FDA PUBLIC WORKSHOP:

ANTIBODY MEDIATED REJECTION IN KIDNEY TRANSPLANTATION

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Page 2

A P P E A R A N C E S

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Page 3

A P P E A R A N C E S (Continued)

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Chris Wiebe, M.D.
E. Steve Woodle, M.D.

Page 4

C O N T E N T S

Page 5

P R O C E E D I N G S

DR. ALBRECHT: As you know, we had a series of session yesterday during which we covered a number of topics. Today we have Session 4 and Session 5 during which we'll cover topics on post-transplant and then on clinical trial design and animal models.

So just to get a couple of reminders for those of you need taxi transportation to airport or other locations please go ahead and ask at the information desk on the first floor.

Also for the panelists, as yesterday, today you may also purchase boxed lunches. The gentleman is sitting outside the conference room and will take your orders. There's a choice of four boxed lunches.

For others lunch will be served in the cafeteria during approximately 11 to 1:00 so you can avail yourself of that. And the Internet access if the same as yesterday.

So with that we're going to start the morning. This morning's session will be monitored by me and Dr. Peter Nickerson and we'll go ahead and get started. Thank you.

Page 6

DR. NICKERSON: Thanks very much, Renata. It's a pleasure to talk about post-transplant monitoring, diagnosis, treatment of AMR. And just before we start we are going to try and keep all the speakers on time today. We're going to try and finish by 1:00 given the fact that this is going into a long weekend and we're wanting -- mindful of traffic is probably going to flow early today. So we're going to try and finish by 1 so if everybody can please stay on time.

Our first speaker is going to be Dr. Chris Weibe, my college from the University of Manitoba. Chris?

DR. WIEBE: Okay. Thank you very much for the invitation to be here. I have no disclosures. And I want to start by actually talking about the prevalence of de-novo DSA and incidence rate as it was mentioned by Arjang yesterday in her introduction.

If you look at the literature it's actually quite heterogeneous. As you can see on this slide we've reported a 2 percent incidence in the first year at the same time other centers are reporting up to 20

Page 7

percent incidence in the first year.

So how can this both be true? Well, if you look at these five selected studies here and you look in the column in blue, you'll see that actually incidence rates have been reported everywhere from 27 percent at one year down to 2 percent at one year. But what's actually remarkable is after that first year all centers kind of agree that the incidence rate is around zero to 5 percent per year thereafter.

So what's the difference in the first year? I think although they're -- we could argue that there are many possibilities, the real driver is how did that center choose to rule out DSA at the time of transplant?

It should be no surprise that if we use an insensitive test or a less sensitive test to rule out DSA at the time of transplant, we are going to either intentionally or unintentionally miss some low-level antibodies that we're calling negative. And when that recipient, then, is re-exposed to the donor antigens post-transplant that antibody can increase in titer and go from being called negative to being called

Page 8

positive.

And I think if you look in the red column where you see the incidence of de-novo DSA being called in the first month that really highlights that this is in many cases a recall response. Whereas if you're very conservative at ruling out DSA pre-transplant, you would expect to see very low rates in the first six months to a year period.

Now I won't dwell on this slide as it's been shown multiple times, but clearly this has to be taken in the context of the patient adherence as well. And why do we care about do-novo DSA?

Well, as we have shown back as early as 2012, there's about a 40 percent lower graft survival at 10 year in patients with de-novo DSA. And this is largely driven by the clinical cohort, which has the worst graft survival seen in the red line here.

But also clearly the subclinical de-novo DSA patents do decline if you wait for it and follow them in the long term, as shown in the blue line here, and have similar rates of graft loss as the other AlloMune and non-AlloMune causes of graft loss in kidney

Page 9

transplant patients.

And it's important to note that 76 percent of this cohort of 508 patients has actually had stable function and was doing well. And I'm going to talk more about what that means specifically.

To get into some of the detail of this we looked at this cohort of 500 patients and we actually had nearly 13,000 eGFR reports that we could really dig down to see how their function was changing over time.

And the first interesting observation is what you see here in the stable group. You can see that their rate of eGFR decline was about 0.43 mils per minute per year which is interesting for the reason that it's actually very similar to what's been reported in the age-matched healthy non-transplant population going back to the studies in the 70s where these types of things were being studied.

And so it does suggest that if you can avoid DSA, you can avoid rejection and obstruction and these other causes of graft dysfunction the kidney transplant recipients can do very well and are

Page 10

declining at the same rate as the rest of us.

The de-novo DSA patient population, on the other hand, even before DSA ever developed you can see that they have a four-fold higher rate of eGFR decline. And after the onset of the antibody this rate is again significantly accelerated and, in fact, doubled. And this makes sense when you think about it pathologically because now we have new arms of the immune system like the antibody dependent cellular cytotoxicity and the compliment pathway that can start to lead to graft damage.

And this was actually very consistent across the subclinical and clinical groups. Both had higher rates of decline early on and accelerated rates of decline later after the antibody was developed. And really the big difference between these two groups is that there's also a step-wise decline in the clinical group.

So what this looks like in pictures is shown here on the top where you can see that the clinic group if you look just in the red line in those first one to two years, many of the patients with clinical

Page 11

de-novo DSA lost their grafts early whereby the subclinical group almost by definition did well in those first two years.

After the first two to three years -- and this is from the time of antibody onset forward, not from the time of graft -- not from the time of transplant. But after those first two to three years you can see that the lines are actually relatively parallel. But because of the early difference the median graft survival is nearly five years different in these two phenotypes.

And in terms of eGFR what this looks like in pictures is we have the green line at the top there where we have the stable graft slowly declining over time and we have the de-novo DSA patients represented by the black line where even before the antibody develops they do have a faster rate of decline. And then at the inflection point when the antibody develops we have a step-wise decline in the clinical group, but after antibody development both the subclinical and clinical group are declining at a faster rate.

Page 12

Now, like many other centers, we have been interested to see if there are serologic predictors that would help us to discern which patients are going to progress more quickly to graft loss after DSA development. And we've looked at titrating these antibodies down to figure out their relative strengths and we've also looked at the C1Q status.

And as you can see here both in all comers on the left or just the subclinical cohort on the right titration and C1Q status were both kind of weak to moderate univariate predictors of post-DSA survival.

However, after when we actually adjusted for some of the more robust predictors like non-adherence or the clinical/subclinical phenotype these serologic predictors fell out of the model. And I should frame my comments to say that many of the C1Q studies done to date have either been in a mixture of pre-transplant and post-transplant DSA or in some cases when they have looked exclusively at de-novo DSA it hasn't been in the setting of monitoring. It's been in the setting of at the time of graft dysfunction so

Page 13

many -- there has been a discrepancy in the literature in this regard.

So one of the things we're interested in doing with this data is really looking at how we could design a clinical trial. And I'll start by talking about graft survival.

If we were to design a trial that was a five-year study looking at graft survival assuming a sample size power of 80 percent, an alpha of .05 and a drop load of 10 percent and we took all DSA patients who I've already showed you have a median five-year graft survival of 60 percent and assuming we had a treatment that could reduce that risk of graft loss by either 25, 35, or 50 percent, you can see across the top of this table that we would need 600, 300, or 150 de-novo DSA patients in that study.

And keep in mind that only 5 to 10 percent of the average tacrolimus-treated patient population will develop DSA at five years so this is a substantial cohort that would be needed.

On the other hand, if we wanted to enrich that population we could look at the de-novo DSA

Page 14

patients that have clinical dysfunction. And I'm showing here that their median five-year graft survival rate is 28 percent and so you can decrease the number as needed to study by about a third in each of those scenarios.

But the caveat that was mentioned yesterday and is certainly true that in this patient population 90 percent of the patients have either subtle or overt nonadherence making them less than ideal patients that you may want to enroll in a multicenter, multi-million dollar clinical trial.

And I'll, again, frame those comments that if you actually had a good intervention to address nonadherence that may be a very good idea.

So what about other potential surrogates? eGFR, as you know, has been already considered by the FDA to be a valid surrogate endpoint in the CKD literature for predicating and staging of disease. And here doubling of serum creatinine which is a 57 percent decline in eGFR as a valid surrogate endpoint. And also in a consensus paper by Thompson, et al, they discussed the 40 percent decline in eGFR over two

Page 15

years assuming a baseline eGFR of 50 mils per minute, which is actually quite consistent with the average transplant patient.

And we did look at this in our cohort and we saw that for each 1 mil per minute decrease in the eGFR at three years post-DSA onset that there was a hazard ratio that was highly significant for graft loss.

And so crunching the numbers what that looks like is if you were to do a two-year study you would expect an eGFR decline of around 7.8 mils per minute in that two-year period. And if you had a therapy that could reduce that eGFR decline by either 50 or 70 percent, you can see you need 550 or 282 patients. And the numbers in brackets there are the expected risk reduction in graft loss.

And if you extended that study to three years you can see the numbers do go down slightly. So these represent slight decreases from using the gold standard of graft survival, but probably the major advantage here is just that the duration of the trial could be shortened somewhat.

Page 16

What about the pathology scores? One of the things that we did to create this table we used over 1,000 biopsies of which 371 of those biopsies were from the de-novo DSA patients. And we were interest in what were the multivariate (mic drops) and so what you're seeing here (mic drops) the CG -- each row here is its own independent multivariate model.

So you can see for the CG scores in the first three rows that after adjusting for time post-transplant and nonadherence and cellular rejection de-novo DSA is a very strong predictor of the CG scores as been reported previously. However, de-novo DSA does not predict the IFTA scores. These are, in fact, driven by cellular rejection time and nonadherence.

Furthermore, if you actually look at CG and how it can predict graft survival, this is looking at just biopsies done in de-novo DSA patients. And the X axis here is time post-biopsy. And there are 70 patients included in this analysis and I actually just created this figure last week so it's unpublished data.

But you can see that using CG score at the

Page 17

time of the biopsy the red line is the CG score of zero, green is a combination of CG score 1 or 2, and blue is CG score of 3 that clearly these do separate out and that's highly statistically significant.

So the rationale to use a Banff CG score, then, as a potential surrogate endpoint is, first of all, it does correlate strongly with de-novo DSA. Secondly, it's actually quite infrequent at the time of de-novo DSA.

We've reported in our cohort where we are routinely monitoring at least annually for de-novo DSA that 87 percent of those patients will have a CG score of zero at the time of the DSA onset. And this is important because it suggests that we do have time to intervene in these patients before they go on to develop scarring.

And that when we follow these patients over time we're seeing an increase in the CG grade of approximately one point for every three years thereafter. And as I showed you already in the last slide CG score does have prognostic significance.

The caveat, of course, is that we would

Page 18

first need to validate that preventing the progression to CG would also correlate with improved graft survival.

And I think also mentioned yesterday is that perhaps this isn't even the best way to look at this question since we now have electron microscopy. Looking at peritubular basement membrane multi-layering may be both, number one, a more sensitive method to define this evolution and, number two, it actually provides you potentially a wider range of values to study since you're not stuck in the CG score of zero, 1, 2, or 3. You could actually maybe have a score of zero to 10 or even higher if you're looking at peritubular basement membrane multi-layering.

So to summarize some of the important points -- and I know that this figure from our review paper last year was shown yesterday, but it's important to highlight that the AlloMune causes of graft loss really are driven by IFTA and CG. And IFTA is largely driven by TCMR.

As we talked about yesterday, this can be clinical or subclinical. And the reality is that this

Page 19

is smoldering in many patients. And just to give you an example of that from the de-novo DSA patients we have published that in the early time points in the zero to six-month time points patients who will eventually go on to develop de-novo DSA actually have higher rates of both clinical and subclinical TCMR in that first six months.

And when we went on and biopsied those patients for protocol or surveillance at six months we observed higher levels of tubulitis, interstitial inflammation, and, as mentioned yesterday, peritubular capillaritis in the patients who don't yet have antibodies but are destined to go on and get them at a later time point.

And as I mentioned, TCMR even after these time points is an independent multivariate predictor of de-novo DSA. And lastly, when we find de-novo DSA sometimes at four or five years post-transplant or longer and we biopsy those patients right at the onset we again are seeing a high level of mixed rejections and both -- this is driven by both the interstitial inflammation and the tubulitis. But specifically

Page 20

tubulitis was an independent multivariate predictor at that time point for progression to graft loss.

So, in other words, at all time points that we actually look for TCMR in these patients we're finding either subclinical or clinical TCMR in many of them. And this should probably be no surprise because both the ABMR and the TCMR are really driven by the same two major risk factors.

We have the AlloMune risk which as Peter mentioned yesterday is likely most precisely defined by the degree of epitope mismatch. And we have the degree of immunosuppression which, as mentioned multiple times, can in many cases be under immunosuppression which is either nonadherence at the patient population or in cases physician guided.

So to summarize what I said, the enrichment strategies that we may be able to use to increase the endpoint frequency to study this patient population are DSA titer, medication nonadherence, tubulitis, and CG scores.

And really the best endpoints are still graft loss, but I think Delta eGFR or Banff CG scores

Page 21

can be considered. I'll stop by saying thank you very much for invit- -- the invitation. And thank you to my mentors, especially Peter Nickerson and David Rush. Thanks.

DR. NICKERSON: Thanks very much. Our next speaker is Dr. Lakhmir Chawla who will be talking about the best ways to assess renal functional status.

DR. CHAWLA: Good morning and thank you for the invitation. I'm going to take you through something that's a little bit different regarding the assessment of kidney function. It's a little old and some things are a little new

These are some important disclosures. I'm currently on sabbatical at a company in San Diego, California, which has absolutely nothing to do with the kidney at least for now. And I do have some other relevant disclosures due to companies that work in the biomarker space.

So when we think about stress testing clinically we're all very deeply aware of what this is. If a patient comes in with angina we know what to do, if they have intermediate assessment or

Page 22

intermediate biomarker we ask the question do you have a critical lesion, we put them on a treadmill with dobutamine thallium in them, we stress them. We have like 19 flavors of cardiac stress testing.

We have precisely zero for the kidney and so we set out to make the assessment as to whether this might be valuable.

Now as everyone here is aware whether you're talking about acute kidney injury or chronic kidney disease or patients with disease in transplantation the tubule is very important and the interstitium is very important. And I hope to convince you that this might be a relevant way of approaching them.

Now thanks to Andy Levey and the MDRD group and CKD-EPI we now have data of the transplant population. I ask a fellow what is someone's GFR through the day and this is the line they will typically draw, right. That's your eGFR, it's 75. Everything is great in the world.

But that's actually not your GFR through the day. This is what your GFR looks like through the day. So your GFR is not a straight line. It's not a

Page 23

pulse rate of 55 all day long. Your kidney responds primarily, as it turns out, to protein. And what you're looking at are three healthy meals a day with a reasonable protein balance.

And as it turns out vegetarians because they eat less protein have lower GFRs and they have no kidney disease. So I don't want to attack eGFR. I think it's incredibly useful. It is a good surrogate marker. It does work in large populations. But for individuals creatinine is a 60-year old test and we should probably do better.

Now in cardiology we have robust assessments of pathological stress, physiologic stress, and we have testing to look at those things. We do not do as well with the kidney. And this is just to show you what your baseline eGFR is and this is what happens when you get a large slug of protein.

This is 1 gram to 2 grams and what you can see is after about 1 gram of oral protein loading your GFR goes up from a mean of around like 110 to about 150. And you can see in some individuals this number goes well above 180. And if you look at the actual

Page 24

range there are individuals who will go above 200.

So if you have a nice ribeye at Ruth's Chris plus or minus the butter on top and you have healthy kidneys you will have an amazing GFR. You should call Guinness and go, "Check this out, I hit 200."

But you won't notice it and no one will really care, but it does tell you about your renal reserve. Now you can do this intravenously, you can do this through an oral load, but all this is very effective and it does tell you about your reserve. What reserve do you have?

Now the reason why this is relevant is because you can test this in individuals. You can look at this baseline GFR. As you work your way up on the protein load you hit a plateau. I refer to this as the Bosch limit because Juan Bosch is the one who actually discovered or revealed renal reserve.

And if you have a patient who has an allograft or a kidney donor is a better example and you stress them, what you will see is they do not, in fact, have this ability to increase their eGFR. Their GFR does not go up after a big protein load. It stays

Page 25

flat because they do not have reserve because they've lost 50 percent of their renal mass.

And this is how we can measure this. And we also know that patients with subclinical kidney disease lose their functional reserve before they begin to dig in. So once a regular patient bumps their creatinine they have lost 50 percent of their reserve.

This is lost on most of my colleagues who take care of patients in the floor because the creatinine goes up .3 and it's much to do about nothing, but I make the point that if you'd lost your kidney you'd probably notice.

So the issue about glomerular reserve has been known since 1985. And to be honest with you the reason why this hasn't really taken off is because as a society taking care of patients with kidney disease we really are not interested in pre-CKD in the way we are prediabetes. We probably should be, but we're not.

So if you are someone who think you might have prediabetes they'll bring you in for an oral

Page 26

glucose tolerance test, we'll look at your hemoglobin A1C. If it's off, we begin to intervene. It's almost certainly the case that would work with patients at risk for CKD and hypertension, but it's just too much work and too much trouble to do this assessment. And no one has really done the science yet, but it makes sense that this would likely be the case.

But in addition to glomerular reserve there's also a construct of tubular reserve and these two things are not the same. Now this is a study done by Herrera and Rodriguez-Iturbe in Venezuela. And what they did is they took cohorts of patients to healthy patients and patients with CKD and they gave them a test meal which basically is a large protein slug.

And what you see here these are your patients with -- who are healthy this is the increase in GFR. So you see an increase. It's not massive. But this is the increase in the CKD patient. CKD patients also have a mild reserve and this is probably because they took a baseline in the morning and this is their reserves for the day.

Page 27

But this is what happens with their tubular reserve here. It -- and this is your eGF- -- I'm having a little bit of -- here we go. This is what happens when -- that's inulin. This is what happens to your creatinine clearance. This is much bigger jump up and you see a flattening here.

And I'll show you the next slide which will put this into context. So then what they decided to do is the same group then gave a cohort of patients who were healthy kidney donors and CKD patients intravenous creatinine. This has never been done before that I can find in the literature.

So they got a bunch of people. Where they got their creatinine, how they made it safe and GMP for intravenous I don't want to know and I prefer not to ask, but they did it. And this is what you see. What you're looking at on the left is inulin when you give someone a lot of intravenous creatinine and nothing happens to their GFR.

So the fixation on creatinine is interesting since it doesn't do a thing to your GFR, but that's neither here nor there. But what does happen is your

Page 28

creatinine clearance goes up dramatically and that's because your tubule secrets creatinine and it does it very effectively and it can be induced.

And these are healthy patients. These are patients with a single allograft. These are donors who have given a kidney and these are your CKD patients who have no tubular reserve whatsoever. They have no change.

And so we asked the question, well, if tubular reserve dissociates from glomerular reserve this might be informative for both acute and chronic diseases of the kidney. And so if you set out to look at this we know that we can assess glomerular reserve. We probably should, but we don't. But we know how to do this. This is well established. There's hundreds of protocols if not thousands that have been done in the last 25 years.

But the question is is can we assess tubular reserve? And the answer is almost certainly yes and I'll show you some of those data in a moment. But also and importantly the question is is does reserve matter? And I would argue strongly that it does and

Page 29

that we do it for every other disease and we have made enormous gains when we do this. We should consider doing this for the kidney, but that is a different talk and we don't have time to delve into that right now.

We decided to assess and develop a tubular reserve test for patients with acute kidney injury. And if you think about acute kidney injury the vast, vast majority of pathology is considered to be tubular. And the area of the kidney that we're most interested in is the S1, S2, S3 segment and the loop.

And so if you think about the interest level this is really what you want to test. This is what you want to be able to functionally assess in real time. Now there's some very fancy things and if you have a mouse you can do everything, but humans at the bedside who are critically ill on vasopressors with nine tubes in them are not amenable to this kind of intervention.

So we decided to use furosemide as a way to test the kidney. Now furosemide is uniquely suited for this role. The reason why is furosemide is not

Page 30

filtered at all. Furosemide is very tightly bound to albumin. And the way it gets excreted is by active secretion through the proximal tubule.

Furosemide is not filtered at all so in order for you to get furosemide out of your body and in order for it to be a diuretic it has to go from the blood side of the kidney, it has to be actively picked up by the human organic anion transporter and actively secreted into the proximal tubule.

And we basically made the assessment, well, if this is glomerularly independent we can test the tubule and the readout's very straightforward if the furosemide can get from the blood into the lumen and get to the loop there's a nice readout. It's urine output.

So what we did is we basically developed an assessment where we gave a standardized dose of furosemide in a highly controlled fashion and we looked at the urine output. And very simply we gave 1 mg per kg and we replaced the urine that you put out so we didn't hurt anybody and make the volume depleted.

Page 31

And this is the study which is in your packet. I'll let you read this at your own time and leisure. But we basically looked at the standard KDIGO criteria at the time they were called akin. And what you see here -- and I hope this is projecting well -- is the people who progressed have the stripe bars. They basically lose and do not have the ability to increase their urine output. The patients who did well are the ones who could actually increase their urine output.

And there's no special genius to this. This was being done for 50 years in nephrology and critical care medicine, right. Usually the story goes something like this: Hey, I got a patient, they're really sick. I want you to dialyze them and it's like Friday at like two in the afternoon. And nobody wants to put a catheter and dialyze somebody Friday at 2:00 in the afternoon.

So the attending tells the fellow to tell the intensivist to give a giant slug of furosemide and they do. And they pick some random dose depending on your decile of training it goes from 500 milligrams to

Page 32

50 milligrams. The older you are the more you're willing to give for reasons that we can't get into now either.

So you give this giant slug of furosemide, nothing happens. And they go, oh, boy, put a catheter in. And it works. It's actually quite effective. It's incredibly crude, but we basically took that insight that has been sort of floating around critical care medicine and nephrology for 50 years and we standardized it.

What's cool is how well it performs. It gives you an AUC curve of .87. And for those of you who don't live in diagnostic land .87 is really good. Troponin lives at .91. So, you know, we were alarmed that physiology worked. It was quite stunning, in fact, but, you know, there you have it.

So, you know, you can use a simple test that's 10 cents for the furosemide, it's $1 for the saline. The medical student depending on your institution is free or costs you millions so it's your call on that one. But nonetheless it's a simple test. It gives you a nice readout and it performs well.

Page 33

This has been validated now on multiple sets.

And we then actually looked at this head to head against biomarkers. I won't take you through this, but it's .87. Every other biomarker was under .7. This was not good news for the people I collaborate with in industry by the way. They did not like this.

Anyway, it performs well. And the key take home point is that you can do this, it does work, and it has a high performance level. And I apologize for blowing through this so quickly but it's in your packet.

But I want to show you why else I think it's important. We've actually done this now in DGF so as many of you are aware surgeons love to see urine come out of the patient. It makes them feel very happy and warm on the inside.

So what most transplant surgeons do is as they're closing they give 100 milligrams of furosemide. They do this for two reasons. One it kind of tests to see if things are working. The second thing is that it makes them look really good

Page 34

when a patient arrives to the ICU after an allograft like, look, I put the thing back together again, there's blood flowing, there's urine coming out, everything is better.

So some colleagues of mine, Blohm Macleman, took advantage of this and basically said, well, let's use this as a furosemide stress test and see what the urine output after an allograft does and looked at the two hour and six hour, you know, random FST for lack of a better word, it wasn't perfectly standardized by weight, and these are your results.

There you see a curve value in 300 patients. This is a convenient sample. This isn't even standardized it's .84 and .86. This is holding up in DGF, as well.

So for the acute management of patients a simple physiologic test if highly valuable. I don't have time in this talk to go through it, but this pairs well with biomarkers and they work in conjunction with one another.

It also pretty good length of stay. And that's not entirely surprising because if you don't

Page 35

get better and if you're on dialysis you're not going to leave the hospital sooner, you're going to leave it later.

I want to spend the last two minutes of my talk trying to impress upon you where I think the value is in chronic kidney disease and allograft. So there's an important issue which everyone here is aware of who takes care of patients and that is if you have fibrosis in your interstitium the kidney is toast. That's always bad.

No matter how good or bad it may look if the interstitium is bad everyone knows bad things are going to happen. And the big problem is we largely don't know what to do about it.

So following someone's interstitium is something we would like to do and we would like not to have to do it with a biopsy all the time. Now for certain centers that take care of transplant patients they have protophized (phonetic) biopsies, they're very good at it. They promise me they never hit an acrid artery in their entire life. I'm sure that's true, but I'm not 100 percent sure that's true. And

Page 36

there are risks with biopsies and if you could do it a better way it'd be worth investigating.

Now there's an important question which is when you have fibrosis do you, in fact, have loss of tubules or do you have secreted matrix?

This is an animal study which basically show you -- shows you what happens after acute kidney injury. If you look out a couple of months, and this is what happens in people, the kidney shrinks. Well, if the kidney is shrinking the likelihood is this matrix being secreted is unlikely because when that happens and like amyloidosis the kidneys get bigger.

So most pathologists -- and we don't know this -- suspect that most of the fibrosis you see is, in fact, loss of tubules. Now no matter how good your glomerular look if you're not connected to a tubule you're not filtering anything.

And this (indiscernible) looking at what's called a tubular glomeruli so there are beautiful, lovely glomeruli that are fully intact that are decapitated. They do not connect to their corresponding nephron tubule and they are non-

Page 37

filtering glomeruli. And they do not contribute to GFR and they don't do anything to help the kidney.

And those tubules are not online and they don't work. And this is likely why fibrosis is such a disaster for these patients because there's no way to reconnect them.

So in patients with CKD we are also now looking at not just the urine output, which is surrogate, but also measuring the furosemide in the urine because it allows you to assess proximal function from distal function. And we think that this may represent a way of having a noninvasive functional assessment.

We do not think this should replace biopsies but we do think that if you get a biopsy and you index it to a thoughtful assessment of tubular function you could probably track that over time.

And basically I'm finishing. Thank you. These are the next steps which is we need to do these studies and many of these are underway so stay tuned. And with that I'm happy to take questions during our session. Thank you.

Page 38

DR. NICKERSON: Okay. Our next speaker is going to be a double header here with Dr. Mark Haas talking about diagnosis of acute and chronic using the Banff and pathologic correlates of graft survival and the utility of molecular diagnostics.

Mark?

DR. HAAS: Thank you, Peter. Thank you to the organizers and for putting together such an excellent and informative workshop.

I'm a pathologist. I'm going to spend the first part of my talk reviewing the pathology of transplant rejection correlates with graft survival. So I'm going to talk about the pathology of acute of active antibody mediated rejection, chronic active antibody mediated rejection, and a little bit -- just a little bit about pathologic factors influencing graft survival following treatment of antibody mediated rejection. These are my disclosures.

So as we've learned a lot in the last day and a quarter, antibodies are very important in terms of the prognosis and outcome in patients who receive renal allografts. But it wasn't always known that

Page 39

this was the case or appreciated that this was the case.

Very early on antibody -- preexisting antibodies were a big problem in transplantation. You'd put the kidney in and it would essentially stop functioning right on the operating table and turn blue or pale and you would have hyperacute -- a process called hyperacute rejection due to preexisting antibodies in the recipient against either blood group antigens or HLA on the donor kidney and this would inevitably lead to rapid graft loss sometimes just right there on the table.

But shortly after that cross matching techniques were developed that prevented hyperacute rejection. And essentially for the next 20, 25 years after that with a few exceptions -- and Paul Terasaki was clearly one of those exceptions -- antibodies were really forgotten about and the focus really became diverted to cell mediated rejection.

And the first few Banff classifications for acute rejection in the kidney barely mentioned antibodies. It was all about cell mediated rejection.

Page 40

But this started to change in the 1990s and this was one of those early studies that came out of the Edmonton Group of Phil Halloran in which they studied and compared rejection patterns in patients who did and did not have antibody HLA. And these were class 1 antibodies.

And this was not at the time of transplantation, but this was subsequent to transplantation. So this was acute rejection, not hyperacute rejection. And what they found in that patients who did not have antibodies was the typical finding of T-cell mediated rejection characterized by inflammation and tubulitis.

But that in patients who did have anti-HLA antibodies this was seen in only about half the cases. And the findings that were predominant in these patients were those of microvascular inflammation -- glomerularitis, inflammation of the glomerular capillaries, fibrin thrombi, the result of inflammation in vessels, peritubular capillaritis, margination of neutrophils and of monocytes in the peritubular capillaries.

Page 41

And all of this microvascular inflammation was reminiscent of the early findings of hyperacute rejection and this really seemed to be sort of a hyperacute rejection like kind of a picture.

And so what the kinds of findings that they were seeing were here thrombosis in the glomerular capillaries, margination here of neutrophils in the peritubular capillaries, margination of leuko- -- of mononuclear leukocytes in the glomeruli, so called glomerularitis, and peritubular capillaries, peritubular capillaritis.

And these mononuclear cells were predominantly CD68 positive monocyte macrophages, not lymphocytes which are CD3 positive. And, in fact, CD68 immunostaining is actually used in diagnosis of antibody mediated rejection in heart allografts as a very -- excuse me -- important tool in these allografts.

However, none of these findings is specific for antibody mediated rejection. These are markers of microvascular injury, endothelial injury, and can be seen with just about anything that injures the

Page 42

endothelium.

So pathologists were quite pleased when C4d came along. C4d is a split product of complement factor C4 which is part of the classical pathway of compliment activated by antigen antibody interactions.

And when C4 is cleaved C4d is formed. And what makes C4d special is it binds covalently at the sight of its formation and thus it's a relatively long lived -- and when I say "long lived" I'm talking about two weeks here -- marker for humeral immunity.

And what we see in allografts is we see C4 that are undergoing antibody mediated rejection and are exposed to donor-specific antibodies in many cases is linear staining by immunofluorescents of C4d in the peritubular capillaries.

The glomerular staining is actually quite non-specific. And it's the peritubular capillary staining which is really indicative of an antibody reaction.

The first evidence that C4d might be prognostically important in the kidney and might be related to humeral activity came from Helmut Feucht in

Page 43

Germany who was really way before his time in 1993 who studied 93 for cause renal allografts and looked at peritubular capillary C4d deposition and noticed that those biopsies that had peritubular capillary C4d were associated with a very poor graft survival at one year compared to those biopsies that were C4d negative.

Furthermore, C4d was associated with re-transplants and an elevated PRA suggesting its association with antibody. And this was subsequently confirmed in quite a number of studies that were done around the year 2000 showing that C4d was highly specific for the presence of donor-specific antibodies with ranges of specificity in the 90 to 100 percent range.

Pathologists became so enamored with C4d that when the first Banff classification for antibody mediated rejection was published in 2003 that C4d staining in the peritubular capillaries was one of three findings that was required for the diagnosis of antibody mediated rejection.

There had to be histologic evidence in terms of microvascular inflammation as I showed you before,

Page 44

there had to be serologic evidence in terms of donor-specific antibodies, but there also have to be immunohistologic evidence, generally C4d staining, within the peritubular capillaries.

And we were very happy with this and this lasted a number of years until we started to find that we seemed to be missing cases of what appeared to be antibody mediated rejection. That these biopsies occurred in patients who had donor-specific antibodies, who had microvascular inflammation, but were being called no antibody mediated rejection because the C4d was negative.

And there were two key studies, one I'll point out here and one in my next talk, that really established the presence of C4 -- the viability that antibody mediated rejection could occur in the absence of C4d.

And this is a protocol biopsy study done by Alex Loopy and colleagues in Paris where they do three month and one year protocol biopsies in all of their DSA-positive patients. And they looked at the findings at one year based on the findings at three

Page 45

months. And the findings at three months could be classified into three categories. These were all protocol biopsies of stably functioning grafts.

So there were those with subclinical antibody mediated rejection that were C4d positive, they were DSA positive, and had microvascular inflammation. There were those with clearly no antibody mediated rejection, C4d negative, and no microvascular inflammation. And there were those that were suspicious C4d negative but with microvascular inflammation.

And at one year predictably the patients who had subclinical AMR had a low GFR, had frequent tubular atrophy and interstitial fibrosis of IFTA, and 43 percent had transplant glomerulopathy. Patients with no antibody mediated rejection had a good GFR. Some had mild IFTA, but usually not. There was no TG.

Patients who were suspicious looked more like the subclinical antibody mediated rejection -- low GFR, frequent IFTA, and even some transplant glomerulopathy. So apparently antibody mediated damage to the kidney resulting in fibrosis and

Page 46

transplant glomerulopathy could occur even in the absence of C4d.

So for the sake of time I will skip this -- the next slide and just go right to the revised Banff classification which now includes C4d, but does not require C4d for an absolute diagnosis. You still need to have microvascular inflammation, you still need to have donor-specific antibodies, but now the C4d requirement has been replaced by evidence of recent antibody interaction with the allograft which can be in the form of C4d but can also be in the form of more severe microvascular inflammation. And I'll talk about this a little bit more in the next talk, but again there are C4d positive and C4d negative forms of antibody mediated rejection.

So moving to chronic antibody mediated rejection the classic lesion of chronic antibody mediated rejection is transplant glomerulopathy characterized by double contours of the glomerular basement membrane evidenced on a silver or PAS stain which highlight this basement membrane.

But like microvascular inflammation, TG is

Page 47

not absolutely specific for antibody mediated rejection. And in this study here by Bonu Sys (phonetic) they found evidence of either C4d positivity, donor-specific antibodies, or both in about three-quarters of patients who developed C- -- who developed transplant glomerulopathy.

Meaning that approximately 25 percent of the cases seem to be associated with something else other than donor-specific antibodies. And what were these? Well, this is a study from Bob Colvin's group that was published awhile back in Kidney International showing that Hepatitis C can be associated with transplant glomerulopathy, forms of thrombotic microangiopathy such as acute or persistent calcineurin inhibitor toxicity, and there's evidence that TG can be a manifestation of cell-mediated rejection as well, but most of the time antibody-mediated rejection.

We've seen a number of curves like this showing that TG is associated with poor graft outcomes. This is a study from the Mayo. And that TG is associated with the presence of donor-specific antibodies particularly anti-class 2 donor-specific

Page 48

antibodies.

But note this, that TG at least by light microscopy is rarely seen in the first year post-transplant. And the unfortunate thing about that is that once we seen transplant glomerulopathy it's generally accepted that this graft is going to fail. Whether it's going to fail fast or whether it's going to fail more slowly is of some debate, but transplant glomerulopathy means that the graft is eventually going to fail and fail faster than a graft without transplant glomerulopathy.

So is there a way to diagnose or at least predict TG faster than just diagnosing it be routine-like microscopy? And clues to this came from a study that came out of Sidney, Australia, by Wavamunno and Nankivell in 2007.

And this is also -- and I'll again defend the use of surveillance biopsies here even though I know that they're -- they are very suboptimal in terms of the patient experience in transplantation.

But in Australia they do do quite a few surveillance biopsies. And what Brian and his

Page 49

colleagues did in this study is they looked at patients who ultimately were diagnosed with transplant glomerulopathy back two to five years post-transplantation. And they went and they looked by electron microscopy at their -- excuse me -- early biopsies one to three months post-transplantation and was there anything on there early biopsies that predicted transplant glomerulopathy.

And what they found were three findings by electron microscopy that seemed to be associated with subsequent development of TG -- endothelial cell swelling. And, again this is the glomerularial capillary, the basement membrane, and the endothelial cell which is swollen. There's a sub endothelial electronic loosened widening. And if you can appreciate here very early wisps of new basement membrane formation not apparently by light microscopy, but apparently very early on by electron microscopy.

And when you take patients with these early findings plus donor-specific antibodies who have had subsequent biopsies but have these early findings and DSA within their first three months post-

Page 50

transplantation if these patients are not treated for antibody mediated rejection they are virtually certain to develop transplant glomerulopathy within the first two years post-transplant.

DR. NICKERSON: Mark, I've just got to caution you. You're already near the -- you're over the --

DR. HAAS: Okay.

DR. NICKERSON: -- you're over your first talk. So I just want make sure --

DR. HAAS: Okay.

DR. NICKERSON: -- you're not going to run over overall.

DR. HAAS: All right. And so -- and this can be prevented by treatment for antibody mediated rejection. So just to -- so just to summarize the value of Banff 2013 this is a study that came out of Quebec City basically showing that by the additions that we made to Banff 2013, particularly C4d negative antibody mediated rejection, that this not only increased the sensitivity for diagnosis of antibody mediated rejection, but also increased the association

Page 51

of the diagnosis with subsequent graft loss. So basically the sensitivity and the specificity was increased in Banff 2013.

So now I will go on and talk about some molecular markers in -- and molecular diagnosis in diagnosis of antibody mediated rejection.

Again, my disclosures. This is, again, the initial allograft antibody mediated rejection classification which required C4d in the -- in diagnosis of antibody mediated rejection. And molecular studies were important in addition to the protocol biopsy study of Alex Loopy, et al., in terms of identifying C4d negative antibody mediated rejection.

Because here we have this study of Bonu Sys who used molecular markers in the biopsy tissue identified by genechip analysis. And these were endothelial-associated markers and found that these were predictive of graft loss.

And here are some of these endothelial markers. Von Willebrand's factor was the most associated with graft loss. But what they

Page 52

specifically found was that if one had these molecular markers, these ENDATs, endothelial associated gene transcripts, plus C4d, plus donor-specific antibodies there was a high rate of graft loss.

However, if one did not have C4d but still had the ENDATS and the donor-specific antibodies there was still a high rate, albeit somewhat reduced, rate of graft loss again indicating that one could have antibody-mediated damage to the graft without C4d deposition. And, again, this was again one of the earlier markers of C4d negative antibody mediated rejection.

The Banff classification specifies that if C4d is not present that a higher level, a higher threshold of microvascular inflammation is required to diagnose antibody mediated rejection than if C4d is present with a threshold -- a microvascular inflammation threshold or glomerulitis plus peritubular capillaritis score of at least 2 rather than 1 simply -- and this was put in rather empirically simply to prevent us from over diagnosing antibody mediated rejection.

Page 53

But we weren't really sure if the 2 threshold was really the right threshold. But this study again using molecular techniques from the Albert Einstein Group studying biopsies with different levels of microvascular inflammation validated this threshold of 2.

So here we see this is -- these are these gene transcripts associated with antibody mediated rejection. And the numbers in red, the P values in red indicate an increased level of these gene expressions of these inflammation-associated transcripts.

And when comparing a microvascular inflammation score of 1 versus zero there was no difference. But this difference became significant with a threshold of 2 versus zero or 2 versus 1 suggesting that this threshold of 2 was, in fact, appropriate for diagnosing C4d negative antibody mediated rejection. And we see here that C4d, itself, did not influence these molecular markers. So microvascular inflammation score of 2 appeared to be validated in the Banff classification.

Page 54

A very important topic that came up at the last Banff meeting was what to do when we have microvascular inflammation but no donor-specific -- no detectable donor-specific antibodies. How do we know if this is antibody mediated rejection or some kind of non-specific effect because we know that microvascular inflammation is not, itself, specific for DSA?

Well, the obvious thing would be to check for non-HLA antibodies, but this is not done in all laboratories and can take time. And you don't want to delay treating somebody with antibody mediated rejection.

And one possibility would be a molecular test that might determine the likelihood of antibody mediated rejection. And for the sake of time I'm only going to refer to one of these tests here which is the molecular antibody mediated rejection classifier score.

And this is based on gene analysis within the biopsy tissue was also developed by Phil Howard in the Edmonton Group based on 30 non-redundant gene probes selected from comparisons between biopsies

Page 55

showing histologic antibody mediated rejection in the presence of donor-specific antibodies.

And predictably most of these probes are associated with cell types that have been associated with AMR -- endothelial cells and NK cells -- as well as macrophages. And what Phil found when they looked at the antibody mediated rejection score versus histology is they found that approximately 90 percent or close to 90 percent specificity for this molecular classifier for determining if a biopsy showed antibody mediated rejection.

More importantly, adding this classifier to the histology increased the predictive value of the biopsy in determining which patients did and did not get graft failure after the biopsy.

So we see here that this is based on the biopsy features alone and this is the probability of developing graft failure in patients who did not and did develop graft failure.

And in those patients here in the blue adding the molecular score to the histology identified and lowered the probability of predicting graft

Page 56

failure in those patients who did not ultimately develop graft failure and increased the predictive value of the biopsy in the majority of those patients who did develop graft failure.

And to put this another way here looking at biopsy correlates with graft loss at three years. When the molecular test for ABMR was negative and the biopsy showed no evidence of antibody mediated rejection the graft survival was good at three years. When both of these were positive the graft survival was poor.

But, again, adding the molecular test here improved on the prediction of graft loss compared to just the conventional biopsy data alone. And, in fact if one had to use just one of these two, the molecular classifier actually seemed to be superior to histology in predicting graft outcomes.

And I guess that's my last slide. I thought I had a final slide, but -- oh, yes. So, anyway. So this is just sort of a table summarizing how molecular studies can be employed in addition to histology in terms of aiding the diagnosis of antibody mediated

Page 57

rejection.

So if we have no histological evidence of antibody mediated rejection at all, we probably don't need molecular studies. And if we -- but if we have an inadequate biopsy then the ABMR classifier which actually works on medulla which cannot be used for histologic diagnosis of antibody mediated rejection, but does seem to work in giving a molecular score can be used in terms of increasing the probability that we're dealing in a biopsy with antibody mediated rejection.

And here if we have microvascular inflammation but no donor-specific antibodies if the molecular score is greater than this cutoff value we might want to treat the -- consider treating the patient with antibody -- for antibody mediated rejection. Whereas if the molecular score is less than 2, probably -- probably refrain from treating the patient for ABMR.

And these molecular tests are being refined all the time. Their specificity is constantly being increased since the days of ENDATs. And hopefully

Page 58

these might be incorporated into the classification in 2019.

And with that I apologize for going over and I will stop. Thank you.

DR. NICKERSON: Thank you very much, Mark. Our next speaker is Dr. Steve Woodle from the University of Cincinnati talking about the treatment of AMR updates since 2010 standard of care and emerging therapies.

Steve?

DR. WOODLE: So I'm not sure how I can do all of this in ten minutes or so, but we'll give it a shot.

DR. NICKERSON: You have 10 minutes on the schedule because it's 9:30 to 9:45.

DR. WOODLE: I'm sorry?

DR. NICKERSON: You have 15.

DR. WOODLE: Oh, okay. The schedule said only 10, but that's okay. Okay. So I just wanted to mention this. Over 50 years ago Tom Starzl was one of the groups that was doing kidney transplants. And back then they didn't know about ABO compatibility,

Page 59

but they found out real fast when they had some antibody mediated rejections.

These are described in this book which copy right is 1964. And he and Ken Porter, and outstanding renal pathologist, described antibody mediated rejection in ABO incompatible transplants. And that description is what I went to the first time when I started dealing with these clinically in the late 1980s. And they described the presence of substantial edema, mixed infiltrates with polys, transmural arteritis, and endofelial targeting.

And so for any of those interested in the history or particularly interested in understanding histopathology, Ken Porter and Tom Starzl's descriptions 50 years ago are very instructive.

The talk -- I've divided the talk into complement inhibitors followed by immunoglobin as a target which we've already discussed a little bit. Some of these areas I'm going to go over it quickly because we've had a lot of discussion about them yesterday. And then wind up with plasma cell targeted therapies primarily based on protease inhibition.

Page 60

There are two primary ways that antibodies can damage the graft. We know of the third way which Elaine Reed has described, but I'm not going to -- I'm going to leave that aside for now because that's difficult to measure.

But the important point here is that the focus has been almost entirely on compliment inhibition for years. But it's important now that we understand that there are complement independent mechanism and rejections that actually present in patients to consider the FCR mediated effects. And for that we needs tests that can diagnose and tell us when an antibody's capable of binding an FC receptor and also tests of being able to identify when FC med- -- FCR mediated injury is occurring.

And I think that's part of the future of where the field needs to go to move beyond the almost unifocus on complement.

So we heard yesterday about the issues of distal complement inhibition with eculizumab. We'll talk a little bit about that story. But the field is actually moving in the direction of proximal

Page 61

complement inhibition and we'll talk about three agents that are being used that are targeting the C1q, C1r, C1s complex.

This targeting is asking a fundamental question. And that fundamental question is is can one prevent the TG that does not get prevented by eculizumab?

And critical to that is whether or not the alternative pathway is also involved. Because if it is there's a possibility that these classical pathway inhibitors at the level of C1q, C1r, and C1s may not work.

So eculizumab binds the C5, prevents conversion C5A, it prevents membrane attack complex generation. It's approved for PNH at 2007 and atypical HUS in 2011. It is a very expensive drug with cost estimated $400,000 a year or more. And this is made -- made -- played a major role in diminishing the use of that drug in this meeting. If this drug didn't cost that or cost a fraction of it we'd be talking about it a lot more today.

The first study that came out came out of

Page 62

Mayo. It was a single-arm study with historical control that showed substantial reduction of AMR from a historical rate around 40 percent to around 10 percent.

Follow-up studies showed that the AMR still occurred despite terminal complement inhibition. And beyond one year outcome showed that transplant glomerulopathy will still occur even if you do this. And that was a major negative effect on this particular strategic approach.

So there have been two major trials sponsored by Lexion. This is one we talked about a lot yesterday. It was a strategy to prevent antibody mediated rejection in living donor kidney transplant recipients that required desensitization.

I won't move on for it -- I'll move on from this trial just to say that the drug worked. It just didn't work as much it was predicted beforehand. And I don't view this as a failure of the drug. It's a failure of the trial design and, more importantly, trial execution. But really the ultimate responsibility lies in the leadership of the company.

Page 63

There's another trial that's going on. This is primarily in Australia and also in Europe. It's in deceased donor recipients. And it's sensitized deceased donor recipients. A total of 80 patients, 15 sites. It was last updated in Clinicaltrials.gov in October. Estimated study completion is June of 2017 so we're looking very much forward to hearing about the results from this trial.

This is an interesting study that's been published. It was done by Sanjay Kulkarni and Jordan Pover at Yale. And it looked at eculizumab for chronic antibody mediated injury. And it was a pilot trial.

And as such, although it didn't show an effect, this study highlights the real difficulties in conducting a trial in this setting. A primary endpoint was actually estimated GFR.

This slide doesn't project well, but it's actually a spaghetti plot of the GFRs in both control and treated patients. And what it shows is the -- what the study wanted to show is a change in the rate of decline of GFR.

Page 64

And for those of you who are interested in doing studies in chronic AMR it would really be instructive to read this study and understand all the trials that they went through to get this. Enrollment was a major problem. It is difficult to enroll patients when you have really strict inclusion and exclusion criteria.

So let's move on to proximal complement inhibition. It's important to understand one of the reasons why the C1q test is negative when antibodies are low. When antibodies are not in saturating conditions you cannot get a hexagonal array of C1q. So C1q's this inverted umbrella. It has six globular heads that need to attach to the complement binding regions in the FC portions of antibodies.

Once that's stabilized adequately the molecule can then engage C1r and C1s. So you need saturating conditions with antibody to activity complement at least in vitro. In vivo I don't know that this is absolutely true. We do see C4d staining in some patients whose antibody levels are considered to be less than saturation in a single antigen BSA.

Page 65

So what happens with C1 inhibitors is that they bind to and disrupt the interactions between C1r, C1s, and C1q and that prevents activation of the classical pathway.

There are three of these agents in development that we can tell so far. Two of them are actually plasma derived products and one of them is recombinant.

There have been some publications. This is -- there have been two pilot studies published with the CSL bearing product. This is from Stan Jordan's group, 20 patients. And then there's another smaller study that was from Denny Gladson, Carmen Lafalshay (phonetic).

There's also the Shire product. There's a phase 2B randomized, double blind, placebo-controlled study, yes, in a complement inhibition trial. A randomized, double blind, placebo-controlled study and 18 patients.

And so I won't talk much about these pilot trials because you can't tell. They're small numbers of patients. But these are important drugs to follow

Page 66

up because they're asking fundamental questions about the role of complement and how we -- how we employ drugs to inhibit complement mediated injury.

So -- and I've already mentioned these.

So we talked a little bit yesterday about immunoglobulin. Now immunoglobulin, itself, is a target for drugs. And this is the IdeS molecule. It's been talked about. It's a product derived from strep pyogenes.

It's assisting proteinase so it really attacks the disulfide bonds that are present that are necessary to stabilize and link the heavy chains and light chains and add further stability to the antibody molecule.

The degree of cleavage we're not really exactly sure about. It appears that certainly the heavy chain undergoes further cleavage other than just the disulfide bonds.

Humans during infection normally produce neutralizing antibodies against this and they decline over time. These anti-IdeS neutralizing antibodies are commonly found in humans because most of us had

Page 67

strep infections when we were younger. And so this anamnestic xeno response against this drug is going to be something very important to pay attention to as this drug is developed.

The structure of this molecule has been worked out in a collaboration between the proponents of the drug who are from London University and also from Max Planck. This is data that I referred to yesterday about Jill showing the degree of degradation of the antibody molecule.

This is actually an in vivo study in rabbits. You can see the level of IG does down quickly over the first few days, but importantly that antibody rebounds. So starting within about four to five days completely back to the exact levels that were present prior to treatment within a week or less.

So a fundamental issue in this strategy which is a fundamental issue in a lot of clinical trials that we have is can you leave the antibody at the levels it was when the patient had rejection or before they were transplanted and expect to have good long-term function or not have further injury.

Page 68

So a fundamental question in all of this work is do we have to eliminate the antibody? And if we don't have to eliminate it, how much do we need to reduce it? And it's a fundamental question that is at the development of therapeutics in this area.

So, anyway, so this is the phase 1, 2 trial that Stan Jordan has. Obviously also Bob Montgomery is using this drug. So and I think the other -- so I've mentioned one of these questions.

The other question, of course, we talked a little bit about yesterday. What was going to happen with renal function when it's suddenly faced with a requirement to excrete large volumes or grams of intervascular protein.

So for plasma cell targeting we're going to talk. Mainly the focus to date has been on distal inhibition of the protease. That is inhabitation at the level of enzymatic proteolytic activity.

The constitutive proteasome inhibitors are what we're going to focus on. And we'll a little bit of data in irreversible inhibitors with -- as primarily evidenced with carfilzomib. We'll also talk

Page 69

about studies ongoing that are targeting deliberately the plasma cell niche and also survival factors. And then at the end talk a little bit about other combinatorial approaches.

So the innovator drug here was bortezomib. Everybody knows that. It works by inhibiting the enzymatic activity in the 20S core. The primary mechanism by which proteasome inhibitors are thought to work in multiple myeloma which we think also exists for their use in targeting normal plasma cells is by the induction of ER stress.

When a protein is synthesized the first thing that happens when it enters the interior or the endoplasmic reticulum is met by chaperones and foldases which correctly fold that protein.

If it is misfolded it exposes hydrophobic residues that are toxic. If this is not dealt with and they've built up to a certain level, the cell will commit suicide.

The natural response is to induce hundreds of proteins in a process called the unfolded protein response. And this is a response that's meant to

Page 70

refold the protein, fix it, or if it can't be fixed ubiquitin label it, shunt it out, and degrade it the proteasome.

If one blocks the proteasome all of this builds up. And it's like having -- you know, you've seen pictures of the garbage in New York City when their garbage workers are on strike. That's kind of what happens in the ER when you use a proteasome inhibitor.

I ran a search back to 2010 on proteasome inhibitors and kidney transplant and I turned up about 250 papers. And I'm just going to summarize some of our papers and what they've shown.

Following our initial paper we went and showed the proteasome inhibitors work as primary therapy, not just as rescue therapy for antibody mediated rejection.

To date the results from proteasome inhibitors for antibody mediated rejection in my opinion the results for early AMR and the results for late AMR are equivalent to those for IBIG-based regimens. Therefore, I think they should be

Page 71

considered as a standard of care equivalent to that of IBIG. And with 250 papers and literature I think that's pretty substantial.

We've shown that the variability in results is a result derives at least in part and we think predominantly from the differences between early and late antibody mediated rejection.

We've also shown that there are actually improved results in pediatric recipients. There's data from our group with hearts, but there's also data from other organs suggesting that there's a fundamental difference in plasma cell biology and B-cell biology in infants as compared to adults. And we've also outlined the toxicity profile.

There are two new proteasome inhibitors that are considered second-generation proteasome inhibitors. They also work on the same set of three enzymatic activities in the 20S proteasome. The difference is carfilzomib is fundamentally different because it's an irreversible proteasome inhibitor. So once you bind the proteasome that proteasome is irreversibly damaged. It can't do any work anymore.

Page 72

And so the way you recover from that is that you have to make new proteasomes. With bortezomib it's gone in 12 hours. Proteasomes back to business as usual after about three or four half-lives. I mean, I'm sorry, the half-life is 12 hours.

And so this is actually a data that -- a paper that's come out recently on the use of carbilzomib to treat antibody mediated rejection in a pulmonary allograft. It is from Pittsburgh. Advise you to read it and take a look at it. It presents preliminary evidence. The problem is it's also combined with IBIG in this regimen so it's hard to sort out the differences.

One area in which we have not -- don't have a problem sorting out differences is a desensitization trial we're doing on carfilzomib. And we have data from carfilzomib model therapy out to four weeks. This is a proof-of-concept trial. It has an iterative design. It also has an adaptive enrollment that's based on precision estimates using abrasion statistic. And we have biologic assessment of bone marrow niche resident plasma cells in the study.

Page 73

This is recovery of CD138-positive bone marrow plasma cells. We have shown that if you take CD138-positive plasma cells and culture them in vitro, take the culture supernatant and do a single antigen BSA, the profile from that culture soup is identical to what's in the circulation.

That means the first -- if the first bar is 82 and the second one's B7 and the next one's DR51, they're exactly aligned. So these are the cells that are responsible for long-lived permanent antibody production in the marrow.

We see a 70 percent reduction in these cells with three -- just over three weeks of carbilzomib therapy, mono therapy alone. So clearly this is unequivocal evidence that we are depleting the long-lived plasma cell population in the marrow.

We've taken this further and done single-cell RNA seq analysis. This is 2,000 cells where the messenger RNA in each individual cell was measured 2,000 cells across this way, about 1,000 genes in this direction.

One of the things we found very interesting

Page 74

is there's a population of cells that's actively proliferating in the marrow. And when you treat with a proteasome inhibitor this population expands. So it suggests that these plasma cell populations are capable of repopulating once -- from their own population once you delete them.

This is interesting work that you're going to hear in a few merits -- minutes from Stuart Knechtle's group which is doing very interesting work in primates suggesting that there may be another source by which plasma cells may regenerate.

We've also shown that there's an induction of immunoproteasome which is a mechanism why which resistance may be achieved in the remaining plasma cells.

A little bit about targeting the plasma cell. When it sits in its niche in the bone marrow which confers years of survival to these there are a number of factors involved in keeping the cell there and also keeping it alive.

One of the cells that keeps -- one of the -- the primary mechanism by which plasma cells are

Page 75

thought to be homed to the marrow and tethered is by an interaction between CR4 in the plasma cell and CL12 on the bone marrow stromal cell.

Blockade of this with an FDA-approved drug called mozobil or plerixafor made by Sanofi can potentially mobilize these cells. This is the same drug that's used to mobilize CD -- CD34-positive stem cells for stem cell transplantation.

We've shown now and will show at the American Transplant Congress that there's a progressive mobilization of these cells into the peripheral blood plasma cells from the marrow out of their niche. And when they get into the peripheral blood they start to die. They go from 13 percent dead rate to a 37.9 percent. Proof of concept that if you mobilize these cells from the marrow you can kill them.

We've also shown -- I'm looking at the known pathways for death that there's certain mitochondrial factors such as noxa that are probably driving this and it's counter balanced by an increase in BCL2.

So it's possible that BCL2 inhibitors may be

Page 76

synergistic to proteasome inhibitors. And what this type of analysis has done is given us multiple pathways by which we can start to achieve synergy in the future.

The future is going to exist in looking at proximal proteasome inhibitors such as deubiquitanse inhibitors and ubiquitin binding protein inhibitors, ER stress inhibitors, autophagy inhibitors.

One of the things that happens -- one of the major reflexes that protects plasma cells from death is that if you can't degrade protein in the proteasome you can possibly degrade it in the -- by a process called autophagy.

And there's massive induction of autophagy genes in humans treated with carfilzomib in their bone marrow plasma cells.

And this just shows here's prox- -- this is distal complemented inhibitor where the bortezomib and carfilzomib work. But there are inhibitors in the proximal portion of the proteasome proximal that can give you blocks and series to achieve synergy.

DR. NICKERSON: Steve, if you could wind up?

Page 77

DR. WOODLE: Okay. All right. I'll move -- skip through this. The only thing I would leave is that there are BAFF inhibitors which are major growth factors. We have a trial of bortezomib and belimumab that is ongoing so this will actually be a BAFF blocker.

There are IL-6 blockers. I don't know how much interest there is in Genentech to use tocilizumab in this area. Stan Jordan has done some work. It's very interesting to follow.

There's also another interesting IL-6 blocker that's coming out by a company called Viteras that is actually an FC-engineered product.

So in conclusion, I would say there's a number of innovative approaches that have emerged since the last meeting that we had here seven years ago. The classic complement cascade early inhibitors are being developed. That story's going to be written.

There are drugs that can actually attack the antibody and degrade it and completely eliminate fully-formed antibody for a period of up to a week.

Page 78

There are newer proteasome inhibitors, irreversible inhibitors. There are -- there are proximal proteasome inhibitors and plasma cell niche components that can be targeted.

We believe the future -- and this has been said before. But we believe the future in development of antibody humeral therapies is going to be combinatory regimens. It's not going to be individual drugs, but it's going to be drugs that are specifically designed in targeting the biology in a rationale way that will move this field forward. Thank you very much.

DR. ALBRECHT: So before we go to discussion we're going to have one public presentation. Dr. Robert Woodward of Caredex will be speaking about donor-derived cell-free DNA in AMR.

You have eight minutes, please.

DR. WOODWARD: Thank you to the organizers for the opportunity to discuss donor-derived cell-free DNA in the diagnosis of AMR in kidney transplant recipients.

Cell-free DNA in the circulating blood is a

Page 79

product of physiological cell turnover and pathological cell death such as necrosis. The fraction of plasma cell-free DNA originating from an allograft called donor-derived cell-free DNA is higher in situations of active allograft injury than in healthy, stable transplant recipients.

We and others have developed methods to quantify the differences between the genomes of the donor and the recipient that are both represented in circulating cell-free DNA.

Recent publications have used these methods to demonstrate several characteristics. First, the level of donor-derived cell-free DNA is very low in stable transplant recipients.

In heart transplant patients the median fraction of donor-derived cell-free DNA is only 0.07 percent. In kidney transplant patients the median fraction of donor-derived cell-free DNA is 0.21 percent.

Second, donor-derived cell-free DNA is elevated at the time of rejection. Usually man fold higher than in stable transplant recipients. There

Page 80

are also indications that this rise may be identified earlier than clinical or histopathological signs of rejection.

Third, donor-derived cell-free DNA decreases following successful treatment of rejection returning to the level of stable transplant recipients.

There are numerous publications that demonstrate increased levels of this biomarker in allograft rejection. Publications not listed here showed a proof of principle from single centers in small numbers of patients.

The publications selected here represent studies with a large number of patients or samples with significant group sizes for analysis.

The AlloSure method that we have developed from measuring donor-derived cell-free DNA has been described in three major publications that have appeared in the last six months. This method amplifies a panel of sequence variants and then uses clinical-grade next-generation sequencing to count the recipient and donor alleles without the need for genotyping the donor or recipient.

Page 81

Gerscovich published the clinical validation for heart transplantation last November increased donor-derived cell-free DNA with rejection and decreased donor-derived cell-free DNA with treatment for rejection.

The Bloom and Bromberg publications this March demonstrate clinical validation of donor-derived cell-free DNA in kidney transplantation which I'll discuss more in the next few slides.

To be considered for clinical use a molecular diagnostic assay should be rigorously analytically validated. The AlloSure analytical validation study was published last November in the Journal of Molecular Diagnostics.

The established lower limit of quantification of AlloSure is 0.2 percent donor-derived cell-free DNA. Results below this level are reported not as a quantitative value, but as a valuable result indicating cell-free DNA could be measured successfully, but the level of the donor contribution was indistinguishable from zero.

The quantifiable range of the test is 0.2

Page 82

percent to 16 percent covering the range of critical values observed in all publications in heart and kidney transplantation and including the decision point for kidney transplant of 1 percent donor-derived cell-free DNA.

All of the studies were performed with reference materials that have been validated on an orthogonal technology.

The Bloom publication in the Journal of American Society of Nephrology demonstrated that AlloSure donor-derived cell-free DNA discriminates AMR from no AMR. The reference standard for diagnosis of antibody mediated rejection was histological findings meeting the BANFF 2013 criteria for chronic active or acute active antibody mediated rejection. And the control group was all other diagnosis found on all other biopsies.

The area under the curve of an ROC plot is 0.87 demonstrating a high level of accuracy to discriminate between AMR and no AMR. At a threshold of 1 percent the sensitivity of AlloSure for AMR is 81 percent and specificity is 83 percent.

Page 83

The negative predicted value is very high, 96 percent, calculated using the overall prevalence of AMR in this multi-center study population which is representative of UNOS.

In this study, 75 percent of for cause biopsies were negative for rejection. And a test with a high NPV can reduce the number of unnecessary biopsies.

An AlloSure test result above 1 percent in a patient who also has DSA means a patient is likely to have AMR. A low AlloSure result can be used to rule out AMR or when measured serially in a patient be used to indicate recovery or response to treatment of rejection.

The Bromberg publication defines the AlloSure performance metrics in the stable transplant recipient population. In contrast to the median level and AMR of 2.9 percent donor-derived cell-free DNA reported in Bloom, the median value of 390 samples from 93 stable, healthy patients is 0.21 percent.

The 96 percentile is again 1 percent indicating that values outside the range of normal

Page 84

biological variation match the above threshold range in AMR.

A case study is shown on the right. This patient had low levels of donor-derived cell-free DNA in the first few months and biopsies at 30 days and 60 days were non-specific.

At five months post-transplant de-novo DSA were detected and a third biopsy diagnosed AMR. The donor-derived cell-free DNA was also significantly elevated up to nearly 4 percent.

The serum creatinine is high, about 1.7 to 2.1, but little changed over time. This suggests that AlloSure could have picked up the AMR earlier if it had been measured in the month prior to the AMR.

So donor-derived cell-free DNA provides a quantifiable direct measure of allograft damage. The results of the studies in kidney transplantation provide a clear indication of clinical utility to reduce unnecessary biopsies in patient management and provide clinical utility for several aspects of clinical trials such as the CTOT 19 in which it is currently being used.

Page 85

As a marker for antibody mediated rejection donor-derived cell-free DNA can provide a prognostic tool to forecast the likely course of disease, a tool that estimates the extent of injury, and a predictive tool for forecast the likely response to treatment.

A potential clinical trial application would be evaluating safety and efficacy of a treatment for antibody mediated rejection. Existing endpoints might be biopsy-based histological findings for evaluation of transplant glomerulopathy six months after treatment.

But measurement of donor-derived cell-free DNA can provide non-invasive measures of the degree of AMR active injury that can be followed at relatively narrow intervals.

AlloSure testing would also ensure consistent baseline status or allow stratification of study subjects at the time of the initial biopsy-based diagnosis of AMR.

AlloSure testing could also provide an accurate and precise measure of recovery from AMR injury that can be performed frequently to follow the

Page 86

trajectory of the response to treatment.
So in summary, AlloSure is a clinical testing service to quantifying donor-derived cell-free DNA which is clinically validated by our marker in AMR. AlloSure is available for Caredex Clinical Laboratory both for patient management and for support of drug-developed clinical trials.
Diagnostic donor-derived cell-free DNA complements the knowledge provided by biopsy, the prognostic qualities of DSA, and other tests useful in AMR studies.
AlloSure offers a new dimension by providing a quantitative measure of ongoing injury which can be repeated at frequent intervals. Thank you for the opportunity to present these data.
DR. ALBRECHT: Thank you for your comments. Could the questions for session 4 please be projected?
DR. NICKERSON: And if there's any -- before we get to those is there any clarifying questions of any of the speakers? I'd invite those now from the audience or from the table.
Yes?

Page 87

DR. OZDEMIC: My name is Handan Ozdemic. I'm a renal pathologist and a transplant pathologist from Baskent University, Ankara, Turkey.
My question is for Dr. Mark Haas. In my daily routine I'm staining HLA-DR in parallel to C4d. I noticed that the patients who have C4d negative d there's loss of peritubular capillary HLA-DR expression in the areas of C4d negative areas.
Could it be possible the peritubular capillary HLA-DR expression -- the loss of HLA-DR expression can be the sign of antibody mediated rejection especially in patients with C4d negative?
And second question is I have a -- for example, we have a biopsy. And in this biopsy you have only minimal tubulitis in few tubules. And interstitial inflammation lower than five persons and vascular rejection.
What will be your final diagnosis? Is this vascular rejection pointing out a (indiscernible) rejection or a (indiscernible) rejection? Thank you very much.
DR. HAAS: Okay. I'll take your second

Page 88

question first. You have an isolated endoneuritis and so it's -- the question is, you know, what does an isolated endoneuritis mean?
There is -- I think a lot of that depends on when the biopsy was done. The data that come from Edmonton suggests that a late isolated endoneuritis, that is more than one year post-transplant, is more likely to be antibody mediated than cell mediated.
So I would suspect that if it's a later biopsy, that is more than one year post-transplant, I would definitely test for donor-specific antibodies. If the DSA are positive, I would treat that patient for antibody mediated rejection.
If it is an early isolated V-lesion, the data from Edmonton suggests that it is more than likely either, one, cell-mediated rejection or, two, perhaps non-specific.
The -- there's no real, you know, DSA type of test to determine that so if it -- if you're dealing with an indication biopsy associated with an elevation in serum creatinine and there's no other explanation on the biopsy for the elevation in serum

Page 89

creatinine, perhaps treatment of this with a short pulse of steroids might be appropriate or you may simply just want to follow the patient and if there hasn't been a decline in graft function and see what happens from there.
But if there has been a decline in graft function then maybe treatment as T-cell mediated rejection would be appropriate. And the studies from Bonu Sys suggested that these early isolated Vs do frequently respond to steroid therapy.
The second question is a lot more complicated and because you're dealing with a number of different factors. One of the problems that we deal with in terms of evaluating peritubular capillaries is that in patients who have chronic antibody mediated rejection PTCs are lost over time. And this is one of the factors contributing to interstitial fibrosis.
And tubular atrophy is the loss of PTCs. So is the decline in HLA-DR staining, you know, reflecting a loss of PTCs or endothelial injury, itself.

Page 90

One way to show that might be, you know, to also stain for an endothelial marker. If there's -- clearly if there hasn't been a decline in the level of PTCs, it's possible that it could be a marker for early endothelial injury. In DSA -- if the DSAs are positive, possibly antibody mediated endothelial injury. But obviously that would need to be, you know, validated in some kind of a controlled study.

If there's clearly decreased peritubular capillary density then -- you know, then you're dealing with a chronic process and probably not something you necessarily want to treat.

Does that answer your question?

DR. OZDEMIC: Yes.

DR. HAAS: Okay. Thanks.

DR. NICKERSON: Other questions? Yes, Stuart?

DR. KNECHTLE: I wanted to ask Dr. Woodward what is the ability of the cell-free DNA assay to distinguish between antibody mediated rejection and cell mediated rejection or between acute tubular necrosis from either preservation injury or drug

Page 91

toxicity?

DR. WOODWARD: It seems pretty specific for rejection. Sorry. It seems pretty specific for rejection. When we looked at other types of injury that were no T-cell mediated or antibody mediated rejection there didn't seem -- there wasn't a reproducible or significant increase.

In the studies so far the lowest level of T-cell mediated rejection, a grade 1, did not have high levels of cell-free DNA. But other levels of T-cell mediated rejection as well as antibody mediated rejection had high levels of cell-free DNA.

Whether it could distinguish between them we'll need much larger studies maybe, maybe not.

DR. KNECHTLE: Why do you think that the assay is specific for antibody mediated rejection? Why should it be?

DR. WOODWARD: Based on the concept that it is looking at DNA that's being produced by injury from the graft we think it's probably mostly it's reflecting microvascular injury.

And all of the talks that we've seen about

Page 92

microvascular injury association with mediated rejection may be why. There may be other injuries that also have some microvascular that aren't rejection, but why it wouldn't be associated with them I'm not sure.

DR. WOODLE: I could have missed it, but what are the markers that you're looking at to designate the donor? Are they actual HLA gene sequences that you're -- that you're amplifying or are they other gene low SI expression markers?

DR. WOODWARD: They are a set of single nucleotide polymorphism that differ between the donor and recipient, but are not associated with disease. They're just snips that are different between the donor and recipient.

DR. WOODLE: And those snips you're looking at how many different genes do they -- are they analyzing?

DR. WOODWARD: They're not located within genes. They're in non-genetic parts of the geno.

DR. HAAS: Yeah. So you mentioned that the donor-derived cell-free DNA did not seem to be

Page 93

increased in 1A rejections. Was it increased in 1B cell mediated rejections do you know?

DR. WOODWARD: Yes.

DR. HAAS: It was? Okay. So clearly these are cell mediated rejections so it -- I guess one of the problems is that in some cell mediated rejections, particularly the more severe cell mediated rejections like a 1B, there is quite a bit of peritubular capillary inflammation.

So, again, microvascular inflammation is not specific for ABMR. So it would -- obviously something needed to be perhaps combined with another test, but, you know, all -- but if it can be used to eliminate some biopsies, that's a good thing.

DR. WOODWARD: Yes.

DR. NICKERSON: Chris?

DR. HAAS: Not necessarily for me, but.

DR. NICKERSON: Chris?

DR. WIEBE: One more question while you're up there.

DR. WOODWARD: Uh-huh.

DR. WIEBE: As you've heard from a number of

Page 94

talks there's this concept of smoldering rejection especially in high-risk patients which can be defined as recurrent rejection or high AlloMune risk.

Have you noticed that there's a different baseline in these high-risk subsets versus low-risk subsets?

DR. WOODWARD: We haven't looked. And I'm going to take what we've learned today and see if we can go back and identify any of those high risk or smoldering categories in patients in the population of the dart study and see if we can identify a difference.

It's something that we've been interested in, but haven't looked at yet.

DR. NICKERSON: I believe I just have my own question to -- oh, sorry.

DR. MANNON: I'm sorry. I have like a zillion questions, but, Anita, go first.

DR. NICKERSON: Okay.

DR. CHONG: In a related question -- thanks, Ros -- how about smoldering CMV infection of polyoma? Could you tell the difference or are you just looking

Page 95

at injury?

DR. WOODWARD: So in -- so far we've had two BK virus infections and they both had elevated donor-derived cell-free DNA. We have not yet observed any CMV.

DR. NICKERSON: Ros?

DR. MANNON: This is on a completely different scale. Okay. So different question. So question number 1 for bachelor number 1, Dr. Woodle, you indicated -- and I sense a lot of optimism about complement inhibitors.

But you also mentioned in one of the other comments about IDS was removal of antibody. So how do you sort of put those two together because obviously the complement inhibitors mitigate injury, but they don't, you know, intrinsically remove antibody.

So do you think they're going to be monotherapy or dual therapy?

DR. WOODLE: I don't know. I think it's -- the -- if you look at the approaches in general that are being taken for AMR, it's either elimination of the antibody or inhibition of its function or a

Page 96

combination of the two.

And I think that if you're going to inhibit function, then the question is are you going to inhibit short term and expect that to have an effect on the long-term outcome or are you going to have a long-term maintenance therapy that will go along with it?

I think it's a fundamental question if you're therapy leaves the donor-specific antibody in the circulation for prolonged periods of time, what is that going to do to graft function?

Most of what I'm hearing today is that -- and what I've heard all along is that the presence of a DSA is deleterious to the graft. And so that's one of the reasons just -- it's just my personal intellectual bias that elimination or reduction of antibody is -- is a preferred approach to take if you're developing drugs for this particular indication. Otherwise I think that you may be looking at long-term maintenance therapy.

Now even with plasma cell deletion approaches you're going to prob- -- it looks like now

Page 97

the field is headed towards the need for long-term maintenance therapy to inhibit the reflexive responses that occur. And you're going to hear more about that later on today in merian model and a primate model.

I'm not sure if that answers your question. And if it didn't --

DR. MANNON: No. I mean I --

DR. WOODLE: -- can you restate it?

DR. MANNON: -- kind of wanted to get an opinion because my sense from you is this -- and my own personal optimism of complement, but it doesn't really effect DSA. And I know several years ago we really debated that.

And I think it's either going to come down to multiple approaches, we salvage grafts with AMR and hyperacute in the cases of incompatible. And I just kind of wanted to get your opinion. I'm just --

DR. WOODLE: Yeah. So I think complement -- you know, for almost the entire history of AMR complements almost been a singular focus. And I think now with the C4d negative forms of rejection and understanding that you really need saturating levels

Page 98

of antibody to activate complement that we have to be considering the non -- the complement independent mechanism for rejection because I think once you wipe out complement those are still going to be there if the antibody remains present in the serum.

The issues with the IdeS I think is IdeS, as I see it, is almost like a plasmapheresis.

DR. MANNON: Okay.

DR. WOODLE: That you eliminate the antibody for a period of a week to ten days or so, but then after that the antibody is back in full force at the same levels.

It's not clear to me whether or not you're going to have reflexive responses from that sudden elimination of antibody. We know that when you reduce antibody there are homeostatic mechanisms that come into play. And you'll hear more about that I think from Stuart and Anita.

And so it's really going to be an interesting experiment. I mean, IdeS is really a very intriguing molecule. And, you know, we got two guys, Stan Jordan and Bob Montgomery, who have as much

Page 99

experience as anybody in this field, really a couple pioneers of the field, looking at it. So it's going to be -- it's a really fun time I think.

DR. NICKERSON: Do you have more?

DR. MANNON: Yeah. They're unrelated, though, to this so I don't know if you want to go to those questions. I was actually going to now --

DR. HAAS: Can I say something about the IdeS?

DR. MANNON: Yeah.

DR. HAAS: Oh, with -- just a brief comment with regard to IdeS. At least, you know, for what a lot of the purposes what people are looking at IdeS for right now, including Stan Jordan, is that IdeS not necessarily as a long-term treatment for antibody mediated rejection or prevention in any way of antibody mediated rejection, but as a means for desensitizing patients who cannot be desensitized through more sort of standard of care means like rituximab plus plasmapheresis plus IVIG that these patients can be desensitized using IdeS and be allowed to have a transplant.

Page 100

But as Steve noted and others, there's a rebound of antibody following the IdeS treatment. So you get the antibodies down and you get the transplant in, but then you're going to have to allow your other therapies to keep the -- to limit the amount of rebound. And whether that's proteasome inhibitors, whether that's anti-CD20, that's -- you know, that's the question.

But I think IdeS is more of a, one, desensitization-type therapy and possibly, two, a rescue-type therapy in patients who develop high levels of DSA and very severe AMRs. But I think that's going to be its primary usefulness.

DR. NICKERSON: Dr. Colvin?

DR. COLVIN: Yeah. I'd like to get to another topic which has run through many talks yesterday and today. And that is transplant glomerulopathy. We heard from Dr. Wiebe and Dr. Haas how important this is in terms of a measure of antibody mediated rejection and as a prognostic aspect, a prognostic surrogate.

What I want to emphasize is how poorly the

Page 101

Banff system scores transplant glomerulopathy. It's an ordinal system. It has four categories. And it's based on the findings of one glomerulorist, one most affected glomerulorist.

And I would urge those of you who are proposing or doing clinical trials to develop a more accurate, more objective way of scoring transplant glomerulopathy.

And one way to do this which I've been involved in is to use digital whole slide images which Bonu Sys has shown that increases the reproducibility of pathologists even scoring with the Banff system.

Use whole slide images which can be archived, individual capillaries can be scored not just the whole glomerulorist globally, and the system is auditable. There's an audit trail of the scores of individual pathologists for each individual capillary.

So I do think this is an important endpoint or at least an indicator, a secondary endpoint of these clinical trials for chronic antibody mediated rejection.

And there are methods that have been

Page 102

developed to do this in a way that's beyond what Banff is using.

DR. NICKERSON: Maybe just a follow-up question, then. Bob, what about electron microscopy in that regard?

DR. COLVIN: I love electron microscopy and I love electron microscopists, but their sample size is even more dismal than what we see in a light microscopic biopsy.

I agree that they can see early signs. They can see early signs of things that don't end up to e antibody mediated rejection. And the studies that Mark has shown you I think those are mostly pre-sensitized patients where you'd expect the early signs to be there in a few months.

We always do electron microscopy to evaluate antibody mediated rejection so I am supportive of that, but I don't think it's the way to score it. It's the way to detect early changes, but probably not the best way to quantitate just because of the sampling problem.

DR. WOODLE: Just as a non --

Page 103

DR. COLVIN: But I would welcome Mark's comments on that.

DR. WOOLDE: Just as a non-pathologist how many glomeruli do you normally examine under an EM?

DR. COLVIN: Well, one to two typically, sometimes three or four. Depends on the sample and depends on what you're looking for.

DR. WOODLE: Okay. And do you usually look under -- do you look under light and choose one to actually -- or do you look at the specimen to choose which one to look at?

DR. COLVIN: Yes.

DR. WOODLE: And do you usually choose the most pathologic one -- so is it a worst-case scenario that you're doing under EM?

DR. COLVIN: Well, not necessarily. The way it's done is a small portion, as you know, is put in the EM fixative. And then thick sections -- what we call thick sections are made of those.

And we use those to select the glomeruli. We obviously don't want to study a sclerotic glomerularis and we obviously do want to study any

Page 104

glomerularis that has some notable feature otherwise we just choose a representative glomeruli.

But if you take three, four, or five little pieces you'll be lucky to get three of four glomeruli. Mark, do you want to comment on that?

DR. NICKERSON: I think Renata wants to move on to the questions --

DR. COLVIN: Oh, okay.

DR. NICKERSON: -- at this point. I think the pathology discussion you could have at the coffee break so let's keep moving.

DR. MANNON: Can I just ask a very quick clarification from Dr. Chawla's presentation? Because I have a feeling we won't get a chance to talk about it in these questions.

But has anybody done this furosemide stress test in either brain-dead donors or candidates for donation that may be DCDD before they donate, yes or no?

DR. CHAWLA: Here we go. Sorry. Not that I'm aware of. And we obviously want to look at that because that could be a nice way to decide, you know,

Page 105

on a marginal donor does it work or what kind of opportunities do you have.

DR. ALBRECHT: So I actually think that's question 3. And if there are other comments about --

DR. MANNON: I'm sorry.

DR. ALBRECHT: No, no. This is --

DR. MANNON: Renata, I didn't even pay attention.

DR. ALBRECHT: No, no. But it's actually one that we would like to have some discussion on so please go ahead and share your views and comments.

DR. NICKERSON: So I have a couple comment -- maybe one comment about the deceased donor. It's going to be very hard to separate that out to some degree for someone who has brain death because you're going to have diabetes insipidus basically with the lack of ADH.

DR. CHAWLA: Yeah. That's exactly right. So I think from a urine output standpoint it's not useful. But the other thing we did and I didn't have time to go into it is we actually measured furosemide concentration in the urine.

Page 106

And so that allows you to actually look at direct tubular transport. And for those of you who are interested in horse racing, furosemide testing is routinely used in horses and I won't get into why, but it's cheating if you don't do this in horsing race -- racing.

So the assays are very good because there's a lot of money in horse racing and they're quantifiable. And what we've been able to demonstrate is that the furosemide concentration is not always linear with the urine output. And what that suggests in at least some patients is that their proximal tubular functions intact, they can lose furosemide across but they don't respond.

And there's others who move relatively less furosemide across but have a really brisk response. And that might be informative because you may or may not have the opportunity to look at tissue. You get on the back table but by then you're kind of committed to some degree.

And if there are -- if the test was done as a convenience sample and then you did a back table,

Page 107

you could probably then marry that data later on and see if it's informative.

But in general I want to be very clear that, you know, functional testing should never be done in isolation, you know. Everything we do clinically at the bedside when you take a functional test and you add it to something else you typically get better fidelity of information.

And I think that it's important that we not get really sort of, you know, siloed in our thinking. You know, look at what the cardiologists have effectively done. They use functional testing and they use other biomarkers, they put the two together. And additionally if you can have imaging that's your trifecta.

In nephrology we are weak in our imaging at least at the bedside. We do better with allografts because they're more accessible, but in a native person it's very challenging. And we don't have good functional tests and so we need to do a lot better in all those domains in my view.

DR. NICKERSON: Mark?

Page 108

DR. HAAS: With regard to furosemide and acute kidney injury, I mean, furosemide as we know acts by inhibiting the transporter in the thick ascending limb in the apical membrane. And in pa- -- and the thick ascending limb is very sensitive to ischemia.

And when patients develop ischemia -- and this maybe relate to Ros's question -- when there's either ischemia reperfusion injury or whether there's cold ischemia these transporters in the apical membrane actually are -- become incorporated into the cytoplasm and there's a loss of -- there's a loss of transporters in the apical membrane.

So how does acute -- so one might expect furosemide to have a lesser increment on urine output in patients who have ischemic acute kidney injury. Does acute kidney injury affect the -- you know, the usefulness of the furosemide assessment of reserve?

DR. CHAWLA: Yeah. So this is a very important question, but I think that one of the things that we've come to realize is that the acute kidney injury which was formerly viewed to be ATN is not ATN.

Page 109

And we know -- we know that this notion that decreased blood flow drives acute kidney injury is wrong.

Most patients with sepsis inflammation who are resuscitated in ICU have increased blood flow, not decreased. They have a primary microcirculatory defect. Blood flow is maldistributed in the kidney and it's maldistributed in a medullary and a cortical fashion and so you have a very heterogeneous injury.

And must of that is localization of ischemia and a lot of dysfunction primary mediated through what appears to be a stunning phenomenon which is probably highly adaptive.

And so what you don't know in any of these individual patients is time of injury, depth, and severity. And that functional test gives you a rather blunt readout. And I think there are a manifest -- manifold reasons why you have poor response.

And I think we need to step back and recognize we just don't know on a person-to person, individual-to-individual basis what's happening at the cellular level. And we need to prognosticate and make thoughtful decisions to give therapies.

Page 110

I think many of the reasons you've outlined could be why there's some resistance to furosemide, but we just don't know. And there's probably at least ten reasons why this occurs.

There was also a question over there.

DR. VELIDEDEOGLU: I just want to comment on the same subject. Before moving onto the furosemide or other types of stress testing I think the fundamental issue is that -- is the preservation of renal function, the ultimate goal. I mean, that's what we are striving for to preserve renal function, to prevent any nephron loss as a consequence of rejection mediated or other types of damaging kidney transplant patients.

As Dr. Chowla explained, there's quite a bit of renal reserve in healthy people. So one of the questions probably that needs to be answered is that how much renal reserve if there's any do transplant patients have?

And I know that there are some studies performed on this, but in my opinion probably needs some further work. And is that also dependent on the

Page 111

type of immunosuppression that they are receiving?

For example, if they are on a CNI-based regimen because of the constriction, do they have more renal reserve compared to patients on non-CNI regimens?

And this also ties to the concept that we've been seeing especially in old papers that while patients had an acute rejection episode, but the creatinine returned back to baseline value or the GFR return.

Does that really mean that those patients did not sustain any permanent damage or is it simply because that the remaining nephrons are compensating for the ones that are lost?

So if anybody wants to or Dr. Chowla wants to --

DR. CHOWLA: Yeah. So I think that's a really important question. And let me address the first one first which is that I think the medications do matter. If you're on a calcineurin dependent inhibitor I'm sure your ability to alter filtration fraction and increase blood flow, which is the primary

Page 112

means through glomerular reserve, is mitigated. And those -- that -- those studies have not been done at least from a glomerular standpoint.

I also think that your second question is very important that if you do have injury, what we have clearly demonstrated is that you have sustained a hit even if you're creatinine comes back to normal.

So it is very uncommon to rare that an episode that results in a brief episode of acute kidney injury does not actually result in durable injury whether you can measure it or not.

And what the kidney does very effectively and why I am very anti-creatinine -- I'm in the ABC camp of anything but creatinine because I think that it's old and it needs to be updated -- is what the kidney does is that it says, oh, there's so much creatinine around I can't increase my filtration. I'll just take my tubular reserve and secrete more.

And we sit there and see the creatinine go from 1.2 to 1.5 back to 1.2 and we're happy. But the kidney is durably injured. It's managing. And we think everything is fine and nothing is fine. And I

Page 113

think that this is a huge problem in an intellectual approach which is damaging because creatinine is a lousy marker in my view. And our continued dependence on it I think is enormously problematic.

I would concede we have nothing better now, but it's a lousy marker. And the fact that we haven't updated it in 60 years I think is enormously problematic.

DR. GASTON: So we did those studies. And this is -- seems like ancient history, but it has published in JASN in 1995. And we studied renal functional reserve in patients on and off cyclosporine and transplant recipients.

And basically -- and the control group was Imuran treated patients then for those of you who remember Imuran. And the -- there was substantial functional reserve in the patients on Imuran that was related to renal blood flow that was abrogated with cyclosporine. And these were chronically treated patients on stable doses of cyclosporine.

To my knowledge it was done with Imulan clearance, with PAH clearance, and so on, very labor

Page 114

intensive. And those studies have not been repeated to my knowledge with tacrolimus.

Based on differential impact between cyclosporine, tacrolimus, an renal function, long-term renal function my bias would be that the affect that we saw with cyclosporine would be less with tacrolimus, but I'm not sure of that and that work has not been done.

And then I would expect the patients on BELA and not on CNIs to behave much like the Imuran patients even though that's not been done either. So I -- that's always made me suspicious of the GFR as an endpoint because as a nephrologist I know I can manipulate GFR all sorts of ways and so that I think of an endpoint as more of a fixed kind of thing.

That said, watching the BELA data evolve and with data out to seven years that shows this differential between CNIs the duration of that effect over time has become more compelling to me in thinking about it.

But clearly the patients retain renal functional reserve. And it is affected by the

Page 115

mediations they take or can be affected.

DR. WOODLE: So, Bob, is it possible to do these tests when patients are on drug like a CNI that reduces renal blood flow by 30 to 50 percent? Is it applicable test?

DR. GASTON: Well, the way you do it is with -- or the way we did it was with a fixed infusion of amino acid that was known in normal people. And basically we used L-arginine and we got all into nitric oxide and so on in the studies. And it was interesting.

So that, yeah, it's fairly standardized and very doable I think or reproducible, but it's very labor intensive. And I think you have a difficult time even getting Imulan these days to do that sort of thing so.

DR. CHAWLA: I would agree with all that. I think that's very important. I would just point out the one piece of good news is real time GFR in a non-invasive fashion is coming soon. And we will have that at the bedside within the next three to five years in 510(k) and everything quite clean and very

Page 116

non-invasive and non-toxic.

Not our company. I mean us as a community.

DR. NICKERSON: So just a follow-up question with the Lasix the furosemide stress and looking at reserve. So can that be done as an out-patient?

Is this a requirement that that come in and be clearly monitored?

DR. CHAWLA: Oh, yeah. It can certainly be done as an out-patient. It can be done with Gatorade is the ideal replacement solution. And you just have them sit down and you just keep track of them.

And there are colleagues of ours who are doing this and they're doing it as a convenient sample. People will come in for a biopsy for whatever reason and they're basically getting furosemide post-biopsy which in some ways is useful to see if they have hematuria and basically wash them out if there's a concern about that.

And they're just tracking it as they go and they replace them CC per CC at the bedside with -- with Gatorade which is, you know, a nice, balanced salt solution at the bedside.

Page 117

And so far I think they've enrolled over 85 patients. It's been very safe. As to whether the data are meaningful or not it remains to be seen. That's to come.

But it is very straightforward in so long as you have it in a reasonably monitored environment. I don't think this is something you do, you know, without someone checking in on them, you know, but certainly in any kind of reasonable hospitalized setting or a clinic setting would be fine.

DR. GASTON: Oh, just the addition that I would say about the CNI effect is if you can look at a myriad of studies from Chris's and Peter's to all sorts of other things and see that those patients in that top curve have very stable GFRs. They're adherent and don't have DSA.

They have stable GFRs over 10 to 12 years basically and they're all on CNIs. And so that -- there's a lot to be said for that that there is not built into CNIs a decline in GFR independent of other things.

So that to do -- even though CNIs may

Page 118

inhibit this renal functional reserve business what we're after is not preserving renal functional reserve necessarily. It's preserving kidneys. And I don't know if Dixon's still here. Dixon taught me that years ago, Dixon Kaufman, that we're not about preventing diabetes or maximizing GFR even though that's -- those are both good things. We're really about making grafts work for long periods of time for the patients that we serve.

DR. ALBRECHT: Dr. Colvin?

DR. COLVIN: Yes. Just a follow-up question to Dr. Chawla on the furosemide test. One of the things we're not very good at as pathologists and I think probably also as clinicians is predicting which patients with acute kidney injury are going to recover or not.

And does your test allow this distinction to be made?

DR. CHAWLA: Yeah. So thank you for referring to it as my test, but it's certainly not my test. This is a conglomeration of knowledge from people who are much older than me and a simple step of

Page 119

standardization.

There's two studies now where they have looked at this later in the course of acute kidney injury and it does predict. And, you know, this is not rocket science. We've all done this clinically. Someone's sort of in their recovery phase, you give them a big slug of furosemide, they respond. You sign off on them Friday and you don't see them ever again hopefully.

And we do this all the time. This is just a standardization of bedside practice. But it does work. It does work with metrics. And the general metric is that if you make more than 100 CCs an hour for 1 mg per kg so the math is easy to remember you tend to be okay. If you're under 100 CCs an hour for 1 mg per kg assessment you don't do as well. And obviously there's gradations depending on how far or below that threshold you are.

So it does work. It should not be an exclusive test because many patients have variable effects of furosemide based on furosemide itself. And it doesn't have to be furosemide. If someone wanted

Page 120

to get very cute they could use probenecid or anything else that uses human organic transporters. This just happens to be super cheap and convenient which is why we selected it, but it does work on both ends. It works to predict worsening. It works to predict recovery.

There's no traction in DGF. And given the cost constraints we're all feeling I think it might be an opportunity to marry it with other thoughtful diagnostics that we use and maybe improve fidelity overall.

DR. NICKERSON: I'd like to move to question 4 which is based on the information on diagnosis, treatment, what do we know about the ability to select control therapy?

And I'd like comments on this please because I think this is a really critical question for us going forward as we think about clinical trial design.

So what do we -- what are the comments that we have and do we -- what do we know about the ability to select control therapy?

DR. MANNON: So, Peter, I was -- you know, I

Page 121

was not involved in the design of the Alexion equlizumab trial that Steve showed the data for. But the way the control arm was allowed was sort of substantial flex- -- I would say flexibility in the terms of it was an HLA incompatible living donor study with central laboratory.

So they wanted you to use relatively high-risk patients that were both DSA positive and flow cross match positive. But they used a central lab to sort of ascertain whether your level of risk was similar to someone else's.

And then after the enrollment it was randomized. You could, you know, do the incoming treatment is whatever, plasmapheresis, whatever the standard of care of your center was. And not every center gave pre-transplant immunosuppression and some centers did.

And so I don't know if that's the kind of question you're asking, you know, from an acute -- you know, I mean, we have -- we have three different uses -- desens, AMR, and CAMR.

And so, you know, from that equlizumab study

Page 122

that was a desens application. And so I think there needs to be some standardization because I think it hurt the study in the end.

From the AMR study, again, there's lots of ways peop- -- you know, I know on the calls we said, oh, this is the standard. But there's -- every center has a little bit of a tweak, you know, whether they're using IDIG or low dose IVIG or cytogam and then the CAMR.

So I don't want to monopolize the conversation, but I -- because there's other people who are in here that do this work, as well.

DR. NICKERSON: So maybe make -- just I'll -- before I let you go, though, talk about CAMR and what you would say you would think as a control therapy in CAMR.

DR. MANNON: So, I mean, I think what we do is, you know, we sometimes give the option about -- we typically will give a one course of IVIG to see if we can make any dent into the DSA because our assessment is that the persistence of DSA is persistence of injury.

Page 123

Similar to one of the other speakers yesterday, if we see fairly advanced IFTA, grade 3, we typically reserve that. If the eGFR is below 30 it portends doom and so I think we sometimes use the IVIG just to give patients a sense of hope.

ACE and ARBs are standard of care for proteinuria unless the eGFR is too low or there's hyperkalemia. Steroids have been used in our group in modest doses and then intensification of immunosuppression is tolerated. And, again, it's based on the eGFR when you try to push tack up.

And then we get into a debate about rituximab and use of rituximab dosing based in some of the other studies. And it's not used consistently.

So those are -- those are the things that a standard big center -- and, you know, I'm welcome to hear my other colleagues.

DR. WOODLE: So I think that very clearly if you're going to do an AMR trial -- so an AMR therapeutic trial you've got to either focus on early or late. And the late by whatever term you define it, you know, has to be carefully defined.

Page 124

And but if you take an AMR that's past six months, most of them are going to be mixed rejections that have the molecular signatures of both T-cells and AMR. And I think that because with early AMR the therapeutic results are really good, okay.

And so based on diagnosis if you're going to allow both early and late into your trial I think you need to stratify and I think you need to control for it both at the entry level and at the endpoint analysis level.

As far as treatment it's my opinion, and, of course, those opinions are always subject to bias, but I -- my interpretation of literature between IVIG based regimens and proteasome inhibitor based regimens, which I think are the two major options that you have right now, that the results for early AMR are equivalent and the results for late mixed rejection are equivalent. And that's in terms of IDSA reduction, in terms of histologic improvement, and in terms of renal functional outcomes.

And I think those are the three major endpoints we've looked at and analyzed when we've

Page 125

looked at our results and then sort of compared them to what the historical literature is.

That being said, I did not show data at the end of my talk, but, as I mentioned before, IDSA reduction by at least 50 percent in 14 days is the most powerful predictor in our analysis of outcomes of graft survival.

Death censored graft survival after an episode of AMR treated with a proteasome inhibitor. And that's in a group of about 100 patients with AMR treated with a single proteasome inhibitor based regimen. And that's a pretty big experience.

DR. NICKERSON: And just to clarify because I think the Banff language is hurting us still. Is this acute AMR where we actually have graft dysfunction that you're talking about?

DR. WOODLE: Yes. Yeah. So that series was exactly that, Peter. As I mentioned before, we're starting to try to move to where we're treating AMR before we have renal dysfunction.

And I think that that -- that's an important thing to consider when you're looking at entry

Page 126

criteria.
DR. NICKERSON: So if -- and maybe just going to push you a little bit more. So if you have chronic AMR, the smoldering, chronic active AMR where you have some early DG and you have some active lesions, microvascular inflammation, C4d positive, and you're creatinine has really just got this sort of niggling rise, what's your approach as standard of care in your center?
DR. WOODLE: Yeah. So our general feeling -- so we look at not in terms of trying to put things into a basket. We look at the biopsy, we look to see if they have proteinuria, we look to see if what their renal function is and how much inflammation they have in the graft.
That being said, we treat them all the same. We don't have a specific treatment for one of those lesions. What we try to do is you try to get rid of the antibody as much as possible, okay.
And we don't -- what we haven't included in our therapies is a long-term maintenance concept for keeping that antibody suppressed. So we don't have

Page 127

that. We give a two-week course of a proteasome inhibitor based regimen.
Now and so for our outcomes we basically look -- we want to see the antibody go down by at least 50 percent. If it does that, to me, suggests it's a treatable lesion. It may be the biology rather than the treatment. It may be not what you treat with, but just the fact that patient's particular biology is treatable.
We also like to see proteinuria stabilize. We like to see the creatinine come back down to baseline. But, as you know, with these chronic lesions it's hard to know what the baseline is so.
DR. DJAMLI: Peter, if you -- oh.
DR. NICKERSON: Go ahead.
DR. DJAMLI: I just wanted to say that we published last year in human immunology the -- a rather large series of chronic rejection. Of course this was observational in 126 patients with CAMR just to see how we had progressed our management over the past 15 years.
And, in fact, the first round of them got no

Page 128

treatment because we didn't know what was the best thing to do. And progressively we escalated therapy to steroids and steroid IVIG and additional rituximab and in some cases (indiscernible) and there was a mixed component.
Given all the limitations of this observational study we have come out of it with defining our control being steroids and IVIG because that was -- after all I just mentioned in multivariate the single determining factor in improving outcomes by 50 percent in terms of graft loss.
And if there is no significant scoring on the biopsy or is serum creatinine is less than 3, then we add rituximab. So that has become our new standard.
MR. HAAS: You say chronic antibody mediated rejection. Do you mean chronic active antibody mediated rejection or just chronic?
I think it's very important to distinguish between the two. I mean, if you have just TG by itself, that's just chronic antibody mediated rejection at least most of the time and if you have a

Page 129

history of DSA.
But if you have TG plus active microvascular inflammation or C4d, that's chronic active antibody mediated rejection. One is just can almost be considered a bland scar and the other can be considered an inflamed process that is in the process of undergoing scarring, but, you know, it's still an active process.
So I think it's important to distinguish.
So do you know if these also had active inflammation?
DR. DJAMLI: Yes. The common denominator was CG. And the vast majority of them had active lesions. And we looked at the independent impact of those variables including microvascular injury and so forth and the DSA and some of them panned out significant in univariates. But in multivariates the only variables that were really retained were retox-- not -- is IVIG and the functional value, I'm sorry to say, but serum creatinine at the time of biopsy.
DR. NICKERSON: I'm going to call the session to a close. We're about five minutes over time. We do want to try and stay on time so we are

Page 130

going to ask people to reconvene in ten minutes at 11. So it'll be a brief break and we'll start up at 11 again. Thank you very much.

(A brief recess was taken.)

DR. BALA: Thank you, Dr. Albrecht. So we'll start with the first talk and our speaker is Dr. Gregory Knoll. And he will be talking about potential primary endpoints in clinical trial of antibody mediated rejection.

And he will be covering examples like desensitization, prevention and treatment of acute AMR, and prevention and treatment of cellular rejection -- chronic rejection. Sorry.

DR. KNOLL: Thank you. This is my disclosure slide. So allograft survival, and more specifically the one-year allograft survival has really become the main endpoint that we use to evaluate therapies in kidney transplantation.

And this figure's from the 1983 landmark trial of cyclosporine and you can see the patients who got cyclosporine clearly had a better one-year graft survival than the comparator Imuran and Prednisone.

Page 131

And in the decade following this trial the one-year graft survival I think has really become the most important clinical endpoint we've been using in kidney transplant.

So what are some of the other outcome issues we can use? So, first of all, we can use clinical endpoints. These are also called patient important outcomes. This is simply a characteristic that reflects how the patient feels, functions, or how long they survive. And in kidney transplantation examples of this include graft survival, patient survival, and quality of life.

We also have biomarkers and these are just characteristics that are objectively measured as an indicator of a normal biologic process, a pathogenic process, or some response to therapy. And typical ones in transplant are creatinine and GFR that we've heard about in the talks.

And finally we have surrogate endpoints and these are simply biomarkers that are used as substitutes for our clinical endpoints. And a true surrogate is really expected to predict both benefits and harms of any intervention.

Page 132

So what are the advantages of using surrogates? Well, these are usually measured earlier in a trial compared to our clinical endpoints that allow for shorter and cheaper trials. This results in faster decision making about treatment which is very important in this topic area.

Also typical surrogates are continuous variables so that all patients in the trial will, in fact, have an event and this greatly reduces sample size, increases power, and reduces cost.

So what are the disadvantages? Well, the major thing is that most biomarkers are, in fact, not valid surrogate endpoints. And it's actually quite difficult to properly validate a surrogate outcome.

First of all, the surrogate needs to be prognostic for a hard clinical endpoint. Changes in the surrogate with treatment must predict changes in the occurrence of the clinical endpoints. And finally the full effect of the treatment on the clinical endpoint should be captured by the surrogate. And invalid surrogates may misrepresent really the true consequences of an intervention.

Page 133

And in the literature there's a variety of examples where we've had bad surrogates where in well-done clinical trials the surrogate measures were moving in a favorable direction whereas when we looked at the clinical endpoints -- and many of these are mortality -- they were going in an unfavorable direction. So we just have to be careful when we choose our surrogate endpoints.

So what then clinical endpoints would be important to transplant patients? Well, I think obviously patient survival. But I think also allograft survival in my mind perhaps is more important as it accounts for both patient death and graft failure.

I think it -- I look at it as marker of quality of life if you think of time off of dialysis with the graft still functioning. It's also a marker of cost given the cost differentials of the treatment.

And, as I mentioned, the one-year allograft survival has really been the one that's most commonly used. But as I'm going to show you it's become more

Page 134

difficult to use as our success has improved.
So if you think of overall now one-year graft survival is in the range of about 94 percent. And if we think of in the setting of ABMR what's one-year graft survival it's not an easy number to find, but it's probably in the range of 90 percent as most of these grafts fail beyond the first year.
So if we think of sample sizes needed to try and improve on this 90 percent one-year graft survival you could see if you just wanted to get them back to sort of the average of 94 percent with a new therapy this is going to require 1,400 patients in that trial.
If you had a dramatic -- you know, sort of a blockbuster-type drug that you thought might have a dramatic improvement from 90 to 98 percent at one year, this would only require 276 patients.
But I've highlighted the reflux era study which many of you know about, but this is one of the largest published randomized control trial in ABMR. And this had only 38 patients and it took 21 centers to get those 38 patients. And they didn't even get their sample size of 64. So although that sample size

Page 135

of 276 looks fairly reasonable in the context of the condition we're talking about it may be very unrealistic.
Now what about late graft survival? Could this be a possible endpoint? These are just some figures from some different studies. And you can see that the outcomes are highly variable. And we know this depending on when the ABMR is occurring, is this associated with nonadherence, is this an early or a late lesion.
But I think when I looked at it the average seemed to be in the range of a five-year survival of about 50 percent. So if we took that as an average benchmark if we wanted to show a small relative increase, say, of 10 percent, so going to 50 to 55 percent, this is over 3,000 patients in that kind of trial.
A more typical trial might look at an increase of the endpoint of 25 percent. This would translate into an absolute change of five-year graft survival of 63 percent. And this would only require 456 patients which again seems much more reasonable,

Page 136

but in the context of this condition it may be a trial that's just not feasible.
So I don't think graft survival on its own will be a useful endpoint for ABMR trials. It's going to be difficult for new interventions to show a reasonable treatment of fact using realistic sample sizes. And I think most of our interventions are likely going to produce more modest incremental improvements. And these sample sizes are probably just not feasible.
So what, then, might be the ideal endpoint for ABMR trials? Well, as we've discussed over the past two days, I think markers of histology are going to be very important such as freedom from ABMR or its components or perhaps freedom from TG.
We need to relook at our conventional biomarkers. And finally we'll probably need to encompass some of the new biomarkers such as DSA and gene transcript expressions.
And I do want to point out that these outcomes are all surrogate endpoints. And most kidney transplant trials, in fact, do not measure clinical

Page 137

endpoints. So this was a systematic review we did a number of years ago where we looked at all kidney RCTs in a fixed period of time.
And you can see that the surrogate -- a surrogate endpoint was the primary outcome in 78 percent of these trials. So we're using surrogates a lot. I think we just have to be careful in how we select them and how we use them.
So, again, getting back to the candidate endpoints for ABMR trials, if we talk about the hard endpoints of patient and graft survival I think for feasibility issues these are going to be difficult. Quality of life is obviously an important endpoint, but I think this is going to become much more relevant once we have some proven treatments to choose from.
So I think we are faced with looking at some surrogate endpoints. And I think the key ones that need to be looked at a little more closely are some marker of kidney function, histology, DSA, gene expression in the graft, and proteinuria. And I'll expand on these in the rest of my talk.
So, first of all, is kidney function a valid

Page 138

surrogate outcome measure? Well, again, kidney function measurements are used a lot in kidney transplant trials. This was a systematic review that we did awhile back that showed some measure of kidney function was using in trials about 80 percent of the time.

And eGFR which has become very commonly used was a primary secondary outcome in 61 percent of these trials. So obviously very common in the field of kidney transplant.

So first of all I think the first question you want to ask is reduced kidney function associated with worsening graft survival? And this is one of the oldest papers to look at this where they looked at the one-year serum creatinine and then looked at graft survival over time. And you can see that there was a clear association with a higher serum creatinine at one year with worse graft survival.

And the authors of this study concluded that the quality of renal function should be implemented as a newer endpoint for comparative trials.

So the rationale for using kidney function

Page 139

as an endpoint would be that you improve early renal function and you also improve long-term graft survival. And that was clearly true in that observational study, but is that rationale true in the setting of a randomized trial when you have an intervention?

So I'll just give you one example. This is from the Symphony trial many years ago where they looked at a variety of immunosuppressive regimens. And the primary outcome of this trial was, in fact, the one-year GFR. And you can see that low dose tacrolimus was, in fact, the best regimen and produced the best one-year GFR.

Low dose tac also had the best one year graft survival in that study. And when looking at validation of the endpoint you want to know is this full effect of the treatment on the clinical endpoint. In this case would be graft survival being captured by this surrogate. And in this trial it was not entirely clear.

As you can see on the right these are the acute rejection rates from that same trial. And you

Page 140

can see that the low dose tac arm also had the lowest acute rejection rate. So perhaps it was the pathway of reduced rejection rather than through GFR that was leading to the improvement.

And this is just a schematic that you'll see when people are looking at validation of surrogate outcome measures. You have an intervention here low dose tax that led to an improvement in GFR. And what's being hypothesized that less toxicity from this regimen was leading to improved graft survival.

But I just showed you that there was a possible alternative pathway for this treatment to work. And that might be better immunosuppression leading to fewer rejections and improvement in graft survival. So in this particular trial it's not clear that GFR is, in fact, a valid surrogate outcome for graft survival.

So here's another study that was looking at eGFR and the relationship between graft survival and mortality. And you'll see this a lot in these types of studies where there's a strong association between the outcome and in this case both death and graft

Page 141

failure.

But then when you look at the discriminating ability how well can this predictor actually tell you who will and will not eventually have graft failure? It doesn't perform as well.

In this particular ROC curve this is the six-month eGFR is the predictor looking at three-year censored graft survival. And it doesn't matter how you calculate these eGFR you can see there's a bunch of different formula there that the C statistics are in the range of .5 to .6, so not very good at discriminating who's going to get graft failure.

Here's a study from the Mayo Group where they divided the patients into their GFR at one year being above or below 40 mils per minute. And what I wanted to highlight on this first slide was that the patients who had a GFR above 40 mils per minute at one year, 49 percent of them in this series eventually lost their graft, so patients who you thought would do well intuitively at one year.

And when they looked at this a little bit further they broke down that group of patients with a

Page 142

high GFR into those that progressed and those who did not progress. And you can see that lower orange brown survival curve. The group that had progressed but had a high GFR at one year actually did worse than the patients who had a low GFR at one year. So although not very intuitive, early renal function tells us little bit -- little about the risk of late graft failure in many of our patients.

So why is the GFR at a fixed time point off and poorly predictive of long-term outcomes? Well, perhaps the creatinine may be a poor marker of true GFR as we've heard in some of the earlier talks today.

And, two, GFR may also not reflect the severity of the disease that's going on in the graft if we look at the pathology. One serum creatinine value may not reflect true baseline or steady state and that may be an issue with these calculations.

And a lot can occur after 6 or 12 months. And a lot of these studies are early measures of function and looking at events well down the road. And we clearly know lots of stuff can happen in that time period contributing to graft loss.

Page 143

So what about declining kidney function? Could this be more predictive? Well, this was looked at in a very large series of patients. These are non-transplant CKDP patients. And this I'll remind people that in nephrology -- many nephrology trials doubling of serum creatinine has become a standard endpoint and that's equivalent to minus 57 percent decline in GFR.

And this is strongly associated with ESRD and that's why this endpoint is used. But the big drawback is that it occurs very infrequently and especially in the short frame of most clinical trials.

So in this particular study they wanted to see if they looked at lesser declines in eGFR would this also be associated with ESRD? And you can see here the figure over here that if you have a minus 30 percent decline in GFR there was still a five-fold increase risk of ESRD.

And you can see the association at ten years 64 percent of these patients had ESRD. And really the important thing was that this event occurred in 6.9 percent of this population. This study had 1.7 million patients in it compared to less than 1 percent

Page 144

of the patients who had a doubling of creatinine.

So the author said or certainly suggested that maybe this is a better endpoint because it occurs more frequently, but is also associated with our hard clinical endpoints.

Now this exact same study and analysis was done in a large set of transplant patients. This is from Steve Chadban's group in Australia. And you can see whether looking at overall or death censored graft failure that a 30 percent decline in eGFR was strongly associated with these endpoints.

And what they also showed was smaller declines in GFR occurred more commonly. So if you see if even you took a cut point of 20 percent, this occurred in 19 percent of the patients. Importantly, the decline in GFR was associated with both death and graft failure.

Just highlighting the C statistics this really appears to be no cutoff for different declines in GFR. And finally I do want to point out that the C statistics are in the range of .7 so these are good, but not great in diagnostic performance measures.

Page 145

But, again, these studies are suggesting that perhaps a decline in GFR is an improvement as a marker of long-term function over a GFR at a fixed time point.

Now what about DSA, is this a valid surrogate outcome measure? Well, we've heard from Dr. Woodle about this and this is his -- one of his earlier studies looking at this where he had a group of patients that had DSA measured day zero and day 14 following therapy and broke them down into a group of responders and non-responders.

And you can see here in the graft survival curve those that had a reduction greater than 50 percent there were no grafts lost. This was a small series, but the patients at a less than 50 percent reduction had a significant improvement in graft survival.

Here's a second study from Dr. Woodle that was looking at a scoring system -- a histologic-based scoring system following proteasome-based therapy that I'm going to talk about in a little more detail in a minute.

But within this trial he also had his DSA

Page 146

data. And, again, if you see a greater than 50 percent reduction in DSA, 100 percent one year graft survival compared to only 57 percent for those that did not have a less than 50 percent decline. So these two studies suggesting that decline in DSA may be an important surrogate measure.

Now what about these histologic markers? Are these valid surrogate outcome measures? Here's the study I just mentioned from Dr. Woodle. So what they did was they created an acute composite score and a chronic composite score based on the components of the Banff scoring system.

And you can see on the left this is the acute composite score. And it didn't matter if this was earlier or late AMR, you could see that there was a nice decline in the composite score following a treatment.

And as you might expect on the right -- the figure on the right there didn't appear to be much change in the chronic composite score.

In looking at this a little closer, again, the acute score is on the left. You can see that most

Page 147

components of this acute score fell nicely with treatment. And, again, really in the chronic scores there was really no major effect.

So perhaps this acute composite score or some type of histologic measure like this could be used as a possible surrogate outcome measure, but obviously we need longer follow up with graft failure data.

Here's a figure from Loopy's paper that we've seen a few times basically showing that the one-year protocol biopsy if you have evidence of subclinical antibody mediated rejection, that's the figure in the red, you have the worst outcome long term.

And what they then did was they put the presence of ABMR into their multivariate model. And you can see that there was a three-fold increase risk of graft failure in the presence of subclinical ABR. And it's important to point out that this is also independent of GFR and proteinuria, other strong clinical factors that we use.

So perhaps the absence of ABMR or the

Page 148

presence of ABMR on a protocol biopsy would be a possible surrogate outcome measure.

Now here's a trial from Dr. Montgomery's group looking at the C1s raised inhibitor. And in this particular study they developed a score card based on histologic criteria as the primary endpoint.

And you can see the score card has a glomerularitis score, a vascularitis score, et cetera based on the variety of findings on the light macroscopy.

And what they did was they measured this -- or used this score card at entry into the trial and on day 20 in the trial. And unfortunately in this particular trial there was no real improvement in any components of this particular score card.

But what they did show in a subset of the patients who had active therapy on a six-month biopsy none of them had transplant glomerulopathy whereas 43 percent of the placebo patients had TG.

And as has been suggested by others, including Dr. Colvin, that perhaps the presence of TG would be an important surrogate outcome measure.

Page 149

Now here's another study from France looking at the other C1s raised inhibitor and they didn't use a score card, but they looked at components of the Banff scoring system at entry into the trial and then six months later.

And you can see except for C4d there was really no improvement in any of these histologic components of the score card. But getting back to our discussion on kidney function you can see that there was a significant improvement in GFR despite any real improvement in histology.

So, again, suggesting that perhaps both of these are using them somehow together would be the better way to go.

Here's the papers that we've seen from the Mayo Group on the equlizumab trials. And, as you recall, the primary outcome was just the occurrence of ABMR using our standard diagnostic criteria.

And as been discussed by others, these are the long-term follow up that where maybe viewed as being not as promising as we thought. But if you look the occurrence of TG in the eck treated patients was

Page 150

45 percent and then the control group it was about 64 percent.

And this was not statistically significant, but these are two also I would say fairly relatively small numbers. And I think the trend anyways might be that this is a promising therapy again suggesting that TG may be a possible surrogate outcome to use.

Now what about the molecular microscope Dr. Haas has given us an introduction to this? Are these -- or could these be potentially used as valid surrogate outcome measures?

So this is one of the original papers from Phil Halloran's group where they took a bunch of kidney biopsies and gave them conventional a diagnosis using histology. And those are labeled along the X axis.

And then they applied the micro array to the biopsy samples and determined a classifier using discriminate analysis which really is just a number reflecting the probability that ABMR is operating in the biopsy.

And you can see in this particular study

Page 151

they've chosen this cutoff of .2. And you can see that the high ABMR scores are nicely clustering around the histology of ABMR. And when using that threshold they've got an excellent AUC in this particular study of .89.

And Dr. Haas already showed the study so I'll just go through it briefly. But basically they used that scoring system in a whole new different cohort of patients. And as was pointed out in a previous talk the slides here -- or, sorry, the (indiscernible) curves here of the blue and red one, those are the patients that had the worst outcome. These are the ones that were S positive so they had an ABMR score that was positive and greater than .2.

And this was independent really of whether there was histologic evidence of ABMR being present. So perhaps this ABMR score could also be used as a surrogate outcome measure.

I just want to touch briefly on composites as I think this will be discussed by Dr. Irish in the next talk. But really why do we use composite endpoints? It's really so that we can combine

Page 152

infrequent events together to allow sufficient sample sizes.

But there's always caveats with composites. And, first of all, is that the composites are often -- the components are not of importance or the same importance. So in our particular example is the persistence of DSA really the same as graft loss if you had those together in a composite. Probably not. And you see this a lot in other fields of medicine.

Also the components may not occur with a similar frequency. And it's often the less serious one that occurs the most often. And this is really common in the cardiology literature. If you look at it there's often, you know, admission to the hospital is the main thing driving it rather than mortality.

And then the final issue to think about is this relative risk reduction. So really you want the biology of all your components to be working in the same direction so that they have similar relative risk reductions. And really what the worst thing you want is when they start going in the opposite direction.

So keeping that in mind just as we talk

Page 153

about composites this was a study that looked at the -- I'll say the composite in a different way. This was not a randomized controlled trial, but looking at combining different areas to see if we can improve prediction.

And what they did was they took our typical clinical factors and added on histology data as well as DSA data to see if they could improve prediction of graft survival.

So, first of all, what they did was they used this risk calculator on the left which uses GFR and age and gender and typical things we would use clinical factors to predict outcome. And it calculates a five-year graft survival.

And what they did was this was created in a different set and then applied. This was a large group of patients from the Mayo Clinic. And you can see that it performed very well in this second group of patients. The AUC for the death censored graft failure was .84 which was very good.

And what they then did was they added histology into the model so they added the

Page 154

glomerulitis and chronic interstitial fibrosis scores. And you can see that the C statistic improved quite nicely from .84 to .9. Excuse me.

And then finally they added DSA to the model and it really unfortunately didn't add much prediction. But at least started the idea that if you add histology in addition to our clinical factors this may improve our clinical prediction.

Here's another paper using the ABMR score from Halloran's group. And you can see here at the time of treatment for ABMR if the score was positive this was associated with a two-fold increase risk of graft failure.

And the important thing of this is this is independent of the humeral histologic score. So, again, using the score in conjunction with histology gave a better prediction of outcome and improved model discrimination in this particular study from .77 to .81 again suggesting that this composite of clinical factors, histology, and the ABMR score may be a better predictor of outcome and may be a better way to look at endpoints.

Page 155

Finally, I'm just going to touch on proteinuria. There's a ton of literature in the non-transplant population, but I'll just show you one study recently published in a large cohort of transplant patients where they measured proteinuria at the time of a biopsy.

And you can see in that top box that increasing the amounts of proteinuria as we know are strongly associated with graft failure down the road. And important in this trial was this was independent of all the different histologic parameters they saw on the biopsy at that time.

And it was also fairly discriminate. You can see the ROC curve on the left with an AUC of .73 for the presence of proteinuria at one year and late graft failure.

But I think interestingly the figure on the right this is in the cohort of patients who had transplant glomerulopathy. And you can see that increasing the amounts of proteinuria within the patients who had TG was strongly associated with graft survival again suggesting that perhaps proteinuria

Page 156

within this population would be an important surrogate to consider.

So which outcome measures should we use? Well, I think it obviously depends on the type of trial that we've -- we've been talking about different trials, but is this a trial to prevent ABMR or are we talking about a trial once someone already has established ABMR and were going to treat it. Is this an early event or is this in a late event, as well?

I've basically been focusing on (indiscernible) what we always have to remember that safety endpoints are going to be crucial in these types of trials such as our overall infection rates and cancer rates.

And I'm just going to give a couple of examples really to stimulate discussion in the QA period. And these are just opinions because none of these have really been validated in any trials.

So this is a potential composite endpoint that you could consider for a treatment trial. It doesn't have to have all of these components, but certainly these components are what I think are

Page 157

important.

Some functional outcome, histology outcome, a molecular outcome, DSA outcome, and some damage or proteinuria marker. So if we look back at our functional outcome I think perhaps if we looked at a greater than 30 percent eGFR decline and it's always important to know when the start and end point is. And I think it would be obviously the entry into the trial is the start point and I think the convene time would be one year following -- sorry, following therapy to look at the functional decline throughout that time period.

If we look at a histology outcome perhaps we may want to look at -- and I'll just call them bad features on the protocol biopsy at one year. And I would let the pathologists work this out in a little more detail, but we could look at persistence of inflammation, persistence of C4d, or the presence of TG.

I think the molecular score is very interesting and perhaps the most novel edition to what we've been talking about in the last few days. And

Page 158

perhaps the presence or persistence of positive ABMR score on a protocol biopsy could be a nice component to a composite endpoint.

As far as DSA less than 50 percent reduction of DSA may be a surrogate, but also as was mentioned yesterday perhaps a more significant reduction or elimination would be something you want to look at.

And then finally I put in proteinuria and I just picked an arbitrary cutoff. But I also stated that if TG was present because I think we only want to add proteinuria here if we can ascribe it to the TG because it doesn't make much sense if it's due to something else.

And I do want to point out again that this is just an arbitrary selection of outcomes with a bunch of arbitrary cutoffs. But what we need to do as a community I think is start measuring similar outcomes pre and post-treatment to see what is responsive and what is predictive similar to what Dr. Woodle's paper did when he looked at the change in that composite score following an intervention. I think that's where we really need to start measuring

Page 159

these.

So here's a potential endpoint that we may want to use in a prevention trial and it has a lot of the same sort of themes. But I think as far as prevention obviously I think just the diagnosis of clinical ABMR would be enough as a key part of that diagnosis.

Then we could look at, again, some bad features on a protocol biopsy, the molecular score, from a DSA perspective perhaps the development of a de-novo DSA would be important, and, again, some proteinuria or damage marker.

So to summarize, I think it's difficult to use patient-important outcome such as graft survival alone in ABMR trials given the sample size required to show realistic treatment effects.

Surrogate endpoints are commonly used in renal transplant trials especially measures of GFR. And while convenient from a sample size and a power perspective most of the surrogates are not well validated.

Surrogate outcomes, though, and composite

Page 160

measures involving these surrogates in my opinion will be necessary for ABMR trials. And likely candidates that I've gone through are GFR, histology, molecular transcripts, DSA, and proteinuria, and some combinations of these.

Validation of the endpoints will need to occur and we need to begin measuring these outcomes before and after treatments. And finally long-term follow up will be needed for all ABMR trials using surrogates to evaluate their eventual effect on hard clinical endpoints such as graft survival.

Thank you for your attention.

DR. MANNON: Thanks, Greg, in particular for putting in so much information in the allotted time. Our next speaker is Dr. William Irish will be talking about performance of clinical trials and low incidence conditions.

DR. IRISH: So how do you advance this? Oh, yeah. Okay. Thank you. So today I'm going to spend a little bit of time talking about scientific challenges and study design considerations of studies in low incidence and rare conditions.

Page 161

So just by way of disclosure I'm a full-time employee of CTI, and international contract research organization. And by way of further disclosure I'm a statistician by training. And so I'm going to discuss these issues from a statistical perspective.

And as the cartoon says, statisticians aren't always right. And I think my wife would agree with that one.

So scientific challenges. So there are very few epidemiologic studies describing the occurrence of AMR and reporting incidence varies. And we've seen that depending on, for example, different diagnostic criteria at the local practice center level, different patient populations studied which is really a reflection of geographic regions. And this presents a challenge when designing a clinical trial.

Studies of AMR require multicenter, multi-country participation that have inherently different healthcare systems, treatment options, and management approaches.

And coupled with that is the type of study or the intended indication. And Dr. Knoll sort of

Page 162

discussed that whether it's prevention or whether it's for treatment both are potentially hindered unless there's a crystal clear on the diagnosis or resolution following treatment.

And there are regulatory challenges. For example, the choice of endpoints, the choice of comparative group. This is a very complex question and it may be an eliminating step.

For one, how do you get subjects to participate and enrolled if there's potential for harm?

The use of historical controls. This requires access to reliable, valid data. And temporal bias is always an issue especially in a disease area where management practices are constantly evolving.

And sample size. We need a sufficient number of subjects to show a treatment effect with a certain level of power. And this is a question I get asked constantly. How many subjects do I need?

So looking at the incidence of AMR so this table is based on a brief lit review that I performed. And we see that a lot of this data is investigator-

Page 163

specific which creates a problem.

The best that could be said is that AMR occurs after transplant and the occurrence depends on pre-transplant amino status, post-transplant diagnostic criteria, and type of AMR be investigated.

And this is an important issue and this has been discussed at length when designing a study. AMR is not an it, AMR is a they.

So let's, for the moment, assume that we're investigating the same AMR. So based on my lit review I calculated the incidence of AMR at one year post-transplant to be approximately 9 percent. This is a weighted average based on sort of a non -- a random effects model.

If we assume treatment reduces AMR by 50 percent and we need -- we need approximately 478 -- 487 subjects for a group with 80 percent power. Now this is a big trial. A study of this magnitude would take years to enroll even with a large number of centers.

So to overcome these challenges we need creative strategies at the design stage. There are

Page 164

creative ways that we can look at the data analytically, but for the remainder of this talk I'm going to talk about the design stage.

And so, for example, there are enrichment strategies. These are used to decrease variability and maximize power. Adaptive designs. Making planned well-defined changes in key clinical trial design parameters as data accumulates. And willingness of the regulatory agency to consider the creative use of surrogate and composite endpoints that were discussed in the previous -- previous talk.

And these strategies are not necessarily mutually exclusive. For example, we can use an adapted design to change the enroll- -- the enrichment strategy or we can incorporate phase in methods to help guide decisions. For example, dull selection, sample size re-estimation, futility, or assessment of a biomarker's predicted probability of response.

So I'm going to talk about these -- some of these issues in more detail in the next series of slides.

So enrichment strategies. Have I picked a

Page 165

population that's most likely to be able to show an affect? So this is a very important question.

The main reason for enrichment is study efficiency. Increasing the chance of success often with a smaller sample size.

So one approach to enrichment is to decrease heterogeneous 80, so include subjects that have certain characteristics that put them at risk. But we need to be careful with this approach. It may require a long time to recruit if the study population is too narrowly defined. And this could potentially impact the type of AMR and subsequently the incidence rate.

Exclusion criteria is relatively straightforward, but that depends on whether the indication is for treatment or for prevention.

Prognostic enrichment. Here we want to select subjects with a greater likelihood of occurrence of an event like AMR or substantial worsening of a continuous measurement like change in eGFR.

So this approach increases the absolute difference between the treatment groups, but will not

Page 166

alter the relative effect. And the characteristic or measurement process, for example, a biomarker, needs to be validated and agreed to by the regulatory agency.

So here's an example. Could DSA relative intensity scale be used as a viable prognostic enrichment strategy? So this data's based on results that were published in 2015 in transplantation.

So the figure on the right suggests good discrimination with an AUC of 79 percent although the DSA relativity intensity scale is much more variable in patients with AMR based on the figure on the left-hand side. Since this data is based on a single center it's not clear how this association would translate to other centers.

What about pre-transplant HLA DSA level? So this figure based on data that was published in 2010 suggests a positive correlation of peak pre-transplant DSA level and risk of AMR. Oh, sorry. So here we're looking -- we have this gradient in terms of the DSA and we have this nice linear relationship in terms of the risk of AMR.

Page 167

So ideally if we have a reliable, validated biomarker that can predict the relevant type of AMR be it DSA relative intensity scale or the pre-transplant DSLA -- DSA level or the Banff CG score, we can have a study with a reasonable number of subjects.

For example, to show a 50 percent relative reduction in AMR with treatment if we enrich so that we have a background rate of AMR 50 percent, then all we need is 58 subjects per group as opposed to 487 subjects per group without enrichment. So that's an 88 percent reduction in the number of subjects required for the same power.

So this is a hypothetical example of the potential benefit of a prognostic enrichment strategy using a simulation program Nr. So the idea is to enrich the study population with patients that have increased likelihood of AMR based on a biomarker.

So in this example I'm using the peak transplant DSA level. So this enrichment strategy is based on the ability of pre-transplant DSA to distinguish between AMR and no AMR which is summarized by the area under the ROC curve which is .9.

Page 168

So this higher event rate, and this is in figure -- figure 1, translates to a lower sample size for a clinical trial depicted in figure 2, which can have both practical and ethical advantages.

The benefits of this strategy, however, needs to be weighed against the cost of screening and recruitment, et cetera. And for those that are interested, the simulation was conducted using the bio-PET program Nr.

Predictive enrichment is another -- is another option. So with this strategy we choose subjects more likely to respond to treatment. I.E., these are probable responders.

So the advantage of predictive enrichment is depicted in this table and this is based on results of a talk by Dr. Temple in 2014. So here if 25 percent of patients have the biomarker that predicts the effect and marker negative patients have no response, an unselected population would need 16 times as many patients.

Even if 50 percent of negative marker patients have a response, an unselected population

Page 169

would require almost three times as many subjects. And, again, the key issue here is having a valid biomarker.

So this is a schematic representation of an adapted two-stage population enrichment design. This was published in the New England Journal of Medicine. So the idea here is the population is stratified before randomization into two sub groups, S and S prime according to some binary biomarker like the presence of DSA pre-transplant.

An interim analysis occurs when a specified number of patients -- this is denoted a N-not in this figure -- have been enrolled in each sub group. At that time there'll be a specific number of event in each -- into each group, AMR events in sub group S, for example, AMR events in sub group S prime.

So the data are analyzed at this interim juncture and the trial may be terminated for futility, continued as planned, or continued by enrolling patients only in sub group S.

In this design there's a biological basis for assuming that the biomarker may have predictive of

Page 170

response in sub group S but not in sub group S prime. And the purpose of the interim analysis is to verify this assumption if it's true. And, if so, to enrich the remainder of the trial with patients with sub group -- with the sub group S only.

The randomized withdrawal study this is an example of a predictive enrichment strategy. So in this design all subjects receive active treatment for a specified period of time. All subjects who respond are then randomized to continue treatment or to placebo. So they're withdrawn off treatment.

And any difference emerges between the group receiving continued treatment and the group randomized to placebo would demonstrate the effect of the active treatment. So this is sort of the general schematic representation of a randomized withdrawal study.

So this is actually a unique design. This is a schematic representation of a three-stage study design that was published in Statistics of Medicine. Not too sure if you can see this clearly.

So this unique design has three stages. Stage one consists of an ordinary, randomized,

Page 171

placebo-controlled trial. Patients who responded to treatment in stage one are subsequently randomized to continue treatment or placebo or withdrawn in stage two.

While patients who did not respond to placebo, non-responders in stage one, are placed on after treatment and the responders are then randomly assigned a treatment, continue therapy or placebo withdrawn.

So these sort of three-stage study designs are denoted by the rectangular boxes in this figure. And if we take the P values from stage one, the P values from stage two, and the P values from stage three these are combined statistically to test the overall efficacy of treatment.

So for studies of rare events like an AMR where patient numbers are limited this three-stage clinical trial design may be a more powerful design option than the traditional randomized trial for conducting a clinical benefit.

What's nice about the design you can incorporate stopping rules for futility or for

Page 172

efficacy. So you can -- you can use sort of an adaptive design within this -- within this trial design schematic.

So the benefit of this type of design however needs to be weighed with any sort of logistical issues, recruitment, length of follow up, et cetera.

And what's nice about this is that the statistical sort of operational characteristic, the validity of this design was studied extensively in this paper.

So biomarkers and surrogate endpoints -- and we had a really nice discussion earlier about biomarkers and potential surrogate endpoints. And, as discussed, a surrogate is a biomarker that's used as a substitute for a clinical endpoint and is expected to predict clinical benefit.

And there are three key questions one needs to ask. Is the biomarker able to accurately and precisely be accurately and precisely measured? So this is sort of analytical validation.

Is the biomarker associated with the

Page 173

clinical endpoint? This is qualification.

And what is the specific context of biomarker use? So this is sort of the utilization of it.

And in 2015 an FDA workshop was conducted to discuss surrogate endpoints and biomarkers in kidney transplantation. And Dr. Knoll summarized some of the potential endpoints in his earlier talk.

But here is just a few examples of potential surrogate markers in AMR and these can be used for preventative and treatment trials. But, again, more studies are needed to sort of validate their clinical benefit.

So what about composite endpoints? What if we combine these composite -- composite surrogate endpoints, would this work?

So when planning a trial with a composite endpoint one should ask does the composite endpoint really measure a disease?

Does the use of a composite endpoint solve a medical problem or is it just for statistical convenience?

Page 174

Are the individual components of the composite endpoint valid biologically plausible and of importance for patients?

Are the results clear and clinically meaningful?

Do they provide a basis for therapeutic decisions?

And does each single endpoint support the overall result?

And, importantly, is the statistical analysis of the composite endpoint valid? Is it adequate?

So this figure sort of illustrates one reason why a biomarker in general could be of correlative clinical benefit, yet might not be a valid surrogate.

First, there are usually multiple pathways through which the disease process influences the risk of the clinical efficacy endpoints. If the proposed surrogate endpoint lies in only one of these pathways and if the intervention does not affect -- actually affect all pathways, then the effective treatment on

Page 175

the clinical efficacy standpoint could be over or underestimated by the effect on the surrogate endpoint.

Second, the intervention, itself, could have mechanism of actions that are independent of its intended effects on the disease process. So this was discussed in the 2015 workshop and was discussed at length by Dr. Flemming.

So just in summary, the therapeutic development AMR presents many challenges. There's a need for alternatives to the traditional randomized control trial, for example, an adaptive design. Need for more creative outcome measures. Perhaps some non-biological measures such as quality of life which was alluded to or quality adjusted life years. Difficulties of recruiting a control group. And this may depend in part on the type of study whether it's for prevention or for treatment.

And the solution requires multi-collaboration among stakeholders including the transplant community, sponsors, regulatory agency, and I might add specifically statisticians all need to be

Page 176

on the same -- all need to be on the same page.

An acceptance of biomarkers as well as creative or non-traditional endpoints is another solution. Alternative trial design such as the adapted designs.

And these designs are complex both statistically and logistically so there needs to be a certain level of education in the transplant community. And perhaps this could be done via the ATC.

Lastly, leveraging existing resources. For example, transplant registries or individual level of clinical trial data. So pooling individual level of data could help inform clinical trial designs into a value -- to evaluate and validate potential biomarkers and surrogate endpoints. So I'll leave it at that.

DR. BALA: Thank you, Dr. Irish. Our next speaker is Dr. Anita Chong from University of Chicago. And she'll talk about animal models in AMR, how can they inform clinical studies.

DR. CHONG: Okay. I want to start by thanking the organizers, especially for not putting me

Page 177

as the final speaker. That honor is for Dr. Knechtle.

Okay. All this work in mice are the work of post-doctoral fellows and students in my laboratory. And the clinical work is done in collaboration actually completely by Dr. Ron Pelletier at Ohio State University.

I have no conflict of interests to declare, but there is some off label use from (indiscernible) by Dr. Pelletier's studies.

Okay. As you have heard today and yesterday that there has been a lot of work trying to target antibody mediated rejection. And I think that these strategies can be categorized into those that target the early prevention of antibodies using a lot of the conventional immunosuppression and also a lot of interesting limiting antibody mediated damage that has been extensively discussed by Dr. Woodle earlier today.

But I think that there is actually a sweet spot and a Goldilocks phase where the ability to target and stop ongoing antibody production. And there are some approaches that may perhaps be used to

Page 178

be considered as targeting this, but these are pretty I think straightforward. The use of IVIG, depleting B-cells, depleting plasma cells.

But I think that none of these strategies really leverage the huge amount of information that we're gathering and we're understanding of this phase of this B-cell expansion in the germinal center where B-cells proliferate extensively, undergo class searching, undergo sematic hyper mutation so that they generate high affinity antibody cells, release the cells from the germinal center as antibody secreting cells as well as memory B-cells.

And so our goal in starting these mouse studies was to develop a therapy at least in mice for treating ongoing B-cell responses and plasma cells responses that result in a rapid depletion of antibody responses and long-term suppression.

So when we started our studies about five years ago there was a rationale for using and starting with CTLA4 IG. That the high affinity mutant belatecept was approved for kidney transplantation.

And while we knew that there was a slightly

Page 179

higher rate of acute rejection, the salutary effects on antibody was not apparent at that time. However, in mouse models there were some data to suggest that blocking another co-stimulation pathway with anti-cd-154 can be used and was successfully used to disrupt established germinal center B-cell responses.

However, because we know that CTLA4 IG not only inhibits the cd-28 co-stimulatory pathway, but it can also inhibit the co-inhibitor or checkpoint pathway. And there was a possibility that the use of CTLA4 IG, especially late in the antibody response, may actually be enhancing the antibody responses as opposed to inhibiting it.

And so the first series of experiments we did were really simple. We wanted to delay CTLA4 IG and see how late we could -- for what were the effects of delay CTLA4 IG on inhibiting and ongoing antibody responses.

And so in the mouse, a B6 mouse, what we did was immunized with a BALB/c spleen cell donor-specific transfusion. And this allows the antibody responses to be accurately measured and we didn't have to be

Page 180

concerned about the antibodies binding to the graft and kind of delaying the appearance of antibodies in the serum.

And so with CTLA4 IG as we expected if you start treatment continuously twice a week from the day of immunization you saw strong inhibition of the antibody responses.

If we waited till seven days after immunization when we could already see a significant increase in DSA, we found that when you start treatment within a week the antibody increase is immediately halted.

And then the third what we did was to treat from day 14. And you can see that this -- when we start treatment on day 14 we could no longer inhibit that antibody response illustrated in blue.

So there could be two reasons for why this very delayed day 14 CTLA4 treatment fails. Firstly, it's because the late germinal center B-cell response has now become resistant to CTLA4 IG or, secondly, that the germinal center B-cells had already exported antibody secreting cells and that these cells were

Page 181

then resistant to CTLA4 IF.

And so to be able to address these two possibilities we develop a technique to track allo-specific B-cells that was described in brief by Dr. Gebel yesterday. And we used a double -- double fluorocrome single tetramer approach because we know from the observations by Mark Jenkin's Group in Minnesota that there is a very large population of B-cells that can actually recognize the fluorocrome.

And while this does not completely eliminate other components of the tetramer that the B-cells are -- may be recognizing, it's significantly enriched for the MHC-specific B-cells.

And the flow plots for gating these cells, especially for gating the germinal center B-cells which express fast and GL7 illustrated below.

So then what we did was a very similar experiment is we -- as I've previously described which was to treat CTLA4 with CTLA4 IG either from day zero to day 7 or from day 7 to day 14 and day 14 to day 21 and then the mice were sacked at those indicated days.

And in each time point that we treated with

Page 182

CTLA4 we observed a significant decrease in the number of tetramer positive, so allo-specific germinal center B-cells whether you treated them from day zero or day 21. So the germinal center even at the late stage remains susceptible to collapse in the presence of CTLA4 IG.

So which allowed us to conclude that it's likely that the reason for why CTLA4 IG starting on day 14 fails to reduce the DSA levels was because the germinal center had already exported the antibody secreting cells.

The other export cells from this germinal center is memory B-cells. And certainly it's clear that if you can inhibit germinal center B-cells it's a good thing for transplant.

So what we did was use the same B6 model, however, we were -- we used this -- a mouse that could report on B-cells that had entered the germinal center. And these are B-cells that then activate the enzyme cited in deaminase which is AID cre.

And so when the B-cells enter this germinal center they turn on the expression of XYFP and we can

Page 183

detect these cells and enumerate these cells. And, in particular, the B-cells that are donor-specific. And in this case instead of using the class one donor-specific tetramer we validated and are using a class-two specific tetramer system.

And what we find is that in mice that are sensitized with this BALB/c spleen cells you can see that by day 43 there is a significant increase in the total number of memory B-cells.

And when you treat the mice with day 7 with CTLA4 IG you can see that this memory frequency of cells is significantly decreased. Whereas if you treat them on day 14, then the memory numbers are not significantly different from the untreated controls.

And these differences in memory B-cells of significant because if you then challenge these mice after stopping CTLA4 IG for two weeks with a secondary challenge of BALB/c spleen cells you can see that the DSA levels that we see in the absence of CTLA4 in this rechallenge mirror the frequencies of these memory B-cells.

So allowing us to conclude that the delayed

Page 184

CTLA4 IG at least from day 7 post immunization inhibits -- significantly inhibits allo-reactive memory B-cell generation.

And more importantly we also show that if you delay CTLA4 treatment till day 6 post-heart transplantation in the model of BALB/c to be six mice, which is the full MHC mismatch system and in which the hearts are completely stopped beating on day 8.

And so you can see that by day 6 post-transplantation there is significant C4d deposition as well as T-cell infiltration in the grafts. And in this case we can show again that the delayed treatment of CTLA4 IG can reverse -- or can inhibit and collapse the germinal center response.

Such that if you look at the data here which focuses on class one specific germinal center B-cells in the absence of any treatment the germinal center B-cells increases from day 6 to day 14; however, if you treat them from day 6 to day 14 with CTLA4 IG the germinal center B-cell numbers are completely collapsed.

Importantly, this is also associated with a

Page 185

significant prolongation and treatment of acute rejection such that about -- about 60 percent of the grafts go on to survive long term with treatment under CTLA4 IG starting from day 6.

And if you look at the hearts that are sacrificed on day 60, you can see that there is a significant reduction in the amount of complement deposition in these grafts.

And importantly as an additional control if we add that hyper immune serum to these mice that were treated with CTLA4 IG we abrogate a lot of the effects of CTLA4 IG.

Okay. Thank you. Okay. Then the last experiment that we wanted to do was what happens -- what is the effect of CTLA4 in fully-sensitized recipients?

And so in these experiments what we first -- what we did was to sensitize the recipients with BALB/c spleen cells and then wait 10 to 20 weeks later and then we transplant these mice with BALC/c hearts.

And then we analyze initially the quality of the memory recall response. And if you remember in a

Page 186

primary B-cell response the majority of the B-cells enter a germinal center response. And it's in that reaction where there's significant proliferation of the B-cells in class switching and affinity maturation.

However, in a memory response we see very little germinal center response as illustrated in this figure. In contrast, what we find is that there is a rapid differentiation of the memory B-cells into antibody secreting cells independently of the germinal -- a strong germinal response.

And you can see it here in an elispot assay. This is an elispot assay that quantifies the number of antibody secreting cells that are specific to donor class one molecule KD.

And this is illustrated here in numerically that you see that there is a very strong increase in the total number of antibody secreting cells in -- upon heart transplant in a sensitized animal and that this response is very quick and also very rapidly reduced.

We show that if you treat these mice,

Page 187

sensitized mice, with CTLA4 IG that it completely abrogates the antibody differentiation and the total number of antibody secreting cells that you can recover from the spleen. It inhibits total DSA responses which is illustrated in the next graph. And, importantly, it prolongs graft survival with CTLA4 IG alone in these sensitized recipients.

So collectively these mouse studies show us that CTLA4 IG is indeed an unexpectedly potent inhibitor of germinal center and memory B-cell responses. And that it can affect -- it can collapse an ongoing germinal center and importantly it can also inhibit memory B-cell reactivation in differentiating into antibody secreting cells.

And I think that these data are in retrospect very congruent to the clinical observations reported recently by Vincenti, et al., that despite an increase in the frequency of acute rejection the antibody titers are significantly reduced compared to calcineurin controls.

But what -- as I mentioned earlier, what CTLA4 cannot do is inhibit antibody production by the

Page 188

exported short-live antibody secreting cells. And so we reasoned that if we combined CTLA4 IG with bortezomib as you've heard previously that this would be a reasonable protocol to rapidly reduce antibody secretion and sustain long-term inhibition of antibody responses.

And so to this -- to test this very briefly in a mouse model what we did was to do to indeed immunize mice with BALB/c and then wait before we start treatment with CTLA4 IG 14 days after the immunization either alone or with bortezomib along given two doses or bortezomib in combination with CTLA4 IG.

And you can see that the combination group was significantly better at reducing antibody titers at 14 days later compared to the monotherapy group.

And importantly we also what we did was to challenge these mice with a secondary immunization and then in the mice that were treated with CTLA4 IG we continued that treatment.

And we showed that in the bortezomib group which was only given two doses on day 14 and day 16

Page 189

these mice responded in the secondary response very comparably to the untreated animals. Whereas mice that were maintained on CTLA4 IG did not respond to the secondary immunization.

So with this in mind we started a collaboration with Dr. Ron Pelletier who's a transplant surgeon at Ohio State with the hypothesis that belatacept in combination with velcade would be effective at inhibiting or controlling acute AMR.

And so this is an institution in which the standard of care is ATG induction, everolimus, neural, and a rapid one-week steroid taper.

So after I visited his institution about a month or so later in 2015 Ron -- Dr. Pelletier encountered this first patient who was a 39-year-old male receiving his third kidney transplantation. So this is a highly sensitized patient, but did not have DSA.

This patient had graft failure 12 hours after post-transplantation and was -- DSA was detected about 11 days post-transplantation. And then a positive acute AMR biopsy was proven about day 18

Page 190

post-transplantation.
He started treatment with belatacept about day 18 post-transplantation together with velcade treatment. Two treatments day 26 and day 35 post-transplantation. And what he saw was that there was a rapid decrease in donor-specific class one as well as class two. And that these titer remain suppressed for over a year. I think this particular patient is a year and a half post-transplantation.
So I see that I'm running out of time so I won't go -- but the second patient he treated had very similar reduction and maintenance in long-term survival. And he has now five patients with exactly the same course of clinical outcomes.
So I want to conclude by saying that animal models can inform on clinical trials but that there are certain limitations and future directions for us. We don't know what -- whether the effects of CTLA4 IG on B-cells are unique to CTLA4 IG or can be recapitulated with other immunosuppressive drugs.
We don't have a very good model of chronic antibody rejection so we don't understand the

Page 191

processes and, therefore, what would be the best drug combination for acute versus chronic rejection. And certainly I think it's very important we don't have a good model for belatacept or CTLA4 resistant T-cell mediated rejection.
So with that thank you for your attention.
DR. MANNON: We're running a few minutes behind, but our last speaker is Dr. Stuart Knechtle from Duke University. He'll be talking about animal models that are pre-sensitized.
DR. KNECHTLE: Well, thank you to the organizers for including me in the program and thank you all. You're either very curious, very polite, or adherent to FDA policy for being here so thank you.
So we've all heard at this excellent conference over the last couple of days that we lack a reliable, durable therapy for antibody mediated rejection. We have difficulty desensitizing patients to allow them to be successfully transplanted.
And although we have some therapies, we really don't know how to treat do-novo DSA and how to -- what guides our therapy.

Page 192

So that is matched by a new opportunity due to many new drugs that target C-cells, plasma cells, or cytocons. And what I hope to talk to you about is the value of animal models for helping us understand mechanisms of the allo B-cell response and antibody activation and B-cell maturation.
Models that hopefully can guide us in developing a rational approach toward the application of novel drugs to this challenging clinical problem.
So the goals of the non-human primate models that we have used in our laboratory are to try to mimic human HLA sensitization and antibody mediated rejection. This is a challenging problem.
We're able to measure it in monkeys a positive cross match to class one and class two antigen. And the histology of antibody mediated rejection in the monkey models closely parallels that that is seen in humans.
And Bob Colvin and the group at Mass General also working with cynomolgus monkey models have demonstrated very elegantly the very close parallels between non-human primate and human renal allograft to

Page 193

pathology.
We want this non-human primate monkey to be a robust model that's actually challenging. We don't want it to -- the challenge of some of our research, of course, in rodent models is that it sometimes doesn't translate or predict what happens in humans. And since non-human primates are about 97 percent identical to humans there tends to be a much greater parallelism. And, in addition, many of the human drugs and reagents developed only work in either humans or, in most cases, non-human primates.
I actually backed into this area. Antibody mediated rejection is a long-standing interest of mine, but I backed into this because I spent a fair bit of time studying T-cell depletion with either alemtuzumab in humans or CD3 immunotoxin in monkeys.
And we demonstrated and Doug Bloom in Wisconsin wrote a paper showing that homeostatic repopulation happens when you deplete T-cells profoundly and that there is a compensatory activation of a B-cell response associated with a high BAFF level.

Page 194

And so allo-antibody is an accompanying problem typically when you're using profound T-cell depletion. And Jenny Kim Page is the first author on this first of our models in monkey. And this was to use that T-cell depleting immunotoxin in monkeys intentionally to study de-novo allo-antibody production.

And we demonstrated first of all that we reliably develop allo-antibody after four to six weeks post-transplant and that the histology of these kidneys closely parallels antibody mediated rejection as seen in humans.

And then we were -- the focus of this work was to study the usefulness of co-stimulation blockade to prevent de-novo allo-antibody development and antibody mediated rejection.

And we published this first paper which effectively demonstrated that either a belatacept in blocking CD28 or a 2C10 which is an anti-CD40 blocking the CD40, cd-154 interaction effectively prevented rejection as shown here by the blue and the purple lines which show the monkeys treated with co-

Page 195

stimulation blockade is that they maintain stable era function without rejection over time. And, as shown here, the treatment effectively blunted a development of allo-antibody which occurred in all controls.

Jean Quan at this time really moved us well ahead by suggesting that we should be looking at a lymph node morphology and not just the morphology, but the amino chemistry to look at what's happening in the germinal center as a result of T-cell depletion and B-cell activation and to get a handle on what co-stimulation blockage was doing at the germinal center level.

And by a co-staining for B-cells with CD20, T-cells with CD3, and KI67 as a proliferation marker what he demonstrated then was that in the co-stimulation treated monkeys either with bela- -- treated with either belatacept or 2C10 that we were disrupting this proliferation of B-cells in the germinal center.

So, in other words, co-stimulation blockage is substantially disrupting the activation of B-cells in the germinal center. And that is shown graphically

Page 196

at -- on the right side of the slide showing that the treatment severely blunted KI67 or a B-cell proliferation in the follicle.

So I think that work effectively demonstrated that co-stimulation blockade could block out any production of B-cell isotype switching, germinal center reconstruction, and t follicular helper cells in the germinal center.

And that work served as background work also for the clinical trial that was sponsored by the FDA that Allen Kirk performed carrying this into human kidney transplantation with a cocktail of alemtuzumab induction, sirolimus, and belatacept to maintenance therapy that has turned out to have excellent results without allo-antibody production. And excellent graft function and survival.

While we move from that concept of trying to prevent de-novo allo-antibody production in the monkey to trying to model the highly sensitized patient. A different problem, but also related, of course, to allo-antibodies. So how do we take our highly sensitized patients and desensitize them more

Page 197

effectively?

And in order to model this in the non-human primate we wanted a difficult model. We wanted to make it tough to succeed. So we exchanged skin grafts between MHC mismatched non-human primate rhesus monkeys and we'd give them two successive skin grafts that reliably results in a very high MHC class one and class two allo-antibody level. And this reaches a peak and then a decay over time.

And we wanted to then try to desensitize them when they're on the shoulder of this curve, not when they're at the peak of sensitization because this would be more analogous to our human patients who have relatively stable allo-antibody levels over a long period of time.

So we do two phases of the -- or three phases if you will of the experiment. There's the sensitization phase, then we desensitize, and then we do the kidney transplants using the same donor as the recipient is sensitized by skin grafts.

That can be argued as an even more difficult situation than we face in the clinic because our

Page 198

patients are usually across sensitized by a third party sensitization or other means of sensitization, but they're usually not sensitized to this high degree.

So that is a tough model and I won't tell you about all kinds of things that did not work and how we beat our head against the wall for a long time. But I'll focus instead on a therapy that has looked very promising and that is the combination or proteasome inhibitors and co-stimulation blockade.

So what we do is give that therapy over a four-week period when we're on the shoulder of this -- of the sensitization curve here. And what we found was that the combination of these agents resulted in a reliable decrease in bone marrow plasma cell secreting allo-antibody and about a 50 percent reduction in donor-specific antibody.

In contrast, when you give either bortezomib alone or belatacept 2C10 alone we did not see any significant change really in allo-antibody compared to a baseline.

So following that triple therapy or what

Page 199

we're calling now dual targeting desensitization we then went on to perform kidney transplants. And the regimen that we used to immunosuppress the monkeys was a depleting -- a T-cell depleting induction regimen with anti-CD4 and CD8 and conventional maintenance immunosuppression, if you will, with tac MMF and steroid.

And this is the overall survival result. You can see that controls reject at a mean of about 27 days and the monkeys treated with the desensitization protocol did not succumb to rejection.

However, we did see in these longer surviving monkeys a significant issue with CMV infection. Rhesus has their own unique CMV species and it behaves similarly to humans. And despite prophylaxis we had significant challenges.

So, in other words, depleting plasma cells targeting co-stimulation molecules and T-cell depletion is profoundly immunosuppressive. And while we were able to prevent graft rejection and AMR, this was a daunting combination of immunosuppression.

I think the conceptual breakthrough that we

Page 200

made was that -- and this is published just earlier this year by Jean Quan and really based on his observation of what's happening in the germinal center -- is that if you treat with proteasome inhibition alone we are actually activating the germinal center.

And as shown here germinal center B-cells are exposed -- they are expressing BCL4 at higher levels. The follicular helper T-cell is substantially activated as shown in the upper right. And that's shown pictorially here where this quiescent B-cell follicle is activated following bortezomib treatment alone. And this was also associated with an in- -- a significant increase in serum BAFF levels.

So now returning to the cohorts treated with the triple therapy as shown pictorially in the upper left, this dual targeting regimen was able to actually substantially lower donor-specific antibody. And mechanistically we've been able to look at bone marrow plasma cells which are substantially reduced in the monkeys, lymph node germinal center B-cells are also substantially reduced as are lymph node follicular helper T-cells and were blunting substantially the

Page 201

isotypes switched B-cell proliferation.

And that is shown a little bit more graphically here. And on the left are the control lymph nodes and the red here is staining for the B-cell follicles. And this is post-treatment on the right here.

And you can see that these b-cell follicles are essentially empty on the right. So there is a profound effect of co-stimulation blockade in combination with proteasome inhibition in altering the germinal center morphology. And that is summarized graphically on the lower right for you.

In order to aim for a more tolerable immunosuppressive strategy at the time of kidney transplantation we backed off of T-cell depletion induction and gave them basilizimab instead, an anti-CD25.

And that was much better tolerated and the overall graft survival is shown in the upper right here with 3 of 3 monkeys having rejection-free survival. And the histology is summarized in the upper right with an absence of antibody mediated

Page 202

rejection. And, again, that is paralleled by complete disruption of the lymph node follicles.

So that I think is a clinically applicable strategy that we can take forward into patients. You've just heard from Anita Chong about a very -- a similar strategy minus the anti-CD40.

But I think it's encouraging that this works not only in a mouse but in a monkey. And we're working with Steve Woodle to, you know, think about how we will carry this forward in the clinic.

I think some of the questions that we're interested in addressing and using the monkey model to try to address is would plasma cell targeting more specifically with monoclonal such as daratumumab or elotuzumab or plerixafor accomplish the same type of donor-specific antibody reduction plasma cell depletion with less toxicity than proteasome inhibitors.

We're also interested in looking at whether BAFF or IL-6 receptor targeting in combination with plasma cell depletion could be just as effective in terms of the clinical outcome in the non-human

Page 203

primate.

Another pressing issue is how durable is this sensitization? The type of strategy that I've just outlined for you would probably apply to desensitizing if you have a living donor, but for deceased donor transplantation you'd have to desensitize in a durable manner that would last three years or so for most programs.

So we're interested in considering how durable is that approach and can we extend that by a maintenance phase of desensitization therapy.

So in conclusion we have found that the non-human primate model is an invaluable tool for developing better immunosuppressive strategies and drugs for transplantation and can lead us to more appropriate clinical trial design. Thank you.

DR. MANNON: Can we go ahead and get the session questions up? Any questions for clarification of the four prior speakers from anyone?

DR. WOODLE: I would ask this question to Anita and to Stuart both. I think that if, indeed, you're inhibiting a germinal center response the

Page 204

question is the end result would be a couple things.

One is do you prevent the formation -- the end result of germinal center is either mature B-cell or plasma blast. And what I didn't see I think in your talks were do your treatments with belatacept blockade or CTLA4 IG prevent the generation of new plasma blasts that are antigen specific?

DR. CHONG: So in the mouse studies we know that even if you have memory B-cells or naive B-cells if you introduce antigen in the presence of CTLA4 IG you will inhibit those B-cells form differentiating. So it always requires T-cell help at least for allo-specific antibody responses.

And we also show that if you give CTLA4 IG late you can still -- there is a window in which you can inhibit the germinal center output of memory cells. So you can actually inhibit B-cell sensitization or at least in terms of, you know, memory B-cells.

So all the outputs that are germinal center dependent, time dependent within the germinal center you can inhibit and you can inhibit the recall.

Page 205

DR. KENCHTLE: So, Steve, we have not been able to look at allo-specific B-cells, but we do bone marrow aspirates to look at plasma cell that secrete antibody and use an elispot to look at them. And there is a substantial reduction in antibody secreting cells by those bone marrow derived plasma cells.

DR. WOODLE: I just wanted to congratulate both of you. I think that there's always been a question in our mind as to why there's resistance to proteasome inhibitor therapy. And it's clear now from your work and it just substantiates a lot of prior work that if you deplete the source of antibodies there are reflexes in place and mechanisms to replace that in the immune system.

And now we know how to go after this. And I think this is probably the most significant development in the history of proteasome inhibitor treatment for human responses. And my guess is that this is going to be a big step towards solving the problem.

I don't know of a more promising approach in the field right now to treat human responses in

Page 206

transplant recipients other than what you guys are proposing.

DR. ALLOWAY: I'd like to kind of compare and contrast the toxicities that you reported in the monkeys compared to what we've seen in the humans.

We've used T-cell depleting induction in combination with tacrolimus and MMF for a long time and treated a lot of people with velcade. Knock on wood, essentially we -- although we have prophylaxed effectively for the viral effects we have not paid the price of infectious complications in that regard.

However, as we move onto treating with belatacept despite the prophylaxis we do -- we are paying the price in terms of toxicity and being able to handle and manage the viral infections that do occur.

So I guess I'm interested in your -- you essentially assigned the over immunosuppression that was related in your monkey model to your -- your induction -- potent induction depletion and potentially the PI.

But I would kind of offer an alternative

Page 207

reason for that.

DR. KNECHTLE: In other words, you're suggesting that it's the co-stimulation blockade --

DR. ALLOWAY: Yeah.

DR. KNECHTLE: -- that may be responsible? Okay. That's fair enough. So since I'm giving all these drugs I can't tease them apart. However, I can tell you that the CD4 -- the depleting Cd4 monoclonal that we give that comes from Keith Ryman (phonetic) that is a profound deplete and those monkeys don't reconstitute their Cd4 cells for many months. The CD8 is also very effective.

There's some variability by lot for this CD4, but in general we have significant problems in our monkeys when we get profound T-cell depletion. I mean, we've had -- we've learned that from previous experiments.

Of course, the -- you may be correct, though, that co-stimulation blockade is a significant cofactor in that issue. So we addressed it by backing off. And, frankly, as you know, a T-cell depletion plays a very important role in successful

Page 208

transplantation of sensitized patients. That's one of our better tools besides tac.

So we were surprised that giving simulect we didn't see more rejection early on. So I suspect that the combination of bortezomib is not only -- and a co-stimulation blockade is also affecting the T-cell, of course.

In fact, we've also shown that the naive T-cell component is enhanced by this therapy.

DR. ALLOWAY: In humans a similar story that we saw when we had EBV mismatch I think we're seeing a similar story when we have CMV mismatch. I mean, maybe this is oversimplification, but what is the serial status of the monkeys? Are they all positive?

DR. KNECHTLE: Yes. All the monkeys are positive.

DR. ALLOWAY: Okay.

DR. KNECHTLE: Yeah.

DR. MANNON: Any other comments about the clarification? Otherwise we'll turn to the questions. And Shukal indicated she'd like us to walk through them. We don't have to be -- some of this may be

Page 209

rhetorical because -- I'm sorry, Steve.

MR. WOODLE: Just one more comment. I think this highlights -- so one of the reasons why we wanted to get away from IVIG is so that you start to specifically target known biologic pathways. And that's what's happening here.

And I think to further emphasize to the FDA we're going to be coming with combination regimens both of them off label. They may be actually outside of the field of transplant. And this is where I think the field is moving and agency should be considering this and be prepared for it.

DR. MANNON: No. I think that's an excellent point, Steve. And, you know, based on you have rodent data and non-human primate data mimicking each other and I know we've been sort of honestly let down sometimes by those models not coming through, but I think in this case the biology is mimicking what we're seeing in people.

And there are differences in depletion. We can't use ATG. Rabbit ATG is ineffective in NPH. So I think that they're trying to adopt those agents, but

Page 210

I do think that, you know, a number of us in the room have seen these data and they're very interesting. And, you know, people looking at an agent which unfortunately commercially is difficult to get now, you know, and I wish -- I don't know if there's anybody from BMS here, but I'd hope they'd be encouraged by these kind of data.

And the sad fact that they may not be here is unfortunate. They may be listening in, but I hope that they're encouraged by some of these presentations.

DR. WOODLE: You know, sometimes you get market share through the back door, you know. And this is sort of an end around approach to getting that but certainly, you know, if this works out, I mean, this means that patients are developing humeral responses post-transplant will be converted to bela. That's what I think.

DR. MANNON: And I have my own conversion stories of people, but I'll get in trouble. But I have no stock options, blah, blah, blah. I have nothing to do.

Page 211

So if you guys are okay we'll move onto the first question in terms of what we know about endpoints and using them. And the one comment I feel comfortable making is in terms of histopathology.

So Mark Haas presented a lot of information and there's been comments about this. I think one of the challenges I think for the BANFF working group is some clarification of some of the definitions to be clear that there are clinical entities associated with the pathology.

But also that maybe one of the working groups has to really redefine the CG TG -- you know, CG as a potential endpoint because, as you point out, it's the worst glomerulus of the number of glomeruli you see.

DR. HAAS: Yeah. I mean, the glomerular lesions as a whole do not have great inter-observer reproducibility. So, I mean, that's a problem. The -- the current -- the current definition of CG is a very low threshold kind of defi- -- definition.

And so I think we need -- and clearly increasing amounts of CG have association with changes

Page 212

in outcomes as we saw in some of the talks this morning.

So I think we -- as Bob suggested, we probably need a more granular assessment of CG. And, you know, this is something that Bob and Michael Mangel are working on and to look to see if this is adopt -- adaptable to a clinical trial.

And ultimately if it is this'll have to be incorporated into BANFF. I must say, though, BANFF is a consensus, it moves very slowly. Watching BANFF move is kind of like watching the grass grow.

We had evidence for C4d negative antibody mediated rejection in 2009 and it took until 2013 to incorporate it into the classification.

We've had evidence that IFTA is bad since 2010 and it still hasn't been incorporated into the classification. So admittedly these things take time.

One thing that I would perhaps suggest and advise is that although BANFF is the consensus maybe for the purposes of clinical trials to try and develop and validate histologic endpoints outside of BANFF is not necessarily a bad idea. And this is coming from

Page 213

somebody who is heavy invested in BANFF.

But, you know, in order to make the field move faster, move faster we can't really depend on something that moves very slowly. And if we have data then that might give a push to BANFF to try and move its process along at a little bit faster rate, too.

DR. MANNON: And an alternative strategy with this transplant therapeutic consortium investigating biomarkers, histo- -- you know, having -- I mean, part of the issue is that BANFF is a voluntary organization. You don't get paid and in your spare time of the thousand other things you're doing, you know, you have to do it.

So and that's kind of why I sort of don't want to throw stones because I know how long it takes me to look at things. But I think that that might be an opportunity for the liaison between a private public partnership and the BANFF working groups are individuals interested that want to look at this because now they obviously realize -- if the group doesn't realize that, I think these histological endpoints are really quite critical. And some of the

Page 214

definitions have been really improved.
I think along the same lines the molecular endpoints group has to come up with. You know, it's more than the indats and the intercomex. There's other things that people are working on in this room that haven't been published yet that look quite promising.
DR. HAAS: Yeah. And certainly, you know, I mean, the ABMR classifier is a massive improvement over the indats. And I think the slides that were shown, you know, that Greg showed this morning really show that quite well.
And the onus is very much to try and incorporate some of the molecular diagnostics into the classification. There was a very early attempt to do this back in '13. Excuse me. But I think it really sort of needs to be moved along at a little bit faster rate.
But, again, it's the -- you know, it's a consensus. It moves slowly. And it'll ultimately be done and I expect that BANFF 2019 will have an ABMR classifier in the classification. But I actually

Page 215

proposed at the 2017 meeting just a few weeks ago that we try and do it this year, but it kind of met, you know, a great deal of resistance.
And so but we need to really I think see studies that use this as perhaps an alternative endpoint. And if this is found to be valid as an alternative endpoint then that will facilitate its incorporation into BANFF.
DR. MANNON: I guess the only other comment I wanted to make is five years ago we tore each other alive. Not us necessarily, but some of the labs tore each other alive about donor-specific antibody and the validation using that as an endpoint.
And I think we've heard a lot of clinical data that's encouraging in terms of seeing drops in DSA or the most immunogenic. And, again, that might be another TTC project where the HLA labs, you know, in conjunction with all the ongoing issues of monitoring might be interested. I'll just throw that out there. I'm not, you know -- anyway.
DR. HAAS: And just one, you know, sort of point of caution about DSAs again is that I think we

Page 216

heard yesterday, I mean, how one measures DSA in terms of what constitutes a 50 percent drop is important. There are limitations to MFI which seems to be the current standard, but we've heard that MFI has its own limitations.
And also all DSAs are not created equal. And some DSAs are harder to get rid of than others and we've seen this in desensitization. So that you may have -- for example, I mean, it's easy enough if you have a single DSA, but many of these patients will have more than one donor-specific antibody. And one of them might be quite amenable to current therapies to lower DSA and the other might be, you know, a DR51 that's harder to get rid of.
So a 50 percent reduction per se might be a good start, but I'm not sure that's necessarily the gold standard. And I think we really need to go farther than just, you know, lumping all DSAs together.
DR. WOODLE: Mark, I was wondering if I could just take a -- make a general observation on that. So our drop in DSA is using the immune dominant

Page 217

DSA. And that is defined as being either class one or class two and it's the highest in the five level, okay.
And so what we find in both desensitization and in antibody medicated rejection is that if that antibody drops almost all other antibodies drop with bortezomib.
The old history about the public epitopes of DR Beta 3, 4, and 5, that is DR51, 52, and 53, they are more -- they seem to react quite well to proteasome inhibitor therapy.
Your point is well taken though that in general class two doesn't respond as well as class one. So those are the caveats that I would just add to your comments.
DR. GEBEL: I'd like to add one thing to your comment, too. And that is with DSA -- we've been talking this entire time looking at one half of the equation.
In order for DSA to have any effect there has to also be a target. And what we don't know -- it's just unknown at this point -- is what is the

Page 218

level of target expression especially for things like HLADQ which we know at the onset at least on peripheral blood is expressed at a ten-fold lower concentration than HLADR.

In vasculature I just don't think we have enough information. I think there needs to be more dedication towards looking towards what that target is.

DR. TAMBUR: I'm sorry that I'm going to bring you back again, but we are talking about MFIs that are close to 20,000 and we're outside of our scale to be able to differentiate which of them is stronger than the other.

We're lumping them together and I believe I am the only one who's currently doing titrations. And I can tell you DQ titers are significantly higher than class one titers. And maybe everything that we're seeing right now is a result of this.

So obviously it's only my data, but I think we need to start looking at antibodies in a little better resolution of how we quantify them. And right now we're looking at this range and we have patients

Page 219

have antibodies some were up there and we're totally missing that.

So I think before we jump into conclusions we owe it to ourselves to test whether that might be the case. And I challenge the centers -- and this is why I approached Montgomery at the time -- that had differential responses to treatment to look at how strong that antibody truly was prior to treatment, not by MFI but something else that they can truly quantify the antibody.

DR. HAAS: And that was actually the point I was trying to make, but not nearly as elegantly because I'm a pathologist.

DR. MANNON: Uh-oh, somebody leaning in the back. So any comments about question 2, the pros and cons of composite endpoints?

Is everybody just like burnt out? Maybe the presentations were --

DR. LENNON: Yeah. So one question or more comment towards these composite endpoints. One of the ques- -- words that keeps running around in my head is value here.

Page 220

I know we got to find something that works. That works towards improving for patients. In my opinion there's three pieces. Longevity, which I think we talked about; health, health outcomes, decrease cardiovascular disease, all sort of the side effects that actually affect the medical aspect management of the patient; as well as the quality of life. And quality of life came up briefly here.

But what's the value of these treatments that we're proposing and what's really the cost here? To me there's two parts of the value equation and right now we're looking at does it work. And ultimately we've got to find something that does work.

But then the other side is going to be what are the costs? Is there healthcare utilization measures? Are there pieces that from a patient perspective this is more -- you know, this is from me adherence simplifies or makes harder adherence. It does -- makes me feel cruddy the day I get it, et cetera, et cetera.

So just a push that as we work on trying to find something that works clinically and meets either

Page 221

our surrogate or our clinical endpoints that maybe we look at some of the sort of process measures of how is this actually interacting with the patient and the provider as they interact to make it work so.

DR. MITTELMAN: I also have a question on composite endpoints because I know we've talked a lot -- you've talked about it today. But don't people kind of just cheat to use composite endpoints?

I mean, I've been around the clinical trials a lot and you kind of do it to just get your study properly powered basically. And -- and then you end up combining a lot of events that are of different importance levels to patients and then it kind of screws up your data the way I understand composite endpoints.

I'm kind of confused why people are still looking at them. So I guess I would challenge you guys, I mean, to think about this. I've planned a lot of trials in my day. I used to do pricing and market access at some point so I'm confused why there's -- why it's on the board.

DR. WOODLE: Composite endpoints doesn't

Page 222

necessarily mean that you introduce a degree of subjectivity. You can have composite endpoints in which the individual components are objective and measurable and verifiable.

And I think that those are always preferable to an endpoint that's subjective.

DR. BALA: Dr. Knoll, do you want to --

DR. KNOLL: No. I was just -- I mean, that's very good points. Obviously we -- in this field we're using them because we're struggling to get enough patients, enough endpoints to show any meaning effect to some of the therapy.

So as I did point out, you have to be very careful about what you put into the composite. And one of the key issues is relevant importance to the outcome. And you just can't have things that are small.

I mean, there used to be a phase in industry trials and some of the trials we did many years ago where loss to follow up was part of a composite which like made no sense to me with graft and patient survival being part of it.

Page 223

So I think we've thought about it a little more and I just brought up -- no. I don't think anyone has the answer of what the endpoints should look like for the trials yet, but I think we're going to have to have a few things in there. And we do have to realize that they have to be important for everyone, patients and providers.

DR. MITTELMAN: Yeah. And, I mean, so Jack mentioned this value point, right. And I've been to a lot of conferences recently where, you know, people are trying to understand what are important outcomes to patients without necessarily looking at these intermediary secondary endpoints.

And so I guess one of the challenges I would ask you guys is is where are you in thinking about some of those things particularly around something Jack mentioned, quality adjusted life years which I know they do in the UK and other countries. And I used to do it also for planning for purposes.

So does the FDA and do you guys ever think you'll begin looking at that more, those kinds of metrics, endpoints?

Page 224

DR. ALBRECHT: So there is actually a group at FDA that's involved with patient reporting outcome. And one of the challenges is that developing those kind of patient reported outcome measurement scoring systems is very challenging.

It does involve -- just like many of the biomarkers that you're hearing about today it does involve working with patients. It's important, but, again, it's time consuming. It involves working with patients, having groups of patients discuss what is and isn't relevant to their health and their -- the benefit that they gained from the treatment that's being proposed.

And then again the same thing validating it and assuring that other groups of patients can use that same score to score whatever the parameters are that are objectively determined to be of importance.

So, again, just to summarize there's a group and certainly I think in the field of transplant we could start talking about that.

MR. MITTELMAN: Yeah. But what about the other point that Jack mentioned which is value, right?

Page 225

So icers and those kinds of things that people look at. So this value based which is where in the hospital work that I do, you know, it's value-based models are all the realm now. And, I mean, I remember pricing drugs that you guys passed that were ridiculously expensive. I feel bad about it.

DR. BALA: Dr. Irish?

DR. ALBRECHT: From the agency we look at the benefit and risk to patient. Actually the FDA does not involve itself in the pricing, although that has been a topic recently.

MR. MITTELMAN: Yeah. I just mean can it -- can it become one? I mean, we're talking about it, right? So is it something the FDA's going to consider, start looking at?

I mean, why not?

DR. ALBRECHT: So the FDA follows the laws passed by Congress and so if that's in that equation then obviously the FDA would participate. But that's the scope of where we get our authority.

DR. ALLOWAY: I would like to use that opportunity to make a comment about PROs, however. So

Page 226

we all have used the common toxicity criteria for adverse event reporting that has been developed in oncology. And they are attempting to develop a similar strategy for PROs.

So I hope that we can get closer to using a PRO in a way we use the common toxicity criteria for importing adverse events because historically, as you know, the price of validating a PRO has been more expensive than the price of developing the new drug.

So I think that if we could come to that agreement with adverse event reporting, hopefully we'll be able to do the same with patient-reported outcomes.

DR. ALBRECHT: Well, and as I mentioned, the 21st Century Act does include patient-focused drug development. And I think we're still, you know, taking the first baby steps, if you will.

But it's true that when the FDA is looking at products and saying, well, here are the benefits, here are the risks and someone says based on whose criteria. And the important point is it's got to be on the patient's criteria.

Page 227

So not someone else deciding what's important to patients, but actually having those public types of for -- where that information can be gathered and then studies where it's tested and so forth.

So, no, I agree with what you're saying. It's just the scope of FDA is the risk benefit of the product and how the patient then ultimately feels, functions, and survives.

DR. BALA: Dr. Irish?

DR. IRISH: Yeah. I just wanted to -- you alluded an important issue with respect to a composite endpoint. You have these individual components and when we do analysis we -- we equally weight them.

And this is -- this is a challenge. And there's a lot of research now that's being done using composite endpoints, but providing sort of a clinical utility value to the -- that's not equal. And that's a really important area for research in terms of the composite endpoint.

DR. BALA: Dr. Knechtle?

DR. KNECHTLE: If I could make a comment.

Page 228

Sorry. If I could make a comment about your question on value. I'm sure you recognize this entire two-day discussion involves exceptionally expensive therapies. So the denominator in the value equation is going way up, right, so the value is coming down.

And I think for that reason it's interesting to me that you were the one that made the comment because certainly as money is sucked out of the healthcare sector it's going to be harder and harder to do this type of work.

And transplant centers are evaluated on their overall outcomes. So if you do a higher-risk patient, you're potentially hurting your results and your program. So I think there's a real premium on coming up with affordable strategies that work and applying them very carefully in a way that not only preserves a reasonableness to the cost of the therapy but also reasonableness to the risk and the side effects to the patient.

DR. WOODLE: In terms of overall long-term healthcare cost the drug costs are only a part of that. One of the major advantages if you do economic

Page 229

advantages of these approaches, if you do salvage a graft and keep a patient off dialysis there are huge cost savings overall to the industry. So that's a major factor.

But I think knowing that that saves a lot of money to the healthcare system in the long term combined with the fact that this is a small population when drug companies go to do their calculations to determine what the market price will be those are factors that are going to wind up being higher prices for these drugs rather than lower prices.

DR. BALA: Dr. Knoll?

DR. KNOLL: Yeah. I just wanted to say, again, just a follow-up to your question about the endpoints is there's another initiative called the song initiative which is a standardized outcomes in nephrology that myself and Dr. Nickerson, Dr. Mannon have been at some meetings.

They're looking at developing a core set of endpoints for all kidney transplant trials. And it's an international group. Half of the people involved are patients so it's a very -- it's a true partnership

Page 230

with patients and providers.
And there is a meeting that's going to be held at the ATC coming up so if you are interested in that I can certainly get you linked up with that group.
DR. MITTELMAN: That'd be cool. I know nothing about it so that'd be -- that'd be cool. Thanks.
DR. BALA: Okay. So if we could just spent couple of minutes -- couple of minutes on the last question. What are the major limitations to the applicability of the animal models of AMR to clinical transplantation?
DR. KNECHTLE: Well, I'll try to answer that I guess. Certainly the non-human primate experiments are extraordinarily expensive so funding is a major limitation. Then there's always the question of how translatable is the animal data to humans.
There's been a trend toward disbelieving rodent data in the last five years I think that's fair to say. I'm not trying to say that in any way disparaging the value of rodent data. It's -- there

Page 231

are obvious benefits that we do not realize in outbred models that are available in the rodent data.
On the other hand, non-human primate data is presumably more applicable. But even some non-human primate data has not translated into human so there are inherent disadvantages of course of modeling.
The obvious advantages of the animal data is that you can do much more in-depth mechanistic analysis and have more rational design of human clinical trials.
DR. CHONG: Yeah. I think, you know, in defense of the mouse model I think that there are just so many reagents that allow us -- and also in terms of cost -- to really nail down on fundamental mechanisms that hopefully will translate in large part in terms of the immunology of the B-cell development, plasma cells, and at least set a framework of a hypothesis that can then be selectively tested in humans given their limitations or in non-human primates given the limitations of especially in humans the access to the lymphoid organs which is very, very difficult and the only the access to either a limited number of biopsies

Page 232

and peripheral blood.
And so if you just -- my take from the last two days is that if you just monitor without underlying hypothesis you -- it's very difficult to separate the chance -- especially with limited numbers the chance correlations from something that actually makes some sense.
And if you have a hypothesis then perhaps the design to either prove or disprove that hypothesis that you have formulated and nailed down in a mouse model I think makes it perhaps a little bit more powerful than just randomly monitoring as many parameters as you can.
DR. MANNON: I know these non-human primate studies how expensive they are and painful. I think the very positive aspect of them is that they do develop transplant glomerulopathy. It's harder to see that in the mouse.
We have our old MHC mismatch model that's un-immunosuppressed. And those we got terrible IFTA and vasculopathy and glomerulitis we never were able to really pull up glomerulopathy.

Page 233

So I'm positive that a -- and, you know, all the old tolerance studies that, you know, when the kidneys failed in the primates they do develop it. And, again, understanding the transition from glomerulitis to glomerulopathy to me is like a complete unknown of why that happens.
I mean, we've been looking in in vitro and looking at, you know, co-stimulation -- I mean, stimulation of antibody and glomerular endothelium and cannot get -- you know, we can get cell activation, but we don't see that kind of pattern.
So I think that animal mod- -- you know, though it's a lot of money and very tedious. It's got a lot of merit to being more human-like than, you know, than we know -- than we realize.
DR. WOODLE: I think one of the things that's important is you're exactly right, Ros, complement in the mouse is not worth studying if you want to translate to human.
OKT3 or NECU3 that model was beautiful, recapitulated what happened in humans tremendously. CTLA4 IG is very translatable from the rodent to human

Page 234

as is it looks like the proteasome inhibitor work may also be very translatable to human.

But what's really exciting now is for the first time in transplantation with this proteasome inhibitor work we have mouse models directly relating to human therapies. We have primate models directly related.

We didn't describe it today, but Jim Driscoll at our institution now has an in vitro model that keeps human plasma cells alive for 14 days or longer which is the first time people have been able to keep human plasma cells alive long enough to study them.

So much of the drug interactions in future synergistic studies of drugs we can now do in vitro in humans. And you also saw our data today we're actually taking plasma cells from humans treated with these trials and studying them with modern approaches.

And so now we have all of those models directly focused on a mechanistic way to develop these. And so that's what's -- that's the exciting development in the last couple years that exists.

Page 235

DR. MANNON: So I'm going to have to -- oh, I was going to cut everybody off to let Renata finish us up.

DR. ALBRECHT: Well, I was just going to say that at the FDA part of drug development we look at in vitro data, we look at non-clinical data, and then we formulate how to do the clinical studies.

And so I think we value when there is information both in vitro and from animal models. That actually helps inform how to take a product into human.

So although there is a cost I think there's also clear benefit in being able to design better studies, better understanding process. And I don't know if Dr. Bala who reviews a lot of these wants to add, but we invariably have those discussions of where's the proof of concept and what's informing our clinical studies.

DR. BALA: I think we are -- we can close this session and return it back to you right on time.

DR. ALBRECHT: Okay. Well, we're just past 1:00 so let me keep it very brief. I want to thank all the speakers. Thank you. Very excellent presentations, very informative hearing the latest science.

Page 236

Again as I said yesterday we are so pleased that the patients were able to join us. And thank you so much for your comments. I think it helps us keep -- you know, helps keep us honest and realize that the work that this whole group is doing is to benefit the patients that have transplantation.

I want to thank my FDA colleagues who made sure things ran smoothly. And, again, to the audience. Thank you for joining us.

And with that, thank you very much. Have a safe trip back.

Page 237

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