Due to unexpected technical difficulties, audio quality is compromised until 12 minutes and 13 seconds into the recording. The transcript starts at this time. We apologize for the inconvenience.

Beth Eaby-Sandy: Which we haven't really done in the past and that means there's advances, which is exciting.

Rasheda Persinger: So, this just further breaks it down for us in terms of adenocarcinomas, the most likely to harbor a molecular biomarker. Most common type in non-smokers. Squamous cell. Generally more centrally located. And other enlarged cells we have the neuroendocrine cancers, the carcinoid tumors, and so forth.

Rasheda Persinger: I would like to make note, and I know we're making note of it in a later slide, is that even with squamous cell, just don't not count them, or discount them in terms of doing molecular testing on them. If their clinical picture shows otherwise, such as if they're a never smoker, so forth.

Rasheda Persinger: Biomarker testing. Who should we test leads right into this next slide. And so molecular testing based on the NCCN guidelines for non-squamous histology, primarily found in adenocarcinoma, but should test all nine squamous lung cancers. Per the NCCN guidelines is asking us to test those that are driver mutations, meaning that there is a medication that's FDA approved for the treatment of that mutation, such as EGFR, ALK rearrangement, ROS1, BRAF, PD-L1. Testing should be a part of broad molecular profile.

Beth Eaby-Sandy: And I think it's interesting, because people say, "Well, what's the point if nothing else is approved except for what's listed in number one?" But NCCN does make a statement saying the goal is to find any other molecular drivers that you could enroll in clinical trials. Just gives you a better clinical picture, and possibility of trials, even if it's not in the first line, but in other lines going forward.

Rasheda Persinger: So, the NCCN Guidelines on squamous cell carcinoma consider, as I mentioned before, EGFR and ALK testing in never smokers, mixed histology specimens or small biopsy specimens. Consider ROS1 and BRAF broad molecular profile, and PD-L1 testing recommended. This is just what we already know. Yes, there are guidelines, but we also need to align that up with our clinical picture of when we assess patients.

Beth Eaby-Sandy: And I think this is for the patient that, you get a pathology report and it says, "Metastatic non–small cell lung cancer, predominantly squamous." And sometimes the pathologist will even say, "80% squamous or adenosquamous and only 20% adeno," but if there is that adeno component to it, we should be testing for it.

Rasheda Persinger: Absolutely.

Beth Eaby-Sandy: And certainly the clinical picture. Do you have any squamous EGFR mutated patients in your practice?

Rasheda Persinger: Yes, we do, and we got it. They came in for a second opinion. They actually had testing done prior to coming. Well, not NGS testing. They were squamous, and the recommendation was just to go right to chemo and then we did additional testing, because they were a never smoker, and it showed that they had an EGFR mutation. And they are still in therapy today, and responding.

Beth Eaby-Sandy: Yeah. I've had five in my practice, squamous patients with EGFR mutations. But what did they all have in common? They never smoked.

Rasheda Persinger: Never smoked. So, that's the clinical picture.

Beth Eaby-Sandy: Yep, so we can't just say we won't test them, and we should definitely test them, as well.

Rasheda Persinger: And in your practice, are you seeing that they are responding just as well as those who are adenocarcinoma non-smokers.

Beth Eaby-Sandy: Yeah, absolutely.

Rasheda Persinger: So, the biomarkers in non–small cell lung cancer adenocarcinoma. We've kind of mentioned them, but this is a whole list. What I didn't see listed here that I just wanted to bring attention to was the [inaudible] exon 14 that I'm skipping that's not listed here that is a driver mutation that we can treat. And then also, I did not see [inaudible]. That is another one that we're testing for too also, because we know that there's new FDA guidelines, approved drugs for that particular mutation.

Beth Eaby-Sandy: Yeah. These pie charts change year to year.

Rasheda Persinger: Month to month almost, right?

Beth Eaby-Sandy: There's a lot of them. That's the point.

Rasheda Persinger: Yeah. Yeah. And so biomarkers in squamous cell carcinoma, EGFRvIII and PI3KCA, EGFR, and such as what we listed here. Again, just another pie chart to show you different mutations that have been seen in squamous cell carcinoma.

Beth Eaby-Sandy: Do you commonly do that in your practice? I don't, so it's okay to say no.

Rasheda Persinger: No. No.

Beth Eaby-Sandy: Okay. I mean, I included this, because there's data for it, but other than EGFR, which would be really uncommon in squamous, unless there's a clinical picture for it, we don't routinely test squamous patients for next gen sequencing, so I want to be clear on that.

Rasheda Persinger: No. Yeah. Absolutely. I think it definitely aligns with what is the clinical picture?

Beth Eaby-Sandy: Yeah.

Rasheda Persinger: Recent updates to the molecular testing guidelines. IHC is not an acceptable test for EGFR mutation, and should not be used to treat EGFR TKIs. In ALK testing, IHC is an equivalent alternative to FISH testing. Multiplex broad sequencing panels are preferred over single gene testing, and oncologists may use molecular testing in patients with histologies other than adenocarcinoma who exhibit clinical features that predict for an oncogenic driver. Just what we're seeing in terms of the squamous cell.

Beth Eaby-Sandy: And let's break that down for a second.

Rasheda Persinger: Yeah, absolutely.

Beth Eaby-Sandy: So, the multiplex broad sequencing panels. Again, people might say, "Oh, they might be more expensive than a single gene test." But the problem with the single gene testing. So, if you're going to say, "Okay, I'm going to send this tissue for just EGFR, because that's all I think this patient might have," which you don't know. And then they come back as negative. "Okay, well let's send again for ALK." You don't want to do those single gene, because you're going to waste your tissue after just a couple. And I've seen that happen in some practices, so they should just be doing the broader testing panel.

Rasheda Persinger: And I think it's important to [inaudible] especially in the community setting, where we know that 85% of oncology patients are treated and seen. And so it's very important, again, for those APPs to be knowledgeable, and especially if they have influence on the oncologist in that practice, to let them know why it is more beneficial to get a broad panel versus just a single test.

Beth Eaby-Sandy: And then the first bullet. Just don't be confused by immunohistochemistry or EGFR over expression. That's something that will be reported sometimes, but over expression is not the same as a mutation, so don't be confused by that and say, "Oh, you have an EGFR mutation." A lot of cancers, lung, head, and neck over express EGFR wildly all the time, but that doesn't mean it's mutated in the DNA.

Rasheda Persinger: I know in our practice, I'm not for sure in yours, as well, Beth. If we have a second opinion patient that comes in, and they have IHC testing results, we typically will redo it, either if we'll do tissue in house or we'll do liquid plasma, which we'll talk about later.

Beth Eaby-Sandy: Yeah, it all depends. I mean, if someone has an EGFR mutation from an outside molecular testing, I'm not going to dispute it. I think, usually I will say, "Okay, that was a reputable company. We'll go with that."

Rasheda Persinger: Even if it's negative?

Beth Eaby-Sandy: Well, if it's negative, we may repeat, and again, it depends on the reputability. Was it from a reputable company like Foundation Medicine or something like that, or was it just a very small panel that only tested three things? Then, of course, we would repeat it.

Rasheda Persinger: So, sampling challenges, and we're talking about tissue. Lung cancer biopsies are less cellular than other solid tumors. Remember, lung cancer's a very heterogeneity cancer, so depending on where they go in and get that needle and pull out that sample, will determine of what mutations or what we see in that specimen. Bone biopsies usually not good due to decalcification to read for pathology first. Remember, when you have to decalcify a sample, you are impairing the DNA and RNA, and so therefore, you could get a false negative. Seeing that a patient is a never smoker that presents to you, but the panel came back to say there's no driver mutations. You want to give the patient a benefit, and either get a different type of tissue, or liquid plasma.

Beth Eaby-Sandy: Rasheda's exactly right on that, and what we've done in the past, if we need more tissue and the most obvious is, let's say, a femur met. We already know the patient has adenocarcinoma lung cancer. I don't need them to tell me that again in the femur. So, you would have to say to the pathologists, you don't have to read it for pathology, because if you do that, you have to decalcify it, and then you can't do your molecular. So, you would just say to them, "Don't decalcify it. You don't have to tell me it's lung cancer. I already know that. It's pretty obvious from the clinical picture." And then if they don't decalcify it, then they can use it for molecular testing, but that's a pitfall we run into. Because bone biopsies they're so readily available sometimes.

Rasheda Persinger: Exactly.

Beth Eaby-Sandy: It's easiest place to get tissue, but if you don't need another pathologic diagnosis, then they don't have to do it.

Rasheda Persinger: Yeah. Quality assurance of genomic medicine, multiple platforms, validation. Just making sure that if your institution are using outside companies, what is the literature that supports their validation? Is everything aligned? Beth and I was just generally talking this morning that there are so many pop up companies, because this is such a hot topic, but have they had done validations, repeated validations to confirm that what they're providing us is true? Logistical timing of DNA sequencing can take weeks. Centralized versus send out to distant laboratory. Q and S, quantity and quality non-sufficient need 10% to 20% of valuable cancer cells in sample for reliable results.

Rasheda Persinger: Methods of molecular testing in non-small cell lung cancer. DNA sequencing, looking for activated somatic mutation. Remember, we're looking somatic, meaning what is the makeup of the DNA of the tumor, not germline, what you're born with. Insertions, point substitutions, in frame deletions. DNA sequencing will detect mutations in EGFR, T790M which is the resistance for EGFR, KRAS, BRAF, HER2, and MET. Different methods. You have direct sequencing, PCR, NGS, which is those nice little kits we get, we all probably have in our office. Direct sequencing, only one mutation at a time, and NGS kits can do broad panels. Anything you'd like to add to that, Beth?

Beth Eaby-Sandy: Just to say you've made a great point about a somatic mutation. I can say a lot of times patients who come to us with lung cancer and say, "I have an EGFR mutation. Am I going to pass it to my children? Did I get it from my parents?" The answer is no. These are somatic tumor mutations. They are not germline. They're not familial, and they are not passed on, so you can reassure them of that. As opposed to breast cancer and other cancers where that can be something that's familial.

Rasheda Persinger: Exactly. So, the next couple of slides are just examples. This is what Beth has pulled from UPenn's and how they report in terms of sample DNA sequencing panel at UPenn, and you'll see. To the left, there is a solid tumor. My left, I think. Yeah, your right. Maybe, I guess.

Beth Eaby-Sandy: Their left.

Rasheda Persinger: Their left, too. Okay. Solid tumor NGS report, and it just tells you, if you go on down, you'll see at the bottom where it says BRAF, PB600E, and then on the right hand there, it tells us the variance of uncertain significance.

Beth Eaby-Sandy: My point in showing this is that it's really confusing. These are not easy to read, and I have a couple more of these. So, these are not necessarily easy to read. So, you have to, again, look. This is a solid tumor, next gen sequencing, NGS report. So, you know that this is your DNA sequencing panel. So, we can see that the disease associated variants, BRAF. The first one, I don't know. I have no idea what that is. So, that's great, but the second one is be BRAF V600E, which is uncommon, but targetable in lung cancer. So, that is an important finding in this. And then on the right hand side, you have, because they have to confuse us, they put all of these other variants of uncertain significance, and that's just what they are. I don't know what the significance is. I can look through all of them and say, "Okay, good to know. Word salad." But they're not easy to read. So, you have to really look and find out which one may be applicable to your patients. And sometimes I get one back like this that has 10 things, and none of them are applicable to my patient, and we move on with chemotherapy. So, yes, Anne?

Anne: [inaudible]

Beth Eaby-Sandy: No. The actionable ones would be listed under disease associated variants.

Rasheda Persinger: Did you say actionable or inactionable?

Anne: Non actionable.

Beth Eaby-Sandy: Oh, yeah, inactionable.

Rasheda Persinger: Yes. Yes.

Beth Eaby-Sandy: Right, right. So, of uncertain significance, but, see, in the molecular labs, they don't want to tell the provider they're inactionable. They can't make that statement as a molecular testing platform. So, they'll say it's uncertain. It's uncertain. I don't know what you will do with this. Now, we know I'm not doing anything with that, because it's word salad, and I don't know what they are. But as a molecular testing company or even at Penn in our department, they can't say these are inactionable because, "Hey, maybe I have a clinical trial for EZH2PK4." You know what I'm saying? They can't say those exact words, so they term it uncertain significance.

Rasheda Persinger: And I think that's a great point to make, that today they're uncertain. Five years, they may have benefit, and they may be a driver mutation.

Beth Eaby-Sandy: Yeah, and the patient might be alive in five years-

Rasheda Persinger: Exactly.

Beth Eaby-Sandy: And you can go back to that and say, "Oh, you did have that."

Rasheda Persinger: And especially when you look at certain... Going back to lung cancer being heterogeneity, even within the mutations. If you want to go retrospective and looked at, why did this particular EGFR group of people responded better than this other EGFR? Same EGRR mutation, but when we look at these other uncertain significance, it may provide us with valuable information going forward.

Beth Eaby-Sandy: Yeah.

Rasheda Persinger: Methods of molecular testing in non-small cell lung cancer. RNA fusion panels. It's the... I won't say the new thing, but it's an important thing to know. It looks at the gene rearrangements, so we're looking at that ALK, that ROS1, the RET, the NTRK, the FGFR. FISH and IHC fall in this category. RNA fusions can detect fusions, abnormalities. The UPenn report, RNA fusions panels also reports EGFR, but it's very important to note that, though they report it, it is not the EGFR mutation, because remember, RNA is looking at rearrangement. EGFR mutations are in DNA, okay? Sequencing. So, because EGFR is noted on an RNA panel result, they're not the same EGFR mutation and therefore, would not respond to a TKI inhibitor. If you want to add to that-

Beth Eaby-Sandy: Yeah, so these RNA fusion panels are new. We've started doing them at Penn in the past year. I think you will see more and more of them. The one thing, I mean, ALK, ROS1, RET, you can find on here, but you probably already found it in an IHC or FISH test before this. But the one thing is NTRK. So this is where your sensitive NTRK is found. Not in the DNA sequencing panel, but in the RNA fusion transcript, so if you're not doing them, you're not going to find NTRK. Now, the good news is it's 0.2%, so it's really rare, but this is the one thing that RNA fusion panels are actually good for. But if you go to the next slide, this is what they look like. It looks just like the other one. Right? So, I had a patient, and I don't know if this was the one. It probably isn't, but I had a patient with a fusion transcript panel that she said EGFR and I thought, "Oh, EGFR mutation. This looks like my fusion transcript panel." And they're like, "No, that's RNA." I'm like, "You are confusing the heck out of me." Yes, Anne.

Anne: [inaudible] question. So, when you're ordering these panels, does it automatically... Do you check a box to say oh, check NTRK?

Beth Eaby-Sandy: So, they're all reflexed at my institution, but if you are doing this in your institution or at foundation, generally, most people are just checking a box now for a broad sequencing panel.

Anne: [inaudible]

Beth Eaby-Sandy: If they do that. So, it depends if the company does that test, and that varies.

Rasheda Persinger: And I want to say that Beth works at the ideal lung thoracic institution with all these benefits of RNA and DNA testing. I work at John Hopkins, and we don't have an RNA fusion reflex at all. We only do DNA sequencing and we have to send out. And in regards to Foundation Medicine... I hope I'm okay to say that.

Beth Eaby-Sandy: Yeah, you are.

Rasheda Persinger: Foundation Medicine, you have to check and you also have to call, because that is not something that they routinely do is an RNA fusion. You have to let them know that that's what you're looking for, in order for them to test. And then I think it's the CDx, because foundation platform has different testing that you have to use to get that, and then in addition, I wanted to make a comment in regards to Beth saying that we're really using the RNA for the NTRK findings of a NTRK rearrangement or fusion is, I guess, the proper word to say. I was talking to our thoracic oncologist at my institution. We're wondering. Though, yes, you can get ROS1 and ALK rearrangement with DNA sequencing without any problem, but are we missing a small population of patients, because they are we arrangements,

because we don't have the testing for RNA fusion? Just a thought, no evidence to support it or not. But it's just a thought to think of, because they are more apparent on the RNA versus the DNA.

Beth Eaby-Sandy: And apparently these RNA fusion panels are really useful in other cancers, particularly some of the neuro and CNS tumors, because when I called our molecular department to complain that they call the EGFR and it confused me, they said, "Well, that's actually really applicable to brain tumors." So I said, "Oh, okay. I didn't know." It's less useful in lung cancer, though is useful for NTRK, but it is useful in other cancers, as well, so I think it will become more mainstream as more data emerges for looking at RNA fusions.

Rasheda Persinger: So, methods of molecular testing. Either IHC or FISH. ALK, ROS1 can be performed by FISH. Highly specialized equipment, specially trained staff, ALK, ROS1 can be done by IHC as well, identified as a protein expression. Easier, just as good as FISH, and faster.

Beth Eaby-Sandy: Yeah, and so would you say, in your institution, these are the ones you get back first?

Rasheda Persinger: Yeah.

Beth Eaby-Sandy: Yeah. I mean, these are the ones that are pretty quick to come back, which is nice. Other than the sequencing the whole DNA. Takes weeks sometimes.

Rasheda Persinger: Yes, and sometimes we find that we have to kind of remind them. Did they do this, though it's supposed to be reflex, doesn't always happen as such.

Beth Eaby-Sandy: Doesn't always happen.

Rasheda Persinger: This is a sample for the FISH analysis. I think Beth has already stated. All of them are similar, and so you just need to know what you're looking for and what test was used to provide you with your results.

Beth Eaby-Sandy: That one's positive for ALK, and again, that's one that you can say, "Yes, I can take that to the bank. I can treat on that."

Rasheda Persinger: So, liquid biopsy for molecular testing. So, this is the whole new world of things that has come into lung cancer, probably within the last five years, would you say?

Beth Eaby-Sandy: Yes.

Rasheda Persinger: Blood tests to detect these mutations. Some are starting to also identify PD-L1. Beth and I kind of talked about this loosely beforehand, because it's my understanding with liquid blood testing, they're using DNA sequencing, and so PD-L1 is a protein expression. And to my knowledge, that is not something that you can get from a DNA sequencing. Would you like to expound on that?

Beth Eaby-Sandy: Yeah, so we had this conversation. So, currently you can't get PD-L1 out of blood in the major companies. Now, I was in Chicago at a conference last year, and this company said, "We do it in our blood tests." And I said, "Really?" Said, "Yeah!" So, I took it back to my clinic and one of the doctors I work with, he said, "Let's go Google that company." So, we did. We went to the company's

website, and they had absolutely no published data to support their platform that says they can identify PD-L1 in blood. So, really, anyone can say, "Oh, yeah, I detected it," but are they really detect... Did they correlate it with tissue and say, "Yeah, I can tell you that everything in the..." I don't want to name names, but there are major companies obviously that do this testing, and they have validated it like crazy.

Beth Eaby-Sandy: If you go on their website, you will see article after article after publication that shows how they have validated their tools. So, be wary of companies that say, "I do this, we do this, we do this," and then they've not validated it, because apparently it's not FDA regulated, I'm told. So, really, any company can make claims, and say we can test for EGFR or PD-L1. So, I think the PD-L1 one's still out there, and we were also saying PD-L1 is easy to test for on tissue. You need almost no cellularity, and you can IHC stain it, and figure it out, so it's not-

Rasheda Persinger: And it's usually prioritized.

Beth Eaby-Sandy: It is usually priority.

Rasheda Persinger: PD-L1 and then everything else. Often referred to as liquid, biopsy can be done without invasive procedure and usually requires only about two tubes of blood done as routine phlebotomy. Particularly an attractive option, we're looking for T790M mutation or any resistance mutations. It cannot detect histology, thus cannot detect if there is a small cell transformation from EGFR in non–small cell lung cancer. Liquid biopsy relies on DNA shedding from the tumor into the blood stream, and this can vary, so the sensitivity of liquid biopsies may vary, and I just want to make a point there.

Rasheda Persinger: I think it's very important to know when to use liquid biopsy plasma. You cannot use it or you should not. Let me say that, because I know there are some that do. You should not use it in a patient who may have stage four, but limited burden because remember, we need that DNA that's going to shed into the blood, and if there's not a lot of DNA shed into the blood, you're going to get a negative results more than likely. This is a report of a sample for liquid biopsy report. You'll see that the alterations are on the left side. It listed the little frequency, how much was seen there. I think most liquid plasma companies will tell you if it's listed, regardless of how low it may note it in the little fraction. You still need to treat it. It's true. You should treat it, and then it provides you the FDA approved therapies, along with the clinical trial.

Rasheda Persinger: And so this is just a picture of showing you different other advanced stage solid tumors where cell free DNA is used, and what they have seen and found in that. Sensitivity and specificity. These are two meta analysis were conducted looking at the sensitivity and the specificity of EGFR detection on liquid biopsy. Remember, sensitivity is looking at all the positive with a little bit few of negative, false negatives, and specificity is looking at all the negatives, right? And so with little or few false positive. So, just note that. And so as you can see, the confidence interval is very high, showing that there is a sensitivity and specificity with the use of liquid plasma.

Beth Eaby-Sandy: Yeah. I mean, basically the specificity is telling you they're not calling false positives. They're not going to say, "Oh, I found EGFR," if it's not there. So it's unlikely they would do that. But in the sensitivity in this, was a fairly low, I think. Only 67% were calling EGFR when it was there on tissue.

So, you are going to miss some, and as we go in these slides, it will lead into showing why we think you should do both on everybody.

Rasheda Persinger: And that's a good point. I say that it's still high, because it's 67 is more than 50%. That's just my personal bias, but also you have to understand is that it goes back to what I said earlier. When are they using the liquid biopsy plasma? Because if you're not using it appropriately, you are going to get negative. And as Beth said, we'll see in later slides of where we're talking about that, as well.

Rasheda Persinger: So, here we go. Accuracy of tissue versus liquid biopsy. When evaluating for T790M, one study found that sensitivity for detection in plasma was 70% of patients with confirmed tissue diagnosis of T790M. Shows a concordance there. In patients with T790M negative tumors, T790M was detected in plasma of 31% of these patients. So, you're saying patients that had biopsy done on their tissue was shown to have a negative T790M, but when they correlated that with liquid biopsy plasma or liquid plasma, there show that there was a positivity there. Results. Tumors are heterogeneous and results may be missed on tissue biopsy, as well as missed on liquid plasma. If there is insufficient DNA shedding into the plasma, liquid biopsy may be falsely negative as well. Therefore, in the second line setting looking for resistance mutations, it is reasonable to start with the least invasive tests, liquid biopsy. However, if the results are negative, you need to do a biopsy. You should give that patient the benefit. Anything you want to add to that?

Beth Eaby-Sandy: Nope. That was a kind of pivotal study.

Rasheda Persinger: Yeah, it was. So, this is tissue and plasma testing or both. So, this is a study done by Aggarwal at UPenn. 55 patients who have both blood and tissue testing reported. There was a subset when they looked at it M1A was 13 patients with locally advanced disease in chest. M1B was 32 patients with metastatic disease outside of the chest. And so you'll see this graph here that shows detection of therapeutic targe table mutations. In M1A, you had about maybe 45 or 50 that were found in tissue and plasma, and then only found in plasma only was approximate... Or in tissue only, was approximately maybe 55 or so, as opposed to M1B, because it's metastatic, larger tumor burden, most likely are shedding more DNA in the bloodstream. You'll see here that tissue and plasma was higher and that in the case when you looked at found in plasma only versus found in tissue only, there was a little bit, little bit more actually found in tissue versus in the plasma.

Beth Eaby-Sandy: Yeah, but I think the concerning thing there is the 18% of those patients were found in plasma only. So, if you were only going on tissue biopsy, you would have not found those molecular mutations.

Rasheda Persinger: Exactly. Exactly. So, the NILE study, which was another study commercially available for cell free DNA test as effective as tissue testing in detecting non–small cell lung cancer biomarkers. Clinical practice guidelines recommend genotyping for all patients with newly diagnosed metastatic non–small cell lung cancer. The study aimed to demonstrate non-inferiority of comprehensive cell free DNA versus standard of care tissue genotyping, and this was done from July, 2016 through April, 2018, and so as you can see, patients consented was 307. By the time they got to what patients were actually using this study, it was 282, and so this was the largest cell free DNA study. In previously untreated metastatic non–small cell lung cancer finds that cell free DNA test identifies guideline recommended biomarkers at a higher rate among 282 patients. Standard of care identified 60 patients, and cell free

DNA identified 77 patients. Again, making note of those patients that we potentially may have missed. With high tissue concordance, 80% of cell free DNA clinical sensitivity.

Beth Eaby-Sandy: So, that was better than that 67 I had showed you in the [inaudible] analysis. So, I think that just shows technology's getting better.

Rasheda Persinger: Exactly. Testing is definitely getting better. So, EGFR, ALK, and BRAF had 100% positive predictive value for cell free DNA versus tissue with a higher turnaround time, because we're not waiting as long, seven days. This says nine versus 15 days, but I've even gotten it back in seven days. Using cell free DNA in addition to tissue increased detection by 48%. 60 to 89 patients. Okay, so those are 19 patients that may would have not gotten treated for their mutations.

Beth Eaby-Sandy: Exactly, would not have found their targeted therapy and would have gotten bad chemotherapy that wouldn't have worked for them.

Rasheda Persinger: Exactly.

Beth Eaby-Sandy: So, it's impressive.

Rasheda Persinger: You made a little comment there, and I know it's not an aside, but I must say since you hinted to it. It's important to note. Their studies have shown that patients that are EGFR mutated do not respond well to chemotherapy. They actually respond worse and they have a poor outcome. So, again-

Beth Eaby-Sandy: We will get to that in the EGFR deck. We'll talk about that. [crosstalk] I know.

Rasheda Persinger: And more completely than tissue-based genotyping, immuno-oncology biomarkers for non–small cell lung cancer, PD-L1. PD-L1 expression IHC test usually comes back in three days after ordered on pathology tissue. Often referred to in some clinical trials as TPS percent, tumor proportion score, or in other cancers, CPS percent, combined positive score of tumor plus inflammatory cells. Imperfect biomarker cutoff points vary within clinical trials. And do you have anything to say about that? Okay. Further information on immuno-oncology, pembrolizumab. Only drug relying on TPS PD-L1 score for certain approvals. However, if PD-L1 expression is greater than 50%, or now we even are using it if... It really doesn't matter.

Beth Eaby-Sandy: That's controversial. We're going to talk about that in another slide deck, but...

Rasheda Persinger: I won't go into it, then.

Beth Eaby-Sandy: But there are approvals based on it.

Rasheda Persinger: Yes. Durvalumab and study showed that the TPS have 25% to look at responses. Subset analysis of the PD-L1 negative patients did not have a statistical significant benefit in the stage three setting, and this is stage three unresectable.

Beth Eaby-Sandy: Correct, yeah. I don't want to make it... That was a subset analysis that was not perspective, so we don't use that as a treatment guideline, but it is worth noting that that certain subset did not have a statistically significant benefit.

Rasheda Persinger: So, tumor mutational burden. Tumor mutational burden is the number of mutations per DNA megabase. No clinical guidelines for decision making at this time. Imperfect, difficult to determine what qualifies as high TMB. The assays used to measure TMB have had varied results.

Rasheda Persinger: So, there was a trial, phase three trial of CheckMate 227, study of nivolumab plus ipilimumab in non–small cell lung cancer. First line setting chose TMB as a biomarker. In addition, looking at the PD-L1 found progression free survival higher in patients with high TMB as defined as greater than or equal to 10 mutation, irrespective of PDL one expression. However, the overall survival data updated in January of this year was not different for high or low TMB. There was a MYSTIC trial. Looked at durvalumab and tremelimumab durva and treme not better than chemo in all-comers. More confusion in looking at the TMB status and regards. It is important in this study, which we don't have highlighted here. When they looked at an exploratory analysis of the study, and they separated out those patients who got durva as a single agent and they looked at their PD-L1.

Rasheda Persinger: If it was greater than 25%, there was an overall survival improvement of 16.3 months compared to 12.9 with chemo only. Okay. And then when they looked at patients with high TMB, which when they did this exploratory analysis, they defined high TMB as 16 or more mutations. And when they looked at these patients, the overall survival in patients that used the durvalumab single agent versus chemo, there was still an improvement, 16.5 versus 10.5 survival. But then when they looked at low TMB, when they looked at those patients that had a low, less than 16, overall survival. Once again, when they separated and they looked at durva plus the treatment versus durva alone versus chemo, durva still had a benefit there. So, again, I think what this speaks upon, when they looked at this trial, when they initially had this trial, they looked at unselected. All different types, no matter. They looked at the PD-L1s. They had the patients in the TMB, but when they did further analysis, there was a more distinct improvement which leads, which we spoke about earlier, that just may be not right now. TMB really doesn't hold much credit, but maybe down the line.

Rasheda Persinger: So, clinical pearls. Guidelines recommend testing in certain populations. You cannot treat a mutation that you never found. APPs must understand the methodology to explain to patients. IHC and FISH and using RNA for finding fusions. We have more savvy patients now. I don't know about you all, but they're coming in with journal articles, their cells. They have looked at the commercial with all of this immunotherapy. They are coming in, to their benefit, educated, right? They're not just coming in, and just expecting you to tell them what they need and go from there. So, it's very important as APPs that we have a sense of understanding, so therefore when we see them and follow up, because they're wanting to have it in layman's terms, that we're able to communicate this to them. Interpreting reports. Beth already mentioned how confusing, which I agree that some of these results can be, so it's very important that you know and understand what they mean. Site of biopsy, type of mutation. Liquid biopsy, generally sensitive, less invasive, faster results.

Beth Eaby-Sandy: Okay, so we're going to go back and answer our questions again. So, according to the NCCN oncology providers should be performing molecular testing in which types of patients with non–small cell lung cancer? Minimal or never smokers, never smokers with adenocarcinoma, all non-squamous histologies but not squamous cell carcinoma. All non-squamous histologies and some squamous cell carcinoma, if they are non-smokers, or I'm still unsure.

Rasheda Persinger: Oh, and we still get music, too?

Beth Eaby-Sandy: Oh, my gosh. We've got 100%. that's really exciting. Yeah, so I think we pretty much hit that.

Rasheda Persinger: And the next question.

Beth Eaby-Sandy: Which of the following is a true statement about molecular testing in non–small cell lung cancer? Is it EGFR can be found on DNA sequencing and FISH, but not IHC? ALK can be found on DNA sequencing, FISH, or IHC. NTRK that is sensitive to treatment is best found on a DNA sequencing panel. Using liquid biopsy is a faster way to detect a secondary mutation in EGFR positive, non–small cell lung cancer, such as a small cell transformation, or I'm still unsure.

Beth Eaby-Sandy: Okay. So, while we had a slight improvement, but yes. I mean, number two you can find it on all of those and it's treatable in all those. Number one, so EGFR should not be found on FISH. It is found in IHC, but as a over expression, but treatable EGFR mutations would only be found on a DNA sequencing panel. And look, NTRK, no one wanted to touch, which is fine. And then liquid biopsy is a faster way, but remember, not for pathology. So, you wouldn't be able to detect small cell, because that's a pathologic diagnosis. It's not a molecular DNA. And it looks like less people were unsure, but still somebody or a couple of people were. It was a loaded question. It was hard to interpret all of that, but it's a confusing topic and that's why we spent a lot of time on it this morning. So, all right. Thank you very much, Rasheda.

Rasheda Persinger: Thank you.