that they had put their benchmark, their threshold, at an MFI of 10,000, right? So, you know, we dropped it down. I don't see that that's gaming the system, that's just smart -- you know, that's just understanding -- and, again, it's also understanding the system, but also your capabilities and what you're able to do and how likely are you able to rescue a patient, be able to rescue a patient who gets into trouble after one of these transplants?

What I'm really looking forward to are the data on outcomes. Now, the 2-year data, which I've just looked at, actually looks pretty darn good for these 100 percent PRA'ers in terms of graft loss.

But it's going to be interesting as time goes on to see how suddenly you've got all these patient who have, like this patient, been waiting 20 years, and they have all sorts of comorbid conditions. They haven't been seen by a doctor in years, right? And suddenly they're getting offers. It's frightening actually some of the stuff you see. And I can't

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believe that it's not going to have an impact on outcomes.

DR. CAVAILLÉ-COLL: Thank you. I think Stuart Knechtle has something to say.

DR. KNECHTLE: I just wanted to ask the panel for -- I think it was Mark Stegall who basically discussed this notion of clinical trial design with ongoing modification, and it's basically to try to get at this tough problem. We're not about to do controlled clinical trials of no therapy versus therapy in this area. This is a very difficult area. Here we are with the FDA and this is a good time, I think, to discuss, "How do you take novel agents?"

Bob, you told us about IdeS. We've got some promising results in the non-human primate that we would like to move into human clinical trials. The typical way is you do a couple of patients. You showed us a case report. How do we move beyond one to five patients into a rational design of a novel and high-risk therapy?

DR. WOODLE: So I'll take a stab at it because this was part of the talk that I had yesterday. So my

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talk was on progress in AMR. And in particular, I found the eculizumab trial in the U.S. an outstanding example of how old traditional methods can fail a good drug that's effective. And I know this will be repeating some things, but I think it's important for industry to hear how I think some of us think about that trial.

That trial was designed based on Mark's incidences, historical, based on a flow crossmatch of 300 or greater, with control, basically IVIG desensitization,, to give you an AMR rate of about 40 percent. The estimated effect of the drug was to reduce it to 10 percent. An old tired traditional power calculation was done, and the number of patients was established.

The trial was conducted. Enrollment was slow, very typical for an AMR type of study, and the expanded center is still low. I think there was internal pressure. I'm very careful about what I want to say here. But the inclusion criteria were altered, and they were lowered so that patients with lower barriers of antibody were let in, and that lowered the rate of

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AMR in the controls such that when it reached enrollment, the treatment difference didn't meet the predefined expectations, and it didn't match up to the power calculation.

That trial, had it been conducted under an adaptive trial design where the number of patients enrolled could have changed, would have led to an FDA recommendation for approval of that drug. Instead, now the drug is dead in transplant -- okay? -- in the U.S. right now, at least for that indication. The company, I think, my impression is that it's been abandoned.

I would love to see that trial resurrected under an adaptive trial design, allowed to extend enrollment, and then possibly lead to an indication.

I can tell you right now in my hospital, if I have atypical HUS and I have a kidney that's shutting down, the biopsy looks like it may be TMA or something like that, I can get eculizumab no problem because there is no concern about payers paying for it. But if I've got a kidney that's threatened to either rupture, it's oliguric ATN, it's a threatened rupture, the patient is going to lose the graft, the head of the

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Pharmacy and Therapeutics Committee has to approve it, and I have to beg, borrow, and steal, and then he calls my chairman and says, "Woodle wants to order this expensive drug that's going to cost the health system $400,000. Do you approve it?" And I can only get it maybe for one patient a year or two.

That's the reality we live in. And it's because -- and I think that FDA bears some responsibility for the field being in that position with eculizumab because we need adaptive trial designs and we need more innovative ways. But I think -- and I would be interested in what my colleagues have to think about it, but that's the personal way that I view what happened with that drug in that trial.

DR. MONTGOMERY: Well, Steve, you explained that perfectly. I think that, you know, the -- so the acute AMR rate was the same at the Mayo Clinic as it was in the Phase 2/Phase 3 trial, but it was the control group that was different. And I think the problem with eculizumab is it got a two-punch effect, and that's that it's the TG data from the Mayo Clinic.

Now, the thing about the protocol -- and I

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argued with Alexion forever about this -- is that they didn't have -- so the patient would get desensitized, then they would get the drug, and then they would just get observed, and most of the patients went into the transplant with significant amounts of antibody.

And the problem is if you allow large amount of antibodies to circulate over a very long period of time, even if you have a complement inhibitor on board -- and remember most of these patients it was stopped after the first month or two -- you are going to get TG, right? So I think a much more rational approach would have been to make sure that the antibody was lowered to some threshold after the transplant while the patient was on eculizumab.

The problem with that is you have to redose the drug after each plasmapheresis treatment. Nobody likes to do that. It's an expensive drug. Nobody likes to pull it off afterward. But that, at least in our experience, if you can lower that antibody and sort of protect the endothelium during that period, you get a good outcome and you don't get TG.

So it's going to be hard to resuscitate

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eculizumab because there are two reasons not to -- there are three reasons not to like it.

DR. ALBRECHT: So could Dr. Bill Irish and then the gentleman at the mic, and then Dr. Colvin.

DR. IRISH: Yeah, so I'm going to talk about unique design strategies tomorrow in a little bit of detail, but the adaptive design, that's been well vetted statistically for a while now, but those designs are complicated. They're complicated analytically, and they're subject to a certain level of operational bias. So flow of information has to be protected when you're doing an adaptive design.

So the operational components of that are much more complicated. But there is certainly a viable strategy, especially for studies in which you have a rare condition.

DR. SAMANIEGO-PICOTA: Gentleman, Dr. Dixon Kaufman.

DR. KAUFMAN: Thank you. Dixon Kaufman, from the University of Wisconsin. We're hearing about some really innovative new therapies. And I have a question for the investigators. And as you are thinking about

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where the field is going and new opportunities and some of the great new biologics that are being developed in other areas, can you speak to whether you are finding any obstacles or lost opportunities because of the complexities of trying to get drugs developed for one particular area and trying to then apply it to this particular relatively high-risk area that precludes you from designing trials with some of these really incredible new agents?

DR. MONTGOMERY: I would say actually there is a tremendous amount of openness right now on the part of PhRMA to address this unmet need. And the difficulty is the studies, and the difficulty with the studies is enrollment. And, again, I think if this group can talk in more detail about how to overcome the fact that we're dealing with a relatively speaking rare event at any transplant center, and how to really design an effective study when that's the case, PhRMA is really open to advancing these drugs. So I think that's the problem.

DR. KAUFMAN: Is that kind of a universal experience by some of the other investigators? Steve,

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Arji or others that are --

DR. WOODLE: So one of the problems we've encountered is that -- and this one is certainly understandable -- is that when a drug gets FDA approval, for example, for cancer in an expedited pathway, and it's a nonrandomized trial, you can forget it until the randomized trial is completed. Okay? They really -- the companies are very, very worried that we will see a toxicity that will jeopardize them before they meet the requirements for full approval.

And if there is some way that that level of toxicity can -- that companies can be reassured that if toxicity is seen in another population, it won't count against the population they're really interested in, that might help us. But when I sit back and look at the number of drugs being developed in oncology, and we look at the number of drugs that are being developed in autoimmune disease -- RA has had 10 drugs approved in the last decade or so, we've got 1, every one of them was looked at -- almost every one of them was looked at transplant -- what you're seeing and what you're hearing here, most of what you've heard today, except

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for IdeS, is a drug that's being taken from another area, another franchise area, and moved into transplant.

We're begging, borrowing, stealing, going to PhRMA on our knees begging to get a small pilot to look and show feasibility in a small pilot trial. If I can get $250,000 to do a small pilot, I feel lucky. But that's where most of what you're hearing today and the excitement is, is drugs being brought over.

And young people that don't have track records and don't have street cred that some of these guys have, have no chance of getting drug from those companies. If you walked in and you've got a CV and you've shown you've been doing this for 20 years, you might can do it.

And so where it really hurts is young investigators, much more than it hurts the senior investigators. And as we get old and retire, I don't know what's going to happen.

DR. SAMANIEGO-PICOTA: Dr. Colvin?

DR. COLVIN: Thank you. I want to get back to the eculizumab trial. And a lesson that I think we can

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learn from that trial, and it has to do with personalized medicine, at the Banff conference we heard a postdoc analysis of their studies with eculizumab that indicated that two things predicted the response to eculizumab: C1q fixation by the antibody in vitro and a five gene set from the renal transplant biopsy.

And the lesson I think that makes is that we need to incorporate exploratory phenotyping, if you will, of our patients into these trials so we can identify subsets that may respond to these very, very specific agents.

DR. SAMANIEGO-PICOTA: Dr. Knechtle?

DR. KNECHTLE: I just wanted to follow up on Dixon Kaufman's question because there's an obvious example of what he's talking about and that I'm faced now. So one of the agents I'm looking at is an anti-CD40 monoclonal antibody that's currently in Phase 2 as well as Phase 3 trials, and to put that into a novel indication right now despite very promising preliminary data is unacceptable to the company that owns it because that would potentially result in data that has to be disclosed and might be negative. So as far as

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the company is concerned, no go until they're past their Phase 2/3 clinical trials. I think most people in this room probably are aware of that challenge. But that's a reality. You don't --

DR. SAMANIEGO-PICOTA: Dr. --

DR. KNECHTLE: -- a drug in a novel indication.

DR. SAMANIEGO-PICOTA: Sorry.

Dr. Djamali.

DR. DJAMALI: I would like to echo what Steve was saying. It is extraordinarily complex to combine therapies involving PhRMA with different drugs. It makes mechanistic sense, and you all agree that we need more than two or three agents to handle these kind of complex patients. But try to get three, two, companies collaborate, and sometimes with the NIH, to get the paperwork done, it's just incredible. That's why when you see the studies that we propose or we demonstrated here, to have 10 patients for the vast majority of time, and that's the sad part.

So I think it's going to be tough, but we need support from you guys and PhRMA, if they are here, to

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conduct good, strong mechanistic studies.

DR. SAMANIEGO-PICOTA: I just have a question for the group, and this will be the last Marc tells me. It's time to go on a break. What about the creation of consortia specifically focusing at the combined academic-pharma enterprise looking into this?

I worked with consortia now for about 15 years, and although it takes time, at least with CTOT-09 and CTOT-19, the group of investigators developed the protocol, everybody was on the same line. Although recruitment could be a little painful, recruitment is actually going. So I do not know if you guys think that it's probably time to get a couple of consortia groups prepared and put together for that.

DR. ALBRECHT: I would like Inish O'Doherty to answer that one.

DR. O'DOHERTY: Hi. Inish O'Doherty, from the Critical Path Institute. And in answer to your consortia question, so we've started the Transplant Therapeutics Consortium, between is between the AST and ASTS, and we have eight industry members already. We have our first face-to-face coming up in May. We're

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selecting working group topics to work on. Obviously, there is plenty in the field that can be put forward as a topic, but we're trying to limit our scope to things we can achieve and hope to have success in the first couple of years. I'm happy to share more information as we progress in that.

DR. ALBRECHT: And just on behalf of FDA, as you know, as Dr. Mannon summarized, we've been holding a number of open public workshops trying very much to sort of talk about these areas of unmet need. And the role we have is, again, we are the regulators, and we meet with individual investigators, with companies, under IND meetings, provide advice, help talk about protocols.

I think Dr. Woodle identified a problem that is not new to us, that we see that very good protocols are designed, and then for whatever reasons, they get modified, and sometimes they succeed, and sometimes they don't. And so we're very aware of these challenges and continue to try to provide both public venues as well as through the IND route to discuss these topics. Thank you.

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DR. CAVAILLÉ-COLL: Well, thank you. With that, let's break for 15 minutes and come back here at 5 before 4:00. Thank you.

(Break.)

Session 3: Factors Contributing to Antibodies in the Post-Transplant Period

DR. BELEN: Hello, everyone. This is our third and final session. I'm going to moderate this session along with Dr. Anat Tambur. And my name is Ozlem Belen, and I'm from the FDA.

The name of this session is "Factors Contributing to Antibodies in the Post-Transplant Period."

And our first presenter is Dr. Millie Samaniego, from the University of Michigan. She is going to present, "The Choice of Induction and Maintenance Immunosuppression and their Impact on Preexisting and De Novo Antibodies."

Okay. Dr. Millie Samaniego.

The Choice of Induction/Maintenance Immunosuppression and their Impact on Preexisting and De Novo Antibodies

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DR. SAMANIEGO-PICOTA: Thank you. I want to thank everyone and the FDA for the invitation to this symposium.

These are my disclosures. I don't have any conflicts, but I will be talking about many drugs used off-label. The whole talk is about off-label use.

About 5 years ago, there was a meta-analysis published in Transplantation by Roberts and colleagues using the grade system to assess the quality of the different papers published on the topic. And the goal of the meta-analysis was to analyze all the drugs that had been published as effective drugs in the treatment of antibody-mediated rejection, and these are listed here on this column.

And if you look based on the grade system, the evidence supporting the treatment, only plasmapheresis, plasma exchange, and immunoabsorption, according their methods, had enough scientific background to be used as therapies.

Unfortunately, we know that although this is certain, many of these drugs also have an effect, some in a greater extent than others, but all of them had

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been published at having some kind of beneficial results in patients with antibody-mediated rejection.

This meta-analysis was also interesting because it shows how it has changed the interest in drugs treating antibody-mediated rejection throughout the years. And what is more interesting is that you see that tacrolimus, that you cannot see here very well because of the way the slide projects, one of the drugs that although we know today have a major role in preventing the development of de novo antibodies, was considered to be in the bottom of the pile.

Now, if one goes to a pharmacology class, a transplant pharmacology class, all of the drugs that we use today in the management of antibody-mediated rejection, early or late acute antibody-mediated rejection, have an effect at least theoretically and should have some benefit theoretically in the outcomes of these patients, yet in real life, in practice, that does not happen all the time.

For the purposes of this lecture, I am going to include primarily induction agents, belatacept, alemtuzumab, ATG, and talk primarily about mTOR

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inhibitors and CNIs, will talk briefly about rituximab, and I have left bortezomib to the expert speakers tomorrow so they will have more time to present their data, and hopefully it won't happen what happened to me where all my slides have been shown already this morning.

So primarily the induction agents are being used in antibody-mediated rejection with the goal of suppressing T-cell responses. From that standpoint, we have the depletional and the non-depletional agents, agents that can be used in the treatment of antibody-mediated rejection as well as the prevention of it, and we also have agents that use primarily as depleters of antibody-producing cells, or their precursors.

Thymoglobulin. Thymoglobulin is an interesting drug used off-label, FDA approved for the treatment of rejection, not approved for the -- that we use as an induction agent. However, it's the most commonly used induction agent in the United States.

This is a study that my friend Arjang Djamali published now 3 years ago in Transplantation. This is a single-center retrospective study in patients that

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were transplanted between 2009 and 2011 with a presence of donor-specific antibody with MFIs within this range. Their flow crossmatch prior to transplant was negative. And patients were either treated with rabbit ATG or basiliximab on the basis of the surgeon that was in charge of the patient at the time of admission.

All patients received TAC, MPA, and prednisone-based immunosuppression. And the goal of the study was to look at the difference in these two groups in cellular rejection, antibody-mediated rejection, and development of donor-specific antibodies.

The two figures that we're going to see right now are logram (ph) analysis of the incidence of antibody-mediated rejection in these two groups, and it's clearly evident here that patients treated with ATG had a much lower incidence of acute antibody-mediated rejection than those treated with the interleukin 2 receptor antagonist.

Interesting as well, we see that the development of donor-specific antibodies in the ATG-treated patients is lower than what we see in patients

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that received the interleukin 2 receptor antagonist for induction, suggesting that these patients not only have an early beneficial effect in modulating the immune response, but also may have a later modulating event that at least can be detected up to 24 months after the transplant event and the induction therapy.

Now, this has been replicated in this very interesting paper by Peter Reese's group that just got published in the electronic format at JASN last month where he shows patients registered in UNOS who have also had a Medicare charge for transplantation, they matched multiple pairs of patients that had either been induced with Thymo and alemtuzumab or induced with Thymo and basiliximab. And he goes to show that there is a very mild but yet statistically significant benefit in the survival of patients treated with Thymoglobulin compared to those treated with basiliximab. There was no statistical significance in the survival without sepsis, neither it was in the survival on allograft, without allograft failure, or lymphoma or melanoma, which is not included here.

So Dr. Djamali and his group concluded that

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Thymoglobulin was associated with a reduction in the incidence of donor-specific antibody and antibody-mediated rejection without significant infections, side effects, that can be attainable to this drug, and that he suggested as well that randomized clinical trials were necessary to address this issue.

As far as I know, there is only one randomized trial that was done comparing interleukin 2 receptor antagonist and Thymoglobulin, was done by Bob Montgomery and his group at Hopkins.

And, Bob, if you want to comment about that trial later, please be my guest.

The next one is alemtuzumab. And I have to say that I have bias or my experience with alemtuzumab is colored by the years I spent at the University of Wisconsin. And I don't pretend to be an expert, there are experts in this group, about Campath.

As you know, it's a humanized monoclonal antibody against CD52, which is a pan T-cell marker. It's also expressed by B cells, monocyte, macrophage, and natural killer cell lineage.

Now, it was introduced as an induction agent

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in desensitization protocols in this study that you have heard quite a bit today, was the 2008 paper of the results of the rituximab and IVIG regimen at Cedars-Sinai.

What I never understood about the use of Campath was the predisposition and the reports that induction with alemtuzumab could be associated with the generation of antibodies. Many people in this room had published case reports about the development of autoantibodies in patients being treated with alemtuzumab for other types of autoimmune diseases. It's known to know that they may develop thrombocytopenic purpura, they may develop thyroid disease, so on and so forth.

Jun Cai, after working in Wisconsin with Stuart, went to the Terasaki Foundation, and they looked at some of the patients that Stuart had recruited in the alemtuzumab induction trial. This was a calcineurin-avoidance protocol. And 42 percent of the patients enrolled in this study went on to develop Class I and Class II anti-HLA antibodies that were 60 percent donor-specific and 40 percent non-donor-

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specific. And of these patients that developed antibodies, 40 percent, 4, have gone on to develop clinical and histological antibody-mediated rejection during the period of follow-up.

Those who critiqued this study mentioned that the reason why these patients were at a risk to develop antibodies is because this was a CNI-free protocol. The only study that I really found was published in Transplant Procedures, and the University of Michigan seems to be a little bit snobby, and we couldn't have access to the paper, so this is data from the abstract. These were patients published from a single center in Pennsylvania transplanted between 2009 and 2011, all of whom received tacrolimus and MMF immunosuppression with steroid with avoidance.

They go on to show in this small study that the incidence of antibody-mediated rejection was significantly higher in patients that receive alemtuzumab induction compared to those who receive rabbit ATG, whereas the incidence of acute cellular rejection was comparable.

So what do we know from the literature about

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alemtuzumab? This trial, an initial trial, is the INTAC study group trial that, as we all know, showed no difference between ATG and alemtuzumab in high-risk patients. There was no significant difference in terms of the antibody cellular-proven rejection, biopsy-proven acute rejection. There was no difference in patient or graft survival. There was a benefit of alemtuzumab in the development of early infections compared to Thymoglobulin.

Now we move to the study of Peter Reeves. In this study, he compares all the outcomes of patients that receive antibody induction in kidney transplantation. It's a very well-designed and statistically balanced paper.

At the end, after the exclusion criteria, he ends up with approximately 36,000 patients, about over 5,000 induced with alemtuzumab, close to 10,000 induced with basiliximab, and over or close to 22,000 induced with rabbit ATG, and he matches patients with alemtuzumab and ATG that can be matched on specific criteria that are mentioned in the paper, and as well matches that he makes between basiliximab and ATG.

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The data between ATG and basiliximab you already saw. The data between alemtuzumab, in the solid line, and ATG, in the hashed line, is shown here. There was a benefit in the probability of survival in those patients that were treated with ATG compared to those treated with alemtuzumab. There was also a trend for patients treated with ATG to have a lower -- or a higher survival without -- a lower survival without sepsis, but this did not reach statistical significance. But when we get the survival without allograft failure, obviously the patients treated with Thymoglobulin fare much better than patients treated with alemtuzumab. There was no difference, although a little trend towards the end of the comparison benefiting Thymo versus alemtuzumab in patient survival without lymphoma.

Now, regardless of the induction agent that one decides to use for depletional purpose of the T-cell compartment, we know from the studies from Allan Kirk and Pearl, at the NIH, that neither ATG or alemtuzumab has a great effect in controlling immunological response, memory response. No effect in

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central memory, very little effect in CD45RA memory in the peripheral tissues. And no effect on the effector memory.

Now, we move again coming from the depletional agents in T-cell responses to depletional agents that may have effect in T-cell biology, but are primarily directed to depletion of antibody cells, either precursors or in the plasmablast stage.

Now, we have discussed, and some of you have seen the presentation of this trial. It was the final trial in which the Cedars-Sinai group compared in a randomized fashion IVIG alone versus rituximab and IVIG for desensitization of patients between 2011 and 2012. The goal of enrollment was 90 patients, but this study was stopped early because of the incidence of antibody-mediated rejection compared in the IVIG alone arm of the study compared to antibody-mediated rejection in the rituximab and IVIG arm.

Important aspects of this study, in addition of the higher incidence of antibody-mediated rejection in the IVIG alone, was that in first place there was a rebound of antibody 6 months after treatment and

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transplant in the IVIG placebo group that was not observed in the group that was treated with IVIG and rituximab. None of the patients with IVIG and rituximab had antibody-mediated rejection in protocol biopsies.

And this is what I think the value of rituximab should be nowadays, is in the prevention or control of rebound. You have seen these already today. This is the study of Annette Jackson at Johns Hopkins, where you can see the rebound of antibodies in patients that receive rituximab and in patients that did not receive rituximab.

You can also see that there is a significant change in the MFI in those patients that were induced with rituximab compared to those who did not receive the induction. And although it is true that there was no statistical significance between graft or patient survival, rituximab for patients with high levels of antibody facilitates the management of those patients and reduces the number of plasma exchange treatments that they usually require.

So what happens when we combine all the

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different treatments? This is a study that Mark Stegall published 10 years ago in which he reviewed all the patients at Mayo Clinic that had been desensitized with different protocols: high-dose IVIG; a combination of rituximab, IVIG, and plasma exchange; a combination of plasmapheresis, IVIG, monitoring; and a group of patients that receive all treatments combined. And what is obvious is that as one adds more synergistic agents, the incidence of antibody-mediated rejection decreases substantially, but the group that fares the best is the group that is treated with synergistic agents, but also managed with posttransplant DSA monitoring.

We move now to belatacept. Belatacept, as you know, has been linked to higher incidence of T-cell-mediated rejection, not only in frequency, but also in the severity of the rejection and the need for antilymphocytic therapy. However, even when this is a very well-documented fact, patients that are treated for induction with belatacept tend to develop lower levels of donor-specific antibodies in spite of the higher incidence of rejection compared to the control

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arms.

Move to the complement inhibitors. And you heard from Mark Stegall this morning about these patients, and what he's seen in the early outcomes is that there is a lower incidence of antibody-mediated rejection, that these patients require less plasmapheresis, less splenectomy; in other words, can be rescued in an easier way than patients that only receive plasmapheresis and IVIG. The initial benefits seen with eculizumab stems from protection of the endothelium by inhibition of the distal formation of the MAC complex.

Now in long-term outcomes, there was no difference between outcomes and the initial benefit, as you heard today about that eculizumab had on transplant glomerulopathy disappears. And the reason why it disappears, you also heard from Mark today, is because these patients have continuous microcirculatory inflammation, and the reason of the maintenance of the inflammation is that none of these patients that require eculizumab for a long time had a reduction in antibody levels.

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C1q inhibitor has only been utilized in this day and age for desensitization. This is the protocol that was utilized. No difference was observed between one group or the other, but the important message is that although C1q inhibitor seems to work fairly well in reducing titers of antibodies, when the titers are low, there is very little effective of C1q inhibition in reducing donor-specific antibody levels.

Finally, we have tocilizumab. And tocilizumab has been used right now for the treatment of chronic antibody-mediated rejection. The greatest benefit that we can encounter in this group of treated patients is the effectiveness that tocilizumab has in resolving the microcirculatory injury and inflammation that eculizumab and other complement inhibitors have not been able to achieve.

Finally, we see that the best drugs in inhibiting CD4 memory cells continue to be the calcineurin inhibitors. You have seen this slide several times today of the CTOT-09 trial where obviously patients that were continued on calcineurin inhibitors develop less donor-specific antibody than

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those that were withdrawn and kept on MMF and prednisone.

DR. ALBRECHT: Dr. Samaniego, could you wrap up, please?

DR. SAMANIEGO-PICOTA: Something similar happens when we withdraw patients or maintain patients on calcineurin inhibitors. This is a cohort you saw this morning as well showing patients that had an increase in incidence of DSA when they were withdrawn from cyclosporine and treated alone with MMF and everolimus, something similar occurs in the incidence of antibody-mediated rejection.

So in conclusion, I would say that we do have effective immunosuppression that has been available since the 20th century. In my opinion, CNI-based regimens should be the first choice for patients at risk to develop antibody-mediated rejection. Rituximab should be considered in patients with rebound. And there are much more important issues that still have to be -- need to be addressed, as the number of mismatches, the association of BKV infection, with de novo antibody production, DSA monitoring, and

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nonadherence.

Thank you.

(Applause.)

DR. BELEN: Thank you. Next we have Dr. Arthur Matas, from the University of Minnesota, and he is going to present, "Calcineurin Inhibitor and Corticosteroid Minimization and Avoidance Protocols and HLA Antibodies."

Calcineurin Inhibitor (CNI) and Corticosteroid Minimization/Avoidance Protocols and HLA Antibodies

DR. MATAS: Thank you. Thank you for the opportunity to be here. The nice thing is I got to hear everyone else's talks as well. And it's been a terrific day.

I'm going to try and talk quickly because a lot of what I am going to present has been shown by a number of speakers.

I've got no disclosures related to this presentation, but we do have grant funding from a number of companies to continue our DeKAF study. And I won't discuss any off-label drugs.

When one talks about calcineurin and

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corticosteroid minimization, it's important to recognize that the steroid spearing studies were done before anyone really tested donor-specific antibody, and so the endpoints of those trials were really acute rejection and graft loss, whereas more recently with calcineurin minimization, we had antibody testing to use as an endpoint. And so as I present this steroid data, really there is long-term follow-up, but very little DSA data.

The goals of prednisone minimization trials were really to avoid prednisone side effects. And we certainly heard from our patients this morning the plethora of prednisone side effects that we've been trying to avoid in our patient population. The hope was to do that without an increase in acute rejection and no change in chronic graft loss.

And these trials started in the prednisone-Imuran era with calcineurins and cyclosporine, and there were a number of trials with cyclosporine-prednisone or cyclosporine-Imuran-prednisone with prednisone withdrawal, and essentially with selected, clinically well low-risk patients, these trials

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resulted in increased acute rejection and increased graft loss, and that was shown in a meta-analysis by Bert Kasiske.

When CellCept was brought in, there were two major trials, one in Europe and one in the United States, in which prednisone withdrawal was tried late posttransplant on a background of cyclosporine and CellCept, and both of those trials showed an increased incidence of acute rejection after steroid withdrawal. And, in fact, with late steroid withdrawal there are meta-analysis showing significant increases in acute rejection.

And this led to trials of what I would call rapid discontinuation of prednisone, and Steve Woodle calls early corticosteroid withdrawal, in which prednisone is stopped in less than 2 weeks, and usually within 1 week posttransplant, and essentially there have been numerous single-center studies, randomized and non-randomized, but as well, meta-analyses and registries reports on these early prednisone-stopping studies, all showing an increased incidence in acute rejection rates, or the majority at least showing that,

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albeit some of them are early and mild and easy to treat.

There has been no increase in steroid-resistant rejection, no impact on patient and graft survival, and a number of reports showing that rapid steroid withdrawal is associated with significantly lower rates of new onset diabetes, cardiovascular risk factors, avascular necrosis, and fractures.

In one report by Pascual and Cochrane analysis, noted there was a significant increase in acute reject with rapid discontinuation of prednisone only when cyclosporine was used, and that was not true when tacrolimus was used. But in reverse, when the decrease in new onset diabetes was only seen when cyclosporine was used, not when tacrolimus was used.

It was a very interesting analysis because it said there were a few studies of the benefits of rapid discontinuation of prednisone, and I think those of us that started these trials never thought to try and measure things like skin changes and appearance changes, it was just sort of intuitively obvious that if you didn't use the drugs, you would not have those.

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Dr. Woodle's study was the only prospective, randomized, double-blind study early on. In that study, there was antibody induction with TAC and MMF and prednisone for 7 days versus a taper to 5 mg by 6 months. And the major findings at 5 years were there was increased biopsy-proven rejection in the steroid withdrawal group, albeit in a subanalysis less with Thymo than with IL-2R.

There was no difference in the primary endpoint, which is a composite of death, graft loss, or moderate to severe acute rejection, no difference in the rate of antibody-mediated rejection, no difference in renal function, and the steroid withdrawal group had improvements in cardiac risk factors.

The only study looking at antibody development in steroid withdrawal was one published by Cantarovich in AJT in 2014. In that study, there was anti-thymocyte globulin induction with cyclosporine and CellCept and zero prednisone versus a steroid taper for at least 6 months. The major findings at 5 years, increased biopsy-proven acute rejection in the zero prednisone group again, but no difference in death,

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graft loss, or renal function.

Determination of DSA was actually not planned in that study, but they had the information in 151 out of the 197 patients, and they reported that in each group, about 11 percent developed donor-specific antibody, obviously no difference between groups. And interestingly, their steroid protocol was noted to have increased diabetes, dyslipidemias, and in this particular study, malignancies.

One of the concerns about rapid discontinuation of prednisone has been that late posttransplant graft survival might be worse. And this is our data. And our study is not randomized; it's looking at steroid-free versus historical controls.

We now have 15-year data, and we decided we would look at those that survived 5 years with graft function. So we got rid of all the early noise. And you can see on the left, living donor; on the right, deceased donor; on the top, patient survival; on the bottom, graft survival. In 5-year survivors, there is no difference in long-term outcome whether you are on or off steroids. So early steroid withdrawal and being

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steroid-free up to 5 years and after that does not hurt your long-term outcome.

Turning now to DSA and minimization trials, CNI minimization, where there is more data, certainly the early studies, as shown on this slide, did not have DSA, but the study by Ekberg, et al.; the Caesar Study; the Abramowicz study; the study by Smak Gregoor; all looking at CNI minimization; reported increased acute rejection in CNI withdrawal or minimization. And you can see the data for each of these on this slide.

This slide has been shown before. It's the randomization of patients on cyclosporine, to continue on cyclosporine versus being converted to everolimus. And has been reported before, those converted to everolimus had more donor-specific antibody, more antibody-mediated rejection.

A similar study, the first one being cyclosporine-based, this study now tacrolimus-based, with de Sandes-Freitas, et al., looking at subclinical lesions and donor-specific antibody in patients on TAC, CellCept and prednisone randomized to continue tacrolimus versus conversion to sirolimus. They had a

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whole host of exclusion criteria, so these are low-risk patients that were randomized.

And at the bottom of the slide, you can see at 24 months, there was increased biopsy-proven acute rejection, increased Banff "i" greater than zero scores, and increased donor-specific antibodies in those who were randomized to the sirolimus group.

Don Hricik's CTOT study has been mentioned before. So I think the important part of this study was these were absolutely pristine patients. They had no donor-specific antibody, they had a low PRA, from zero to 6 months, had no rejection, no donor-specific antibody, and at 6 months had a totally clean biopsy, and the goal was TAC withdrawal, and as you've heard already today, the group randomized to TAC withdrawal had significantly more immune events, including donor-specific antibody, and the study was stopped by the DSMB.

In a similar study, not TAC withdrawal, but TAC weaning, by Gatault, et al., using extended-release tacrolimus, patients with no DSA and no acute rejection at 3 months were then randomized at 4 months. And

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again you can see a host of exclusion criteria, so these were low-risk patients who were randomized, and they were randomized to a 50 percent reduction, so not withdrawal, but a 50 percent reduction in their tacrolimus dose -- you can see the targeted trough level there -- versus continuation.

And you can see the results on this slide. There were 188 patients randomized. And in the 50 percent reduction group, there was significantly more biopsy-proven acute rejection, more donor-specific antibodies, and in protocol biopsies at 1 year posttransplant, there was significantly more patients who had a Banff "i" score greater than zero, and the conclusion from this study was TAC levels should be maintained at least during the first year.

Well, this particular study by Dugast, et al., looks at late TAC withdrawal and entitled, "Failure of Calcineurin Inhibitor Weaning." This study was a prospective, randomized trial, multicenter, that looked at patients 4 or more years after transplant who had normal histology, stable graft function, and no anti-HLA immunization. And only 10 patients were randomized

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because in the placebo group, they had three acute rejections, two patients developed anti-HLA antibodies, of which one was a donor-specific antibody, and all five patients were started back on tacrolimus, and even at 4 years in clinically well patients, tacrolimus withdrawal failed.

There are other lists, and I think Ros Mannon mentioned them earlier this morning, and they're shown on this slide, of other studies in which there has been minimization, and in each of these studies, the minimization group had increased donor-specific antibody.

And then, finally, the real calcineurin-free study, which has been mentioned before, is the BENEFIT study, which was a prospective, randomized study with 100 centers and over 650 patients. There were three groups: more intensive belatacept, less intensive, and cyclosporine.

And the important point I think is that early on there was no difference in patient or graft survival. There was certainly more acute rejection in the belatacept group, as you've heard already, and more

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severe rejection in the belatacept group. But when you looked at 7 years at the percent of patients that developed donor-specific antibody, as shown on this slide, the cyclosporine group, in the pink, had had significantly more development of donor-specific antibody.

We all know from the series of studies that the GFR was always better in the belatacept group as compared to the calcineurin group, but this had not translated into better graft survival or patient survival until the 7-year data. So it's certainly in concert with the better GFR and the reduction in donor-specific antibody. The 7-year data with belatacept shows a 43 percent reduction in the risk of death or graft loss as compared to the cyclosporine group.

So to summarize, steroid minimization was done before donor-specific antibody testing. It showed an increased early acute rejection but no change in graft survival. This was mostly limited to low-risk groups, although we certainly in our center apply this protocol to essentially all first or second transplants. And we've looked at a variety of higher risk groups, and

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they seem to be comparable results, the same results, for that same high-risk group that you would get if you continued prednisone.

The CNI minimization studies, both cyclosporine and tacrolimus, there have been studies of minimization or conversion all the way out to 4 years in low-risk or pristine patients, and there has been increased acute rejection, increased donor-specific antibody, increased Banff "i" scores, and in one study, increased graft loss.

And then, finally, in belatacept versus cyclosporine, although there was increased acute rejection in belatacept, there was less donor-specific antibody, and right now that's been shown in better graft survival. So perhaps with this particular drug we're changing the paradigm.

Thank you.

(Applause.)

DR. BELEN: Thank you. Next we have Dr. Rita Alloway, and she is going to present, "Nonadherence -- Definitions, Monitoring, Prevention and Management."

Nonadherence -- Definitions, Monitoring, Prevention/Management

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DR. ALLOWAY: Thank you very much. I would like to thank the organizers for allowing this topic to be discussed here today. While we refer to nonadherence quite frequently, I compel you to actually define, monitor for it, and attempt to develop a strategy that prevents it, or we're able to maintain a good solid adherence plan for our patients.

These are the following disclosures.

The objectives of the talk today are to differentiate medication nonadherence and compliance, describe measures to quantitate medication nonadherence, and discuss efforts towards prevention and management of nonadherence.

So nonadherence is not new to us. I think that basically Hippocrates in 500 B.C. said, "Keep watch also on the fault of patients which makes them lie about taking things as prescribed."

Also, C. Everett Koop quoted as saying, "Drugs don't work if people don't take them."

Now, I think that by the show of hands earlier, we've shown that none of us are very compliant

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or very adherent. However, I think we need to especially look for strategies to intervene in this regard.

While there has been a lot of talk today about nonadherence and the development of new therapeutics, adherence rarely has been incorporated into the therapeutic drug development process. And I maintain to you that transplantation can no longer accept the status quo of the level of nonadherence that we have become to accept.

I think, as been described here today, the first shot that we have at transplant is our best shot for transplant long-term success, and managing and keeping the patient on an adherent regimen at this time is very appropriate.

The other thing is despite millions of investment, the "magic" drug or procedure to render adherence irrelevant is not on the horizon. So it's a thing that's going to continue to be something that's very important for us to discuss.

And hopefully federal mandates are not necessary for us within our centers to properly

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resource adherence initiatives if adherence continues to be neglected primarily as it is today.

So when you talk about medication adherence, and talk to the experts, there is a difference in terms of which they like to use. The two terms that are most commonly used are medication "adherence" and "compliance." "Adherence" is the preferable term. It refers to the extent to which patients take the medications as prescribed by the health care providers. "Compliance" is more referred to as a passive act of which the patient follows the provider's orders. And I think that because this tends to show obedience or passive following of the patient, people have wanted to promote the idea of adherence to improve the knowledge that the patient has to adhere to their medication.

So medication adherence must be recognized as a behavioral process that is influenced by many factors. It assumes that the patient has the knowledge, the motivation, the skills, and the resources to follow what the health care provider's prescription actually is.

When we look at medication nonadherence, we

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all know that there may be intentional medication nonadherence or unintentional. Intentional medication nonadherence is actually defined as an active process whereby the patient chooses to deviate from a treatment regimen; while unintentional medication nonadherence, which I think represents most of the cases, is a very passive process which the patients may be careless or forget about adhering to their treatment regimen.

So the World Health Organization identified five dimensions of adherence for all medications focusing on health system factors, socioeconomic factors, therapeutic-related factors, patient-related factors, and condition- or disease-related factors. And this has been very well studied in the transplant population.

And as you can see by the complexity of components of each of these factors' spectrum, that an adherence or a development of a strategy to provide optimal adherence is going to be complex, it's not going to be the same for everybody, and we will have to intervene and have the patient involved in this.

But I think anytime you do a risk factor

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assessment, you look at which of these factors are modifiable. And I think if you look at the health system factors and the therapeutic-related factors, you'll see that there are actually some ways to improve this area, and there are some factors here which we can modify.

So when we begin to talk about measuring medication adherence, there are objective measures and subjective measures. Objective measures tend to be direct measures that provide the evidence that the medication has been consumed or taken. Luckily, within transplantation, we actually now have a drug, belatacept, where if the patients come and get their infusions, we have direct observation of them actually receiving their drug.

There are also indirect measures that we can look at that can be made objective as well, such as providing evidence suggesting that the medication has been consumed or taken. Pill counts are frequently used. Tacrolimus drug levels we use quite frequently. Pharmacy refill records and medication possession ratios.

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But there are actually subjective measures as well where patients provide testimonials that the medication has or has not been taken, and this can be a self-report or assessment by others.

So, again, the direct observation options in transplantation, the advantages are that they are objective, they're highly specific, and they're non-invasive. The disadvantages are the feasibility issues that occur along with it. They are labor-intensive, and in many cases, they're not practical. They may be expensive. And as you know, they're not an option for all transplant recipients.

Now, fortunately, in transplantation, drug concentration monitoring has been available for our primary immunosuppressants for the CNIs, and it is incorporated as standard of care. The advantages of that are it's objective, it may be part of standard of care, and it is a direct assessment of whether the patient is taking their medicine, at least in the close proximity of when the level is drawn.

However, there are disadvantages. All of our medications do not have a routine drug concentration

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monitoring plan that has been associated with long-term outcomes that we use routinely. The disadvantage, too, as I said, it is just a snapshot of the behavior that occurs prior to the drug being taken. It's affected by many factors other than medication adherence, as well all well know: metabolism, drug-drug/drug-food interactions, poor absorption. While we have become willing to accept the costs that are associated with it, there is a high cost associated of monitoring therapy, and it is invasive.

So recently there has been a lot of work looking at tacrolimus interpatient variability and its impact on long-term graft outcomes. Now, when you look at all of these studies, they basically imply that the interpatient variability that occurs is not only related to nonadherence, but they tend to say that they think that nonadherence is a large predictor in this.

However, I just want to point out that we know that that's not the only factor. But there are a lot of studies out there, I'm just going to talk about three of those real quickly.

This study basically had 310 renal transplant

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patients who had their tacrolimus levels analyzed for 4 to 12 months posttransplant, and their interpatient variability of the trough levels were calculated. They had an arbitrary break of less than or greater than 30 percent IPV.

Roughly, as you can see -- and this is what it's turned out in a lot of studies -- roughly a third of the patients were considered to have a high interpatient variability, and roughly two-thirds had what has been defined as acceptable. DSA was performed at 1, 3, and 5 years. And in this cohort of patients, 17 percent lost their graft, and 12 percent or 13 percent developed a de novo DSA.

Now, when you and you apply the interpatient variability calculations to their primary outcomes of death-censored graft survival here, you can see that the patients had an interpatient variability of greater than 30 percent, had a lower cumulative death-censored graft survival than compared with those that had a lower IPV.

Although the legend is different on the subsequent slide that they have in their paper, when

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you look at the development of de novo DSA, again you can see that the patients with the lower IPV had an improvement or had a lower incidence of the development of de novo DSA in the study.

Another study that looked at this focused more on the late outcomes with the composite endpoint of graft failure, late biopsy-proven acute rejection, transplant glomerulopathy, and doubling of serum creatinine censored for death.

And the reason that I want to point this out is this was a study in over 200 transplant patients where they analyzed the tacrolimus levels between 6 and 12 months posttransplant, and basically if you look at the hazard ratio, the TAC IPV was the highest predictor of this composite endpoint, where you saw 1.4 percent increase in every unit of IPV that was noted in the patients. So as you can see, with the increasing IPV, the composite endpoint was met.

But what is even more significant is when they looked at this and they compared it with the target tacrolimus levels that the patients were on and receiving, you can see that at the lower tacrolimus

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levels, that really we currently target anywhere from 4 to 6 or maybe 6 to 8 posttransplant, the impact of interpatient variability is actually higher when we're targeting these lower tacrolimus levels, as we are in a lot of our regimens today.

This is another study that looked at interpatient variability in 220 renal transplant patients, and essentially they again analyzed their levels between 6 and 12 months posttransplant and divided them into three tertiles with the lowest IPV being approximately 10 percent, the mid mean was 18 percent, and again the highest tertile was 31 percent.

And in this study, in this center that conducted protocol biopsies in 3 months and 2 years, they used this to calculate the change in chronicity score during this time. And basically the recipients with the highest IPV had an increased risk in the moderate to severe fibrosis and tubular atrophy at 2 years compared to those with a low IPV. And, again, this was the single most important predictor of long-term graft survival in these groups.

Now, tacrolimus interpatient variability is

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something that you can implement within your clinics today if you cooperate really well with your IT department. It's difficult, but they can do it. And we're attempting to utilize that on a day-to-day basis in our center.

Now, electronic monitoring is available. The advantages of this are it is objective. It can indicate the actual time and date of the bottle opening. So what this allows us to do is detect poor adherence with a dosing schedule. You can see if someone is taking a medication twice a day, if they miss the dose more frequently in the morning or if they miss the dose more frequently at night. It also can detect pill dumping. And it's noninvasive.

The disadvantages of it are the cost, it's not effective with liquid medications, it can malfunction and lose the data. Sometimes the device is bulky, as you see here, with the MEMS Cap strategies, that basically the patients don't want to carry these around. And it also assumes that the medication that was actually removed from the bottle or the box is actually taken.

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Now, there was a study in Minneapolis in Minnesota that basically looked at the nonadherence utilizing the MEMS Cap with antiproliferative agents -- MMF, sirolimus, and azathioprine -- in 195 kidney transplant recipients. And what was very interesting I thought was that adherence between months 1 and 2 actually predicted adherence at 6 and 12 months. And I think that that's an important concept for us to understand and see.

Nonadherent patients in this study had more frequent and earlier acute rejection and death-censored graft survival. And during the 1 to 3 months, adherence with 4-times-daily drugs, as we know, is 84 percent, 91 percent with twice-daily drugs, and 94 percent with once-daily drugs. And you can refer to this paper for more information.

There's another way to assess adherence, with pharmacy refill records. Again, they're objective, they're standardized, they identify patients who fail to refill their medications, they're noninvasive, and they're not expensive. But there's a big problem with misinterpreting the usage of this information when

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there has been a variety of doses changed.

I offer to you -- and maybe I shouldn't be so specific -- but I think it's almost impossible to use pharmacy refill records when you're evaluating a drug like tacrolimus or sirolimus or any of these others where you change the dose frequently and it doesn't elicit a new prescription. So that is one of the things that limits the usefulness of this type of information.

This type of information is actually used to calculate a medication possession ratio, or essentially proportions of days covered. And these are the two most common formulas that used to estimate patients' adherence to chronic medication. Both formulas use prescription data and calculate the days of which the patient has the medication on hand.

This type of analysis has been incorporated into a lot of the chronic disease trials of diabetes and cardiovascular disease, but what is interesting is we don't know in transplantation what the optimal medication possession ratio for any immunosuppressant is. We obviously tell the patient, "You need to be 100

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percent adherent. Take every dose, every time, on time." But we really don't know what the optimal medication possession ratio is.

There are self-reports. The advantages of these are quick, simple, inexpensive. The disadvantages primarily are that they overestimate adherence. And they're burdensome. And basically patients, when you ask them at the clinic visit, they tend to remember what they've done the last 3 or 4 days, but not necessarily what they've done 3 months or even 6 months since their last visit.

The last thing is in clinician reports, again the advantages are simple, quick, and inexpensive. And to be honest, in a lot of these reports that are in the literature right now, these are clinician reports to nonadherence.

But unfortunately for you guys, you tend to underestimate nonadherence. So if we're talking right now that we have a problem with DSA development and nonadherence based upon clinician information, it's underestimated for nonadherence.

So I know my time is over, but let me just

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briefly take you through this last concept. We need to develop interventions which promote adherence. We need to think about when we intervene, where we intervene, and how.

And I'm not going to spend the time going through the status posttransplant, but it's optimal to find an intervention time that promotes adherence, and it's going to be different from every patient.

Luckily, we have a lot of new interventions to promote adherence with the smartphone apps and the computers, and we have a lot of different types of interventions that I want to point out. It needs to be multidisciplinary, and when we've seen the most success, they have been in multicomponent interventions.

Right now, there have been more randomized, controlled trials that actually look at adherence, but the scientific rigor there has increased, but it's not as it should be. The types of interventions that are tested are heterogeneous. Multicomponent interventions appear to be the most effective.

Intervention effectiveness appears to be

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increased when you actually tailor it to what the patient says they need, and it needs to be dynamic based upon the patient's response.

The degree of the intervention impact is variable. And often trials don't evaluate clinical outcomes, especially when it comes to long-term outcomes.

So I just want to leave you with a new paradigm as you think about nonadherence, focusing on the qualitative measurements. This is really where the field is going. And basically the qualitative measurements provide insight into patients' values, knowledge, beliefs, that influence behaviors and choices in transplantation self-management. And the focus now begins on self-management of the patient.

And when you do this, self-management begins to explore the task that individuals must undertake to live with this chronic condition that we've given them now. They may not have end-stage renal disease, but they have the disease of immunosuppression. They need to have confidence to deal with the medical management of this. They need to know what their role in

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management this is, and the emotional management related to chronic conditions.

So this is references on this slide, but essentially we need to focus on things that strengthen the patients' ability to learn how to self-manage their conditions and diminish the interventions that make the self-management harder.

And what is interesting is the transplant patients basically say that the reason why they try to be adherent and what scares them the most is their prevailing fear of the consequences. And I think that that is something that we have to understand.

When you look at immunosuppression and taking immunosuppressants, basically when this shows you how many domains that taking immunosuppressants impacts, it shows you that it's going to be a complex solution. So just as we're talking about a precision medicine in transplantation, I think that we need a transplant-specific precision prescription for adherence for each individual patient. And this includes putting the patient first, hearing what they have to say, and knowing that this is adaptive over time.

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So with that I would like to thank you for this lecture. Thanks.

(Applause.)

DR. BELEN: Thank you, Dr. Alloway.

Next we have Dr. Robert Gaston, who is going to present, "The Role of Acute Cellular Rejection Episodes in the Development of HLA Antibodies."

The Role of Acute Cellular Rejection Episodes in the Development of HLA Antibodies

DR. GASTON: Thank you again. I think if I were putting this talk together at 5:00 this afternoon, it would be different than the talk I've put together, having heard everything there because I sort of interpreted the mandate as, Is there still a role for acute cellular rejection in the development of HLA antibodies? And so you'll I think tell that from the tone of the talk to follow.

So I can't but thinking, how did we get to having this talk at 5:00 at the end of this symposium on antibody-mediated rejection? I have a book, it was given to me years ago, Amburjay's (ph) book from 1972, and I use it as an easy reference on what people

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thought 50 years ago, and these are several quotes from that book. "There is no doubt that the cell-mediated immune response is a predominant factor in rejection." "These nonthymus-dependent lymphocytes can become actively sensitized against antigens, but in cell-mediated immunity, they participate, if at all, only in effector mechanisms, only in association with thymus-dependent cells." And they did recognize a bit of the future, that, "Circulating antibody against donor cells...have been detected by the use of specially sensitive techniques, while the transplant organ was still in place," because they couldn't find them with usual techniques, "and there seems to be a definitive correlation between this finding and the appearance of progressive lesions in the graft, especially vascular lesions." So lots of looking forward there.

But if you look at the more recent literature, a half century later, this is what you see. And these are three very elegant papers, Professor Loupy's paper, "...lack of association of subclinical TCMR with graft survival thus challenges the historical conclusion that T-cell-mediated rejection increases the risk of future

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graft loss, confirms the findings of recent clinical trials, showing that indolent T-cell-mediated rejection can be adequately treated as not associated per se with graft loss...."

Phil Halloran's paper, "We conclude the main cause of kidney transplant failure is ABMR, which can present even decades after transplantation. In contrast, T-cell-mediated rejection disappears by 10 years posttransplant...."

And then from Cleveland Clinic, "However, B cell depletion inhibited alloantibody generation and significantly extended graft survival, indicating that donor-specific alloantibodies (not T cells) were the critical effector mechanisms of renal allograft rejection induced by memory CD4 T cells."

How did we get from '72 to 2016? And why am I giving this talk? And in the research, I came across a very interesting paper from 1960 from the father of antibodies, Peter Gorer, or one of the fathers, and it was about terminology. And I think a lot of what's been said here today is about terminology. In the Greek "iso" means equal, "homo" means the same. If two

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individuals are genetically identical, grafts exchanged between them are equal, but not the same. On the other hand, if they're genetically different, their grafts are the same, but not equal. It is here that transplantese ceases to be homologous with English or indeed with common sense."

And I think the discussion about the role of T-cell- versus antibody-mediated rejection falls a bit into this category of transplantese, and hopefully in the next few minutes I can bring some resolution to at least some of it.

So how did we get there? I really think we got there in the '90s when we for the first time had specific effective immunosuppression and started to see data like these -- this is from Minnesota -- and the concept that there was a subset of acute rejection that did not associate itself with long-term graft failure.

And in this study, it was the rejections that occurred within the first 3 months. There was really no association with late chronic rejection. Conversely, later rejection episodes, 3 to 6, 6 to 12, 12 to 24, and beyond 24, as you can see on the right

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there -- maybe I can resurrect some of this -- were highly associated with risk of rejection or risk of late graft failure.

I think it was perpetuated with these data, as we had availability of tacrolimus, mycophenolate, and so on. And prior to this, this is the risk of acute rejection, these were largely T-cell-mediated rejections in those days, and you can see with the new drugs, the risk of rejection in the early period, 6 to 12 months, 12 to 24 months, declined, but yet there was no impact at all of reducing rejection on long-term graft survival. T-cell-mediated rejection must not be as significant as we thought it was.

We contributed to this with the DeKAF study, and I've updated the data from what's usually used from that, and that is these are late biopsies in the mean of 7 years posttransplant in people who previously had stable function. Basically, what this study seemed to say is that if you didn't have C4d, if you didn't have DSA, you did pretty well. If you had one, either or both of those, you did quite poorly. And this is again starting at day times zero, 7 years, out to there.

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What is not often quoted is that there was really a high degree of cell-mediated rejection in all of these categories as well, and that's not accounted for in this analysis. But nonetheless, the concept that only antibody-mediated rejection was important. You've seen that in the work from Chris Wiebe and Peter Nickerson, again, that in this protocol biopsy study, the patients who developed DSA did very poorly, the patients who did well, and then nonadherence was a risk factor, and we'll come back to that again in a minute.

Okay. Well, you've seen this a dozen times already, so I'm really disappointed this didn't come through in the transition. But basically the survival -- maybe I can bring it down. No.

This is in protocol biopsies in patients who had subclinical T-cell-mediated rejection. They did just as well as the patients -- this is a French series from Loupy -- they did just as well as the patients who had no rejection on protocol biopsy long term. Conversely, it was those with subclinical ABMR that had the poor outcomes.

The next slide did come through from that, and

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this is basically looking at the patients who -- the incidence of or the probability of developing transplant glomerulopathy in the patients with no rejection, subclinical TCMR, and subclinical ABMR. And what you can see is that the patients who had subclinical T-cell-mediated rejection look very little different from the patients who had no rejection, but you can see that very quickly the subclinical ABMR group developed transplant glomerulopathy.

What is interesting, though, is that in this group, the T-cell-mediated group, the development of transplant glomerulopathy was pre-staged by sort of a transition at some point, and development of de novo DSA over time.

This then, sort of in my mind at least, culminated in a paper by Phil Halloran and the group there in which they looked at both histology and then molecular diagnosis.

Basically if you look here, there is some mixed diagnosis, mixed T-cell, mixed antibody-mediated rejection, normal biopsies in green. This is looking at graft survival. And you can see that the pure T-

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cell-mediated rejection, by histology, had the same outcomes long term as those patients with relatively normal biopsy. Pure ABMR had the worst outcomes. And these are for-cause biopsies, by the way. And then the mixed was intermediate.

When they then added the molecular qualifier to it, the mixed group basically segregated with the antibody-mediated rejection, but again you see the pure T-cell-mediated rejection group did not look any different long term.

And it's real easy from all of this then to come to the conclusion that T-cell rejection doesn't play much of a role in all this, but looking at the molecular transcripts over time, what you can see is the T-cell-mediated transcripts are highly present, early posttransplant, late posttransplant. They diminish and are replaced instead by markers of antibody-mediated injury in the grafts.

And the title of the article was, "Disappearance of T Cell-Mediated Rejection Despite Continued Antibody-Mediated Rejection in Late Kidney Transplant Recipients."

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So why are things more complicated than that? What is the relevance of T-cell-mediated rejection? And I think I'm going to go into hopefully just a few slides, and build a case that is still very important, and it's really a continuum. And back to the transplantese, we need to really think about alloimmune activation as a continuum across the board.

This is a study from the Barcelona group in which they looked at patients who had a protocol biopsy at 6 months, and then subsequently over time required a for-cause biopsy, the patients who had chronic humoral rejection, the patients who had isolated IFTA. And then you see the other characteristics on the biopsy, a total N of 86 only, but some very interesting findings in this over time.

And what they found, they were looking at what on the 6-month biopsy predicted chronic antibody-mediated rejection. And what they found was on the 6-month biopsy were markers of cell-mediated injury, that is, interstitial infiltrate, tubulitis, and arteriolar hialynosis against vascular disease as well. But the suggestion was that the findings most predictive on the

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6-month protocol biopsy of long-term injury were related to cell-mediated mechanisms within the graft.

This is a bit more elegant, again from the same group in Barcelona, looking at T-cell reactivity, as documented by ELISPOT testing, posttransplant ELISPOT testing, at 3 and 6 months, with a 6-month protocol biopsy in it. And basically, even though it's very beautiful, it's a bit complicated, that over here are the patients who had a negative ELISPOT test at 3 months and at 6 months, and over here, the patients who had a positive ELISPOT test at 3 and 6 months.

And what you can see is the patients who had ELISPOT again as a proxy for T-cell activation, that those patients who were positive subsequently went on to have subclinical cell-mediated rejection at 6 months, and the predominance of de novo DSA within the entire group was in the group who had positive ELISPOT testing, evidence of T-cell activation, early in the posttransplant course, translated into a higher risk for de novo DSA at 24 months. Conversely, in the absence of T-cell activation, only these two patients demonstrated evidence of de novo DSA at 24 months. So

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a link between cell-mediated immunity and de novo DSA.

This is a very elegant study, has been referred to a couple of times, in JASN from Anna Volushka (ph) at Cleveland Clinic, and this is a study in mice. And basically they sensitized the mice to donor antigens, and basically you can see the donor responsiveness here. They then treated them with an anti-CD8 antibody or a polyclonal, essentially eliminated that responsiveness, that antibody responsiveness, but it had no impact at all on graft survival when they eliminated the cells that were present via mouse, if you will, Thymoglobulin.

Contrary, on the other hand, you can see the same sort of response, a sensitized memory in the model, donor-specific, that were then treated with Rituxan either at day 7 -- or excuse me, were tested again at day 7 and day 30 after Rituxan, and by eliminating the B-cell responsiveness, the antibody responsiveness, in this model, they then were able to prolong allograft survival the same as in the nonsensitized people, again emphasizing the link between T cell sensitization and the importance of

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antibody and antibody-mediated, presumably B-cell-mediated, effector mechanisms.

We then go back to the nonadherence. We've seen this over and over again. And what's interesting -- I don't want to explain this too much -- if the injury was found that they had subclinical with de novo DSA versus clinical, both had adverse impacts on graft survival with a quicker course in the patients who had clinical DSA, and the endpoints being transplant glomerulopathy and interstitial fibrosis.

What's interesting in this study -- and this is further data from the study -- they broke the patients at the time of biopsy into those who had no DSA, no graft dysfunction, that was the majority of patients; no DSA, but graft dysfunction; DSA, subclinical, so no evidence of graft dysfunction at the time of the biopsy; and then clinical. What you can see is that the nonadherence increases across with the highest degree of nonadherence in those with clinical rejection.

But what you can see is that, as in the antibody, those patients were significantly more likely

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to have experienced T-cell-mediated rejection early in the posttransplant course that ultimately resulted in -- or ultimately in those patients -- I don't want to say evolved into, I want to say the same patients over time developed donor-specific DSA ultimately with consequence on graft function.

And then, finally, I can't do this without referring at least somewhat to the DeKAF study. And this is the prospective cohort, and now after this many years, we're just now completing the database in the prospective cohort. But approximately 4,000 patients at the time of this analysis, 3,300 patients with functioning grafts at least 90 days. We were not interested in early graft loss, we were interested in late graft loss. The baseline status for these patients was established at 90 days.

At the time of this, we had a mean follow-up of 32 months. We termed the index biopsy, the first for-cause biopsy, after establishing this. It was standardized across seven centers as a 25 percent increase in the serum creatinine or new onset proteinuria. Obviously an increase in creatinine

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accounted for most of these.
What was interesting in the patients who met those criteria, the incidence of death was the same. We were not selecting for people who had increased risk of mortality. But ultimately the subsequent risk of death-censored graft failure, 20 percent of those patients who had index biopsy went on to that versus very few, if any, that did, and the people who did not have an index biopsy.
If we looked at then what were the risk factors for the biopsy, basically what these data say is that at 90 days, the patients look the same. There was a significant difference in age between 50 and 46. But I guarantee you I can't look at anyone in this room and tell whether you're 50 or 46. Gender was not significantly different. Race was slight overrepresented, but not largely. PRA was no difference in the patients. Serum creatinine at 90 days was no difference in the patients. There had been a slight increase in evidence of delayed graft function early on. And then very early acute rejection in the patients.

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And what you can see then if you look at risk of death-censored graft survival in the patients, that those early rejections did not seem to have a significant impact on risk of subsequent graft failure. Delayed graft function didn't have a significant impact. What did was something happening to the patient beyond day 90, in this case, that triggered an index biopsy, that is, new onset of some event, and in a larger number of those patients than we would have ever predicted, it was cell-mediated rejection or mixed rejection in those patients.
So the last slide is basically this one, and this is to reiterate the algorithm developed by Chris and Peter Nickerson that sort of pulls this together, that there are some minor pathways that ultimately graft loss is a consequence, late graft loss is a consequence of IFTA, but perhaps even more so of transplant glomerulopathy.
Pathways that contribute are certainly these things that we've spent a lot of time and effort talking about: CNI toxicity, age, ischemia, reperfusion injury, and so on. Many of these things

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then result in T-cell-mediated rejection. There's that word, transplantese, about smoldering.
But ultimately the key link in the pathway is under-immunosuppression, and that certainly this mixed phenotype of cell-mediated rejection in combination with antibody to me is a phenotype of under-immunosuppression in the patient. Unfortunately, it can be physician guided. Many times it's patient guided in terms of nonadherence.
Ultimately, then what may begin as T-cell-mediated rejection then in the same patients then evolves into a picture, the picture we've been describing today, and ultimately unfortunately in graft failure.
So the impact of T-cell-mediated rejection is less than we probably thought it was many years. It's declined in frequency, and if recognized early, is relatively responsive to treatment. It clearly pales in comparison to subclinical antibody-mediated rejection as a predictor of graft dysfunction and failure. It remains a strong risk factor for de novo donor-specific antibody, particularly in the setting of

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inadequate immunosuppression, whether it be minimization or nonadherence.
And then the question that I would raise at the end in thinking about this is, given the role of the T cell in promoting B cell responses, can there be effective prevention control of DSA without effective T-cell therapy? I think the basis of what we do -- and I was pleased to hear some of the discussion today -- to talk about looking at B-cell therapies in the context of what we do and what we know works well in suppressing T-cell responses.
Thanks very much.
(Applause.)
DR. BELEN: Thank you, Dr. Gaston.
Public Comment and Discussion
DR. BELEN: Perhaps we'll take some clarifying questions for the presenters before we go on with public comment and discussion.
(No response.)
DR. BELEN: If you don't have any questions, we're going to go ahead and discuss the discussion questions.

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I'll go ahead and start with the second one since Dr. Gaston already touched upon it a little bit.

Is T-cell-mediated rejection early a risk factor for de novo DSA formation? Does anyone want to start discussing with this point?

Yes, Dr. Haas.

DR. HAAS: Just a point of concern is I think to group all T-cell-mediated rejection together may have some of the same drawbacks as grouping all antibody-mediated rejection together.

I noticed on one of the slides in Dr. Gaston's talk, for example, that while tubulitis seemed to be a potential risk factor for de novo DSA formation, endarteritis was not, yet both might be classified as T-cell-mediated rejection. And it was also not clear if you're referring to very early clearly steroid-sensitive episodes of T-cell-mediated rejection with lots of edema and tubulitis and very little interstitial fibrosis versus more of a smoldering TCMR in which, in addition to tubulitis, there is, for example, i-IFTA.

So I think before we definitively try to

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answer that question, I think we need to consider that T-cell-mediated rejection is not a homogeneous set of lesions.

DR. NICKERSON: I might just add to that comment. And we were talking a little bit earlier. When we did publish our original paper talking about the link of TCMR as a correlate with subsequent de novo DSA formation, one of the things that Chris had observed was in the first 6 months, and, in particular, in the biopsies that we did as surveillance, a lot of these patients who had TCMR who went on to develop DSA, one of the features of their TCMR that was strongly correlated with DSA formation was that they had peritubular capillaritis as a feature of their TCMR.

And so their severity of that score was double that of those that didn't form de novo DSA, and we had the hypothesis in that construct that the inflammation in the microcirculation may be through interferon gamma-mediated pathways upregulating MHC, especially Class II, which we know is interferon gamma-responsive, and that increased expression may be part of why there's an increased association with DSA formation.

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So I would make that comment.

I agree with you, there may be features of the TCMR that we need to learn about that are putting the patients at risk within the context of the graph.

The other comment that I would make, though, is that the TCMR might be a correlate because it's causal in the pathway of DSA formation through inflammation in the graft, but it also may be a flag of allorecognition occurring in the regional lymph node system. That is where we're seeing T follicular helper cells interacting with B cells. And so it may just be that the TCMR itself is a flag of allorecognition activity going on, and that the sentinel event is actually occurring outside the graft in the regional lymphoid system that is leading to B-cell sensitization, and it's flagging that there's a problem here.

DR. HAAS: Yeah, the other, on the same point, is that with regard to TCMR is actually in itself the risk factor, is that your data suggests that TCMR is kind of a flag for under-immunosuppression, and that maybe TCMR is just sort of signaling that the patient

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is under-immunosuppressed, and because he or she is under-immunosuppressed, is at risk for thus developing donor-specific antibodies. And so treating the TCMR per se may not necessarily -- just as an acute event may not necessarily prevent the subsequent development of de novo DSA, but a complete sort of reassessment of the immunosuppression may really be what's necessary.

DR. BELEN: Dr. Mannon, yes.

DR. MANNON: So I think the one dissociation of -- the only case I can think of right now clearly is belatacept. So the high risk of rejection early. However, the reversibility of those episodes has been disssociated from the development of DSA. And that's probably one of the few studies that I've seen that I can recall where you see that dissociation.

Or in the olden days when we would have these very early rejections and they went away very quickly with steroids, and you didn't have to go to other agents, my recollection of those -- and there has been data to show that those patients can actually do quite well.

But the belatacept is a good example where

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there is clearly a significantly higher rate of rejection and a dissociation from DSA. And that's the only exception. I mean, otherwise I think it actually -- it's either chicken or egg, but it's involved.

DR. BELEN: Dr. Nickerson.

DR. NICKERSON: One more comment. I think I want to just build on your last comment, Mark, which is that the TCMR may represent under-immunosuppression. Agreed. That's one possibility. It also may represent dominant HLA genetics that are driving an immune response despite us giving what we think is adequate immunosuppression by whatever definition we give. In other words, there are still probably antigens that will drive an immune response that will break through what we would consider is adequate immunosuppression by the drug levels or combination therapies that we currently use. And so I think that we also must be mindful that even with adequacy and full adherence of our patients, there may be mismatches that are really quite dominant in driving a response.

DR. GASTON: I agree with all of this. I think it all sort of fits together actually and in a

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sense that I think the reason why bela is different is because bela provides -- again, back to this term -- adequacy of immunosuppression and how you define it. It provides at one level or another immunosuppression adequate beyond the acute rejection episode to cut down the immune -- or to keep the immune system in check.

So I think it is a flag for what's there, and I can't help but think that you said twice what we think is adequate immunosuppression, and what you said. And I think that's the fallacy, is that we don't really have a good way to determine what that is until we see the adverse consequences. And it may well be a flag, it may well be something else that's going on.

DR. BELEN: Dr. Alloway.

DR. ALLOWAY: I think that when we talk about the rejections that are occurring in belatacept, we don't really know yet if biologically they're the same as the rejections that we're used to seeing under calcineurin-inhibitor therapy, and Dr. Woodle may want to discuss more about that.

But I want to bring up that even when the acute rejection has occurred and it's fully treated,

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and the patients remain on bela, they're adherent to their medication most of the time, and we know. And if that is now adequate immunosuppression, we know that they're receiving it, and therefore we're continuing to see the positive outcomes long term.

DR. MANNON: Fair enough, but I don't know why people reject on bela, and it's shocking. And I agree with you. I mean, I think histologically, there's a swing towards higher vascular inflammation that's dramatic and the graft dysfunction is dramatic. But it also has impressed me that they've resolved very quickly.

Now, Minnie Sarwal apparently had data with the Immucor or whatever her transcript said, saying, oh, it's very different. That hasn't been substantiated, and there is some information in the literature. I mean, we looked in the CTOT study and unpublished data and we couldn't -- now, we didn't have a lot of control rejecters on standard of care, but we didn't see these upper -- and we did low-density rates, so we weren't doing big chip, and so we didn't really see anything different.

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I think the biological behavior is different, though, because you're not expecting a 2B to go away very easily, and they do with bela for some reason. And then you put them back on it and you treat them, and then they're okay, which is odd, because, again, I don't think -- you know, again, I think just like the other drugs, you have people who said, "I took my drugs, Doc, and I was on the right levels, and I rejected."

And I don't think we understand at a cellular level what the adequacy is based on these troughs and why it's so variable for some patients.

DR. SAMANIEGO-PICOTA: What is your opinion about these CD86 oversaturation story?

DR. MANNON: I'm not sure I can -- I'm not sure I know what you're asking, and maybe Stuart knows better. I'm not sure if you're thinking that -- you mean in regards to -- you mean like there's a loss of a negative signal. Yeah. I mean, I don't know, you know. Probably in animal models, in these small animal models, like rodents, you could probably show something.

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I think it's been -- you know, Allan has tried to look at risk -- Allan Kirk -- tried to look at, you know, sort of risks based on cell populations pretransplant. And we've tried to support that substantiation in the CTOT studies and haven't been able to. So I'm not sure that we know.

And there's another company that has a different pathway that they're interested in looking at, and you do worry that maybe there is suppression of a negative signal that you're hoping to have that isn't there. But we tried it.

But Steve Woodle has a lot of data with belatacept. I'll put you on -- since we're putting each other on the spot, you know.

(Laughter.)

DR. WOODLE: I was waiting for Stuart to go first.

DR. KNECHTLE: Allan Kirk has that data, yes, that CD57-positive cells, as a memory cell subset that doesn't express the CD28 and is resistant to a blockade, and that may be a pathway. I know that's somewhat controversial and hasn't been settled yet.

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And then there are, of course, thoughts that you're blocking a T regulatory pathway, which is probably true, with belatacept. And so that's why they're also looking at non-agonistic CD28s as well.

Steve.

DR. WOODLE: So we've followed this story of the CD28-negative effector memory T-cell population that basically escapes, and watched the literature, and looked in our population. In monitoring peripheral blood, we see a CD28-negative, CD38-positive, CD8-positive cell population that arises under bela that never arises under CNI. Now, that's, say, 20 patients on a CNI, but that population is unique to bela. That's in the peripheral blood.

We've actually seen a patient have rejection in which they had a small population of CD28-negatives in the peripheral blood, but a tremendous number in the graft.

And so there is always the issue -- one of the problems is we always look in peripheral blood for these things, but the answer is really in the graft, and I think from my reading of Allan's papers, I think

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that's the direction that they're headed, and it's certainly the direction that we're in, to try to sort that out.

What I can tell you is that I've never seen rejections of 2A or 2B under CNI that didn't show a response to Thymo. I saw that for the first time in my career under a bela-treated patient, and the responses to Thymo under bela are not what we would expect. And we've used a lot more tacrolimus rescue, which is the first thing we went to. And it's not the same tacrolimus rescue that we saw back in 1995 when we first started doing it, it's different.

One thing that these cells do appear to be -- so there's a story in the literature about these cells being mTOR-pathway-dependent and potentially mTOR-pathway susceptible. We've seen that in a few patients, but even that is not. So putting patients on mTOR sometimes makes these cells go away very rapidly, but not always. And so the picture is complex.

We've looked at the CD4-CD57-positive paper that Allan has published, and it's interesting, but we've got to know the cell and we've got to put it in

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the graft, and you've got to put it in the tubule that has tubulitis to hammer down what cells are driving -- what are the primary effector cells driving this.

Technologies are out that are available, it's just going to be something that somebody is going to have to work really hard and get a little bit lucky to show.

Carla Baan actually has a nice case report of a patient that had a very aggressive rejection that went on to cause graft loss where they had done a fairly sophisticated analysis of these cell populations, and I think that's the type of data we need.

But it's certainly interesting. In spite of that, the patients that don't reject, they just sail. They literally look great, they feel great, and I think this is sort of the one big remaining issue that's out there with bela that we've got to figure out. And once we're on the right track with that, I think that that drug is going to be used a lot more, once it's available.

(Laughter.)

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DR. HAAS: Going back to these individual cases, one thing, as a pathologist, that I would certainly like to see a lot more follow-up biopsies because you cite a case where you're trying to type cells in highly aggressive rejections, and typing of cells may be very difficult if you have a graft that's just overwhelmed by inflammatory cells, but in a follow-up biopsy where you treat, and apparently incompletely treat because these patients don't necessarily improve, you will presumably be enriching the cell population within the graft for those particular cells that are really doing the damage.

And seeing sort of before and after biopsies and seeing which cells seem to become enriched when you treat and can differentiate which cells seem to respond to treatment versus which cells don't may be very, very informative and may also allow us to try and develop therapies that are directed against those particular cell types that seem to be resistant to our current therapy.

DR. WOODLE: You know, Mark, I couldn't agree more. I think one of the things that happens when we

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get these difficult rejections under bela is we're biopsying the patient regularly. And I know there is one patient sitting at the table here that wouldn't like that very much. But we felt like we needed to do that. And what will happen is you'll look under the microscope and you look at this, and you go, "Man, that's a lot of inflammation, that makes me nervous," and then you'll treat them, and you'll come back a couple weeks later and it's not much better. You come back 2 or 3 weeks later, and it's the same.

But what doesn't happen -- and those are things that under a CNI, under TAC, you would see, you would go, okay, the next time I look at this in 2, 3 weeks, I bet you there's going to be a lot of scar. But under belatacept these things seem to persist, but the scar doesn't develop. And so that's another thing that's fundamentally different about bela.

And I don't know if it's the absence of TGF-beta induction that you get with a CNI that you don't get with bela, or what it is, but you see persistent inflammation without the progressive rapid onset of a lot of fibrosis in the graft. And so that's another

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thing about bela that's unique that I think serial biopsies when studied appropriately can give us insight into.

DR. SAMANIEGO-PICOTA: Steve, in those patients you just mentioned, do they have normal function, and the only abnormality is this infiltrate on surveillance biopsies?

DR. WOODLE: Yeah. So, you know, they start out with low creatinines to begin with --

DR. SAMANIEGO-PICOTA: Yeah.

DR. WOODLE: -- like .8, .9, and 1. They'll bump up to like 1.4 or 1.5, and they may sit there for a long time, not clearing the lesions, given Thymo, given TAC rescue --

DR. SAMANIEGO-PICOTA: They stay there.

DR. WOODLE: -- not clearing lesions, they stay there, but what doesn't happen as much is they don't have progressive deterioration of renal function associated with progressive fibrosis.

DR. SAMANIEGO-PICOTA: Have you immunostained those tissues to how they --

DR. WOODLE: We have not done as much as I'd

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like to do. And so one of the things that we've had to do in our institution is basically gear up a program to start to be able to look at this. And so the rules are different. The rules under bela are different.

The other thing that's a little bit different is viral responses. I think that our impression is we have to be more aggressive about your concomitant immunosuppression reduction to clear virus under bela as compared to CNI. Now, we haven't analyzed our data, and we need to do that. But that's our impression. I don't know if Stuart or if you other folks that have used bela have had that same impression about clearance of virus under bela also.

DR. MANNON: I would respond, but I feel that this is not a bela session, and I feel guilty for starting it. So I will discuss after.

DR. BELEN: Okay. So we'll give the last comment to Ergun, and we're going to move on to the next question.

DR. VELIDEDEOGLU: Just one quick question to Professor Woodle.

DR. WOODLE: Bela is a very important drug

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because there are several groups -- there are groups poising to use that in combination as an antihumoral therapy strategy. So I just would leave it there. I think you're going to hear more about that tomorrow certainly from Stuart and possibly from others.

DR. VELIDEDEOGLU: One very quick question, clarifying question. When you say "TAC rescued," I assume the patients are discontinued from bela, is that right? No. They receive TAC plus bela. Okay. Thanks.

DR. BELEN: Okay. So we're going to move on to the next set of questions.

From a posttransplant DSA development perspective -- and we're going to take it in two different questions, the first one being, Should induction treatment strategies be based on immunologic risk? And then the second part, if you could discuss CNI minimization and avoidance of corticosteroids from the perspective of DSA development. But we'll start with the induction question.

DR. SAMANIEGO-PICOTA: Yes.

(Laughter.)

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DR. BELEN: Okay.

DR. SAMANIEGO-PICOTA: Let me see how -- really, all the data we are getting about development of DSA and induction agents is from retrospective data, most of them, from postdoc analysis from previous studies. Logic suggests that, yeah, induction therapy is important and it's useful. Which is the ideal agent for induction therapy? Whether if you're looking at T-cell depletion, is it Campath? is it Thymoglobulin? It is possible that both are equally good depending on what type of maintenance immunosuppression you put that patient on, Campath, (inaudible), and MMF, seems to be doing fairly decently based on the Cedars-Sinai experience.

Thymoglobulin works very well, has certain advantages, and Campath does as well, and is the targeting of natural killer cells. And I do not know how -- Stuart probably knows very well -- how well is Campath at depleting natural killer cells vis-à-vis Thymo?

DR. KNECHTLE: I don't know the comparison of that, Millie, but alemtuzumab does target NK cells

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effectively.

DR. SAMANIEGO-PICOTA: Yeah, absolutely. So I don't know that the answer is clear. That's why in Dr. Djamali's paper it's clearly established that randomized clinical trials need to be done. I do not know that there is an appetite in PhRMA to do these kind of randomized trials mainly because the conflict of interest in Sanofi being they are a company owning both medications that may not be able to ever been done, and not in the United States at least.

Whether we should combine a cell-depleting agent at one point in time, give rituximab, you can really only do that in the setup of desensitization. You know, in a patient that gets the transplant tomorrow -- today I mean, 2 days later have a recall response or a memory response and has AMR, that is really not induction, it gets then into the treatment part of things, but if you think -- and I think everybody here probably agrees -- that for antibody, de novo antibody, these are T-cell-dependent antigens, you need to have T-cell activity. T-cell control of T-cell immunity is essential to prevent de novo antibody

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formation. Induction can help to certain level to that.

DR. BELEN: Thank you. Anyone?

DR. KNECHTLE: Don't you think one answer to that question is, what's happening clinically? I mean, just look at drug in the United States; 70 to 80 percent of patients are getting depleting induction therapy. I think that gives you the opinion of most clinicians in the United States regardless of what the co-called experts today think.

DR. BELEN: Okay.

DR. DJAMALI: If I may add a comment here.

DR. BELEN: Yeah.

DR. DJAMALI: I agree that the vast majority get T-cell depletion. And the main question is, Which T-cell depletion is the right approach for the induction of sensitized patients? One of them is effective, and both of them are effective, but one of the would be much more costly than the other one. And the best randomized trial we have comparing Campath to Thymo in sensitized patients, this is patients with a PRA of less than 20 percent, so they are not that

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sensitized. I would really love to see that.
I don't know. I think you're right, absolutely have to do a T-cell depletion, but which one?
DR. BELEN: So we'll move on to the next section of questions regarding the avoidance, CNI minimization, and steroid avoidance. No? I think this was touched upon a little bit, but --
DR. NICKERSON: So I would say we would all love to do CNI minimization, and we'd like to do it as much as we can, but every attempt that we've tried to do it has failed, I would say, so far, especially as it relates to being at risk for DSA. The data around the HLA matching of donors and recipients I think may be a window into where there might be some selective opportunities.
But, again, and I've made this point, the data that's been generated so far has been largely in a Caucasian-based population. We don't know whether that's going to be true in other genetic backgrounds. And certainly I think there needs to be more study in a more diversified cohort of patients more like what we

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would see in the United States and what we see in our program in Canada.
And then I think to do this properly, it should be done in a prospective, randomized, controlled trial where we use selection of these patients for enrollment into a CTOT-09-like study where we do it under very careful conditions to monitor for immune reactivity.
So I think there are opportunities, and certainly we should be pursuing those in the absence of some new medication that's going to all of sudden show up and replace CNI. Whether that ultimately might be bela, I think we're going to wait and see. So I think there's opportunity, but I think it needs to be done in proper studies and shown in more than just one or two populations.
DR. GASTON: So when I look at question A and question B there, to me, the issue is, as Peter just said, the issue I think is yes, we should base induction on the immunologic risk. We would love to eliminate, to minimize or eliminate, CNIs, but we don't have any guideline. We only seem to know whether we've

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done the right thing after the fact. And until we have ways to decide for point for number A to define immunologic risk better than we do right now, or, B, who to minimize in, then we're going to be sort of operating blindfolded.
DR. HAAS: I think one of the problems we have in addressing the possibility of CNI minimization is I don't think that we really know in the current era of CNIs, that is, tacrolimus at its current dosage versus higher doses of cyclosporine in the past, how much chronic damage CNIs really do to the allograft.
And if you go back to the studies, the protocol biopsy studies, that Brian Nankivell and his colleagues did now 20 years ago, they concluded that chronic CNI toxicity was a major contributor to IFTA and ultimately to graft loss. But this was done, cyclosporine, and it was also done when higher doses of CNIs were done.
Phil Halloran has suggested on numerous occasions, based mainly on molecular data, that in today's environment CNI nephrotoxicity contributes very little to graft loss.

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So before I think we can consider whether CNI minimization is a worthwhile pursuit, it would be worth knowing, how much chronic damage can CNIs do to the graft? And we don't know that.
And just one plug into point C, I can't really speak as to the significance of corticosteroid avoidance regarding DSA development, but my other hat is as somebody who's interested in glomerulonephritis and particularly IgA nephropathy. And one of the biggest problems that at least I perceive with corticosteroid avoidance is recurrent disease, and I think that needs to be a consideration beyond just DSA.
DR. BELEN: Dr. Matas?
DR. MATAS: Well, I can't let the comment about the Nankivell paper go by without pointing out that there was significant inflammation in those biopsies that they ignored when they did the study. I think a preconceived notion of what they were looking for, and if I remember correctly, 25 percent of the biopsies between 1 and 5 years had inflammation, which clearly is hard to discriminate what was cause and effect.

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But I think it's important to minimize the drugs. I mean, we all know that. We've seen the benefit of steroid-free protocols. You can't tell in clinic anymore who's on prednisone -- I mean, who is a transplant patient like you could 20 years ago when people showed up with all the puffy face and so on.

And CNI minimization can't be bad if we do it well. And the trick is going to be how to define the subpopulation. And to give you a reason why, the flipside of Tom Nevin's (ph) data, where we looked at immunosuppression adherence using the MEMS Cap, we recently looked at -- and this was presented at ATC last year -- malignancy in relation to adherence and nonadherence, and, in fact, the perfectly adherent patients had more malignancy. And interesting in just looking at it recently, they also had more CMV infections.

So we really need to find the right balance between minimization and preventing rejection.

To address your comment about IgA nephropathy, I think we have to define who, which subgroups -- and I certainly agree with you about potential recurrent

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diseases. There is just a paper published looking at steroid-free immunosuppression in patients with IgA nephropathy, and I wish I could remember where, maybe JASN, showing increased recurrence in the steroid-free group.

And so I think we're going to need to have to look at individual recurrent diseases one-by-one because it may not apply across all recurrent diseases, to see if steroid-free immunosuppression can be done.

But the definition of "steroid-free" has changed. So when we started steroid-free, we were giving whopping doses of prednisone, and it may be that simply 5 milligrams a day would be enough to prevent that recurrence of disease. I think those are all questions that need to be answered.

DR. WOODLE: Arthur, I would make the point that our experience suggests -- and I think, if I'm not misquoting the IgA data, that there is a higher incidence of recurrence, but the progression to graft loss was not different within the first 3 to 5 years. Is that correct? Certainly that's what our data suggests.

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And, as you know, IgA recurrence is a pretty common thing. Graft loss to IgA recurrence is a different issue.

DR. HAAS: That's because -- I think the problem with IgA recurrence in terms of interpreting the data in the literature, which is widely, widely varied, is how one defines a recurrence. Some centers define a recurrence simply by the presence of IgA in the mesangium. Now, these people have abnormally galactosylated IgA, and this is going to deposit frequently in the mesangium regardless of their immunosuppressive status. And those studies that define an IgA nephropathy recurrence simply by the presence of IgA will state that graft loss due to IgA recurrence is rare because IgA recurrence is so common.

On the other hand, other studies will define IgA recurrence by either mesangial proliferation or by proteinuria, and now we're talking about IgA recurrence rates that are more down in the 10 to 20 percent range or even less, but that graft loss due to the IgA recurrence in these studies is greater.

But one thing that was pointed out, I think it

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was in the Ponticelli in KI now about 10 years ago was that IgA recurrences that lead to graft loss tend to be late recurrences, usually recurring about 7 years and beyond posttransplant. So again when one considers how one wants to deal with steroid reduction in those cases, again, one has to consider the timing of the steroid reduction and also the timing of the recurrent diseases and how one defines a recurrent disease.

DR. SAMANIEGO-PICOTA: I want to make a comment, Mark. We had made the same decision of not including patients with biopsy-proven GN in steroid-free, but there is really not too much data to show that that is the right decision. We assume it is. For instance, I don't think of IgA nephropathy primary disease as necessarily a steroid-responsive type of disease. I would like to hear what the other nephrologists in the group think.

DR. DJAMALI: Actually, I think there is data from maybe Art's group, Aleksandra Kukla was the first author on this paper that looked at all patients with primary GN, and those that underwent steroid withdrawal as opposed to not, and those with steroid withdrawal

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had a higher recurrence rate. So all-comers GNs, now what we do is that we keep them on low-dose steroids.

DR. SAMANIEGO-PICOTA: I agree, but what about IgA? Do you consider IgA nephropathy a steroid-responsive GN?

DR. DJAMALI: If I relied on that observational study, yes, and the more recent study that came out, yes.

DR. HAAS: It depends on the IgA. There are -- I mean, the debate as to whether one uses steroids in IgA and whether it's a potentially steroid-responsive lesion depends on a number of different factors. Endocapillary proliferation is one that seems to be associated with steroid responsiveness. Crescents, certainly associated with steroid responsiveness. And there is some data also out there that graft loss -- this is in Henoch-Schönlein purpura rather than IgA, but I consider them sort of sister diseases -- that crescents are associated with not only an increased rate -- in the original biopsy, crescents are associated with an increased rate of recurrence and an increased rate of graft loss due to recurrent

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Henoch-Schönlein purpura nephritis.

And, finally, there's a paper in KI from the Oxford group this month that podocytopathic segmental sclerosis, which is basically segmental sclerosis with overlying swollen and hyperplastic podocytes, is a steroid-responsive lesion. But purely mesangial proliferation IgA is not.

But then again, grafts are not usually lost to purely mesangial proliferative IgA nephropathy. It's those with the crescents, it's those with the endocapillary proliferation, it's those with the segmental glomerulosclerosis that lead to graft loss and IgA.

So I think you need to consider the high-risk IgA's that do lead to end-stage renal disease as being the same ones that are more likely to recur in the transplants.

DR. BELEN: Well, I think this is a wonderful discussion, but we're going to give the last words to one of our patients, Mr. Michael Mittelman. Please.

MR. MITTELMAN: Thanks. Man, I see this as super cut-and-dry. I mean, the conversation is fun to

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listen to.

But, I mean, A, you know, yes, it should definitely be based on immunologic risk. I don't know why it wouldn't be. It seems to me a pretty cut and straight answer.

I mean, B, I take a CNI. So I would certainly say that it should be applied to all, but with the same extent, I'm almost 16 years out with this ABO-incompatible transplant, and one of the surgeons was here, and another one I think is going to be here tomorrow, and their philosophy, and I agree with it, is sort of if it isn't broken, don't fix it.

I'm on a really low dose, and I can tell you from when I lost the second kidney, they switched me to a drug and it screwed my kidney, because they thought the other one would be so much better.

So if there's a little bit of toxicity or potential risk for that, you're already sort of on borrowed time, so why would you ruin something if it's working well just because of a possible risk of -- right? If you're not seeing it, don't kind of screw with it, but minimize it.

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And, C, absolutely, corticosteroids are the worst. Kids shouldn't be on them. I think you've talked -- I've heard a lot about really only adult usage, but, man, pediatric usage of corticosteroids have screwed me big time, and I don't know why you would ever put people on it, particularly if they're posttransplant if it can be avoided. That's my 2 cents.

(Applause.)

Wrap Up -- Day 1

DR. BELEN: So we're going to wrap it up.

DR. ALBRECHT: Well, thank you very much. We're now at 6:00 and closing. So again let me just thank all the speakers for the outstanding presentations, and, again, especially express our sincere appreciation to the patients for sharing their stories with us.

With that, I think we'll close and we'll come back tomorrow -- and actually my apologies -- and really good discussions, very much appreciate everybody interacting and sharing viewpoints.

And with that, we'll close and we'll reconvene

tomorrow morning here at 8:30.

Thank you. Have a good evening.

(Whereupon, at 6:02 p.m., the meeting was adjourned.)

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