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**Jocelyn Fernandez:**
All right. Good morning, everyone.
My name is Jocelyn Fernandez, and I am one of the Medical Policy Specialists 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 panelists and meeting facilitators. The chat feature within this meeting is used for technical issues only any questions to topic discussed today will not be acknowledged. Please send any comments or questions 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 might impact the quality of our recording, and for our attendees to hear the comments being made. During introductions indicate any conflicts of interest 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 is 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 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 experience technical difficulties during the polling process, the polls will remain open for up to one hour after the meeting ends. 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:**
Wonderful, Thank you. Welcome everyone. We're going to just jump right into this. I am joined by Angella Charnot-Katsikas is a Contractor Medical Director with Palmetto GBA, who is collaborating with us for this CAC as part of the MolDX program. We are, Noridian, is a participant in the MolDX program, and therefore, as this related policy falls under their scope of work, they have collaborated with us on this on this meeting.

So, let's get into the agenda. We've already had some welcome remarks. I'm going to introduce the CAC panelists. I'm going to provide a brief overview of the CAC process, which is a little bit different than it has been traditionally in the past. We'll discuss the evidence along with the clinical validity and utility, and then we'll end with next steps.

So, we're going to have that slide up with the CAC panelists names. If you could please identify yourself and indicate whatever conflict of interests you may have in the order your name appears on the slide, Thank you.

Dr. Mandrekar?

**Dr. Mandrekar:**
Hi. My name is Jay Mandrekar. I am a biostatistician and professor of neurology at Mayo Clinic in Rochester, Minnesota. I generally work in the studies from departments of neurology, divisions of infectious diseases and clinical microbiology and radiology, and I have been at Mayo Clinic for the past 10 years.

**Dr. Anitra Graves**:
Thank you. If you're not speaking, if you could please mute your line.

**Dr. Palak Shah:**
Hi, everyone. My name is Palak Shah, I am a heart failure/transplant cardiologists and I run our cardiac genetics program at the Inova Heart and Vascular Institute, and I'm associate professor at George Washington University.

**Dr. Anitra Graves:**
Thank you. Dr. Agbor-Enoh?

**Dr. Agbor-Enoh:**
Hi, I am Sean Agbor-Enoh. I have two positions. First one, I am the Laboratory Chief of NHLBI of Applied Precision Omics and I am also the lead investigator of the Genomic Research Alliance for Transplantation, which is a NIH collaborative, and I am scheduled to work on cell-free DNA, and I am also an Assistant Professor of Medicine.

**Dr. Anitra Graves:**
Thank you, Dr. Hall?

**Dr. Shelley Hall:**
Hi, I'm Shelley Hall. I am the Chief of Transplant.

**Dr. Agbor-Enoh:**
Is this where we state conflict? Or someplace else?

**Dr. Anitra Graves:**
Yes. You may state your conflict. Can you, can you hear me, OK?

**Dr. Agbor-Enoh:**
I can hear you now, correct?

**Dr. Anitra Graves:**
OK, you may state your conflict.

**Dr. Agbor-Enoh:**
Yes. For conflict, I do not receive financial contributions from any company. However, I am, or have been, lead investigators for studies that are co-sponsored by the federal government and cell-free DNA companies. Thank you.

**Dr. Anitra Graves:**
Thank you, Dr. Hall, I apologize, I skipped over Dr. Khush.

**Dr. Kiran Khush:**
Hi, I'm Kiran Khush, I'm a Professor of Medicine at Stanford University and I'm an Advanced Heart Failure Transplant Cardiologist.

In terms of my conflicts, I am the Scientific Advisor for CareDx, and I'm the PI on a research study sponsored by CareDX.

**Dr. Anitra Graves:**
Thank you, and Dr. Hall, we got your name spelled correctly on this slide.

**Dr. Shelley Hall:**
Thank you. Thanks, I am Shelley Hall Chief of Transplant at MCS at Baylor Dallas, and I am a consultant for both the cell-free DNA companies that are working in the heart space, as well as do research in that area, where both companies have sponsored.

**Dr. Palak Shah:**
If there's a minute, this is Dr. Palak Shah, I'd like to just go back and, and for the purpose of the recording, state my disclosure, so my disclosures would include a paid consulting relationship with Natera for leading a clinical trial. Unpaid work with CareDX for work on a cell-free DNA registry, as well as a site PI work, and then, also work with the NIH on the graft consortium, which is focused on cell-free DNA, and then finally, my employer Nova Health Care System has a patent around other biomarkers in heart transplantation.

**Dr. Anitra Graves:**
Very good. Thank you and Dr. Potter?

**Dr. Steven Potter:**
Thanks. You guys had me muted, can you hear me now?

**Dr. Anitra Graves:**
Yes, we can.

**Dr. Steven Potter:**
I'm Steve Potter, I'm a fellow transplant surgeon at Medstar Georgetown Transplant Institute and Professor of Surgery at Medstar Georgetown University Hospital and School of Medicine. Terms of disclosures, I have been a scientific advisor and consultant for CareDX and involved in several of the registry studies. Thanks.

**Dr. Anitra Graves:**
Thank you so much for all of your willingness to participate in this meeting.
So, the new Contractor Advisory Committee is a fairly old committee. However, as of January 8, 2019, CMS redefined its role as an advisory role to contribute to the policy development process. The CAC is a really nice way for MACs to interface with stakeholders and particularly, those experts and investigators, that have a lot of experience in a particular subject matter. For example, today, Allograft Testing for Acute Rejection.

We will be discussing the evidence and the competence that these investigators and physicians have regarding the literature that has already been published. We did select a series of publications. We selected these publications as they represented the pivotal studies related to the tests that we're to evaluate today. There are additional publications, and those may also be used to inform any policies that may result from these proceedings.

Additionally, I'd like to mention that we take a lot of steps to create policies that are based in what is actually published in the literature. So, while we are wanting the advice and opinion of the investigators that we have participating, much more weight is considered when that information has been verified in a published article that has that peer reviewed. The CAC member's comments and opinions supplement our internal expertise in the MACs, but it also helps us ensure that there's an unbiased and contemporary consideration of the state-of-the-art technology and science. Next slide.

We're going to have a series of topics and the slide somewhat covers the ground that we'll be covering during this discussion.

First up is the discussion regarding Heart Allograft Testing for Acute Rejection and I really want to start off the conversation with the advice or opinion of our SMEs to indicate whether or not the tests that we'll be discussing actually are able to discern the difference between rejection versus injury.

There are four tests will be talking about today: The first test in blue is AlloMap, which is a gene expression profile test, it's a little bit different in technique than the other three tests listed, which are AlloSure, Prospera and Viracor TRAC. All three of those are donor-derived cell-free DNA

tests. None of these tests, other than the AlloMap, are FDA approved. So, AlloMap is the only tests of the four that currently has FDA approval.

So, we will be, I would really like to understand from your perspective how these tests, if at all, could discern between rejection and injury, and I'll open the floor.

**Dr. Kiran Khush:**
Hi, this is Kiran Khush and maybe I can get started. The donor-derived cell-free DNA assays should be able to detect graft injury, whether it's due to acute rejection or another cause, and that's because they're looking for DNA released by cells in the transplanted organ. There's nothing specific about rejection that would necessarily cause cells to release DNA. It just so happens at the most common cause of graft injury after transplant would be acute rejection. Now, the AlloMap test was specifically developed and tested as a marker of acute, cellular rejection after heart transplantation. It was not developed to assess for antibody-mediated rejection or other forms of graft injury. So, I think that's maybe a starting place for our discussion.

**Dr. Sean Agbor-Enoh:**
Hi. Let me, this is Sean Agbor-Enoh from the NIH. If I could add to what Dr. Khush just mentioned, specifically emphasizing the point about injury versus rejection, and allow me to point to lung transplantation, wherein unlike other organ transplantation, the patients are exposed to multiple other causes of allograft injury, infection, acid reflux in addition to the two types of rejection, which are acute cellular rejection or antibody-mediated rejection. If I may add, there are additional pathologies that have seen on biopsy in lung transplant patients, which are not generally rejection, but they are considered injuries as well. In several of these instances, your donor-derived cell-free DNA levels are high, and so it's not just the test that could distinguish rejection, rather, it seems more like a test that could tell you that something injurious is going on in the allograft. Pertaining to lung transplantation, there is, thus far, no test available in the market that looks at gene expression. The only tests that have been used in lung transplantation are the cell-free DNA-based tests and I will stop here.

**Dr. Palak Shah:**
This is Palak Shah from Inova, I think the only thing I would add to the comments of Dr. Khush and Agbor-Enoh, are that, although the donor-derived cell-free DNA assays allow for detection of allograft injury, the most common cause of allograft injury, by far, is rejection, whether that's acute cellular rejection, or antibody-mediated rejection. And furthermore, when graft injury is identified in the absence of histologic rejection, it is often associated with the development of rejection, months later on. So, it proceeds the histologic diagnosis of rejection often.

**Dr. Kiran Khush:**
Hi, this is Kiran Khush again, if I can just point out, there's an error on this slide, the AlloSure test has 405 SNPs. The original version of the assay had 266 SNPs, but the currently used version has 405.

**Dr. Shelley Hall:**

Yeah. This is Shelley Hall. I think that important comparison here is that they're looking at the health of the organ in the organ recipient in different manners. Obviously, AlloMap is looking more at the overall immunologic status of the patient, whereas the cell-free DNA preparations AlloSure and Prospera are looking at the graft injury itself. So, they're analyzing different aspects of the entire relationship between the graft and the recipient. The Viracor TRAC doesn't have much yet, in the way of data with the hearts, there is a small sub-segment of one analysis. But right now, that's probably the newest, but if it follows, it's, it's looking at the same thing, cell-free DNA. So, it would follow that it should have similar results when there's more studies done.

**Dr. Anitra Graves:**
Thanks for that. And actually, I wanted to find out what your opinion might be regarding the differences. So, you mentioned, or Dr. Khush mentioned, the number of SNPs to the newer version of AlloSure for heart testing. Is there a difference in performance between the original version versus the one now? And is there a difference in performance between that and the other two tests on the market?

**Dr. Sean Agbor-Enoh:**
I wonder if I could start the discussion.
That is a very good and important question. Unfortunately, it does not seem we have the right kinds of studies to answer that scientifically, it doesn't seem so. However, there are just a few points that's worth making. There really has not been head-to-head comparison between these tests, so it is really hard to know how they compare to each other. That's point number one. Point number two. It is true that these tests do two things that are different. Number one, the number of SNPs of the cell-free DNA test, and I'm talking about the three tests that you have shown here. The number of SNPs are different between the tests and number two, the thresholds that they report to monitor half transplant patients have some small variation between the different tests as well. The question becomes are the number of SNPs that these different tests show/use, would that impact the results that the test would report? Unfortunately, we do not have that kind of information to be able to report that because to my knowledge, it has not been a head-to-head study that is looking at these different tests, that is number one, and then number two, there are no available standards that are common that these different tests have used and so we can look at how they perform on those standards. Let me stop here and see what my colleagues think about that.

**Dr. Kiran Khush:**
This is Kiran Khush and I agree completely, with what Dr. Agbor-Enoh has said. We don't know what the threshold number of SNPs is, above which, um, an increased number of SNPs would not be helpful. So, in other words, we don't know if there is even an optimum number of SNPs to discriminate donor and recipient to make the test more accurate. So, in other words, we don't know whether 13,000 SNPs is better than 400 SNPs or not, and there has never been any head-to-head comparisons of these assays, so we cannot say, at this time, that one test is more accurate than the other.

**Dr. Angella Charnot-Katsikas:**

I'd like to ask a follow up question if I may, this is Dr. Katsikas from Palmetto. So, if you could speak to, so we hear you, that, that the number of SNPs may or may not really play a role in terms of adding value above and beyond a certain baseline value, let's say, for this type of testing given that there, there are no, to your comment, no head-to-head comparisons between the tests. However, I'd like to ask a follow up to that. Can you speak to the test performance across the board for these various similar tests that are looking at the same type of analyte-like donor-derived cell-free DNA? So, for example, if they are performing similarly across the board, does that not provide evidence to that effect, essentially? So, with that, even in the absence of head-to-head comparisons.

**Dr. Palak Shah:**
Yeah, this is Palak Shah, maybe I'll take that question.
You know, I think we, in the absence of head-to-head comparisons between the different assays because I think there's a lot of nuances that goes into the quantification of cell-free DNA as well as the bioinformatics methods that are used to assess donor and recipient SNPs. So, you really need clinical validation studies that show that this assay has been tested in heart transplant patients and performs to a clinical standard that shows appropriate levels of sensitivity and specificity for the noninvasive detection of acute cellular and antibody-mediated rejection. And, as long as those clinical validation studies have been conducted and published that show that data, then, that's what I think is most relevant for the clinical community, the patients and physicians, and providers who are using these tests, as opposed to the nuances associated with how one company measures cell-free DNA, and, versus another.

**Dr. Shelley Hall:**
This is Dr. Hall, and I would support that.
I think there's been enough out there now that demonstrates similar patterns, not between these and the graft work. They all demonstrate almost superimposable patterns, and so the methodology differences don't really matter. It's for the providers in the patient care, it's about the end result and they've all pretty much, almost, can overlay their graphs of measurements and timings, and so that's really where we're at with this technology. And the individual nuances that may prove to provide additional information, down the line, but we're not there yet.

**Dr. Steven Potter:**
This is Steve, I think I agree with Dr. Hall's comments, and I would point out that the methodology is really not dissimilar, the number SNPs is likely to be irrelevant and the issue of a head-to-head comparison for these tests, which are all very promising in all, I think can find an exciting place in the clinical armamentarium. It's really kind of a question nobody's asking for a good reason. In terms of validation, we know we have good validation data from the D-OAR registry in the case of AlloSure in the heart population. And so, I think, you know that we have good evidence for utilization, um, in the detection of rejection in the heart population. Thanks.

**Dr. Sean Agbor-Enoh**:
If I may add a slight nuance to this logic. For the test that and this is Dr. Agbor-Enoh, for the first tests that have studies and specifically there are two tests that have studies done and show really superimposable performance in my mind, in heart transplantation. Being able to detect the endpoints that was used in the study was rejection, and that was the AlloSure test and the

Prospera test that have done those studies. Now, the question then becomes, and I would like to know, you see whether, we think about these, if another test that have not done that study, there's overwhelming evidence now, I think we can agree to that cell-free DNA used to monitor heart transplant patients, the multiple studies, that have shown that they performed well.

Now, what for a test, for a test, that have not done that validation, in my mind, the methodology of the test needs to be looked, into a little bit, before one can say whether, or not, you can use that. Let me translate that into slightly different way. So, let me, take for example troponin. Troponin is a test that is well standardized, and everybody uses it to monitor patients, maybe having an acute coronary syndrome or heart attack. Now when a company develops a new troponin test, we do not ask that company to go and do a clinical trial, to show that that troponin works, we ask the company to be able to show that the test that they are reporting, they are measuring exactly what they say they are reporting. Because for troponin, the assay has been so standardized across the field. However, in cell-free DNA, that has not been the case.

So, while different tests have shown good performance of cell-free DNA in studies, I worry that if you put that same standard as troponin and you have another test coming in, with a slightly different methodology, that has not been well validated, in a patient population, it may be challenging to translate the findings of one study into another, in which case, the methodologies then vary. So, these assays actually have different methodologies. A few of them have done studies, and they've shown good performance. I worry that translating that performance to other assays, which us slightly different methodology, may bring nuances that may not be scientifically valid. I'll stop there and see what the team thinks.

**Dr. Angella Charnot-Katsikas:**
So, I guess, I could, if I could, just interject here, and maybe, prompt, additional conversation. It sounds like it's similar, similar to, what we were just discussing, right? Like if the clinical validity of the donor-derived cell-free DNA is essentially established, regardless of the number of SNPs. Because in some cases, we're not talking about more SNPs or less SNPs, we're talking about more SNPs in some cases.

Then, then, it seems, that, that there may be a reliance there, apart from methodology, although, although we certainly hear what you're saying, Dr.Agbor-Enoh, you know, there are nuances, but it's, I guess, I'd love, I'd love to hear the rest of the panelists, you know, opinions on, on, this matter given the literature, which, as we said, there are, although there are not head-to-head comparison specifically, there is, there are numerous publications that look at the ability of these analytes to inform on injury, we can, we can say rejection, but we know it's injury and other types of rejection. And so, so does that, does that, not provide confidence in the ability of the performance of these tests?

**Dr. Sean Agbor-Enoh:**
Was that question directed to me or to the group?

**Dr. Palak Shah:**
Maybe I'll try to take this one. So, I think one of the, one of the, the, I guess, one of the goals of this type of testing is to allow for your provider clinicians to be able to take that test result,

interpret it, and then apply it to the patient that they are taking care of, and because of these subtle nuances associated with how SNPs are identified and quantitated, and then reported out, the interpretation of the data that is being provided, the cell-free DNA, and identifying what threshold is concerning for rejection, whether its cellular or antibody-mediated, versus no rejection is actually unique to the individual assay. And so, you know, if you look at the different cell-free DNA studies that have been published, they often report very different thresholds, because the assays are also quite different. And so, it's important then, again, to get some sort of clinical validation data together that allows you to say, based on this assay, in this patient population, this would be a normal cell-free DNA versus an abnormal cell-free DNA, irrespective of how it's being quantitated.

**Dr. Angella Charnot-Katsikas:**
Absolutely, so, if so, the thresholds are certainly unique, to these tests, as you say and so, as, long as the threshold, the test, and the threshold provided are meaningful, in terms of providing the clinical validity information, then, it sounds like that, that's really what we're looking for, and I would love to hear if others have feedback here, but, also to maybe, this is a good opportunity to segway into the specific threshold for the detection of tissue injury and rejection.

**Dr. Kiran Khush:**
If I can maybe add to this, I'd like to emphasize the fact that the threshold is not a definite line in the sand, right? It's where you are trying to maximize your sensitivity and specificity of the assay and different centers may choose to use different thresholds based on their patient population and the amount of risk they want to assume. So, for example, some centers use very low thresholds, because they don't want to, risk missing an acute rejection, knowing that they'll have more false positives and other centers may choose a higher threshold, because they don't want to have as many false positives and so I think, you know, underlying this discussion, it's also, we also need to bear in mind, that's the threshold, there's no one definite threshold for an assay. But a threshold is chosen for the clinical research studies performed, for example, D-OAR based on the sensitivity and specificity at that threshold.

**Dr. Angella Charnot-Katsikas:**
Absolutely, and that's helpful information, so, if you could elaborate on why different thresholds might be used in different institution. So, you know, we, we recognize and part of this discussion that, um, it may be that there is trending, that is involved and so maybe that's something you can include in, in, your sort of elaboration of the concept.

**Dr. Kiran Khush:**
Sure. So, I think the trends are important. So, for example, if we take AlloSure as an example, so the D-OAR study is just threshold of 0.2%, so, but the limit of detection assay is 0.12%. So, the results we get are either less than 0.12% or some numerical value, above 0.12% and so, some centers, will do a follow up biopsy for any level above 0.12% because they don't want to risk missing any episodes of acute rejection.

Now other centers may say, well, this threshold used for D-OAR was 0.2, that's the threshold I'm going to use, or they may say, OK, I have an AlloSure value of 0.18%, It is, above the limit of detection, it's not quite the 0.2% that was used at D-OAR so I will do a serial measurement

within a certain period of time, and if it's stable, I won't do a biopsy and if it's rising, then I will do a biopsy. So, I think, underlying this is the clinician's level of risks that they're willing to assume, I certainly know some clinicians who use a threshold of 0.3 and say, if my patient is doing well clinically, is asymptomatic with normal graft function, I will use a higher threshold because I am comfortable otherwise with my clinical surveillance of this patient.

**Dr. Shelley Hall:**
This is Dr. Hall. I'd like to add to that, too. It's also the clinical context because this is definitely not a one and done test. It's not a one blood test, you get a number, and you know what to do with the patient. The trends are incredibly important, but it's also the clinical scenario. How are the centers using it? Are they using it for asymptomatic surveillance? That's going to drive one type of process and one type of threshold to choose. Are you going to be following up? Somebody had a rejection and determine are they stabilizing? Many rejection episodes, they don't go back to the "negative level". And are they stably, elevated, or they know, rising back up again, and so these are definitely serial tests, and the thresholds that centers are going to choose are going to vary based upon the reason for which they're utilizing the test.

**Dr. Anitra Graves:**
That's really helpful. I was wondering if you can elaborate on the testing.

**Dr. Sean Agbor-Enoh:**
If you don't mind let me interject here for a minute and look at it, I have to say this is so true and I don't think this is any different from many medical tests that we use. It is hard to put a single value and to use that as a threshold for all patients and on all patients within scope, in such that, including this test in the context of clinical practice. I think almost imposes that the patients do vary and that the way one should think about the test and the threshold should vary as well. I think I wanted to highlight that; this is actually standard clinical practice. It is very hard for us to use a single threshold of any test, let me not say any test, for most test. It's hard to use a single threshold for all patients or for all patient populations, and so cell-free DNA should not be, at least, should be considered in that same context as well, and I'll stop here.

**Dr. Anitra Graves:**
Sure, apologies for interrupting Dr. Enoh. I wanted to know if we could also segway into the discussion about surveillance. What are your thoughts, and what has the literature demonstrated regarding the scheduling of surveillance, and how, what frequency you would test patients, whether it be in a surveillance situation or for-cause? And is there a different approach between the two types of tests that we're evaluating, the gene expression profile versus cell-free DNA?

**Dr. Kiran Khush:**
Or maybe I can start.

So, what most centers have done is, they've replaced their previous biopsied-based surveillance schedule with a noninvasive biomarker-based surveillance schedule. So, they use the same time points that they used to do biopsies but now do noninvasive testing.

Now, when they start noninvasive testing, they very, some centers may start at one month with the cell-DNA assay, some centers may start at three months. Some centers may wait until six months post-transplant to start surveillance. But what we do know from the literature is that these tests are most useful for surveillance because of their high negative predictive value for both the donor-derived cell-free DNA assays and the gene expression tests the negative predictive values about 97%.

But they're not used for-cause, because they cannot tell you what type of rejection is present and if rejection is even present, and so at this time, they're used primarily for surveillance, but for-cause is still mainly based on biopsies.

**Dr. Sean Agbor-Enoh:**
So, if I may add to what Dr. Khush just discussed. Looking at the number one, validation and number two, looking at it from the side of the lung. I do agree that, now, if there is for-cause, a cell-free DNA test really may not guide you. It doesn't necessarily guide you whether you should change your clinical decision, because even if the levels are low, I'm sure most clinicians that will need to do something about that. So, it would mostly be used for surveillance at the negative test to rule out that something is wrong.

Now, if you look at it, when you ask the question about schedule. I think for lung transplantation, it has really not been many studies that have reported the use of cell-free DNA as part of routine clinical care. In my mind, I think there have been about 1 or 2 studies, and in those studies, the researchers in that case, or the clinicians, if you may call them, did the test on a monthly basis. So, they did the test every month, they got a cell-free DNA drawn and even though, even though, for lung transplantation, the patients will get five biopsies a year but when they used the cell-free DNA to monitor patients, they did it monthly. So the patients ended up getting monthly, they started to test, the test was only started after the first month of transplantation, so after thirty days, and then they started monitoring and every month they got the test for the initial year. They did not use the test for surveillance for patients who are greater than two years because prior cohort studies have shown that the cell-free DNA levels would come and be low up to two years and then they will start rising again.

So, this study only considered patients with the analysis between thirty days and two years and they on they got the cell-free DNA for these patients every month. Now, I don’t know of another study that have used another schedule, but that is the only schedule that I could, I found, upon review of the literature.

**Dr. Angella Charnot-Katsikas:**
Great. Thank you for that. So, if we could continue the discussion about the for-cause versus surveillance, and maybe we start with the heart and explore that in a little bit in more detail, and then we can move on to lung. Does that, does that work?

**Dr. Anitra Graves:**
OK.

**Dr. Angella Charnot-Katsikas:**

So, if anyone, if anyone wants to talk about heart, let's do that for a moment and try to explore this in more depth.

**Dr. Shelley Hall:**
So, this is Shelley Hall, I guess I can dive into this. I mean, I think that we're very, very comfortable surveillance, as Dr. Khush mentioned, due to the negative predictive values. Certainly, the AlloMap technology has been around for a long time and centers are used to those thresholds understanding that there's nothing absolute about a particular number. On the cell-free DNA, I think that that the reproducibility of this and being able to be comfortable about quiescence makes everybody probably even more comfortable because thought of graft injury versus immune activation is a more concrete parameter. So, the ability to use these tests for their rule out or negative predictive value, I think, is strong, easily adoptable and while the thresholds can vary a little bit, that'll be refined over time. And again, those thresholds are going to have nuances based on clinical scenarios.

On the for-cause aspect, I guess, I will go back and say, what do you mean with that question, are you saying, if the test is positive, how is it used, or you saying, if the patient has an event, how are we using the test?

**Dr. Angella Charnot-Katsikas:**
Exactly. I think both of those situations are what we, what we need to explore.

**Dr. Shelley Hall:**
Yeah, so, I mean, both of them, when they're elevated, their positive predictive value is not as strong as, or negative predictive value, and it's, it's essentially a trigger that something is going on in your recipient that you need to investigate further.

Not all of them are going to end up being bad things, and while I think that we still have much to learn about the nuances of the elevated cell-free DNA, we've got plenty of experience of validated AlloMap to know that a lot of those don't end up turning into graft dysfunction. But they are warning you that there's something going on with the patient's immune system, that it's revved up, and so you want to survey and make sure that doesn't end up causing an event. On the cell-free DNA, we're talking about the graft itself, so when those levels are elevated, something is injuring the graft or causing the cells to die or be injured, higher than expected, and it triggers an investigation, and, and there'll be, I think, further aspects of that are being dived into. Whether, as we said, that trends are very important. Is this a one, and done? We're just barely went over the threshold, and then went right back down? Is this a continual rising? Is this a high, and then low, and then high, and then low that, what we call variability, which has its own potential negative outcomes associated with it. All of those things will trigger further evaluations.

Sometimes, there are something and sometimes there is not and, and more and more experience in investigators hands helps to start to identify that. We know right now that in general antibody-mediated rejection tends to cause higher cell-free DNAs, than cellular rejection. And, that is not, again, when you're talking about a group of analysis versus an individual patient the spread of values sometime overlaps.

But at least those are some of the early indicators that we're starting to see, and as the technology advances, the hope is that those confidence intervals will get tighter and tighter, and will eventually be able to identify, um, what's causing the cell-free DNA release without more invasive tests like biopsy, molecular microscope, et cetera, et cetera.

On the flip side, something has happened, the patient is clinically symptomatic, either their echo is abnormal with graft dysfunction, they're coming in with heart failure symptoms, or your biopsy is lit up like a, you know, a blue Christmas tree. Then, you're going to utilize the test in a different way, mostly, that I think, the cell-free DNA, in the sense of, you're going to intervene upon that event and then you're going know, you're going to use the cell-free DNA as a marker of what I call, getting back to stability. And is there, the patient who gets back down to a low quiescent level versus the patient who doesn't, will drive probably, further treatment decisions. So, those are the nuances I see in using the for-cause from either end.

**Dr. Sean Agbor-Enoh:**
Can I interject? Are we on lung? Are we allowed them to complete their thoughts on the heart?

**Dr. Anitra Graves:**
Oh, whatever, you want to make a comment on heart or lung, that's fine. We are going to focus on the lung, following the polling questions for heart. So, if you want to hold your comments until then, that's fine too.

**Dr. Sean Agbor-Enoh:**
No, I think, I think I would, I would add to that. I completely agree with, with, with that, that, you know, one has to think about the positive test for both the angle of the patients, and on the angle of cell-free DNA. So, one if using cell-free DNA to monitor patients and so therefore, if the test comes back positive, what do you do? Or, if the patients show signs for concerning for allograft dysfunction, what do you do? So, I think those approaches have been my experience as well. So, for the sake of time, I'll stop there. I agree with that approach, as well. I think that also seems to correlate with some of the findings of this study, both cohort study or the studies for which people have used the tests in real-time to monitor patients.

**Dr. Anitra Graves:**
I was wondering if you could also comment on this idea of the test's ability to discern quiescence from injury over time. Are either of these types of tests, affected by that time post-transplant or are you adjust the thresholds, how, how is there a difference? And if there is, how do you address it in your management of these patients?

**Dr. Palak Shah:**
I can jump in on this. This is Palak Shah.
What we know. I'll start with donor-derived cell-free DNA. What we do know about donor-derived cell-free DNA is that immediately after transplantation there is this immediate release of cell-free DNA that leads to large, kind of spikes early, post-transplant, that likely represent ischemia reperfusion injury and injury related to graft preservation, but that there is this

exponential decay that occurs in cell-free DNA. And if you look at when cell-free DNA levels really stabilize, it's somewhere either around fourteen days or twenty eight days, which has been used most commonly and we see that those cell-free DNA, labels, levels typically in the absence of injury or graft rejection are stabilized at that point and that's where a lot of the studies have been conducted as greater than or equal to twenty eight days.

If you look on the other hand at gene expression profiling, what we know is that the gene expression profiling is affected by the overall immune state of the transplant recipient and so that if that patient is on, let's say, higher doses of corticosteroids early after transplantation, the thresholds are different and so from that day fifty five to typically month six, the threshold is different than after six months, when typically steroid doses are weaned, weaned down. So, there are some differences in terms of what thresholds are appropriate, and when the assays can be used.

**Dr. Kiran Khush:**
This is Kiran Khush, and if I can just add on to what Palak said. We also know that the AlloMap, gene expression test, levels tend to rise over time post-transplant. This may reflect weaning of immunosuppression over time. But generally, few centers use it beyond two, maybe three years post-transplant, because the levels rise over time, and then you start getting more false positives. I don't think we have enough data with donor-derived cell-free DNA level long term. If you look at the D-OAR study up to two years, post-transplant levels seem to be quite stable. But we don't know what happens donor-derived cell-free DNA levels in a quiescent state, for much beyond two years.

**Dr. Angella Charnot-Katsikas:**
Great. So, it sounds like the real utility of some of these tests is primarily in the early days, a year to two years maximum post transplantation for, for, all of the reasons that you've outlined. So, can we, can we, talk about that a little bit? In terms of what, in your estimation, moves, moves the needle, so to speak, closer to biopsy or away from biopsy, and if that is the use of one test versus a combination of tests, I would really like to explore that.

So, if we could, if we could talk about how, you might use one test in one situation, another test, in another situation, and the combination of testing it in yet another situation, that would be very helpful discussion to explore, given the evidence that we have for, for the utility of the test.

**Dr. Shelley Hall:**
This is Shelley Hall, I can start, I think there, there is benefit to the combination of gene expression profiling and cell-free DNA in surveillance right now. I think that one of the areas, and it’s still evolving and undergoing debate, is what to do when the cell-free DNA is, is, low and the AlloMap is high versus low and you know, we have to always remember that it's a sequence of events and the body's immune system has to be activated then, it has to potentially do damage, it doesn't always. And then damage is detected, and then damage reflects into clinical signs and symptoms, and it's a series. So, the earlier upstream that you can identify an issue, the sooner you can potentially intervene and so, the elevation of genome expression profiling tells you something's going on with the immune system, and it tells you to be looking at the patient.

If you have cell-free DNA elevation and the immune system is quiescence that has the potential to indicate that it's a non-immunologic injury going to the graft and may lead you down a different diagnostic pathway. I don't think we're yet refined enough to say that absolutely. I think that's more in the intellectual realm in future research. But that's how they're complimentary. Obviously, you're not going to do all three cell-free DNA tests. Ultimately, a center is going to pick one, and I don't think there's anything that, right now, that says that one is superior to the other, as mentioned and so, I think that, there's still a long way to go to determine any one way to recommend that people use these.

**Dr. Sean Agbor-Enoh:**
Can I interject for a minute, please? Um, I agree that one would think intellectually, that the gene expression test, which is a measure of immune activation, could provide complementary additive information to cell-free DNA tests, which measures injury. I think one can think of that then, could field that, however, um, let me point out the test characteristics of both tests and then see how, whether, we could discuss how the addition, what will that addition of the two tests give us?

AlloMap, very high negative predictive value. However, it's a high negative predictive value for acute cellular rejection. It does not pick up antibody-mediated rejection, which seems to occur in, Palak, I think you can/may interject, I think it's about 10 to 15% of patients in some studies. Let me finish it and see if you can interject here, Palak. So, it's a very good test, with a good negative predictive value in the upper 90s, right, 97-99% negative predictive value. The study has the ability to tell you that the graft is quiescent, you and you don't have concern for acute cellular rejection. Now, if you look at cell-free DNA across studies, as we said earlier, you can almost interpose them one over another. A negative predictive value of cell-free DNA, now, it's for both acute cellular and antibody-mediated rejection. The negative predictive value is also quite high in the upper 90%. Now, if I think about these two tests as rule out tests, and they both are showing me negative predictive values that are that high, the question becomes, do we have studies that would show us the value of adding them both, if their performance is quite high. I want to see here. I reviewed the literature, and I don't know whether I saw one and I want to see whether any of my colleagues can interject to add on to that point. I'll stop here.

**Dr. Palak Shah:**
So, just to add to Sean's comments, right, so the, what we know as that antibody-mediated rejection depending on the characteristics of the transplant program effects anywhere from 10 to 15% of transplant recipients with African Americans being disproportionately affected compared to white patients. And what we do know is that gene expression profiling with its high negative predictive value was only developed to identify acute cellular rejection but has no ability to detect noninvasively detect antibody-mediated rejection.

On the other hand, if you look at the number of studies that have been published on donor-derived cell-free DNA, specifically, they have typically combined acute cellular rejection, and antibody-mediated rejection, defined as acute rejection as a whole and that's where we see the excellent diagnostic performance and negative predictive value, which has been noted throughout the meeting. I think importantly, when we looked at this within the graft consortium, we actually separated out acute cellular rejection and antibody-mediated rejection and as Dr. Hall

pointed out earlier, saw even better performance for cell-free DNA in terms of the noninvasive detection of antibody-mediated rejection. So, so, there are some important differences within the terms of the ability to actually noninvasively assess the graft for both ACR and AMR.

**Dr. Mandrekar:**
This is Jay, I just have a question. When you say this can be done by tests…

**Dr. Angella Charnot-Katsikas:**
Oh, I'm sorry, if you're speaking, please get closer to your mic, or phone, or computer because you're very muffled. We cannot hear you at all.

**Dr. Mandrekar:**
Can you hear me now?

**Dr. Angella Charnot-Katsikas:**
Not very well. Let's try one more time.

**Dr. Mandrekar:**
[inaudible 1:07:13]

**Dr. Angella Charnot-Katsikas:**
It's still pretty muffled.

**Dr. Anitra Graves:**
Yeah. Think he might be switching to the phone.

**Dr. Kiran Khush:**
So maybe in the meantime this is Kiran Khush and maybe I can just add on to what Sean and Palak said. So, this data from the D-OAR registry, which looks at the AlloMap and AlloSure test and their various combinations. So, what we know from this data is that, in 56% of samples or cases, both tests are negative, and what we know is that the likelihood of acute rejection in that context is very, very low and most centers won't go on to do a biopsy. Now in about 25% of cases, the AlloMap, or the gene expression test, is elevated but the AlloSure, or cell-free DNA test, is negative or below threshold, and in those cases, often what we find are other causes of immune activation, such as infections, most commonly CMV, viremia, even very low levels of CMV replication.

Now, in about 11% of cases, the AlloMap gene expression test was below threshold, but AlloSure the cell-free DNA assay was above threshold. We now know that this may represent antibody-mediated rejection, or it may even represent development of de novo donor-specific antibodies. Then, finally, in 6% of cases, both tested positive and there's a very high likelihood that acute rejection is present. I think that's kind of helpful to keep some of that data in mind when we're looking at the test results in combination.

**Dr. Steven Potter:**
Hi, this is Steve. I just want to, I guess, ask a question, I so I'm on this panel, I think, in large part as an abdominal transplant surgeon, because I'm the co-chair of the ASTS Legislative and

Regulatory Committee and served as an advisor on molecular diagnostic techniques to the society. And so, I just want to ask a question of the, of the moderator whose I heard you say that both gene expression profiling and cell-free DNA are really of primary utility in the first two years after heart transplant, but in the abdominal transplant world, to our way of thinking, these markers continue to be useful, throughout the life of the allograft and, in fact, their longitudinal use has a lot of value. So, I want to ask that of the panelists and moderator to maybe to clarify that two-year time limit.

**Dr. Angella Charnot-Katsikas:**
As far, as far as the moderator is concerned, it's really asking the panelists for what the, what the evidence really provides. So, I'm leaving this discussion to you, but that that would seem to be what was said earlier.

**Dr. Sean Agbor-Enoh:**
If I may add, I think Dr. Khush provided this, maybe she could jump in, as well.
The reason for these two years, it comes from experiences from published study. Wherein it took patients that did not have rejection, or some clinically significant events. On two studies, the Stanford GTD study, and the Graft study. They measured cell-free DNA, serially, in these patients, studying before transplantation, the day of transplantation and then serially over time, and then, the time continued beyond two years.

What these two studies noted, including the Graft study, is that for all patients or for most patients I think all, you have cell-free levels are quite high immediately after transplant surgery, as expected. The levels then decay and there is some kind of algorithmic decay pattern to reach lower levels that could be usable around the 28$^{th}$. Then at that time buys immunity. Beyond that, the levels still continue to decay but is still way below the threshold of clinically used cell-free DNA, right up to about eighteen months to two years, it starts rising again. By two years the level starts rising and we do not have enough data to know what that means clinically, but because the levels are rising beyond two years and we don't know what it is clinically, these cohort studies did not include patients beyond two years of transplantation, it stopped there. So, I think it feels to me that one would need some studies to look at cell-free DNA beyond two years to try to make sense of what that means.

**Dr. Steven Potter:**
Thanks. I think that's really helpful to clarify for the, for the audience and another way to put that very erudite statement is the lack of evidence after two years is not proof of lack of utility after two years and, biologically, we have a lot of reason to think that these modalities may still be useful, long term. So, let's just, you know, for the state of the recording, I think it's worth having that discussion and clarification and thanks for letting me ask that.

**Dr. Palak Shah:**
And I think the other thing I would add, right, if you look at certain studies that have been published using noninvasive surveillance in heart transplantation, such as the gene expression profiling test, you know, within the Image trial, you know, a majority of those patients were actually enrolled somewhere between 13 months and three years, with another group of patients being enrolled between, year, at year four and year five, post-transplant. So, I think, to Sean's

point, we do not have long term data on cell-free DNA in these cohort studies beyond two years. But there are certainly data that supports noninvasive graft surveillance beyond two years after transplantation.

**Dr. Kiran Khush:**
And this is Kiran Khush, and if I could just add to that. I think most studies use two years as the timeline for acute rejection surveillance, because the incidence of acute rejection after two years is quite low and so, most centers stop doing routine surveillance after about two years, and so, since these tests were originally studied for acute rejection surveillance, they also stopped doing the assays routinely after two years. Again, because most centers don't even do surveillance after two years for acute rejection.

This doesn't mean that they don't have value beyond two years, and I think now, we are becoming more and more aware of late antibody-mediated rejection, or the role that development of de novo donor-specific antibodies long term may mean in terms of allograft health and if I can just add to that, I think noninvasive assets have not been studied adequately in that context. Hopefully, some of the longer-term cohort studies, like SHORE will start to shed light on these issues. But they very well may have value beyond two years. We just don't have enough data at this time.

**Dr. Angella Charnot-Katsikas:**
Thank you, and with that, I'd like to kind of circle back to a moment ago when we were talking about the test combinations used and the value provided in terms of both cellular rejection as well as antibody-mediated rejection, and how you use those various pieces of information to determine, what it might be causing new activation, what might be causing moving the needle from biopsy, versus moving the needle away from biopsy, and feeling there is safety in, in, these values that you can preclude the need for a biopsy? Can you speak to the performance? If you're aware of, of some of these tests that perhaps can provide all of that information, sort of in one shot versus perhaps requiring the need for multiple types of tests to kind of piece together that information.

**Dr. Shelley Hall:**
This is Shelley Hall I don't think there is a single shot test that exists. I mean, you can, even these tests, will sometimes require other tests.

So even if you do the combination AlloMap/AlloSure test that gives you both cell-free DNA and your genomic profiling, as we specified, we think there's benefit to both of those tests. There are still other things that will need to be done. Now, if they're both quiescent and low, the hope is that actually removes things done to the recipient and it helps us minimize testing. But there are other medical problems going on with the patient. There are other issues. So, it's not going to alleviate everything. If they're positive then, as stated, it doesn't necessarily diagnose the problem. It just says that there's a potential problem that exists and so, further testing will be needed. So, none of these combined are individually, ah, are going to be the end, all be all answer for our patients.

**Dr. Anitra Graves:**

I really appreciate those answers.

I was wondering if you could also comment on the fact that, particularly in the Image trial, there really was a significant need to put that reliance on the clinical exam for the signs and symptoms of the patient. Where only six of those patients, actually, where the rejection was identified solely, based on the investigation following an elevation in the test result. Can you speak to the importance of a clinical evaluation in concert with the testing, or do you think, oppositely that, that it's not as significant for maybe one or the other for gene expression versus cell-free DNA?

**Dr. Shelley Hall:**
Yeah, I would say if you had a positive clinical exam, the horse is out of the barn and you've missed the, the best part of surveillance, right? Which was to prevent enough graft damage to produce symptoms and those patients historically, you know, often if it is due to rejection, it's symptomatic they can do worse. So, the goal is that your physical exam is almost pointless. That you're detecting these injuries and addressing them before it produces graft dysfunction and concomitant symptomatology.

**Dr. Palak Shah:**
Just to simply add to that, this is Palak Shah. If you look at the majority of rejection episodes that are identified in these large cohort studies and/or registries. The majority of them are rejection episodes that are identified at the time of a surveillance endomyocardial biopsy as opposed to a for-cause endomyocardial biopsy because to Shelly's point once you have heart failure, graft dysfunction, an abnormal hemodynamics at that point, that patient already has rejection, and you're going to go pursue that.

**Dr. Anitra Graves:**
Really helpful, thank you for that. We're going to advance to the polling questions.
We'd like to, we have opened up a poll question number two and this is really to help us memorialize your responses. You've done a great job at providing us information from regarding the literature. If you can start with question two which is, is there sufficient evidence on the clinical context and specifically, we're talking about for-cause versus surveillance, in which the specified molecular diagnostic test could be used? If you can indicate whether or not the evidence clearly indicates, for-cause only, surveillance only, or it doesn't clearly indicate the specific clinical context, that would be great, and we'll give you time to respond.

**Dr. Sean Agbor-Enoh:**
Do we just click on the screen or how do we respond?

**Dr. Mandrekar:**
You have to open it in the browser on the cell phone.

**Dr. Anitra Graves:**
Oh, yes. Yes.

**Dr. Sean Agbor-Enoh:**

I would like the moderators to know that I'm unable to do that. If you don't mind, contact me afterwards, and then I will be able to do that. I will try putting it on my phone, and I have it on my computer.

**Dr. Anitra Graves:**
Will do.

**Dr. Palak Shah:**
Yeah, I think I may be in a similar situation I have the GoToWebinar up, and I don't see the actual poll questions, I'm sorry.

**Dr. Mandrekar:**
You have to open another browser. Another thing that that they sent long time back. You have to go to the web, CVENT.com, and then tap that.

**Dr. Palak Shah:**
OK

**Dr. Mandrekar:**
That is where you enter your name e-mail, and then they give the verification code that's where you begin.

**Dr. Shelley Hall:**
I don't understand what was said. When I open it up, it says, discussion and polling closed.

**Jocelyn Fernandez:**
Doctor, can you refresh your screen? You would click on discussion and polling.

**Dr. Shelley Hall:**
Yeah, I mean, on my phone, I am, and it just says closed.

**Dr. Kiran Khush:**
I'm able to do it on my phone, I was able to get to the polling questions. There was an e-mail that was sent out with all of the steps to be taken maybe a week ago.

**Dr. Anitra Graves:**
We'll make sure that we keep the polls open, so you have a chance to enter your voting after the discussion. So, don't worry. We will make sure that we get your answers even beyond the hour today. So, we'll, we'll go ahead to question number three.

**Jocelyn Fernandez:**
I'm opening those up right now.

**Dr. Anitra Graves:**
Great. Question number three, is, is in the existing evidence, what is the level of confidence, or certainty regarding test performance data reported without any confidence intervals? You largely discussed regarding the data, and, and you did a really good job on discussing the consistent

results on the performance of these tests. So, we just want to have your opinion recorded regarding, you know, if these tests were reported with performance statistics that did not include confidence intervals would that affect your certainty, our confidence in the results reported, and, or does it have no effect. That is the purpose of this, this particular question. We'll give you a few minutes to enter your voting.

**Dr. Palak Shah:**
So just for clarification, you're asking about the absence of confidence intervals, but in CARGO and in D-OAR there were reported confidence intervals, right?

**Dr. Anitra Graves:**
Yes, that's correct. Yes.

**Dr. Palak Shah:**
Yeah.

**Dr. Anitra Graves:**
OK, we will, advance to the next question and this question kind of goes what you've already spoke to regarding the utility of surveillance, not for-cause with respect to heart transplant recipients. If you could just enter your answer regarding yes or no, we talked already regarding the schedules. So, unless you have another comment, we can just wait for you to enter your answers to the yes, no, and we'll advance to the next slide shortly.

**Dr. Anitra Graves:**
OK, question five.
Again, you've touched on this, but to memorialize your answers, is there sufficient evidence on the ability of molecular diagnostic tests or combination of tests to discriminate acute T-cell mediated rejection from quiescence?

**Dr. Anitra Graves:**
The next question is very similar. That question, number six, is there sufficient evidence on the ability of the molecular diagnostic test, or combination, to discriminate acute antibody-mediated rejection from quiescence, and again, we did discuss this. So, this is just too, document your opinion.
We also discussed the next question, which talks about the thresholds and cutoffs, and we, and we talked about that pretty extensively. So, when you are finished with 6, go ahead to 7A and provide your responses for question 7.

**Dr. Kiran Khush:**
If we don't think there's enough data, should we just leave it blank?

**Dr. Anitra Graves:**
Absolutely.

**Dr. Angella Charnot-Katsikas:**

Yeah, and, I would say, if there, if there's any place to provide text-based comments, you're more than welcome to do that, to explain, you know, like a blank, something left blank, or one of your responses. And we will look at these at length after all polling has completed.

**Dr. Anitra Graves:**
But for the recording, was there, is, there a test that you feel that there's not enough data to respond?

Alright, we'll go ahead and advance to question 8. Which is our next polling question. We already discussed 7B.

So, the question 8, and that question is, is there sufficient evidence to indicate that in patients without signs and symptoms of rejection use of molecular diagnostic tests or combination would preclude the need for an endomyocardial biopsy?

So, would you be confident if without, a positive test and in a patient without signs and symptoms that you would be able to safely avoid an endomyocardial biopsy, that might otherwise have been scheduled.

9 is very related to that. In that scenario, we are asking, is there sufficient evidence to indicate that in patients with signs and symptoms, and I think you did discuss this extensively, the use of molecular diagnostic tests would include the need for endomyocardial biopsy, and I believe all were in agreement, that you would need to do further evaluation for these patients.

Question 10 is a little different. The question is, is there sufficient evidence on the ability of the molecular diagnostic test or combination of tests to guide clinical management without endomyocardial biopsy? And if yes, for any of the above, what aspect of your clinical management would be influenced by the test results alone. Any comments on that?

**Dr. Palak Shah:**
This is Palak Shah, at this juncture, these diagnostic assays help you, um, detect the potential of allograft rejection and acute rejection. But there is no evidence or information that guides the clinical management in terms of whether or not this is cellular rejection or antibody-mediated rejection. Therefore, a biopsy is typically required along with other diagnostic testing to determine what is the appropriate treatment pathway for the patient.

**Dr. Angella Charnot-Katsikas:**
Yeah. That's helpful information.

**Dr. Shelley Hall:**
Yeah. I would agree. I mean, that's what future trials are being designed to do. I think, there, it's a piece of information that helps you make a decision, um, but right now, we need trials to figure out how to, protocolize that, are there standards that are obvious, that’s, we've got a way to go on that.

**Dr. Steven Potter:**
They can help you avoid an unnecessary biopsy but can't replace a biopsy, when needed.

**Dr. Angella Charnot-Katsikas:**
Great. I just wanted to highlight for, for our, for, our SME panelists, that absolutely, you know, again, feel free to provide context to your responses. And if you are, you know, responding one way, because you're taking the lab test in isolation versus responding another way because you're considering context, I just want to make sure that we are encompassing a complete understanding of the responses that you use. So, anyway, please, if somebody was about to say something?

**Dr. Shelley Hall:**
How it right now? When you're on this poll, there is no free text options. It's just click on the answer.

**Dr. Angella Charnot-Katsikas:**
Yeah, I mean, even as part of this recording because the discussion is still ongoing. So, if you respond to certain way and you want to, know, provide context, by all means, please do fill here, and we'll be able to, to kind of consider that in conjunction with your response.

**Dr. Anitra Graves:**
If there are no further comments.
We'll head to question 11. This question is, would you perform an endomyocardial biopsy if the molecular diagnostic test indicates rejection, but the patient exhibits no signs or symptoms of rejection, and that's including any other diagnostic you might characteristically perform?

**Dr. Kiran Khush:**
This is Kiran Khush, sometimes I wish there was a maybe. This case, it's clinically where the test is just about the threshold but the patient's otherwise doing really well, we may choose to just do a serial assay and have them draw again maybe in a week or two.

**Dr. Angella Charnot-Katsikas:**
Thank you, that's exactly the kind of context, what we're also looking at. So, all of these, you know, are helpful statements, even if you can't put them in a box.

**Dr. Anitra Graves:**
That's right. Any other comments there?

**Dr. Jay Mandrekar:**
This is Jay. I'm not a researcher in this area, but at as a patient, if I were to see something they could do, whether the patient is good or bad, I would consider my life to be more precious than $27,000 worth of biopsy, right?

**Dr. Shelley Hall:**
Yeah, I mean, I think that these questions are they are set up as an either or and life is rarely either or. So, I think all of these you know, we're picking the biggest generality in our answer. But the true reality is, it depends. So, the surgeons hate us because we always have all these nuances to something and they want just a yes or no and we're like, it depends.

**Dr. Angella Charnot-Katsikas:**
But that's why we have this two-hour discussion so that we can get to all of those depends on or as many as we possibly can fit in this session.

**Dr. Angella Charnot-Katsikas:**
Dr. Potter, were you going to say something?

**Dr. Steven Potter:**
I just wanted to let Dr. Hall know that surgeons don't hate Cardiologists. It's very, it's a very important point. We appreciate the nuance.

**Dr. Anitra Graves:**
Perfect, and we'll go on to question 12, and we promise, we will not hold you to your answer in stone, we understand that there are, sometimes maybes as well. Number 12, how confident are you in the evidence that for AlloSure, Prospera and Viracor TRAC, an elevation in donor-derived cell-free DNA indicates rejection? So, this is really a statement to that group of tests that are in the category of donor-derived cell-free DNA.

**Dr. Anitra Graves:**
Then, Question 13 that follows is similar, how it applies to the gene expression.

I'm sorry, go ahead.

**Dr. Shelley Hall:**
That's alright, this is Dr. Hall, I was going to say this falls into the depends, too, because there's positive, meaning it's crossed that your whatever threshold you've chosen and then there's the, oh, my gosh, it's huge, and then there's, oh, well, it came back down and they're all elevated, but they all mean different things.

**Dr. Anitra Graves:**
Can you speak to that a little bit regarding if you're testing, I want to get an idea from the panel about the AlloMap and AlloSure as a combination test, we are aware that the marketing is for HeartCare, which presumably combines those tests.

Um, as, as panelists taking care of these patients, are you testing with both tests on the same day? Or how are you using those, those tests in combination, if at all?

**Dr. Shelley Hall:**
Yeah, so this is Shelley Hall, it's part of our standard protocol, they're drawn together. It's one tube of blood and sent off and you get the two pieces of information, and then we have the good old 2 by 2 grid of, "positive-positive", "positive-negative", "negative-negative" and we have protocols in place of how to interpret that and then, but those are generalities we've actually kept within each box some options, open. It's not just, it's a negative-negative you just do this or if it's a positive- positive you just do this, because we recognize is there's the rest of the clinical nuances. Fresh transplant/old transplant, recent AMR that's just been completed their therapy, high risk for rejection, forgot to take their meds for three days. I mean, there's all kinds of stuff

and so, we give, we are right now analyzing those to see kind of where those all fell and determine if we can narrow down those treatment options somewhat. So, that's how we're using it.

**Dr. Kiran Khush:**
This is Kiran Khush, I agree with Shelley, in general, the tests are drawn together with the exception being in months 1 and 2 post-transplant. So, you can start using AlloSure at day 28, but you can't use AlloMap until day 55. So in-between those two days we use AlloSure alone.

**Dr. Angella Charnot-Katsikas:**
And are you familiar with centers that approach, this type of monitoring, using one versus another test apart from the timelines that you just outlined and excluded because of the studies supporting their use only in specific time post-transplant. But, you know, you did speak to the use of the combination tests in your centers and how you have these grids that sort of help you move the needle closer to or away from biopsy or other causes of injury. Are you, can you speak to the alternate possibility where centers may use one or the other type of test modalities at different times, perhaps in a tiled kind of approach or some other way?

**Dr. Kiran Khush:**
I'm not sure if this is answering your question, but I think if you order the CareDX assays, you get HeartCare combined. But, for example, if you were to order Prospera, you would only have a donor-derived cell-free DNA assay.

**Dr. Angella Charnot-Katsikas:**
Yeah, I think that that helps. So how would that, how would, how would a center utilize that information when they don't have the complement, I guess, you know, with something like the HeartCare package, let's say?

**Dr. Kiran Khush:**
I think the donor-derived cell-free DNA, I'll say, in and of itself, has a very high negative predictive value. Centers may feel comfortable just using that assay in and of itself for acute rejection surveillance.

**Dr. Sean Agbor-Enoh:**
If I can interject there, with Dr. Khush, I completely agree. It is really worth noting that the test performance of donor-derived cell-free DNA alone, without gene expression, it's actually quite good. Very high negative predictive value for monitoring for both rejection and for both types of rejection, which is antibody-mediated rejection and cellular rejection. Now, we're talking about upwards of 95 to 99% negative predictive value. So, you know, looking at the data alone, you know, without the combination, I would think, centers looking at that data should feel somewhat confident of monitoring the test, monitoring with cell-free DNA, monitoring itself without the combination, at least the data indicated that.

**Dr. Angella Charnot-Katsikas:**
So, we talked a little bit about the value of providing both types of tests, are either donor-derived cell-free DNA and the gene expression profile, as, in regard to, something like an AlloSure/ AlloMap combination, but if we're talking about something like the Prospera test, which only

involves the donor-derived cell-free DNA, it sounds like you're saying that center, you know, given the test performance with a very high negative predictive value, that some centers may opt to go that route, given that it can in fact, detect acute cellular and antibody-mediated rejection. So, given that summary, um, does this panel believe it is better to do both tests as opposed to the one test, you know, given, given that past performance, as discussed, it, and, you know, does the evidence really support one approach over another?

**Dr. Palak Shah:**
So, maybe I can add some comments here for the group to consider. At this point, there is a lack of data that says surveillance with gene expression profiling plus cell-free DNA, is superior to surveillance with cell-free DNA alone. I don't know if we'll ever have that data. I think the points that Dr. Hall had made earlier, and Dr. Khush, I think, revolve around that, that, gene expression profiling test may provide more information around the immune state of the individual, the adequacy of immunosuppression, the risks for future rejection episodes. If the goal is to simply say, this patient has rejection right now, or does not have rejection right now, then cell-free DNA, based on its performance characteristics that Dr. Agbor-Enoh mentioned earlier, it's sufficient to say, the patient does or does not have rejection with a high negative predictive value, in isolation.

**Dr. Sean Agbor-Enoh:**
Thank, Dr. Shah, thank you for clarifying that. I wanted to make sure that my comments interpreted only, in that context, the context being my interest is looking for allograft injury or looking whether the patient have rejection, what will the performance of cell-free DNA, compared to cell-free DNA plus gene expression profiling? We do not have the data comparing both. So, we're looking at just the performance of cell-free DNA to answer that specific question. Injury and rejection, the test characteristics are very good across studies. If you look at the published studies that are looking at just cell-free DNA alone, the test performance. It's very good.

**Dr. Angella Charnot-Katsikas:**
So, are you saying that data on immune status is, contingent on a gene expression profile or can data on immune activation or status be garnered by a cell-free DNA test as well, and can immune modulation be performed as a result of a cell-free DNA test?

**Dr. Sean Agbor-Enoh:**
Let me trace that and leave it to the panelists that are more familiar with using the test, with these data, familiar with using these tests clinically. If one looks at the test characteristics of cell-free DNA, looking at the endpoint that we monitor patients for, the endpoint that we monitor patients for, thus far, is rejection, clinically. Then the test characteristics of cell-free DNA across studies, the negative predictive value of cell-free DNA is in the upwards of 90%, 95, 99%.

Now, if you were going to then monitor the patients for immune states, however, you want to use that clinical data, cell-free DNA does not indicate immune states, it indicates allograft injury or rejection, if that's the clinical interest, rejection or allograft injury, cell-free DNA, has great test performance across multiple studies that have been published, and it would seem, looking at that data, that if your interest is rejection or injury, it would seem that cell-free DNA can guide you,

as a rule-out test. Now, that's a positive test assay/rule-out test to indicate that the patient, if the levels are low, to indicate that the patient does not have acute rejection, or clinically significant allograft injury. That's what the current data states. If I have misinterpreted something, I'm eager to listen to my colleagues and see how they would approach it.

**Dr. Angella Charnot-Katsikas:**
So, the question is, you know, is it useful, is it reasonable and necessary to do both tests and are there management implications to doing a combination test that includes the gene expression profile, like AlloMap so, you know, as opposed to a cell-free DNA test, which, although, it provides information on injury or, and, rejection, if management can be, is that sufficient? Do you really need to do both tests when you can do one test? Even though we've discussed the different information provided, how does that ultimately impact management and can management for something that includes, even immune modulation and management of immune modulation can that be garnered or, by, the results of a cell-free DNA test?

**Dr. Sean Agbor-Enoh:**
If I may, Kiran, Kiran, let me interject and then, I pass it to you. My answer is not answering that question that you just posed. And for the management of the patient, I would not have an opinion in that case. I was just talking specifically, to monitoring for rejection. So, for that, I would defer to people like Kiran Khush, that use the test clinically to manage patients, let me stop there, and pass it back to Kiran, my apologies for interrupting.

**Dr. Kiran Khush:**
Thank you, Sean. I think the bottom line is we can't answer that question at this time because of the lack of data. There haven't been any prospective studies comparing management, using cell-free DNA alone versus management using gene expression profiling plus cell-free DNA.

If you look at the literature, there's one retrospective study using carefully selected banked samples, showing that the area under the curve for the assay, when both tests are combined, is slightly higher than cell-DNA alone. But I think that if we really wanted to definitively answer that question, there would have to be a prospective study looking at these two assays alone and in combination.

**Dr. Angella Charnot-Katsikas:**
I think that's a critical point that you mentioned, and I'd like to expand on that a little bit more. So, as you mentioned, you know, sometimes immune activation is a consequence of other things like infection, which is also being looked at in these patients, right? They're getting monitored very frequently for various infections, BK, CMV, EBV, all of those viral infections.
So, so given that, that additional, you know, monitoring that's happening, in addition to this testing that's being performed, you know, the question is really how valuable the gene expression profile and really, do you need the combination or can you just really perform one task, the cell-free DNA test, that performs well in an understanding, injury and rejection, and then you have, you know, all of your other, you know, the plethora of tests that include viral detection, donor specific antibodies, all of that to help understand the rest of the information like immune activation.

So, I guess what we'd like to hear, and then I know some of you know, it's not always easy to, to get pinned down to a yes or no. But that's kind of what we're looking for. Is it really reasonable and necessary to do both tests?

**Dr. Sean Agbor-Enoh:**
I think this is a very challenging question, if I may jump in here, because we don't know. I think that Dr. Khush's point on, it is, it is hard for us to say, at least for me, let me speak for myself, it is hard for me to say that the combination test is not good or doesn't give me added value compared to the single test because we do not know. We don't have sufficient data to tell us whether a combination is better. Whether if I manage patients with a combination, it will give me more information, or I would do something else for the patient or better for the patient than if I do it with a single test. So, you're right. That's an important question, that looking at the literature, it is hard for me, speaking for myself to answer that question.

Because the data is not there. Theoretically, thinking about it biologically one may argue, about points, about immune activation, that I think, that is not your question. It not biological or theoretical implications, it is whether there is data supporting that the combination test gives you better information, or have some better performance in, in a way there has been one study that has shown that, I think, as Dr. Khush pointed out, and I also believe that we do need these studies to answer just that question. As of now, I would say, I do not know.

**Dr. Angella Charnot-Katsikas:**
But isn't that the point, exactly? That if the evidence isn't there, to support the utility of, or the added value of both of the tests combined than if it really reasonable and necessary to perform those tests, and should Medicare pay for it?

**Dr. Sean Agbor-Enoh:**
We don't know whether we can say, I'm not, I'm not, I don't know about what Medicare needs to pay for tests, but you're asking people who do science here and do clinical practice, to answer a question that they do not have the facts for. That's where I think I'm trying to be a little bit more hesitant to tell you that the combination test is not useful.

What we're saying here is that if your end point is to look for rejection, the current data would suggest that the cell-free DNA, in studies that just use the cell-free DNA alone, that data, cell-DNA is sufficient to monitor for rejection.
However, we have not studied immune activation, looking at a positive immune activation in the context of cell-free DNA to know whether if you've got that useful information. Actually, I would just tried to stop talking here. I'm sorry, I got to just keep going it, I do not know whether immune activation gives me more information when I'm monitoring patients for rejection, information that cell-free DNA does not provide however, that just may be because I've not studied it.

**Dr. Angella Charnot-Katsikas**:

Right. So, maybe we can open this up for the rest of the panelists to comment. So, does the evidence supports the use of the combination test? It, and, you know, is it really reasonable and necessary, as per what the evidence shows up today, to perform both tests simultaneously?

**Dr. Shelley Hall:**
So, this is Shelley Hall, I think right now, while the evidence is still relatively small, it's positive. So, there's nothing that's come out in the combination testing, that has said, this appears to be useless, we should abandon it. Um, yeah, we've discussed about the additive value of the two technologies together. Now, I think that the transplant community is more rapidly accepting cell-free DNA than they did with gene expression profiling, but gene expression profiling is also, the only technology we've had for over a decade to non-invasively monitor our patients. So, to abandon something that we've had for greater than a decade, that has documented proven utility for the new kid on the block, which may be promising, and it may, we don't know, it, may turn out to be so much more powerful that ultimately, the gene expression profile becomes a selective test for certain scenarios. We just don't know that yet. There's no there's not data out there that says we should stop looking at the more tried and true for the new kid and there is data that suggests that there are additive benefits, and we just have to continue to gather more data to see which way this is going to go.

**Dr. Anitra Graves:**
Well, we are getting close to the top of the hour, and I'm going to try to get some comments on the record regarding the application of the cell-free DNA in lung allograft testing, if you could advance the slide.

In this case, we only have donor-derived cell-free DNA as possible tests with the three manufacturers on the slide, AlloSure, Prospera and Viracor TRAC. I was wondering if I could get some comments from you about the nuances of this testing in lung transplant patients versus heart and also to comment on whether or not you would use these tests interchangeably. In other words, does one perform better than the other in lung transplant patients? Or do you not have that information yet? Let's hear your opinions about this cell-free DNA for lung transplant patients.

**Dr. Jay Mandrekar:**
This is Jay from the pure statistical point of view, I see the publications are available on lung are much in the preliminary stages compared to the heart literature that I have reviewed, that was shared here.

**Dr. Sean Agbor-Enoh:**
I agree. The volume of the publications between heart and lung is clearly different and there are much more publications in heart than there are in lung. However, if I may add, if you look at the features in lung transplantation that mood suggest that the test could be just as powerful and as useful.

Your first question about the interchangeably. It really speaks to the thresholds that are used in lung transplantation. In lung transplantation, the thresholds that have been proposed, by these tests, goes somewhere from around 0.8%, donor-derived cell-free DNA to about 1%.

Compare that to heart transplant, the threshold is somewhere around 0.2, 0.25 it's also a 0.18 to 0.25%, depending on the test to used. So, the thresholds that I use in lung, are almost 3, 4, 5-fold higher than the thresholds that I use in heart.

So, the test variability, as you know, the amount of cell-free DNA that you intend to measure goes up, the variability of the test becomes lower, and such that just technically, the variability of the test is better, at the thresholds that it been proposed in lung. I say this because, despite the lower number of studies available in lung, it would feel to me that the available studies, which currently show similar test characteristics of the heart transplant studies, it would feel to me that this test would just, have just, as good performance in lung transplant as well. So, I would stop there to give opportunity for my colleague and then, hopefully, we'll have additional comments again.

**Dr. Anitra Graves:**
Yeah, one second, as well, as you're gathering your thoughts, I just wanted to also get input about the fact, that these individual tests here, do not all have publications at all. Can you also comment about whether or not you would be comfortable with that conclusion? Dr. Agbor-Enoh in the in the case of Viracor TRAC where there's no peer reviewed publications currently available.

**Dr. Sean Agbor-Enoh:**
Yeah. If I may jump in, then say I think that in my mind, because they methods have nuances that we do not yet understand, it’s going to be hard to translate the data from one test to another test. I think that points to the same thing we said in heart transplant, I would think that it may hold in lung transplant. It is possible that, that is less of the case in lung transplant because the thresholds are 3 to 5 times higher than it is in heart transplant. It has just had me thinking about the methods, genomic methods, to translate to another, from one test to another.

The second point to this is that so if you start to forget about clinical tests and CLIA labs, and you think about that, these are genomic tests, yeah, tests that are based on genomic methods, you ask yourself a simple question. How reproducible are genomic tests? In 2015, and I'll send that publication to you guys, there was a Nature publication that tried to answer just that question, asking that themselves, how reproducible are publications on genomic tests? And that lack of information that the publication did not produce, or they use slightly nuanced methods, only 25% of those publications were reproducible. It gives you pause.
It is hard to translate one test to another, unless you can verify that the methods that I use in that test, you have a way of getting them interchangeably. So, for that reason, I would say, and this is an opinion, I want to make sure it’s stated that way, I have not done any studies to validate that.

It's just, for genomic tests it is hard, to translate one method to another method that is completely different. Unless we establish standards that these methods can show performance to those standards, it becomes hard to translate. The point I am making I'll make here as well.

**Dr. Angella Charnot-Katsikas:**
Yeah, I'd like to add to what you're, what you're standing there. I think that's it. You may mean, we certainly will look forward to whatever information you'd like to send. But that is a very

broad generalization about genomic tests. And so, I need to make sure that we are maintaining contexts and looking at specific tests. Because really, reproducibility is essentially, you know, the same test over time would be used to monitor specific patients in the vast majority of cases that would be something that we would expect, and that those tests would perform reproducibly if performed on the same patient for monitoring purposes.

So, you know, we're not really, really getting into mixing and matching of, of tests right now, for the purpose of patient monitoring. But we do look forward to whatever information you'd like to provide, because, in fact, many, many genomic tests are, in fact, quite reproducible.
So, with that, I know we're getting super close to time, but I do want to open it up to the other panelists, who really haven't had a chance to weigh in on the last two questions.

So, first thing, again, is there, is there evidence to support the utility of the two tests versus one, does that evidence exist, and really, that's the sort of a yes or no answer. And then the second is what Dr. Graves just asked, about the lung allograft testing. So, in our last, we're already over time, but in the last, for our sort of ending comments, if you could provide responses to both of those, before we wrap, that would be very helpful.

**Dr. Shelley Hall:**
This is Shelley Hall, I already replied on the dual testing, so that's on recording. I think that the lung, obviously, is behind the hearts, but is growing, and I think that the lung is a more challenging organ because there are so many more things that can cause lung injury besides rejection. So, I think that, again, the negative predictive value of it is strong. I think that there will be. I don't think we really know as much yet about where the thresholds are going to land and you know, the work ups that will be done in this space. So, I, I feel like there's enough data out there looking at what you had and obviously, I'm a cardiologist not a pulmonologist to say that it can be used more as a screening tool, but probably not yet as a diagnostic tool.

**Dr. Angella Charnot-Katsikas:**
Thank you for that. Any other final comments on the last two points?

**Dr. Steven Potter:**
This is Steve, I mean, I think for lung the data is scant but there are data available in the Keller study was interesting in that it showed with the caveat that you're talking about against historical controls, it showed an ability to really help decrease the need for invasive biopsies. So, I mean it's a nascent field, but I think it's exciting.

**Dr. Sean Agbor-Enoh:**
If you don't mind, I'll come back as lung transplant physician, just to advocate for lung. The lung has many things that can cause allograft injury, and they seem to occur at a higher rate.

Rejection, acute rejection, for example in the studies that have been published, occur somewhere around 25 to 50% in the first year of transplantation, think about that for a minute. Not including infection or other causes of acute allograft injury, that's point number one. Point number two, the thresholds that are proposed for lung are higher, they are three to four times higher than heart. I believe that, and the data supports this belief, not just the belief, the data actually, not just the

Keller study, there have been the graft prospective cohort study on lung transplant, multi-center that shows the same.

Then there's a lung study, which is the study that was done with AlloSure monitoring lung transplant patients monthly. All these studies indicate that there is true value in monitoring these patients with cell-free DNA, using cell-free DNA for its negative predictive value as a rule-out test. So, I believe that the lung, that these tests are quite useful in the lung and the data that's available, and actual cohort studies, are in studies that use this as part of routine clinical care, that, that data supports the use of cell-free DNA to monitor these patients.

**Dr. Angella Charnot-Katsikas:**
Thank you. Dr. Shah or Dr. Potter, do you have any final comments? I hope I'm not missing anybody. I want to make sure we give you the opportunity as well.

**Dr. Palak Shah:**
Yeah, I just I just made mine just before the last gentlemen.

**Dr. Steven Potter:**
I think just to add to it, I think the need is certainly higher in lung transplantation. I think the performance characteristics have been studied for a number of these assays, including the work that we had done in the Graft Consortium and so, I would support utilization, as long as that validation work has been done, which it has been done for some, some of these assays that are being demonstrated here.

**Dr. Anitra Graves:**
This is extremely helpful. I can't express enough gratitude for all of your comments. I know that Dr. Khush had to drop off, but we really, really appreciate the time that you've committed and as we are over time, I'm going to go ahead and close the Contractor Advisory Committee meeting. However, I would like to invite the panelists that are still on to please access the polls for the lung questions, they are identical to the cardiac but in the applicable way, to lung. If you could respond to those poll questions, that would be great. If you have additional comments, we certainly will take them as we have run out of time, we are going to be posting the recording and the polling responses following a QA/QI process that we have internally to make accurate. So, this will be certainly available, the proceedings are only available for the public to review at that time. And with that, I will ask if there are any closing comments before we adjourn.

**Dr. Angella Charnot-Katsikas:**
I would just like to echo, Dr. Graves, and thank you all for your very valuable input today. We truly appreciate your time and expertise.

**Dr. Steven Potter:**
Steve, I just want to say thanks for the opportunity to contribute. Thanks for having us.

**Dr. Sean Agbor-Enoh:**
Thank you so much. Thank you.

**Dr. Anitra Graves:**
And with that, I will release you for the day, and again, thank you all for participating in the meeting.

**Dr. Sean Agbor-Enoh:**
Can someone hang on with us to help us do that polling? If you don't mind so we can answer the polling questions and then just do it. Is there a way or that someone can help us? I can remember by e-mail if that's helpful.

**Jocelyn Fernandez:**
Hi, this is Jocelyn. Dr. Agbor-Enoh, I'm going to send you an e-mail and we can troubleshoot with you after this call.

**Dr. Sean Agbor-Enoh:**
OK, Thank you. Are you going to do it right away so I could do it now or are you going to find another time?

**Jocelyn Fernandez:**
I am going to do it right now

**Dr. Sean Agbor-Enoh:**
OK. Thank you so much for your e-mail, then. OK, Thank you.

**End time: 2:13:32**