Multi-Jurisdictional Contractor Advisory Committee Meeting: Molecular Diagnostic Testing for Acute Rejection in Kidney and Liver Allografts
November 16, 2022

Disclaimer:
This transcript was created with computer voice recognition software and reviewed for accuracy by Noridian Healthcare Solutions' Medical Policy staff. The information provided herein was current when made public and is accurate and complete to the best of our knowledge. The transcription is lightly edited to remove vocal disfluencies. Any discrepancies present in the transcript are deemed immaterial but may occur due to overlapping speech, unintelligible words, medical jargon, inaudible speech due to background noise, or incorrect speaker attribution. Official Medicare guidance is contained in the relevant statutes, regulations, and rulings. This document should not be treated as an authoritative record as it is informational only and posted as an aid to understanding the proceedings.

Jocelyn Fernandez:
Alright, good morning, everyone.

My name is Jocelyn Fernandez, and I am one of the Medical Policy Specialist here at Noridian Healthcare Solutions. Before we begin, I would like to go over a few housekeeping items. All lines are muted except for the CAC panelist and meeting facilitators. The chat feature within this meeting is used for technical issues only. Any questions to topics discussed today will not be acknowledged. Please send any questions or comments you have to cacmeeting@noridian.com

The meeting is being recorded and the recording and written transcript will be available after the call on all participating MAC websites. For the panelists, when not speaking, we do ask that you place yourself on mute to minimize any background noise that may impact the quality of our recording, and for our attendees to hear the comments being made. During introductions, indicate any conflicts of interests for the meeting recording.

Throughout the call, we ask that you announce yourself prior to speaking so that it's clear for the audience and for the record on who was providing each comment. After each discussion, polling will be conducted, you will be instructed on what questions to answer, and we kindly ask that you do not move ahead. Depending on your Internet speed or your location, you may experience a delay in viewing the questions. If you do not see the questions on your device, please refresh your screen.

For those of you that that experience technical difficulties during the polling process, the polls will remain open for up to one hour after the meeting ends and if you continue to experience technical issues, during this time, please e-mail us at cacmeeting@noridian.com, with a contact number that we can reach you at.

I will now turn the meeting over to our Contractor Medical Director, Dr. Anitra Graves.

Dr. Anitra Graves:
Thank you, Jocelyn. Can you hear me, OK?

Jocelyn Fernandez:

Yes, I can.

Dr. Anitra Graves:
Great. Good afternoon, everyone. Jocelyn is joining our broadcast from Hawaii, so it's a bit earlier there. But thank you all for joining this Contractor Advisory Committee meeting. We are really excited regarding the subject matter that our team has worked very hard to make preparations for you.

I am here with Dr. Angella Charnot-Katsikas. She is the Contractor Medical Director with Palmetto GBA, who we are collaborating with under the MolDx Program. Many of these tests are genomic tests and you may not be aware, but these genomic tests require a certain level of specialty, experience, and training, and therefore, we are collaborating with MolDx Program to help us navigate those waters.

Next slide.

So, moving on with the agenda, we're going to introduce our CAC panelists here in a few moments.

I'm going to give you a little overview of the CAC process, because it has changed in recent years. I want to make sure that we're all operating under the same framework for the discussions. Then we'll get right into the evidence discussions, focusing on clinical validity and utility, and the polling will be commencing simultaneously. Then we'll end with closing remarks.

Next slide.

These are our panelists that we have asked to join and provide comments and their opinions regarding the evidence that we have shared. The articles and the listing of publications are located on our websites for your information.

Next slide.

I'm going to ask the panelists if you can open up your mics and introduce yourself beginning with Dr. Huang.

Dr. Edmund Huang:
Hi, my name is Ed Huang. Thanks for inviting me to come participate. I'm from Cedars-Sinai, I'm a transplant nephrologist [inaudible 0:13:44 – 0:13:50]

Dr. Anitra Graves:
Dr. Huang, your vocal, is a little bit muted, so if you can speak closer to your microphone, that will be very helpful.

Dr. Miller.

Dr. Dylan Miller:

Hi Anitra, I am Dylan Miller, I'm a pathologist. I'm in Salt Lake City with the Intermountain Health Group and there are no conflicts.

Dr. Anitra Graves:
Fabulous. And Dr. Brennan.

Dr. Daniel Brennan:
Hi, Dan Brennan, I am transplant nephrologist and the Medical Director of the Comprehensive Transplant Center at Johns Hopkins. And I have Hopkins has grant support from CareDx and Natera and I have received consulting fees in the last 36 months, from both CareDx and Natera.

Dr. Anitra Graves:
Dr. Charnot-Katsikas can you introduce yourself?

Dr. Angella Charnot-Katsikas:
Good afternoon, everyone. I'm Dr. Angella Charnot-Katsikas with, as you heard, Palmetto GBA in the MolDx program. We are happy to host this CAC today.

Dr. Anitra Graves:
And we know we are currently missing Dr. Zarrinpar. Dr. Zarrinpar is tied up in a NIH meeting, so we hopefully will be able to have him join a little bit later. Dr. Miller and Dr. Ekwenna are delayed and hopefully they will be able to join at some point during the meeting.

We will go ahead and however get started with the introduction about the Contractor Advisory Committee.

This committee has been in existence for a long time, Medicare Administrative Policy contractors typically use this committee as a launching point for policies in the past, however, that changed as of January 8, 2019.

The Program Integrity Manual has redefined the position of the CAC as the opportunity for the SMEs that have the specialty expertise in training in a particular subject matter, to discuss evidence and literature of that topic relevant to today transplant acute rejection testing.

The role of the CAC members is really advisory in nature, and it allows us to hear their comments and opinions on the evidence and literature, which assists us in determining if a proposed policy should be developed at all or if an existing LCD should be revised.

You may know that we do have a policy presently for Solid Organ Transplant Testing to evaluate for the presence of acute rejection. So that will be the subject matter of this CAC.

We did select publications that the SMEs were gracious enough to review in advance. It is not all of the publications that are informing us regarding the subject matter. We actually screened over 500 articles in preparation for this meeting. The additional publications may be used to inform any policies that may result from these proceedings. However, we selected those that the panelists have reviewed, because they are a very good representation of the evidence that has been published and is available presently. The CAC members, opinions, and comments

supplement the Medicare Administrative Contractors, internal experience, and we use them hopefully to ensure unbiased and contemporary consideration of state-of-the-art technology and science.

I want to remind us all we're, we're here for the Medicare beneficiaries and in particular, transplant recipients, which are a particularly medically fragile group of patients.

Next slide.

So, to frame our discussion, I just wanted to go over the topics that we hope to cover.

There are multiple tests and multiple organs, so kidney and liver, are the focus for today.

We're going to start with kidney, as the lion's share of publications are related to that, and then we'll end with the consideration of liver transplant tests, of which there are significantly fewer in number.

We're going to look at the patient population that has been published in which these tests are to be used. We want to examine the clinical context, for example, testing for cause as it, as if a patient is presenting with global signs and symptoms that rejection or surveillance as a reason to use these tests.

We're going to look at the test precision, looking at the SMEs opinion of the data with or without confidence intervals and giving us an understanding of how they would interpret that.

We're going to look at clinical utility and in particular, we're interested in how our experts are using these tests on serial testing, if they are at all, and the timing of these tests. We examine the cut off points.

Well, talk about whether or not these tests have the ability to discern the difference between acute T-cell mediated rejection and antibody-mediated rejection from quiescence.

Look at the clinical scenarios when testing might preclude the need for a biopsy. So, in particular result occurs, a physician may not choose to move forward with the biopsy at that time. We'll ask those opinions.

We also want to know what the predictive value of the test results are with respect to guiding clinical management when a biopsy is not performed, would it modify decision making on immunosuppression or not.

We also are going to assess the level of confidence that these tests accurately indicate whether there's rejection or quiescence. Then ultimately, we are going to use all of this information to understand what specified patient-centered outcomes is supported by the use of these tests.

Dr. Anitra Graves:
Dr. Ekwenna, have you joined us?

Dr. Obi Ekwenna:

Yes, I have, good afternoon.

Dr. Anitra Graves:
Good afternoon. Can you just give us a quick introduction?

Dr. Obi Ekwenna:
OK, I am Obi Ekwenna. I'm a faculty at the University of Toledo in Ohio. I'm a transplant surgeon; the Associate Professor of Transplantation of Urology here.

Dr. Anitra Graves:
Dr. Ekwenna, I believe you do have some disclosures. We went by the slides, but I'll just announce it and if you could confirm, that would be appreciative. You have speaker fees and funding through Natera, is that correct?

Dr. Obi Ekwenna:
Yes, I have an investigator-initiated study that I'm doing, and I also have patients who are registered in the registry.

Yes.

Dr. Anitra Graves:
Wonderful. Next slide, please.

So, this is really a summary slide. There are a lot of tests, so, we're going to help kind of guide the discussions, since there are so many.

There are three colors that you may see on the screen. The first three tests in blue, are genomic expression profiling tests.

The second set in green are those tests that use donor derived cell-free DNA and the last test is called QSant and it is a test, that is actually a multi-analyte test. It does use cell-free DNA as one of the analytes, but it also uses some proteomic markers creatinine total protein clustering and CXCL10 as a test to evaluate for the presence of rejection. However, is that test, urine is the specimen. The rest are blood. To be clear, we focused this meeting on those tests that are considered minimally invasive, meaning that they do not require a biopsy to render a result.

So, let's jump right into it. Next slide, please.

Our first slide is related to discussion question, and I'm going to talk a lot less as we continue. Of the tests that we have listed, that last test we have is OmniGraf, which is actually a combination test, on the previous slide, which we will see again, there are some tests that have an asterisk and that asterisk indicates that those tests can be or there has been published articles, where those tests can be used, in combination to render results consistent with either rejection or quiescence.

This first question is: Is there sufficient evidence to identify the patient population that the molecular diagnostic test could be used in?

And, what I'd like to hear from our SMEs are some comments regarding the generalizability to the Medicare population since that is our focus.

How they relate, or how they, how you would use them in different risk populations of patients. For example, those patients that might have a higher risk of development of rejection, versus those that do not.

How these tests might be affected by various illnesses, acute infectious illnesses, in particular, such as COVID, HIV, the flu, and even sepsis from bacterial infections.

I also would like to hear some comments about the application in patients with more than one transplant. Or in patients that have no medical, non-compliance, will you use these tests in that patient population?

Well, I'd like to also understand how these tests will be affected, or how your testing might be affecting, depending on how long after the transplant has occurred.

And then, on the surveillance versus for-cause.

So, let's start off with anyone that has the thoughts together regarding how these tests might be used in various patient populations.

Dr. Huang, Dr. Ekwenna?

Dr. Edmund Huang:
It's a pretty broad question, so, it's, it takes a little time, to think about exactly how to answer this.

The test are all a little different. So, I am not sure what I can give you one answer to this question. There are different tests that have been tested in different contexts so, I think when you ask this question, one of the first questions, easiest to answer is can this be done, surveillance versus for-cause?

Some tests on this list that primarily that have been tested in surveillance, and that would be TruGraf and OmniGraf. Then, you have other tests that have been tested, for both surveillance and for-cause. That would include cell-free DNA tests, as well.

So, so I think that there is probably pretty good evidence for TruGraf and OmniGraf surveillance and cell-free DNA probably can be used in both contexts.

Dr. Angella Charnot-Katsikas:
Dr. Huang, I thank you for your response. I do want to tell you that you're still a little bit muted, so I think we can make up most of what you're trying to say. If there's any way to get closer to the mic. It would really help us in the discussion.

Dr. Edmund Huang:
I can try and switch the audio to my phone.

Dr. Angella Charnot-Katsikas:
Dr. Ekwenna, it sounds like you were trying to say something?

Dr. Obi Ekwenna:
Yeah, I agree with Dr. Huang's assessment.

I think the cell free DNA tests have been there's sufficient evidence to show that they are pretty good in, both for-cause and for surveillance. Um, you know, that's my experience, and that's sort of some of the data that I've been able to review.

And as far as, you know, what patient population, I think all patient populations, the tests have been shown to have some utility. There is still emerging evidence as far as repeat transplants or patients who have repeat transplants, and I think that the evidence there's still sort of emerging across demographics, I think it's, there's definitely some utility across all demographics, risk, immunological risk for most patients, I would say. And that there is sufficient evidence for TCMR and ABMR for both, for the cell-free DNA tests, for sure.
So that's sort of my comments on that.

I don't know if, you want me to, you know, as far as, you know, I don't know what the next questions are. But in terms of the data, you know, the negative predictive value for some of these tests are probably, you know, above 90%. Sensitivity is a little bit on the lower side, maybe 80, 80-something percent for, the cell free DNA test and still better than looking at some of the other markers that we usecreatinine, you know, doing biopsies on patients that have significant risks.

So, yeah, at least from my standpoint, and from what I read in the literature, there's sufficient evidence for its utility in for-cause and surveillance. As far as, I think rejection, you know, obviously in the first year, or in the first six months to a year, that the risk of rejection is higher in most patients; subsequently after that the risk is probably a lot less, however, I do, again, the evidence is still emerging for later patients, but I suspect that, you know in terms of surveillance, it may be helpful in predicting patients who, um, will continue to do well, or, you know, at least, in terms of monitoring some of these patients, long term.

Dr. Anitra Graves:
Thank you.

Can you discuss what effect that an accelerated immunosuppressant treatment might have on the result of these test or the proximity to testing with these tests, or even the effect of drug toxicity; would that result in misinterpretation of the results, or it wouldn't affect it at all?

And anyone can answer.

Dr. Daniel Brennan:

This is Dan Brennan. The Spaniards have actually looked at this, and they've looked at some of your other questions down there. What's the effect of sepsis? What's the effect of cyanide toxicity in ATN?

Basically, with sepsis, it looks like there's a spike with a cell-free DNA that's released that goes away when you treat with antibiotics. And so, when people are trying to decide, whether the spike is coming from rejection or from the patient, well, you could talk to the patient, you could get a urine culture, those might help. I mean, usually you can sort this out.

I do think that the test is useful to discriminate other things there, that you're talking about, and that is, I think, it's useful the cell-free DNA, and actually the molecular test too, to sort out some of the problems that we have. Rejection, does it matter or not, or what is called rejection. The concordance rate for expert pathologists agreeing is 0.2 to 0.6, so it's less than flipping a coin. And so, if you have a very low donor-derived, cell-free DNA, that takes some reassurance. The flip side is, when you have a high cell-free DNA, and you get a biopsy, and it doesn't show anything. That's confusing.

There's BK virus, and BK virus, the assay is not precise. Copy level, in the same lab on the same day, could be 10,000 copies, or it could be 1,000, or it could be 100,000. So, one of the ways to kind of sort out, whether that BK matters, is whether or not they have an elevated cell-free DNA.

So, and then that's with DSA. We know that 50% of DSA don't matter, ever since Terasaki started showing us what the utility of anti-HLA antibody was. All the reports show 50%, no clinical relevance. So, the cell-free DNA kinda helps to sort that out.

Otherwise, as I think most of the practitioners here, kind of the drill is the creatinine goes up and then you tell them to hydrate and repeat. You look at the tacrolimus level, you get a DSA, you get a BK, you maybe get a CMV if they're sick and then in the old. And now, you're more likely to get a donor-derived cell-free DNA if the creatinine is incredibly elevated, otherwise, you bring them in for a biopsy.

I think that's pretty, pretty kind of kind of what most people do.

The molecular tests are interesting, because the ones you got here are not the ones, I am surprised and you don't have MMDX, right?

The molecular microscope, I think, that's more developed, but it's not a blood test. It's a, it's a tissue test.

Maybe you didn't want to focus on that?

Dr. Anitra Graves:
Yes. We're focused on the minimally invasive, so those that would potentially preclude the need for, for biopsy, if that’s what you would use.

Dr. Daniel Brennan:

The OmniGraf is the one that takes a molecular gene expression panel test and a donor-derived cell-free DNA tests together [inaudible]

Dr. Angella Charnot-Katsikas:
I'm sorry. If you're not speaking, can you please mute your line? I know there are folks who are perhaps speaking, not realizing that we can hear you.

Go ahead, Dr. Brennan.

Dr. Daniel Brennan:
So, I think that, looking at your levels, risk level. What's a risk level?

So, I don't think people realize that, if you get a living donor transplant, the last SRTR data, which is dated, shows that the rejection rate is basically the same as if you had a deceased donor. Despite the better, usually the better HLA matching not so much in America since we do so many unrelated. I'm not aware of any ethnic culture, demographic differences. I am aware of some age differences. If that matters, I mean, kids matter a little bit. But it's older people that are a little bit different because they might have a falsely elevated donor-derived cell-free DNA because most of them are, it's relative, it's the percent of the donor-derived cell-free DNA divided by the recipient's cell-free DNA and if this recipient is small and elderly that might artificially raise the percentage. And that's where TruGraf is saying or Natera Prospera are saying, well, we do a quantitative test to sort that out. Trying and paying attention to that, I'm not sure how useful that is, but then that's what people talk about.

Retreat, repeat transplant recipients, that's been pretty well looked at. It's a little bit more elevated when you have 2 or 3. But, it's, it's usable. And that gets on to, I think you're going to have a later question, oh there it is, multi-organ? What about multi-organ?

Well, with multi-organ. I think where it might be useful is establishing a baseline for that individual after the initial transplant when the cell-free DNA would be, have gone down to a baseline. Usually, by two weeks or so. That's where I think it'd be useful and it's looking like simultaneous kidney-pancreas' their baseline might be a little bit more than then what a kidney is, as opposed to a lung transplant, it looks like they've got about the same 1% cutoff.

Liver is different. Heart is different, heart is a lot lower.

So, we'll get into those, maybe a little bit.

Medical noncompliance? I don't think that matters.

I mean, it. I mean, you would think it would raise it, but it's the fact that they would have injury from the noncompliance. But the noncompliance itself, I don't think would affect it.

Time post-transplant is important. There is an elevation from ischemia reperfusion that goes away.

Surveillance verse for-cause. Um, we don't have a lot of data there. From the Dart study, there were some programs that did surveillance biopsies and I think that might help to sort out, because I see there's that many programs 18% of kidney programs do a surveillance biopsy. I would advocate against doing any surveillance biopsy because we already know it's bad data. So now you get bad data that makes you get cell-free DNA, or maybe not. Or you start treating someone based on histology, which we know isn't very good. So, I have a problem with that.

Proximity to pulse. I'm not sure that that matters. Maybe the other panelists know something about that. I already talked about the drug toxicity.

Dr. Angella Charnot-Katsikas:
Can I ask a follow up question to that? So, this is Dr. Katsikas, you mentioned protocol biopsy and the evidence surrounding that. So, so, can you talk about that for a moment?

Because, you know, if some of these tests are used to preclude the need for biopsy in the surveillance setting or in the for-cause setting, then, you know, it begs to question, certainly, how, how useful are these protocol biopsies to begin with, and how informative then are these tests at the at the time point?

Dr. Daniel Brennan:
Well, I think the proof is in the practice, and I'll let the other panelists talk about this.

But protocol surveillance, biopsies used to be done by a lot of programs, and then I think it's as they got more experience and more complications they decided, maybe that's not such a good idea. And so, maybe this, and the cell-free DNA came along not too long after that. And I think most programs have felt that this is a better way to do that.

I mean, we looked at creatinine, which we all know is not a very good marker, but it's cheap, it's easy, and we're in love with it.

As opposed to, if you are putting bronchoscope down someone, that's not, that's not cheap, that's not easy, 50% of the specimens they don't even get about an adequate specimen.

So, it's just, programs that are thinking, forward thinking programs in lung are getting rid of their protocol biopsy. Similarly in heart, you damage the tricuspid valve, I mean, it's inconvenient and who can read, really read a heart biopsy.

So, I know Stanford has pretty much gotten away from protocol, cardiac catheterization and endomyocardial biopsies. With liver, we'll talk about liver a little bit, but, with Kidney, um, I mean, as I said, if this costs a dollar, everyone would be using it all the time.

It doesn't cost a dollar, and it might not be going to the patient and might not be coming out of my pocket, but we are all members of society, and it just bothers us to use spending $2800 a pop to get this information. Until you biopsy someone and they have a seven unit bleed, then you think, shouldn't spend $2800.

So, I let the others comment on what they, what they think about that.

Dr. Anitra Graves:
Yes, I would, I would love to hear the other panelists' opinions regarding this, this particular area of discussion. This surveillance versus for-cause and the differences in center use of surveillance versus only biopsy for-cause and how these molecular tests play a role in the differences between the centers.

Dr. Huang I think I saw you?

Dr. Edmund Huang:
Yeah, I think largely, I would agree with Dan and what he's saying about protocol biopsies and that the vast majority of transplant centers don't do it, at least kidney transplant centers for the reasons that he described. On the other hand, you know, there is data that does, indicate or suggest, that a rejection found in protocol biopsy may lead to things like de novo DSA later on. It's not as conclusive that if you find a rejection, you treat it on protocol biopsy that you're going to improve graft survival, so that's one issue. But, because there's conflicting data and so I think that given the effort that it takes, the risk that it takes, I think a lot of centers just don't do it.

Well, with regard to whether that means that we should, or we shouldn't, use these biomarkers for surveillance, I think that's a different question. That still at least the long-term data, in terms of whether or not if you use one of these surveillance tests and you find a rejection and you treat it, does that make an impact on long term survival? That has not been worked out.

But certainly, what you can see, at least from some data, is that although these tests are not going to find as many rejections as just doing a protocol biopsy in everyone. You're going to find more rejections than you would if you didn't do these biomarkers. So, there is probably some value to it in terms of whether it's going to impact long term outcome, but we still need to know that.

Dr. Anitra Graves:
OK, so, if I just want to have you expand on that?

How often would you do that, then? How often will you use these tests, if you also endorse the fact that protocol biopsy is probably less helpful?

Dr. Edmund Huang:
I wouldn't say protocol biopsies are less helpful. I think that there's just a lot that goes into it in terms of risks, and in terms of whether, or not, the question of whether, or not, you're going to improve graft survival. But, you know, I think that it's a lot more complex, or whether the question of whether you do it or you don't do it, I mean, some of the considerations should be, who do you do it in, right? Are you going to do it in a low-risk patient? Are you going to do it in a higher risk patient? Someone who has a positive cross-match, for example, and I think that there's probably more support for trying to consider these types of interventions or these types of surveillance protocols in people who are higher risk. Like, you know, like I said, like if

someone, who is sensitize, maybe, or certainly someone who has a positive crossmatch, you might consider it.

Acknowledging that we are, at least for us, I am in a program where we don't do protocol biopsies. But we do, do, surveillance, donor-specific antibodies, to give us some sense of whether, or not, how well the patient has immunosuppressed, whether there is antibody-mediated rejection, and if we do see antibodies that are either de novo or are persistent, we do follow those up with biopsies.

Dr. Obi Ekwenna:
Yeah, so I definitely agree with all the comments that have been made. One additional thing is that for kidneys, we are using, you know, kidneys that are at risk for DGF (delayed graft function). Yeah, my centers probably devoted to, you know, close to 50%. We import kidneys and we use, kidneys are, you know, what, the allocation system has changed. A lot of our kidneys have more than 25 hours of code time. And so, we're seeing a lot of DGF. But not every kidney that, you know, the recommendation is that, you know, if a patient has DGF you should biopsy those kidneys. And so, you know, you have, a lot of our patients are anti-coagulated, they're at risk for bleeding. So, we do not minimize the risk of a biopsy on these patients.

And so, if we have 50% of our patients, who have DGF and you know that means you're doing at least 200 biopsies, if you are a program that does 400 kidneys. And so, then, you look at the risk of that, and so we have a lot of centers that are using this, these tools, are able to decrease the number of biopsies and so that's, I think as Dr. Brennan had mentioned, you, know, you, have one of, these patients have bleeding or lose their kidney from a complication, you know, that the value, or the cost of this, of avoiding a biopsy when you have a negative predictive value of 95% or 90% on some of these tests, makes it worthwhile, in my opinion, and based on what we've seen.

We do survey patients with DSA very frequently, and as mentioned, sometimes the DSA goes up, and we biopsied patient and there's nothing, there's no rejection. And we know that some of these tests are better than DSA.

From, the evidence that, we have seen so far. Obviously, you know, with time we'll see if this translates to improve survival or, you know, improve long-term survival, I mean, you know, we, we're sort of being judged on one-year survival, know, what about, you know, maybe 10-year survival in these patients. Maybe we'll capture a few more rejections or a few more graft dysfunctions down the line with surveillance.

So yes, the data is still emerging for that, but I think in the immediate experience that most of us have, it shows that you can avoid, um, certain risks to patients, by using some of these tests.

Dr. Steve Potter:
This is Steve Potter. Can I weigh in? Can you guys hear me?

Dr. Anitra Graves:
Yes, Thank you. Yeah.

Dr. Steve Potter:
So, you know, I think cost is going to be a big issue, and we've seen clearly that some of these tests are, um, clearly able to outperform traditional noninvasive tests in diagnosing rejection.

So, we have some good data, particularly for AlloSure testing, that this is a better test than, looking at proteinuria or doing DSA and in fact, to follow on the last comment, we have some data that would argue that your AlloSure goes up before your de novo DSA develops and that's, that's intriguing, I think. So, the unknown is really how much do we save in dollars by prolonging renal allograft half-life? How effectively are we prolonging allograft half-life by earlier intervention for rejection? So, I think those are unanswered questions that will determine, you know, sort of where does this fall, and sorts a population health issue, but in terms of our toolbox, as transplant surgeons, we find that these emerging technologies are incredibly promising in the real-world right now are very, very useful.

Dr. Ali Zarrinpar:
This is the Ali Zarrinpar. I want to provide a slightly different perspective that is in agreement with pretty much what everyone says. But, 25 years ago, people thought, you know, putting a camera on a cell phone is ridiculous, and completely unnecessary, because not only are the, the images not good enough, but also, everyone has digital camera and you know, whatever. I think this technology here provides us the opportunity for repeated measurements of very specific allograft injury.

And I think we've largely focused, so far, on detecting rejection, and treating rejection, maybe early. But what we haven't really focused on is not seeing rejection when we're trying to alter immunosuppression. And I think that you know, we shouldn't forget that the drugs we give these patients, for immunosuppression themselves are never toxic. And in trying to save them from rejecting, we're actually injuring their kidney chronically.

So, having a tool that allows you to safely go down on immunosuppression and be, you know, relatively certain without biopsying it, all the time, that you are at a safe level of immunosuppression, allows you to actually prolong graft function and graft durability.

So, you know, I think that this technology really hasn't seen its full use yet. Yes, of course, we have to show evidence that, that, you know, it can detect rejection and whatnot. But I think that really, the future is going to be monitoring surveillance, not just for-cause, but surveillance of stable patients.

Dr. Angella Charnot-Katsikas:
So, I'd like to, if I may, kind of, maybe we've talked about a lot of this all, all at once and, you know, it sounds like there is a fairly general consensus, that you all have positive views, for the utility of a donor-derived cell-free DNA test and the monitoring of the transplant recipient. What we haven't really gotten into too much, because we've said a lot, and I think it's been a great introduction so far.

But in terms of the, as we said, you know, high-risk versus low-risk population. Those with DSA, those without DSA positivity, how this type of testing so the information garnered from a donor-derived cell-free DNA test would be used to either A. move toward biopsy or B. move away from biopsy.

So, if we could take the high-risk and low-risk group and then talk about, what would, how this information could be used, again to move forward and move away from biopsy? I think that would be a very helpful approach toward, our further understanding of how you use these and what the evidence provides. What the evidence shows for the utility of these cell-free DNA tests and later, we'll get into the other types of tests as well, because we haven't really touched on the gene expression profiles yet. But, but I would like us to focus, as we have on the cell-free DNA test.

Dr. Edmund Huang:
That's a good question.

I think, when you look at how diagnostic tests work, the sensitivity and specificity should be the same across contexts. That's, that's true representation of the test characteristics.

So, whether you test it in Johns Hopkins or Cedars-Sinai or some other program, it's gonna perform similarly, at least in terms of sensitivity and specificity.

What's different in terms of what you're going to get out of that test, is based on whether that risk is high or low. So, meaning that your positive and negative predictive value will be influenced by the patient mix.

And so, in the end of the day, if you're testing primarily, a low-risk population, highly unlikely to have rejection, rejection prevalence is very low, you're mostly going to get negative tests, and then, the overall value of that test will be lower.

If you're testing in a group where the rejection prevalence is high and, you know, and you have reasonable sensitivity and specificity, you're going to have a lot more yield.

So, in terms of whether or not it can or cannot be used in these populations, the answer is, yes. I mean, the sensitivity and specificity should not change. But, what you're going to get out of it and what actionable events are you going to get learned from using these tests is going to affect, be affected by how much rejection you're going to expect in that population.

Dr. Angella Charnot-Katsikas:
Perfect. Thank you. Would our other panelists like to discuss this issue?

Dr. Steve Potter:
Yes, this is Steve, I would, you know, you asked a really profound question, and I think there's really a couple of questions in there. So, if, to focus on, can we avoid a significant proportion of that renal biopsies that we currently attain using cell-free DNA, I think that we can, I think, there is some evidence from the papers by view and the paper by Bromberg, that, you know, if

you have a stable population, your cell-free DNA levels can be very low and that affords you a very high negative predictive value.

So, in that general population, not a subset of high-risk patients, we have pretty good data that that we can successfully and safely forgo a lot of biopsies.

Dr. Anitra Graves:
Thanks for that and before I advance to a polling question, I wanted to make sure that I took the time to introduce Dr. Potter as well as Dr. Zarrinpar, as they were a little bit delayed.

If you both could just introduce yourselves and provide your disclosures as we don't have that slide up, I would really appreciate it.

Dr. Steve Potter:
Sure. Hi. I'm Steve Potter. I'm an abdominal transplant surgeon at Medstar Georgetown Transplant Institute in Washington, DC. I do kidney and kidney/pancreas transplants.

I am a consultant for CareDX and that is my only disclosure.

Dr Ali Zarrinpar:
I am Ali Zarrinpar and I am surgeon at the University of Florida.
I, my disclosures, I've gotten ASTS grants that are sponsored by Natera.

Natera and CareDx have also worked with me on the investigator-initiated trials and I've given a talk on behalf of CareDx at ATC.

Dr. Anitra Graves:
Alright, Thank you very much.

So, we're going to go on to the first poll question, so that we can get through this body of questions that we have. The first poll question, now I want to make sure the panelists are ready to enter into their answer.

The first poll question: Is there sufficient evidence on the clinical contexts in which the molecular test diagnostic test could be used? And this applies separately to AlloMap kidney, AlloSure kidney, Prospera, Viracor TRAC, QSant, kSORT, TruGraf, and OmniGraf.

Again, that question is, is there sufficient evidence on the clinical context in which the molecular diagnostic tests could be used?

And while you are entering your and thinking about your answers, I'm going to just quickly go through a couple of slides that we prepared to tickle your memory.

Next slide.

This is a quick overview of some of the selected literature that we have on AlloMap. Actually, for AlloMap, we identified two studies that were published and the one issue that I'd like to point out is that the patients in these studies, were typically patients that were coming in for

biopsy. They were paired with a kidney biopsy specimen. A lot of these studies were validation studies and in the 2021 study, these were a source of patients from the Dart study. But there were a lot of for-cause testing as opposed to surveillance. So that's for AlloMap.

Next slide, for AlloSure.

We also have a patient population description for AlloSure and in these patients there were patients excluded that had delayed graft function as well as calcineurin inhibitor toxicity so that it's not a population that this particular group of studies also excluded patients with pregnancy, transplanted organs with other kidney, so maybe kidney/liver type transplants. The other limitations that were associated with the results. In other words, those patient populations were excluded from the studies, just to make you aware.

Next slide.

Again, Prospera also had some exclusions. This would really mostly pertain to patients that were pregnant or less than two weeks post-transplant. So, there were some additional considerations regarding the patient population that they felt appropriate to test.

Next slide.

This is also the other thing for kSORT, just to jog your memory. Again, there were, these, are mostly patients that were stable, so in our discussions regarding cause or for-cause. This particular test is obviously very heavily studied in surveillance or stable patients, as opposed to those that are, that were for-cause, and they definitely combined transplants.

Next slide.

QSant really focused on really real-world evidence, based on the literature that we saw, not only adults, but also pediatric testing, so they have, fairly broad population of adult renal transplant patients, that were used to validate the test.

Next slide.

TruGraf was interesting this particular publications tended to test folks that were greater than 90 days post-transplant, so a little bit further along.

Next slide.

And we'll just hold right here for a moment to allow the panelists to enter their answers.

Dr. Angella Charnot-Katsikas:
Also, I want to make sure, for our panelists, that as you are providing your polling responses, if there are points of discussion that you would like to highlight, as you see these questions, you are more than welcome to do so. So, please go ahead and you know, if there's a prompt here that you want to talk about. By all means, we do want to hear what you have to say.

Dr. Anitra Graves:

So, we'll go ahead and broadcast question number three and have a little discussion while they have a little bit more time to enter into their polling answers.

For question number three. The question is: in the existing evidence, what is the level of confidence or certainty regarding test performance data reported without any competence intervals?

I'm just going to again scan through our study slides

Next slide.

In respect to confidence intervals, we were impressed by the number of publications that published results, of test performance that did not have confidence intervals.

For AlloMap, this is one example, again, two publications, less than a lot of things tests that we are evaluating but as you can see, the confidence intervals are not recorded.

Next slide.

This is for AlloSure just to jog your memory. Again, some of the data is limited with respect to confidence intervals.

Next slide.

This is a study that does have pretty extensive reporting of confidence intervals, but there are also publications are more recent ones that did not include confidence intervals.

Next slide.

This is TruGraf, and in the table, there were two publications that did not report intervals, confidence intervals rather, and then, the trial that did the intervals are as displayed, a fairly broad display.

Next slide.

kSORT again, no confidence intervals are recorded. In some of the publications, there are two that we found confidence intervals corresponding to the data.

Next slide.

This is for QSant. We have one publication that had no confidence intervals. However, the other publication that was selected does.

Next slide.

And OmniGraf, this one was a little bit more limited with respect to the confidence intervals, as well. But they did include the performance statistics with confidence interval, for these, this is a combination test.

Next slide.

So, if you could, after jogging your memory a bit on these publications, if our panelists could enter into the record, the level of confidence regarding test performance were reported without confidence intervals for each of these tests, I would appreciate it. If you're available to comments for the audience, I would also appreciate just getting a window into your thoughts on this.

And it just occurred to me that maybe you do not assign value to confidence intervals, comments on that would also be appreciated.

Dr. Angella Charnot-Katsikas:
Yeah, definitely, more than just a poll, we want to have a discussion about some of these questions. Can we provide your feedback.

Dr. Daniel Brennan:
I don't think you assign valid confidence intervals to gene expression panels, right?

I mean, it's reads, I don't think they have a confidence interval.

Dr. Anitra Graves:
So, would that affect your level of confidence in the data reported?

Dr. Daniel Brennan:
Not really, it's just a different, it's a different type of data. I don't think it's amenable to confidence intervals. I may be wrong, but I don't think you do.

Dr. Edmund Huang:
I think you're referring to like, the sensitivity and specificity, and those kinds of performance characteristics, when you talk about confidence intervals, right not the expression of data?

Dr. Anitra Graves:
Yes, yes, exactly

Dr. Daniel Brennan:
So, so I'll tell you, I actually think the three tests, the three DNA tests are very similar. So, I think that the, the AlloSure has the best studies that have been performed, the original people involved with it, I mean, that the chief medical officer was a PhD. He is very rigorous about making sure things were done appropriately. And they all come from the same platform, from the Stanford Group, from De Vlaminck. So, I kind of give the other, the other companies, the benefit of the doubt, in terms of the confidence of intervals. And yet, we do see they are there are, there are some, some outliers. I mean Ed knows this, right? There are some real outliers. What do you do with that?

Dr. Angella Charnot-Katsikas:
Now can you be more specific?

Dr. Daniel Brennan:

If you look at the box and whisker plots that are published, you can see that you know that they've got the 25% and 75% confidence intervals and those are pretty tight. But then you've got some people that are way outside um, with high values, not so many low values and see, see unusual high values, and we see this clinically, I don't know what to do with them. And then they're doing fine. It's a test, and sometimes you have to put on your hat and try and interpret that, just as it's not a sodium.

Dr. Ali Zarrinpar:
I think the clinical context obviously matters, right? So yeah, like you said, it is exactly a test. You do with it, what you do.

Dr. Edmund Huang:
With respect to the question about confidence intervals, I do think they're important, right? That's just a standard way of statistical reporting, However, if you have multiple studies showing, the same thing, then your confidence, even in the absence of confidence interval, does increase but yeah, in general, I think confidence intervals are important.

Dr. Anitra Graves:
There was a comment that talked about how, um, the, at least, the cell-free DNA tests were similar, or you consider them to be similar. Can you speak a little bit about that because we did notice that the tests are, are different in terms of their description, the genes or the SNPs that are evaluated? Tell me what your opinion is regarding that, and also, if you could also comment on terms of the data and the performance, would you consider all of the tests similar, or assign the same performance for all test if only one of those tests demonstrated that performance, or are there differences that you've seen between these tests, specifically, the cell-free DNA?

Dr. Daniel Brennan:
Since I brought it up, I think that I think they are similar, like I said, they all start, they all licensed the same technology from Stanford. They all use SNPs, whether it's 231 or it's 13,000 SNPs, or 100,000 SNPs, talking to the molecular biologists that I know that doesn't really seem to matter. It doesn't add much value. So, I think that they're similar and we have done little internal studies looking at the different ones, and we don't see much of a difference between, at least between the Natera and the CareDx test. And then that was actually published, it was published by Keith Melancon. You know, they're trying to make a big deal about how quickly CareDx could get the results back. But the end, they are really similar. So, I didn't see much difference between them.

The gene expression panels is actually interesting to me because they seem to be very different from wherever you are in the country, whether you're in Chicago or you're in California, or you're in Canada. So, I'm a little bit, I haven't quite figured that out, that there's different genes go up in different regions of the country and different ways at different genes that go down. I haven't figured it out, but taken together they, the gene expression panels seemed to be giving a pattern that would, that, according to the people who developed the tests, are, are

associated with rejection and, okay, yeah, I don't think the answer is, I'm not as convinced with that.

Dr. Angella Charnot-Katsikas:
We haven't really talked about the gene expression profiles yet, and I'm hoping we can have a, you know, we need to talk about the three categories of tests, right? And, four, in some cases, four categories of tests. We have our donor-derived cell-free DNA group, we have our gene expression profile group, we have sort of another group where it's sort of these multi analyte tests, right? And then, we have, also, combination testing, right? Where, where we've got the donor-derived cell-free DNA in combination with the gene expression profile.

So, if we could talk about the gene expression profile a little bit, and the utility of those tests, first, for their own value but, second, in combination with the donor-derived cell-free DNA tests. I think this seems like it's a, it's a good time in our discussion to do, to elaborate a little bit further on this point.

This is really for any of our SMEs.

Dr. Daniel Brennan:
I didn't want to hog the show. But, so, the gene expression panel, the AlloMap, for example, is a kidney set, now focused gene expression panel, that came out of the hearts.

The hearts was really good, it was, it was helpful to try and discriminate rejection from not, or quiescence from non-rejection, it looks like that paperwork, but I haven't seen it being used clinically with that. The TruGraf or OmniGraf gene expression panel, is, again, out of Northwestern, Dan Salomon's kind of work. You have to be a little bit suspect about the centers that develop the data that were used. And John Friedewald makes a big deal about borderline rejection. I think, I think he's leading the transplant lemmings off the borderline rejection cliff, because I think that, I don’t know, there's a reason it's called borderline.

And so, I'm just worried about over interpreting that. I think we can not talk about QSant and kSORT, I don't think they're going to, they're seeing the light of day. So, I learned, I wouldn't waste a lot of time with them. I understand that the company QSant has gone under and that was Minnie Sarwal’s and she'd been part of kSORT and, I, believe, many people have tried to replicate kSORT data and have not been successful. So, I think we can not spend much time talking about them, and I’ll let others talk now.

Dr. Edmund Huang:
Yeah, I agree with Dan I mean, it basically comes down to TruGraf and OmniGraf. I think, if you’re going to talk about gene expression, you, know, I think that the data is, is promising. But we do need to realize as primarily done out of the same biobank. There hasn’t been a lot of independent validation with it and which I haven’t seen any of that data. I think that they are involved in registry studies now. Trying to acquire and, and, and look at data in a larger, more multi-centered type of cohort. But, as of now, you know, you do have primarily just a handful of papers that are really looking at the same dataset on these, on these tests. With that said, I

mean, there is some promise. I think they do you do see, you know, that there is some value there particularly, when you look at the OmniGraf paper and you compare the gene expression to the cell-free DNA, you can see that both of the test's kind of look at different things, right?

We've known that cell-free DNA can be limited in how well it picks up TCMR and it turns out the gene expression might be better at that. And vice versa, where the gene expression might not be as good for antibody rejection, but cell-free DNA may be better for that, too.

So, the two types seem to work better together. But in terms of whether it is going to have, it still needs a lot more, I think, multi-centered validation.

Dr. Steve Potter:
Yes, this is Steve, I think Dan put it much more bluntly and boldly than most would have. But I think he's completely accurate in his description of some of the entrance here and then in this space, in broad strokes. It's pretty clear that the cell-free DNA is just further along, the development pathway, it has more robust data. Um, and, as we think other folks have pointed out, I think, AlloSure, the data is the most robust, but these are all promising.

So, I think we'll, just, kind of, earlier in the process for some of the multi-modality testing and knowing where they're going fit in the market and how useful they are going to be. It's also concerning that some of them, we haven't really seen that those data that they are promising are generalizable to the whole population of the country.

Dr. Ali Zarrinpar:
Think one thing that hasn't been said, I agree with what everyone else has said so far is that the timing and sort of the timeline of when a cell-free DNA essay is abnormal versus a transcriptional assay is abnormal, they are very different. So, they're really measuring different timeframes. The half-life of this of the changes in the signal are different. And so, you know, I think some of that may have to do with the behavior of the approach.

Dr. Anitra Graves:
[Inaudible]

Yes. Let's get to question four.

Another, another high hot button issue, is there sufficient evidence to support the utility of surveillance testing in kidney transplant recipients, and, if yes, what is the appropriate testing schedule?

Dr. Steve Potter:
This is Steve, I'll take a stab at that. I mean, I think the best way to look at it is, sort of, this isn't augment what we've been doing but a better a more sensitive and better way to it to surveil.

So, we should be doing this with some, regular frequency, whether that's, you know, at month 1, 2, and 3 and then quarterly or months you know monthly for the first four months, I mean I don't know.

I think that we need more data, and we need more time to sort that out, and see what the practice patterns are, and understand the cost benefit, because, again, the societal cost is going to be very large, but if we get it right, then the societal benefits can be enormous.

In terms of further out, I don't know. I think, I think that, you know, that we should be testing at some frequency, particularly with cell-free DNA.

I don't know where gene expression profile is going to end up in that sort of spectrum of a normal surveillance of stable kidney patients.

Dr. Daniel Brennan:
So, in my responses for surveillance, I basically said there's a lack of evidence for anything. But I think that that has to be nuanced a little bit.

I think there's evidence for it being useful. The problem is, is it worth it?

So, I think, for the AlloSure test, for example, that there was a, the admiral study, shows that it shows what the cell-free DNA went up, an average of about three months before there is a clinical event, associated with injury, whether that was rejection or something else.

But the percentage of people that had had such an event, were, like, less than 10%. So that means 90% of people are undergoing multiple testing at significant cost, with generating false positives, that might cause you to react, and be associated with cost and convenience, and maybe harm. So, I think that, yes, it, there is evidence that it is, it could be used, but it hinges on the word utility, what, what is it, so is it useful? It depends on what you're looking to use it for.

Dr. Ali Zarrinpar
I'd like to push back on that a little bit. So not everyone who's under immunosuppressed is going reject, right? And not everyone who has subclinical rejection, is going to, sort of have that glowing sort of biopsy.

So it may be that those, were real, but they stayed sort of in that, in that sort of less fulminant state, and maybe having that information would allow clinicians to adjust immunosuppression, and not necessarily go, you know, not raise the fire alarm, but rather actually have a more subtle response to those changes.

Dr. Anitra Graves:
So, would you suggest we, at this point, we're still experimenting, I mean, is there any evidence? When I when we talked about utility, at the top of the hour, we suggested that that utility be a signified by some patient-centered outcomes such as, you know, graft survival, even decrease biopsies. Is there any evidence, to your knowledge, that has been published to indicate that any type of patient-centered outcome has improved as a result of surveillance testing?

Dr. Daniel Brennan:

I am going to amend my prior statement on what Ali said, I think he's right.

So, what we, if we could define a true group, who's at risk, and there are such people such as those who we transplant despite knowing they have a positive anti-donor donor specific antibody, not high PRA. That doesn't matter. We do it anyways. We know they have that. That would be someone that you probably want to surveil with this.

So, there are such individuals that it would be useful. So, I would like this to be available for at least that and maybe other reasons as we learn more about it. I think we are going to learn more, in the real world, that will probably come with real-world experience, outside of large trials.

Dr. Ali Zarrinpar:
The question you're asking in terms of the "reals" or clinical outcome, it's probably, it's probably too early. Not just too early in the technology but too early for us to know what 3-, 5-, 10-year outcomes are going to be given with this approach.

Dr. Edmund Huang:
There was a paper on protocol biopsies from the Paris Transplant Group, Alex Loupy, where they looked at, I think it was a 12-month surveillance biopsy and the findings of antibody-mediated rejection at 12 months was associated with worse graft survival longer term. That association wasn't seen with TCMR but that goes to underscore what Dan Brennan's point was there. There is there are certain patient populations that it is helpful, right, in, particularly those antibody-mediated rejection patients. That it is helpful to do some form of surveillance.

Now, I want to look back at, the there is, that Park paper that was the one OmniGraf paper in CJASN. They only looked at protocol biopsies. They looked at performance with gene expression alone, cell-free DNA alone, and the combination.

In each of these, for gene expression and for cell-free DNA, the sensitivity was about 40%. What you might say is not that great, right? That you're only picking up, you know, you're getting false negatives about 60% of the time. On the other hand, if your comparison here is not rejection, but your comparison is to how many rejections you're going to pick up compared to not doing a biopsy alone, at least you're picking up a certain number of rejections that you wouldn't have done if you didn't send the test.

So, it all comes down to who is the patient, who are the patients who are at greatest risk, where the biggest bang for your buck, and can tests potentially help you to find the sub-clinical rejections if the risk is high enough.

Dr. Anitra Graves:
Great. That's interesting.

So, no one has commented, but I will ask some of our SMEs to comment regarding the testing schedule and those of you that have suggested that that there is utility, although, has not yet

been published, or we're not yet ready to commit to that outcome in public literature, or what would you propose as a testing schedule?

Someone's already commented that the first year is a little bit different from the years post, 1-year status post-transplant. But talk to us a little bit about what the testing schedule might look like in that select patient population that you referred to.

Dr. Edmund Huang:
I mean, if you want to stick to the evidence, I mean, there is no direct evidence to say that a specific testing schedule should be endorsed. But, yes, I mean, I think it would probably, at this point, be center-specific, but in terms of being supported by evidence, there's no specific data there to tell you.

Dr. Steve Potter:
Yeah, I mean, which, I think, is kind of, exactly right, and the points that I raised earlier is we really don't know and the problem that you have from a population standpoint is, the majority of transplant programs are using this promising technology. So, the, the cart is getting out a little bit in front of the horse. You're going to be seeing very widespread adoption without knowing, ultimately, what's the impact on long term allograft survival, which I think is, you know really the big issue that we need to address, but we do know this is better at doing certain things, than what there are sort of legacy platforms. And so, the best guess that I can come up with, that makes sense, is based on the limited extent data, like, for example, what was done in DART?

You know, what testing frequency should you use? The other thing to bear in mind, independence of population like it's true if it's a low-risk or a high-risk population, the important thing is we do know that these are important to obtain longitudinally and so we can see the change over time, in the, and again, I'm talking, I'm not talking about GEP, I'm talking about cell-free DNA. But we want to be able to see the relative change value over time. That's very useful on our clinical decision making, so that would speak towards some regular frequency of surveillance, rather just one and done. Thanks.

Dr. Ali Zarrinpar:
I think the easy answer is it should be more than surveillance biopsies, and that's very center-specific, obviously, as mentioned.

Dr. Anitra Graves:
Yeah, so let's talk about that, because we, at the top of the hour, someone had indicated that only 18% of the centers do that, so, so, how would that affect your opinion? With respect to scheduling, specifically.

Dr. Ali Zarrinpar:
Ours, center is always the best center, so says, everyone, right?

We always do the best method of surveillance. So, I think it's going to have to be very, you know, it just ends up being very center-specific. By and large, because center’s practices in

terms of who gets transplanted are divergent. You know, not everyone does the same thing. Immunosuppression protocols aren't the same and surveillance protocols aren't the same.

Dr. Daniel Brennan:
So, we've tied ours in when we would do DSA testing, but as I said, I'm beginning to think we should stop doing DSA testing and then, if we have an abnormal, donor-derived cell-free DNA, then we should get the DSA rather than what has been, historically been, the other way around. Because, as I said, 50% of the DSAs are not clinically meaningful. I don't know that we can say that 50% of donor-derived cell-free DNA, above 1% are not meaningful. I'm not, just not sure of that. So, the typical surveillance would be at one month, three months, six months, nine, and twelve.

And whether you should do long-term, then that's also, a little bit similar to what we actually do 1 through 6 months testing for BK. Because we do have good data that BK comes up in the first three months. We want to get the stragglers when you don't need to start it before a month. So, we're trying to make it, at the same time that we do our donor-specific antibody or BK testing, but as Ali said, about, how often do people do protocol biopsies, surveillance biopsies, it's changed, but I would say it would have been early on people would get them really at 3 and 6 months and a year and that, I think most people see that that's too much.

When we do studies, we do them usually at six months a year, and a year and maybe two years.

Dr. Angella Charnot-Katsikas:
It seems that the field is somewhat not united in the utility of protocol biopsy, and we talked about that a little bit earlier in our discussion. I know, you've all have expressed your opinion on that, and yet, there are, of course, many centers that are performing protocol biopsies.

So, you know, you can see where there are some questions related to the use of a noninvasive test in the setting of an institution that may be performing protocol biopsies versus an institution that does not, and how those may differ.

Now, I also want to just touch on and hopefully we can elaborate on the timeframe that you all discussed regarding a testing schedule for the cell-free DNA test, and, you know, in some respects it seemed that it was it was hinged on the timeframe of protocol biopsy. So, if you could clarify that a little bit better, that would be helpful. Um, I know you did say some of those schedules are influenced by the timing of other testing? That's, like, BK virus testing and things like that, but, if you could elaborate a little bit further, that would be very helpful. And, at the end of the day, what we want to understand is, yes, how is this being used? But, but, you know, the evidence that supports that use is critical. The evidence from the literature.

This is really open to any of our SMEs, so hopefully, you're not on mute, you can unmute yourself.

Dr. Daniel Brennan:
Hi, guys. Help me out. I'm always talking.

Dr. Edmund Huang:
I think, at least for me, it's hard to answer, because I think we've already touched on that, that, I don't think, at least, if it's going to be supported by evidence, that there isn't that support. But I do think it's probably reasonable to, if you were going to use it, to use it at a time where you would be considering doing things like DSA or in other programs where they would be doing protocol biopsies. So, at least in my mind, something like at six months or 12 months, but that's not supported by data whatsoever.

Dr. Steve Potter:
So, I mean, you're asking a difficult question because to me, what's the data if ask us, what's the data for the frequencies for which you should obtain a serum creatinine? It's, it's more about global practice patterns than it is about data to support it, right? It's all based on guidelines, but how do we really know? And, so, if you look at the literature, we, I think the paper by Bu and the paper by Gupta both useful in showing us that we can identify rejection that we are otherwise would have missed, and so there, you know, and that's a useful piece of information, because those are, for the most part rejections, that are valuable to treat, that benefit patients. But ultimately, we don't know what frequency we should be obtaining surveillance molecular testing and it's to be determined.

Dr. Anitra Graves:
Excellent. I'm going to move us along with the question five, I'm trying very hard to get us through all these questions. Actually, 5 and 6, if we can open, those for our SMEs, that would be helpful. They really are very similar.

So, the question 5 is, is there sufficient evidence on the ability of the molecular diagnostic tests or combination of tests, as in the case of OmniGraf, to discriminate acute T-cell-mediated rejection from quiescence? Would anyone like to comment on that?

So, five is looking at the ability to discriminate acute T cell-mediated rejection from quiescence and then six is specific to the T-cell immediate rejection from the quiescence, and these tests, based on our discussions earlier, they seem to have different abilities to pick up these specific types of rejection, let alone, discern between the two. So, can we have any comments on that?

Dr. Edmund Huang:
The tests are generally non-specific, and they couldn't be used alone to be able to distinguish between T-cell mediated rejection and quiescence or antibody-mediated rejection in quiescence. There is some data that would indicate at least for cell-free DNA if you use it in conjunction with the DSA, it's more likely to be antibody rejection, but even in that case, you still can't rule out that there's a mixed rejection there. So, in the end of the day, you probably going to still need, you're definitely going to still need, to do a biopsy in order to know what rejection you're dealing with.

Dr. Ali Zarrinpar:
Yeah but, I think the question isn't ABMR versus TCMR it's ABMR versus quiescence.

Dr. Huang:
Right, and you can't even say that either, because if you get an elevated test and you're not going to know whether that's quiescence, TCMR or ABMR, so you can't, you just can't know. You can use the test result to tell you this is antibody rejection, or this is T-cell mediated rejection, at least when they used alone.

Dr. Obi Ekwenna:
You will need a biopsy, still.

Dr. Daniel Brennan:
I think that's what it is. I mean, it can help you to know when it's quiescent. But if it's elevated, you gotta figure out why.

Dr. Ali Zarrinpar:
Yeah, I think the question is a little weird, right? So, the question is, ABMR versus quiescence, you can know that it's not quiescent, if it's, the test is elevated.

Dr. Steve Potter:
That's, well, that's well said. Exactly, so, like, if I if I get a level of 4%, I have a very high positive predictive value that there ABMR. But I'm not going to treat a ABMR without a tissue diagnosis and I'm not going to know if there's concomitant TCM. So, right? So, I think that's exactly right.

Dr. Ali Zarrinpar:
Yeah, the question is a little odd. Because if you had just the population of ABMR versus quiescent patients, you should, you would be able to tell.

But because you're throwing in this third group, you know, whatever, right, or however many other groups, you cannot tell between the other groups, but you could definitely tell them from quiescence.

So, I think the question is just, it's hard to know what you're trying to get at.

Dr. Daniel Brennan:
So, if it were quiescence from non-quiescence, that would be an easier question to answer.

Dr. Ali Zarrinpar:
Yes, absolutely.

Dr. Edmund Huang:
Yet, we are still dealing with sensitivities in the 60% range. So, in other words, there are false negatives. So, this question to see you can't say unequivocally can distinguish this from quiescence, because probably on average about 40% of time its false negative, and that's primarily because of TCMR, at least in cell-free DNA.

Dr. Daniel Brennan:
And yet, they're all better than biopsy.

Dr. Angella Charnot-Katsikas:
So, this is kind of what we're getting at, right so I think, you know, you all alluded to it earlier, and we certainly can appreciate that, you know, these are lab tests, and they are performed in a larger clinical context of the patient's care and so, you know, again, kind of going back to where we were prior, you know, a little bit earlier in the call, regarding moving the needle toward biopsy or away from biopsy or moving the needle toward a reduction of immunosuppression versus not. I think those are some critical decision points that we would like to explore in, you know, the remaining, some of the remaining time that we have.

I'll stop there and let y'all address this again. I know, we've kind of talked about, you know, higher risk patients, perhaps, patients with DSA versus not and others, but again, the sort of utility of value of a donor-derived cell-free DNA value in moving that needle, and we talked a little bit also about threshold, but also about trending, you know, we didn't really get into trending too much. So, would anyone like to address that?

Dr. Edmund Huang:
Going back to the earlier part of the question, at least, this is how I would think of this. Yes, you can distinguish quiescence if, if it's low risk, if the likelihood of rejection is generally pretty low, anyway, and you get a reassuring test that, in that context, negative predictive value is high, it's very likely to be quiescent.

But on the flip side, if you have a high-risk patient, creatinine is going up, drug levels are low and you get a negative test or a low-level cell-free DNA, for example, you probably should still biopsy them because the likelihood of a false negative is going to be higher.

So, it does depend on which context you're in to determine your level of confidence of quiescence.

Dr. Angella Charnot-Katsikas:
Yup. Thank you. That's very helpful.

Dr. Ali Zarrinpar:
That context also will feed into your why treat ABMR versus TCMR?

Right. So, you don't necessarily always need to biopsy, would you?

Dr. Edmund Huang:
I would agree with you on that, too. Like, for example, antibody rejection often times, we don't see that necessarily resolve histologically and so if we start on treatment, for us, we tend to end up transitioning to more chronic antibody rejection therapy, is like basically giving people ongoing therapies with different agents. I mean in that way, if I see that in a cell-free DNA is persistently elevated, I'm not going to keep biopsying the patient. I'd probably pretty much assume that the rejection is still persistent.

Dr. Obi Ekwenna:

You may have some more confidence if the cell-free DNA is resolved, is decreasing, what you're doing is working or that the kidney, maybe, is not going to recover.

Dr. Edmund Huang:
Potentially, but at least in antibody rejection that doesn't usually happen, though. It's usually persistent.

Dr. Daniel Brennan:
I've been amazed at how the cell-free DNA doesn't go down for a long time in either T-cell mediated or antibody mediated rejection, if at all, and it begs to question of really, how often do we really reverse this thing and, what, what is the measure for it?

I know that Mayo will do a follow-up biopsy 2 to 3 weeks after they've treated someone for a rejection. I think that's an unusual approach and, but I've seen biopsies, where you'll get a reading that says consistent with resolving rejection, whatever that means.

Dr. Edmund Huang:
Right.

Dr. Obi Ekwenna:
I think that data is still, um, you know pending for the utility of cell-free DNA, in terms of monitoring, or you know, in terms of monitoring treatment after rejection.

We have anecdotal evidence within our center, but not obviously published data, where you see resolution with the cell-free DNA sort of declining. So, that is still pending you know validation in other centers, but I think the utilities is there.

Dr. Anitra Graves:
Fabulous. We're going to move onto to question 7.

We have 7A and 7B that we would like to get your responses for. This is the idea of thresholds. And we want to know, are there currently published threshold or cutoffs affected by the time post kidney transplant? In other words, if you if you do think that they are affected by the time post kidney transplant, how will that affect how you interpret or use these tests, and, if not, indicate that in your comments?

Dr. Angella Charnot-Katsikas:
I was just going to say, if there are tests that you just don't know that that answer for; cause you're not as familiar with the literature, or with the, you know, your personal experience with utility, please do include that. You do have that ability to free text here.

Dr. Anitra Graves:
I was just going to say, let's go to the next slide, because I will give you a few ticklers again. We have some slides with cut points.

Here's AlloSure, we've got publications for both .5 and 1.0, and we know that SMEs, I'm sure, are quite familiar, that based on those thresholds, there are different performance related to that. Next slide.

Prospera. Looks like they have been pretty consistent on the threshold that they've been publishing. However, they certainly have published different thresholds or published the perform data, which is different thresholds, which may influence when, if these thresholds change based on how long post-transplant the patient is, this may influence you. Next slide.

kSORT also has different thresholds. This one's interesting, because I'm not sure what happens if the level is 8 but regardless, this is the published threshold for that test. Next slide.

I am going to go quickly because kSORT and QSant as you recall, you are less confident, in these tests but that is the threshold for QSant. Next slide

TruGraf, Next slide

And we can go to 8, unless anyone has any more comments on that, or any comments on that, I should say.

Dr. Daniel Brennan:
Just where I think the gene expression panel said, that's a little strange, that question doesn't really work the donor-derived cell-free DNA, I mean, people on the panel have written about that and now it's changing. So, one was a powerful number. I think it still is, but .7 a good number, .5 is a good number. So, I think, I think, we're just learning more.

Dr. Steve Potter:
I think the briefest answer is, is yes.

Did we have enough data at this point to preclude biopsy in patients who have favorable test results?

Dr. Anitra Graves:
All right. We have our next question up.

This question is, is an interesting one question 8, actually 8 and 9 are related.

The question is, is there sufficient evidence to indicate that in patients without signs and symptoms of rejection the use of the molecular diagnostic test or combination tests as in OmniGraf, would preclude the need for a biopsy?

So, this first question is, would the use of these tests, or the results, possibly preclude the need or help avoid a kidney biopsy if the patient did not have signs and symptoms of injection? And then, the related question 9 is saying, but in a patient with signs and symptoms of rejection?

How would that affect their decision making for pursuing a biopsy?

Dr. Steve Potter:
Oh, I mean, it depends on what you mean by signs and symptoms of rejection, but if you have like, we've had patients with persistently elevated DSA, who, without the ability of having cell-free DNA, we would, we would repeatedly biopsy those patients, where we are now comfortable enough to avoid additional biopsies. So, that's, that's a reasonable, I think, statement with limited, sort of, it's an anecdotal statement, but it's a reasonable one.

Dr. Anitra Graves:
Anyone else?

Dr. Obi Ekwenna:
I agree with that statement.

Dr. Anitra Graves:
So, I just want to highlight, so you have indicated a specific patient population for that. What about surveillance? Since you've already identified that, really, surveillance biopsying is not done, more often than not.

Would, would you, if someone's coming in without signs and symptoms, would you, would you, and they have a positive molecular diagnostic test that indicates there may be rejection, would you do the biopsy in a patient that would not otherwise have had a biopsy without the availability of this data?

Dr. Obi Ekwenna:
So, yes. If the patient has an elevated cell-free DNA, and they have a normal creatinine, is that what you're asking?

Dr. Anitra Graves:
Yeah.

Dr. Obi Ekwenna:
Oh, ok elevated creatinine?

Dr. Anitra Graves:
Either, or, so, if they have, if they don't have any signs and symptoms of rejection. So that's, in the setting of, if they're totally, without any evidence, including an elevation in creatinine, would you, and they may have been scheduled for surveillance biopsy, would one role of these tests be to use them, and you cancel their biopsy.

Then the other alternative scenario is you have someone with signs and symptoms, and they, they, have an elevated creatinine, would you just use the molecular test, or would you go ahead and biopsy these patients?

Would you have a different approach depending on whether, or not, there are signs and symptoms of rejection present. You've already indicated that, you would monitor somebody on

an ongoing basis to avoid repeating biopsy, but I'm also interested in avoiding biopsy, or how would that affect your decision making with or without symptoms?

Dr. Obi Ekwenna:
So, with, the test definitely helps us avoid biopsies in the scenarios that you've mentioned. One, a patient comes in with an elevated cell-free DNA and their creatinine is elevated or they have symptoms, whatever those may be, that patient will get a biopsy in my center. Let's say that the patient has an elevated cell-free DNA and their creatinine is normal. I feel pretty confident in biopsying those patients as well. Because I feel like, you know, based on evidence, that, you know, there may be something going on with a kidney. Whether it's, you know, may be rejection, and may be BK, something is awry.

If the cell-free DNA is below the threshold, I feel pretty confident that the patient, if the patient has low cell-free, cell-free DNA, and they have an elevated creatinine, that becomes a judgement call. One will need to look at the clinical picture to determine whether a biopsy is indicated or not. So, those are those are those are my thoughts on that.

Dr. Edmund Huang:
For me, philosophically, if you're going to send a test, then you should act on those results. So, for example, if you send an AlloSure and it's high, and you decide not to biopsy, it begs the question of, why did you send it in the first place? So, I think, philosophically you should use the test.

The question is: who are you using it in? And then also that does depend on the patient mix, and it depends on which test you're talking about. For example, in the surveillance question where primarily I guess what you're asking is, can it replace a protocol biopsy? It depends on what kind of rejection, you're, you think that patient might have, and it also depends on which tests you're going to use. So, for example, I know that there's been a lot of talk about how TruGraf, the gene expression, can help us to avoid protocol biopsies and subclinical rejection. But most of these, that, that test was mostly validated on TCMR, I mean, most of the cases in those in that validation study where TCMR and much less so for antibody rejection and they have shown that that test is not as sensitive for picking up antibody rejection.

So, if you have somebody who has a positive crossmatch and you estimate that they're probably going to have about a 35% risk of antibody rejection, and you use TruGraf to decide not to biopsy them, that might not be the right decision. But if you're, on the other hand, if you're looking at a low-risk population that, you know, that, it's probably not at risk for antibody rejection, doesn't have DSAs, it's unsensitized and the rejection you're planning on seeing are probably going to be TCMR. Maybe TruGraf is a good test, right? Or at least as a better test. But then you would be arguing, well, if they're that low risk anyway, why are you doing the protocol biopsy in the first place?

So, there's a lot involved in thinking about how to answer this question. But, you know, the answer really is, it depends, right? It depends on a lot of things.

Dr. Anitra Graves:
That's helpful.

Dr. Angella Charnot-Katsikas:
Yeah, it really is helpful, and it sounds like you are, if I'm understanding you correctly, you know, because you did sort of single out TruGraf in this example, but, as we discussed a little bit earlier, and it sounds like, in terms of the various donor-derived cell-free DNA tests, that those are essentially used in very similar and overlapping ways. Whereas TruGraf, of course, is a different type of test and I do want, I'm hoping, that you can respond to that. But again, I also really want to highlight that if there are tests here, because we've really focused on certain tests and not others, if there are ones that you just really don't have enough information on to provide an opinion, you know, please do state that as well.

Dr. Edmund Huang:
Just, you know, I think all that TruGraf because that is a test that specifically has been looked at and marketed for surveillance for, you know, ruling out subclinical rejection. I think they've expanded their indications now but at least a lot of the validation has been on subclinical rejection.

Cell-free DNA? I mean, I think we extrapolate a lot of that data from the indication-experience to the protocol biopsy-experience.

I mean, there is data on protocol biopsy, but at the same time, I think that, I think, the same things I said about TruGraf are similar for cell-free DNA too. It also depends on, you know, how are you going to react to that test really depends on what the risk was and at least for me, if I think the patient is sufficiently low-risk for rejection, I usually don't send the test, because, number one, it's probably going to come back negative, number two, if it is positive, there's a high chance it's a false positive.

It goes back to why I was originally saying, if you're going to send it, you should act on it, but then you should decide who you're going to send it on first.

Dr. Anitra Graves:
Very helpful. I'm going to move on because these next two questions get to Angie's point about the fact that these tests are different and we want to have an understanding from your, your perspective of the literature, um, if there's a differential utility between classes of tests.

So, for question 12, specifically, we're interested in understanding how confident you are in the evidence that for the tests that are listed below, an elevation and donor derived cell-free DNA indicates that there is a peak T cell-mediated rejection. How confident are you in cell-free DNA tests can identify T cell-mediated rejection?

Dr. Steve Potter:
This is Steve, so I know for the cell-free DNA, I'm very confident that allograft injury is going on, it's the levels are elevated. But I don't have that same level of confidence to specifically say it's going to be TCMR and I don't know how you, at this state, current state of technology, how will

be able to do so. But that doesn't mean that that's not a valuable test, it can't dramatically help patient care.

Dr. Anitra Graves:
Anyone else?

Dr. Ali Zarrinpar:
I mean, I think that's it, that's a good summary.

Dr. Anitra Graves:
Next question. A similar question, what is your opinion in, your confidence in the gene expression, I'm sorry, next question, next slide.

How confident are you in the evidence that, for gene expression profile tests, that the tests can accurately indicate acute rejection?

Dr. Steve Potter:
I think the best evidence is, would be, I guess tomorrow's meeting because it's really about AlloMap Heart, and that that evidence is pretty good.

Dr. Anitra Graves:
As it relates to kidney specifically for these tests?

Dr. Steve Potter:
That's right. That's why I said that. And so, I think is promising technology, but the best extent of data is really for, it is, available in heart patients.

Dr. Anitra Graves:
And if you could enter your answers into the polling for 14, I would appreciate that and we're going to advance. We have a couple of slides for liver testing, and if you can advance to those slides, I would appreciate it.

So, there are two tests that we found that were in the literature published for use in liver transplant patients. That’s TruGraf and Viracor TRAC, again the different colors indicating the differences in the class of tests, with TruGraf being a gene expression classifier in here and Viracor TRAC, a cell-free DNA test. Next slide.

These are the highlights of the literature that we found and quite frankly we did not find very much. There were three publications in total as it applies to liver testing. So, we just wanted to get there, not polling questions here, but we just wanted to hear some of the opinion of our panelists, regarding the evidence, and whether, or not, the evidence is sufficient to indicate that these tests have a role in management of patients with transplants. Liver, I'm interested in your opinion, because it is apparently very different from kidney whereby surveillance biopsy is not typical, it's not part of the standard, typical standard of care for liver patients, at least that's how it's been portrayed in the literature. But some comments about that, and what your opinion of the literature regarding these tests’ utility might be at this point, if at all.

Dr. Ali Zarrinpar:
Yeah, so we've looked at liver and cell-free DNA and we find, and Levitsky is going to be upset when I say this, but there's zero advantage in measuring cell-free DNA, donor-derived cell-free DNA in liver versus AST/ALT/Alk Phos measurements.

The evidence for transcriptional readouts, I think, it needs to be a little bit better. But I don't think that, that it meets, you know, meets the threshold for being clinically applicable yet. But I can tell you, in our experience, there's a direct correlation between donor-derived cell-free DNA and measures of liver injury, AST/ALT/Alk Phos, that you can get within an hour, multiple times a day, for cheap.

Dr. Anitra Graves:
Extremely helpful. Thank you for that. Any, any other comments? We are getting really past time, but any closing comments that any of the panelists might have, something we may not have touched on. I really appreciate that we've touched on a lot here, and we have been very disciplined through these hours. Any other comments?

Dr. Ali Zarrinpar:
Thanks for having us.

Dr. Potter:
Yeah, Thanks for having us.

Dr. Angella Charnot-Katsikas:
Well, we very much thank you and appreciate your participation.

Dr. Anitra Graves:
Yes, absolutely, very educational for me.

Personally, I've learned a lot from you, and I think that all of the audience would agree that these types of meetings are, are extremely valuable in helping contractors to understand, A. what is happening in, in the world of transplant testing and how you see these tests, those of you who actually take care of these patients. So, thank you so much. I know you don't have a lot of bandwidth, but we really appreciate you being with us this afternoon.

Again, we will be posting the results or the recording of this meeting on our websites, as well as a transcription. Thank you so much again and have a great evening.

Dr. Edmund Huang:
Thanks a lot.

Dr. Obi Ekwenna:
Thank you.