Transcript Details
This is a transcript of a continuing medical education (CME) activity. Additional media formats for the activity and full activity details (including sponsor and supporter, disclosures, and instructions for claiming credit) are available by visiting:
https://reachmd.com/programs/cme/optimal-tissue-acquisition-handling-and-processing-ngs-and-pd-l1-testing/13391/

Released: 05/31/2022
Valid until: 05/31/2023
Time needed to complete: 75 minutes

ReachMD
www.reachmd.com
info@reachmd.com
(866) 423-7849

Optimal Tissue Acquisition, Handling, and Processing NGS and PD-L1 Testing

Announcer:
Welcome to CME on ReachMD. This CME activity titled: *Optimal Tissue Acquisition, Handling, and Processing NGS and PD-L1 Testing* is brought to you by the American College of Chest Physicians and supported by an independent educational grant from AstraZeneca Pharmaceuticals, an educational grant from Genentech, a member of the Roche Group, and an independent medical education grant from Merck Sharp and Dohme Corporation. Before starting this activity, please be sure to review the faculty and commercial support disclosure statements, as well as the learning objectives.

Dr. Edell:
Well, hello, good afternoon. Good morning. Good evening. From wherever you're visiting, we want to welcome you to this third webinar in a series of five on lung cancer management. I'm Eric Edell. And it's a real privilege to be here with my co-chair, Dr. Septimiu Murgu, from the University of Chicago. We're honored to put on this webinar with the sponsorship of the American College of Chest Physicians and our sponsors that you can see listed below. As I mentioned, Dr. Murgu is my co-chair from the University of Chicago, and I'm thrilled that he's here with me. We have three expert panelists today that will be presenting on the topic of tissue acquisition and interpretation in its use for managing lung cancer. Dr. Narjust Duma is the - is an Assistant Professor of Medicine and the Associate Director of the Cancer Equity Program at Dana Farber Brigham Cancer Center. Dr. Christopher Hartley is the cytopathologist at Mayo Clinic in Rochester, Minnesota. And Dr. Jeffrey Mueller is a pathologist from the University of Chicago in Chicago, Illinois. We would encourage you during the webinar to please submit any questions that you might have in the portal so that we can bring those to the attention of our presenters.

We'll start today with our two pathologists going through and reviewing the preparation - acquisition and preparation of specimens, followed by Dr. Duma in the application of these specimens for next gen sequencing and their use in immunotherapy, PD-L1, and the management of these patients.

The disclosures you see here.

And with that, I will ask Dr. Mueller to please take the screen. And we look forward to everyone's participation.

Dr. Mueller:
Hello, everybody. I'm Jeffrey Mueller. I'm at the University of Chicago, I'm a pathologist here. So, I'm going to be talking about specimen processing.

We'll start with a question just to get everybody warmed up, so I will go ahead. And alright. Here we go. It's not advancing. Alright. So, I'm going to be going over what happens to a biopsy or a cytology specimen after it leaves the bronchoscopy, or I should say after it leaves the needle. To better understand or how to troubleshoot problems with diagnosis and molecular, I think it's easier if we have a good communication across disciplines. And I think if everyone understands exactly what happens to pathology specimens, it's easier to troubleshoot or understand the problems that we have in making diagnosis or with molecular testing. So, I'll go through what happens to a specimen. It'll be a specimen journey.

But to start, the two common things that we receive in pathology, we get core biopsies, really it should be any tissue biopsy. This can be

© 2023 ReachMD

a forceps biopsy, and some define that is anything that can be picked up with a pair of forceps. The fluid and the cells in the fluid and small tissue fragments could be considered the cytology part. But really, they're not real strict definitions. If you put large tissue fragments into a cytology specimen container, it would still get processed just the same. But in general, tissue biopsies go into formalin, cytology specimens go into CytoLite. Core biopsy, these, usually in most institutions go into formalin. Formalin is a mixture of formaldehyde and water. It's a fixative. It's a little bit different from CytoLite. Some institutions put everything into formalin, they don't put anything into CytoLite. But anyways, most tissue biopsies tend to go into formalin.

And after it's put into formalin, it goes to surgical pathology. And this is one of our gross rooms. This is a pathology assistant. He's standing in front of a grossing batch. He takes what he doesn't gross, and he takes each biopsy out of the formalin, measures it, describes it, and he dictates all of this so it can go into the report. Then he places it into a small plastic cassette. This is what the cassette looks like. These cassettes will have patient information, it'll have an accession, a search path ID number, and it'll have other patient information. Now, a lot of them have the medical record number on there. And then he usually places the tissue in between these sponges, and it gets closed. This is a lid here, and then that gets sent on - it gets put into formalin and then that gets sent to the histology lab.

In histology, that - the - all these cassettes, these are put into baskets, and they get loaded into a tissue processor. And what a processor in general does, is it extracts water out of the tissue and replaces it with alcohol. And this takes place usually overnight in most institutions. It's a long process and - but this is loaded with all the cassettes it can be all kinds of surgical specimens and as well as cytology cell blocks.

And then the next morning or late that evening, a histo tech will come in and what they do is they remove the tissue from that cassette, and they take this – a small metal well, they fill this with paraffin with the tissue in it. This is paraffin wax that's melted, and then they take that cassette that has all the information on it, the patient information, and they set it right on top of this well, and they cool it. And then when they lift it up, you get this tissue block. This is a paraffin block, and it's attached to the cassette. So, all the patient identification information is on this plastic cassette. And here is the tissue block.

And then the histo tech will put that – mount it onto a microtome. This is a microtome right here. The cassette is right here. And there's a piece of tissue, and there's paraffin around it. And they have a handle on the microtome. Every time they rotate the handle, this moves up and down and it moves against the blade, and it cuts a 5-microns thick section off. So, each one of these here is a 5-micron thick section. And they take each one of these sections, they put these in water, they float them in a water bath, and then they take labeled cassettes, and they lift up the tissue section or the paraffin section onto the slide.

And then that slide gets coverslipped - I'm sorry, it doesn't get slipped, it gets stained. It goes on to the staining part. And that's what this is. This is an automatic stainer, some do it by hand, depending on how many specimens they have a day. But they – here, they – we would load all the slides onto a - into a basket like this and it gets loaded onto the stainer. Usually these will get stained with an H and E. That's hematoxylin and eosin. And then they'll be coverslipped.

And then after the slides are coverslipped, they're sent to the pathologist. They're reassembled, the whole case is reassembled, these usually put them on these trays, and they're sent with the paperwork that goes along with the case to the pathologist. And then the pathologist looks at them under the microscope and actually signs the case out.

If it's - if immunohistochemical stains are required for the diagnosis or for classification, what we do is we order the stains on a computer that goes back to the histology lab. The histology lab will cut an additional 5-micron thick section from the block for each immunostain that we ordered. So, if we ordered 10 immunostains, they would cut 10 unstained slides. They would not stain those with H and E, and they send those slides right to the immunohistochemical lab for a staining.

Cytology specimens. This - though most of you are familiar with this, what we do especially for rapid on-site, we put a small amount of liquid or fluid from the aspirate onto a slide. And there are many ways to make smears. I'll just go over what the most common way that we do. We put a small amount. We try to put a tiny amount because we put too much, this is going to have to be air dried to be stained in the room with Diff-Quik. And if there's too much on there, it takes a longer time for the air dry and a thicker - if there's too much material, it's difficult to interpret because it's too thick and cells are on top of each other. So anyways, we put a very small amount on. The rest is going to go into CytoLite. After we put the material on the slide, we put another slide face down on top of it and with gentle pressure, we slide them apart, and you usually end up with two slides with material on it.

So, you have two different slides, one of those is going to get fixed and stained the next day, the other one's going to get Diff-Quik stain for rapid on-site. And the way I'm describing things is typical for most institutions, it's not at - every place may have its own little variation. The cytology, the rest of them - the fluid will be pushed up into, we use CytoLite. That's the most common fixative. Formalin can be used. CytoLite is a methanol-based fixative, and it contains hemolytic agents. So, the advantage of this is if you have a very

© 2023 ReachMD

bloody specimen, this fluid will lyse the red blood cells in the background. See on a core biopsy, it doesn't matter if the specimen is really bloody, because once it gets to the gross room, they're going to lift the tissue out of the formalin. And the formalin can contain a lot of blood, that's all going to be discarded. Whereas in a cytology specimen, this is going to be spun down. So, we don't want to have too much blood in there. Or if there is, a CytoLite fixative is much better to use. It will get rid of all that blood.

That second slide that's not going to be Diff-Quik stain, that's usually fixed. We use a spray fixative. Some places may have a bottle with fixative in it, and they just put the slides in there. Those get stained the next day.

This is a Diff-Quik stain. This is the most common one that's used for ROSE. It's very simple. There's a fixative, there's a nuclear – I'm sorry, a cytoplasmic stain and a nuclear stain. So, it's just those three parts. And then this final bottle is usually water just to rinse it. So, it's very easy to use, it's easy to maintain. You only have these four bottles, three with these liquids.

And this is what a diff Diff-Quik stain looks like. Diff-Quik is great cytoplasmic detail, poor nuclear detail. It's really good for rapid diagnosis, but it's not the best - it's not near as good as an H and E stain. The reason we don't use H and E stain for ROSE, although some places do, it's much more involved. It takes much more time and it's I think 13 containers, there's many alcohol levels so it's harder to maintain it would take longer.

Another stain that we use as a Papanicolaou stain. This is usually that fixed slide that is not stained in the room during ROSE that will often be stained with Papanicolaou stain that's a cytology stain. It's almost identical to an H and E stain. But anyway, you can see the nuclear detail in here is much better than in the Diff-Quik.

Cell blocks. I think a lot of people don't really - don't even know what a cell block is. And so, I'm just going to briefly go over it. The fluid that is sent that has the tissue fragments and cells in it, that gets put into a conical tube, it's spun down in a centrifuge, the supernatant this poured off, and then the pellet that's left, that is going to become the cell block. And that gets put into a piece of tissue like a mesh bag, and that would get put into one of those plastic cassettes, just like the core biopsies. It gets put into this cassette, it has the patient information on it just like the core, and then that - those cassettes get put into formalin and they go to histology for processing overnight. Everything else is identical to the tissue fragments.

So, when a cell block is cut, it looks like this. This is a paraffin block. Instead of a piece of tissue, it has that - this is that pellet. And when those slides are cut from this, the 5-micron thick sections, it looks something like this. So here we have two, 5-micron thick sections on one slide. Usually, we just have one but sometimes people will put two on one slide.

So how much is in here will determine how much material you have for whatever testing is needed. And we cannot predict with any great deal of confidence while we're in the room during ROSE, how adequate the cell block will be until the next day when we actually see the cell block. And we actually see a slide from the cell block. That's one of the disadvantages to not having ROSE, or I'll go into the molecular part in a minute.

So afterwards, we take all of our air-dried Diff-Quik slides, along with our fixed H and E stains or our Papanicolaou stain slides, and our sections from the cell block so these might be the paired slides, the Diff-Quik that we looked at in the room, the fixed slides that were fixed the next – or stained the next day. Here's our cell block and the case is reassembled with the paperwork and the case is signed out. And again, if immunos are needed, we can order those on the cell block just like we did with the tissue.

Then, as far as what the difference in the way they look, a cell block, which here, this is a high-power view, we can see tumor clusters here. And in the background is this proteinaceous material. Sometimes it's single cells, sometimes it's these clusters like this. This is what a core biopsy looks like. And the difference is in here, there's infiltrating tumor, and it's in a stromal background. So, here, if we wanted to do molecular off of a cell block, we would have to have, it's typically 20% of the nucleated cells have to be tumor or the tumor has to make up at least 20%. So, it's not a problem like in this case, but if you had abundant lymphocytes in the background, it could dilute out the tumor cells and it would not be adequate. On a core biopsy, you have the same issue, you need at least 20% of the nucleated cells to be tumor. But one of the advantages on the core is we could do microdissection if we need to. So, say you just had a tumor down here at the tip of this and the rest of this was benign, we could dissect out this piece and, therefore, have a higher percentage of tumor. But cell block, it's a little bit more difficult because the cells are more dispersed throughout.

And I won't go into a lot into this, but immuno stains, what they are is you have an antibody that's targeting say TTF-1 is targeting a protein in the tissue or in the cell block. And then you have another antibody that binds to those, if it binds, and that has a chromogen attached. And what you end up with, this is what a TTF-1 looks like, which is common in adenocarcinomas. It's a nuclear stain, that's all these dark nuclei that are staining, these are benign cells in the background. So, if we were looking at this, and we need at least 20% of the nucleated cells to be tumor, we'd probably have about 40% here, just roughly guessing.

And this is a cytology specimen where this is lymphocytes mixed with tumor. And this is a PD-L1 stain. You can see the PD-L1 is

© 2023 ReachMD

staining most of the tumor cells, that's a membranous and cytoplasmic stain. And someone else is going to go into that later.

Again, I already talked about cell block tumor cellularity. Here's one with a lot more lymphocytes in the background. And these are the tumor clusters in here in the spaces. In here, it looks like the tumor cellularity is probably about 30%. So, we're over the 20% mark. But again, we can't determine that until we actually have the cell block, until we actually see the slides. So, this is one of the problems with sometimes pleural fluid, you know, we get a malignant fusion. But if there's so many lymphocytes, and mesothelial cells that dilute out the tumor, we may not have enough - or high enough cellularity to be able to do molecular on that.

So frequent barriers to optimal diagnosis and molecular testing. Sometimes it's inadequate specimen for diagnosis. A solution, I think, being able to work with the pathologist to either establish ROSE if they don't have on-site evaluation, or to improve what they already have, is sometimes the reason ROSE is not implemented. There may be reimbursement issues. I don't really know how - what the reimbursement actually is. We're in an academic center, so we're not as involved in that. There's other alternative evaluation methods that can be used, maybe a cytotech telepathology. And also reviewing slides for feedback on techniques. I think that would really help a lot. We're fortunate that we have a screen in the room, a shared screen where the proceduralist or the pulmonologist can see what we see. And they can immediately change their technique based on, you know, what they're getting. Whereas if you're just putting everything into a jar, you may not ever see what the reason is, why you're not getting adequate material. So, I think being able to view the slides if you don't have ROSE.

Inadequate specimen for comprehensive molecular testing, minimizing immunohistochemical staining, right now, the recommendation is to do just two immuno stains to further classify a non-small cell carcinoma. We use TTF-1 for adenoma and p40 for squamous cell carcinoma. If we can tell which it is without doing those, then we don't do any immuno stains, other than PD-L1. PD-L1 is a stain that we're commonly doing on all cases. But there are some places where they're doing a whole panel, and they do this whole panel upfront and say it's TTF-1 positive if it's a primary lung adenocarcinoma, but they did 20 stains, there may not be enough then for molecular. And so, the recommendation is to minimize the number of immuno stains that are done. And we use smears for next gen sequencing, so it's something to consider if the cell block is inadequate or if the tissue biopsies are inadequate.

Long turnaround times, I think working with pathology, to identify what the source of the delays are, and discuss discussions at a multidisciplinary tumor board. I think it makes a world of difference when people get together and actually talk about what the problem is and how, as a team, we can overcome the problems.

This is our workflow here at U of C. We're very fortunate we have the resources, so we always have a cytotech and a set of pathologists on call, I review all the cases, when I'm going to be on call for procedures, I review the histories ahead of time. And the cytotech and I both go into the room, the tech makes the smears, I review them. When we have adequate material for diagnosis, the next thing we do is we do adequacy for molecular since we're going to be using the smears for next gen sequencing. And we place the order immediately after the procedure and hand off the Diff-Quik stain slides right to the molecular lab. I think there's one more question.

Dr. Murgu:
And those people are answering and we're transitioning to the next slide deck. For participants, please, if you have any questions, as you're reflecting on the first talk, please use the chat box, either the chat room or the Q and A box there and then we'll address them after Dr. Hartley's talk. So, Dr. Mueller, if you want to stop sharing, and we move on to Dr. Hartley's presentation.

Dr. Hartley:
Thanks again. Again, I'm Chris Hartley, I'm a cytopathologist. I'll be joining you here from my office in Rochester, Minnesota.

Again, I have nothing to disclose.

And I guess we'll start things off with a question here. All right, great. All right. Moving on to the outline, I'm hoping to hit a few high points here, but mainly focusing on kind of demystifying the process from when we make a diagnosis to when the order comes through for NGS and PD-L1, and where some of the pain points are in terms of turnaround time and accuracy. So, along the way, I'll be sharing the specific experience of Mayo, but give some sense of the variability in practice.

But just some things to think about, who orders the test, you know, oncologists, pulmonologists, pathologists, some places the pathologists kind of have a standing order just to go ahead and order the relevant molecular studies. What tissue to use there's smears, there's cell blocks, there's core biopsies. What assay do you use, and so you can think along the lines of DNA or RNA extraction, whether a specimen is fresh or whether it has a stain. All of these require different validations and formalin-fixed paraffin embedded, which has kind of been the standard for a long time, but there's somewhat of a push towards - shifting towards using smears more exclusively. And then hit a few points relative to PD-L1.

So, here's kind of the black box of the lab that we all deal with. I deal with it myself, I'm not entirely sure what's going on in here all the

© 2023 ReachMD

time. But Dr. Mueller discussed kind of the procedure of the ROSE, from the tip of the needle on the slide to the diagnosis. And so, I'll be covering the secondary circuit.

After that diagnosis made, someone is going to put in that order for NGS and PD-L1 for non-small cell carcinoma, or adenocarcinoma, and in some cases, squamous cell carcinoma, but that will be covered a little bit later. So, the order goes back into the lab, and then you get a result back out. And hopefully that result is accurate. And hopefully that result comes quickly. But as we know, there are often snags along the way. And of course, ideally, you get a targetable mutation. Sometimes, you know, there's unfortunately no actionable mutation. And I think a K-RAS in particular, although I think there now is some targeted treatments for that. And unsat, which is probably the most frustrating because now you have to come up with another specimen and spend another two weeks to get a result.

All right, so just to briefly cover diagnosis. As Dr. Mueller was mentioning, the two main antibodies to use TTF-1 and p40. So, if you see a nice carcinoma here with TTF-1 positivity, there's some suggestion of gland formation here as well. You can probably just call this one adenocarcinoma, but some people argue for hedging a little bit and saying non-small cell favor adenocarcinoma to acknowledge the fact that, you know, sometimes there are hybrid more adenosquamous carcinoma, sometimes there's a small cell component in these tumors and we're just biopsying one part of it. So, who are, you know, how do we know what the whole tumor represents?

Over here on the right is a more archetypal or canonical squamous cell carcinoma, and hopefully it will stain strongly for p40. But some equivocation can occur if the staining is absent or a little weak. So, you might see something like non-small cell favors squam. This often can still be an indication for pursuing NGS. You will get a PD-L1 on all of these. So, and just to quickly mention, TTF-1 is negative in approximately 10% of adenocarcinoma. So, there is some variability, and not every case reads the textbook, of course. So, if you're ever kind of puzzled over a particular phrasing in a diagnosis.

So, the workflow here at Mayo is we get the diagnosis of adenocarcinoma, or non-small cell carcinoma. And here, the oncologist is the one that orders this lung panel, which is this abbreviation here at Mayo for our NGS panel. Oops, sorry about that. And PD-L1 testing will be ordered as well. So once that order comes through a cytotechnologist, and this is pretty unique to Mayo, I'm not aware of this happening anywhere else. We have a team of cytotechnologists who normally they go to on sites and things and identify tumor on the smears on site, but they actually can identify tumor in other contexts. So, they'll pool, in this case up in the right-hand corner, cell block, and they'll circle what they think is the most rich area of tumor, and has the least contaminant, meaning normal. And then a pathologist will quickly come along and kind of check off on that and send it to the lab. And so, this is our way of kind of quality assurance. There's quite a bit of variability across institutions. And there's some places it's the fellows are doing this. Some places, it's the pathologist kind of thrown into the middle of their workload, they're like circling tumor and hopefully, identifying the most accurate. So anyways, there can be a lot of variability. But here it's very streamlined.

So, our turnaround time for the NGS panel, which is typical in most places, is 12 to 20 days, two weeks is kind of a good rule of thumb for NGS. And here are the targets in our panel. You can see it also includes the fusions ALK ROS. So pretty broad coverage, NTRK also is kind of emerging target as well.

So, I want to briefly just as a kind of thought experiment, you consider a span - a scant specimen, why not just try it? You know, sometimes we'll call the clinician and say, 'Look, you know, we don't have a lot here. You know, we're not sure if you want to so proceed testing.' And I think kind of the natural inclination say, 'Yeah, please, just go ahead and push it through.' So, if you think about it, you know, there are nuclei here, you know. And then of course, in nuclei, there's DNA and their normal gene. So, if you just push a specimen through with cells, you'll get no mutation detected. But if you do it at a place like Mayo and place with quality – QC measures, you'll get unsat they'll say, 'Look, there's not enough tumor here.'

So, one question you can ask instead of just saying push it through, you can say – or ask, 'Are there smears?' Not every place will necessarily think of smears as being useful for molecular testing, or they may not - the laboratory might not be validating for that, so they might not tend - they prefer to do testing on a cell block, which is here on the left. This is FFP. So, they may have to send out which a lot of labs may not like sending out testing. But you know, if you ask if there's smears, you can get a lot of yield, in terms of NGS testing, just off a one smear. And of course, there's a route to send it to Mayo for testing.

And here's what our lab lists online for this information. So, you can send one to three slides. And you should have ideally, greater than 5,000 cells, but you can get by with just 3,000. And there's even a nice note about cover slipping. If you have a plastic cover slip, that can actually add several days to the turnaround time. So again, just kind of thinking about things that can hold up the turnaround time. There's the darn cover slip that's on there can cause trouble. And it's also worth noting these cytology slides will not be returned, because the all the material will be scraped off for extraction. But we will take a whole slide image, you of course, can take your own whole slide image before you send, so that will be preserved digitally. And that was actually added to the College of American Pathologists. It's acceptable to destroy diagnostic material, provided that you preserve it as a digital image and use it for molecular testing. And I think that was in 2013. So that's actually been around a while.

© 2023 ReachMD

So, I think one other issue to think about is if you have plenty of tissue, tissue is not the problem, but you don't have a lot of time. So, a patient with brain metastases, for example, and you want to know if there's a targetable therapy. And this is something you could start by thinking about anecdotally, has this ever come up?

How often does it come up? And then you can start to move towards institution level committees and things and decide what - should we have a little in-house assay that can process as, for example, EGFR sequencing rapidly? Or should we consider a route for sending out for such a thing again, in a really urgent clinical context. Obviously, the most relevant thing would be the patient volume and determining whether you'd get an in-house assay or send it out.

You can also pursue immuno stains for EGFR. I don't recommend that. And I don't think there's any guidelines that recommend that. But if you're - I've had a few times here at Mayo, where you're really in a pinch clinically, and if there's positivity for one of the EGFR mutants, you can actually move forward with targeted therapy, but that's kind of a niche example. Some assays, real-time PCR assays can actually give a result in just a few hours, provided that you have FFP tissue in the lab ready to go.

And just for reference, I think this is a great paper here, buried in a pathology journal archives, that kind of gives a great review of molecular testing on different cytology specimens. So, it's for your own consideration later.

Moving on to PD-L1, so mostly 22C3 is used. And here at Mayo, that gets routed either to the cytopathology service or pulmonary service. The pathologist can usually read these out from order to result in about one to three days, five to seven, if you're sending it here for the stain. So, there's a little addition. One to three in house, so if you have your own in-house PD-L1. Again, that's more volume based if you have the volume to support that, you can do that. But just so you know - but you might notice that turnaround time is quite a bit faster than NGS. So, you'll often get a PD-L1 result before NGS, which is a scenario I think, Dr. Duma will discuss in a bit.

But so just as an example, here's a tumor with TPS of 50. So, 50% of the tumor cells are positive here, and this is the lowest end of that high cut-off where you're eligible for immune checkpoint inhibitors.

So, here's another example of a cell block, which I had actually shown earlier. And you might have to trust me, but there's a nice tumor here, here's higher power. This is a nice adenocarcinoma, you can see the vacuoles here. Here's a TPS less than 1%. If you notice some cell staining here, these are actually histiocytes. And so, we're supposed to completely ignore histiocytes. And sometimes it's hard to tell a histiocyte from a tumor cell. So, but just to give you a sense of some of the variability in our practice, which I'll get into a little more, but less than 1% staining here, you know, this would not, I guess, lead to an indication for immune checkpoint inhibitor.

So, this is a paper of mine that's buried in a small pathology journal. But just as a quick high point, pathologist one over here called all of these cases high positive, greater than 50%. But pathologist two called some of those low positive and negative. So that's a pretty dramatic difference. And you may not be surprised, any human activity has quite a bit of variability in interobserver variability. But the main take home point is that if you get a PD-L1 result that's at the border the thresholds, you can request - my pathology colleagues might not like me, but you can request to repeat or consensus review. And actually, as a matter of practice, I routinely will show things when they're right at the cutoff because I know how critical that can be for eligibility for therapy. So, I will routinely do that myself without being asked. So that's another thing you can set up with a lab, and communication with your pathologist in your lab and advance so.

So just to summarize, think about what tissue you can use. Remember, you can use smears, if you validate instead of the assay in house or you can send it out for testing. And just remember, you can scan the smears and ahead of time because there'll be scraped - all the material that we scrape for DNA extraction. And then you can request or, if asked, smears are better for NGS and the cell block for PD-L1, because the PD-L1 kind of competes with NGS because it's taking some of the tissue. And PD-L1 can only be done on the cell block whereas NGS can be done on the cell block or the smear. So, one of the drive - big take-home points is to use smears for NGS when possible.

So, the other thing to think about is what assay to use. Again, rapid targeted testing is possible. There are real-time PCR assays that you can set up. We have an EGFR, EGFR-1 here at Mayo. IHC is really not advisable but can be used in urgent scenarios. Again, you would have to have that antibody set up and validated ahead of time. So, and, as I mentioned here, these kind of require more institution or committee level preparation, close communication with the laboratory.

And then the last few summary points for PD-L1 can be done on FFP only so that cell blocks or core biopsies, not smears. It doesn't require nearly as much tissue only 100 cells whereas NGS you need 3,000. It has a faster turnaround time about half, even at the high end compared to NGS. And you can again consider requests for repeater consensus review in borderline PD-L1 results.

And then finally, in terms of results, unsat. In trying to make a case, unsat can actually salvage a case, because if you get an no mutation detected result, that might lead you to think the patient isn't eligible when in fact, there just weren't enough tumor nuclei there, depending on the QC of your own lab or where you're sending it out. Actually, a great way to test out a lab is to send a blank specimen

© 2023 ReachMD

and see what they tell you. If they tell you negative that's the wrong answer. The wrong - the right answer should be unsat. So, you could try that out sometime if you want. And again, consider repeat collection or testing if you know the specimen showed scant cellularity. So, I think that's a really important point to keep in mind. And I guess we'll end here with a question.

Dr. Murgu:
And there's people are answering now. We'll stop the screen sharing, and then we'll take a few minutes for Q and A, mainly among panelists, and but if participants have questions, please put them in the chat room.

Jeff, I will start with you. You talk a lot about estimating the number of cancer cells on the slide or in the cell block. And I'm also seeing in Dr. Hartley's presentation, you know, the - these percentages in terms of stains. But back to the rapid on-site evaluation, when you look at a slide, how can you tell if there is more than 20% tumor cells on the slide, or less than that? How do you assure on site the adequacy of those slides for molecular testing? And then, you know, as you're reflecting on that, one question that was submitted prior to the webinar by one of the participants, if you don't have ROSE, then how can you tell macroscopically, looking at the material, if you actually have enough tissue?

Dr. Mueller:
Well, for the first question, how do you estimate tumor cellularity? It's usually, believe it or not, you're talking about the 20%.

Dr. Murgu:
Yeah.

Dr. Mueller:
It's usually not that hard, because what - we get pretty good at estimating one thing, but most cases, are one extreme or the other. Most cases, you know, you look at and it's wall-to-wall tumor with very little of anything else, or it's very few tumor cells and a ton of everything else. So, most of the time, you can tell right away without having to think too much about it. I'd say there are rare cases where it's right on the borderline, and I'm having a difficult time being able to tell. But if I was - I'm usually very upfront, I would say, you know, we're right at the border, we'd better get a few more smears just to see if we can improve that. And or the other question was?

Dr. Murgu:
How can you tell without ROSE if you have enough material, I guess for those of us who just deploy a specimen into the Cytolite, is there anything macroscopically that could be reassuring for the procedure list?

Dr. Mueller:
It - I mean, I there are people that have done studies on this. There's nothing that's really reliable. I mean, the more tissue chunks that you see going in there, you know, the higher the likelihood that it will be adequate. But there's nothing reliable. We've tried doing this just macroscopically looking and - because originally, we were doing things off cell block. We were doing molecular off of cell block. And we are having just as many frustrations as everyone else. But when the - in the absence of ROSE, I don't know any way around it. I guess just, you know, put as much as you can into the fluid and then just, you know, hope for the best. But always I think getting any kind of feedback too, reviewing material with the pathologist, that's going to impact how you do things in the future and figure out a way to improve like that.

Dr. Edell:
Do you see the question in the box?

Dr. Murgu:
Yeah, there is a question in the box that I will have one of you answer. As we asked Dr. Hartley a question before we move on. Chris, you mentioned that in your institution at Mayo, the order for NGS is placed by the oncologist. I suspect that is done once the final results are listed in your electronic medical record. And given the fact that the turnaround time for large panel NGS could be in the range of a couple of weeks, I wonder, can you comment on the role of reflex testing? Because the University of Chicago has the reflex testing in place. And I don't know if there is any literature in regards to when results become available to the treating team. Like Dr. Duma, is it better to do a reflex test? Or should the order be placed by the oncologist? And can you comment on that at all?

Dr. Hartley:
Yeah, I mean, I think reflex testing is great. Again, I think we're like Dr. Mueller's practice where he's in the room, he can see the smears and he knows there's adequate, you could send that instantly to the lab for extraction. There - an option to is to, you know, if you can prelim - we do prelims the same day, sometimes the next morning, depending on when that happens. But you could do an extraction and just hold that material. And then when the final result comes through and the oncologist orders, you could go ahead and queue up that extracted material. Again, that's kind of a - you'd really have to coordinate with the lab and set up that workflow. But you know, just in terms of decreasing the delay, that's an option. And reflex testing, of course, helps because every once in a while, unfortunately, we'll

© 2023 ReachMD

do it - we'll send out a result, and I've seen it maybe more in other places, but they - the result won't be noticed right away, and maybe a day or two passes before they put in the order. And you know, now things are getting kicked down there. So, again, just – yeah, I think it's great for improving the turnaround time. So -

Dr. Murgu:
Yeah. And ultimately, you know, the reason we do all this, the reason we try to get better at what we sample and how we sample and we work with you guys is to assure enough material for our colleagues from medical oncology, so they can act on it appropriately. And that's a good segue into Dr. Duma's talk on what kind of tissue do we need for medical treatment and clinical trials? Dr. Duma?

Dr. Duma:
Hi, everyone, I'm delighted to be here. It's quite nerve racking for a medical oncologist to give a talk with two pulmonologists and two pathologists. I often am the majority when we talk about lung cancer, but here I'm the minority.

I want to present some perspective for you. So, you know, everything and all you do is so essential to get these patients to the right treatment. We're going to start with question number one, which of the following is an alternative to tissue biomarker testing in lung cancer? We're going to start these we are real patient story. So, this is one of my patients. This is a 44, non-Hispanic, white female, software engineer, married, never smoker, some distant secondhand smoke while growing up. She came to the ER after a trip to Jamaica because she had pleuritic chest pain. So, the CT scan show a 4-millimeter lesion on the upper lobe and many multiple bilateral small nodule. And this - usually in these patients that I noticed tend to be in the ER or tends to be after they have significant symptoms because they're never smokers. Subsequent after discharge from the ER, the PET scans show the pulmonary lesions and nodules as well as the liver lesion. So, prior to biopsy, we know she already had a stage IV disease. She - I had a wonderful pulmonologist that helped me make some other diagnosis. And she had a stage IV lung adenocarcinoma.

So, we usually in these cases, the patients may present to the clinic to the oncology clinic. And sometimes we see the PD-L1 right away. So, these patients are never smoker, we have PD-L1 of 90%. So, what do you do next, right? Like you get so excited about this 90% PD-L1. Should you do more? And the question is always, yes, we need more, we need more than that PD-L1, we need to find - this is what I told my patients, we need to find the key for your lock. And that key is the genomic biomarker testing NGS. It has so many names, but we need to find that key for the lock. Because otherwise we're going to try to push on immunotherapy in a lock that may not open. In this case, the patient had EGFR exon 19 deletion. We're going talk quickly about these mutation, but that completely changed treatment. You saw that PD-L1 of 90%. In this patient, when we put it into the treatment perspective, doesn't really matter. Because patients with EGFR mutation are less likely to respond to immunotherapy. We have seen data, the patients with EGFR mutation can have a response rate of 0% with immunotherapy. And that's why it's very important to hurry up and wait for NGS. And it's very anxiety provoking, right? You just told a young woman with two healthy children that she has metastatic lung cancer.

This comes from a paper in JAMA Oncology in which shows the difference in survival. And those who don't count for quality of life, the difference in survival in patients that receive EGFR inhibitors versus patients that did not. I have two patients that ran the Boston Marathon three weeks ago, with metastatic lung cancer and EGFR mutations. So, the quality of life is also very different. Not only do they live longer, but they live better. And how can we assure that? Tissue, and not only tissue, but the right tissue test.

Lung cancer used to be seen as one disease, only one. And eventually it was the evaluated as adenocarcinoma and squamous and others. But now we're going to a phase in which lung cancer is actually many. And genomic testing allows us to do that.

I had a patient in clinic yesterday. I specialize in younger women with lung cancer. And based on her presentation, I already had an idea what are some other biomarkers she may or may not have. So, it's not only about the biomarker, it's also the idea of how these patients present. Our positive patients tend to present with brain metastases, EGFR patients at higher rate of leptomeningeal disease, ROS1 patients have a lot of bone metastases. So that's just one example that lung cancer is no longer one disease. And when everything that you're doing to get the tissue to that hungry medical oncologist that always wants more tissue, it has a reason for it.

So, mutations change by geographic location, right? So, when the biggest difference between Asians and EGFR mutation. So, if you can see in this graph, you can see how EGFR is around 40 to 50% in certain areas of Asia. New data has suggested that there's high EGFR also in Latin America, and particularly in Brazil. And the United States tends to be a mix, K-RAS is the second most common after EGFR sensitizing mutations. And it's important to mention that not all EGFRs are the same. So, stay put for other webinars about it. And we also see difference in mutations based on ethnic and racial background. We talk about the Asians, you can see that teal graph that goes all the way up. And what most people don't know that the second group that's most likely to have an EGFR mutation are our Hispanic Latinx patients. Here in Boston, I actually have several patients, mostly Central American with EGFR mutations. Then we see more K-RAS mutations in the white population, because it's linked to smoking, and higher prevalence of ALK in Hispanics and whites.

© 2023 ReachMD

So molecular management, one of the things that I want to come across we as a medical oncologist is that first, all adenocarcinomas should be tested, regardless of race factors. It doesn't matter if the patient has smoked one pack in their entire life or has a 50-year back history, because now we have targeted therapy for K-RAS G12C, which is very linked associated with smoking history. And we also have target therapy for BRAF, who's also associated with a smoking history. So, risk factors, you know is through a patient with adenocarcinoma. So, all of these should be tested for biomarkers.

What about next gen sequencing? I'm a big proponent of next gen sequencing because the field of lung cancer is changing rapidly. In 2020, despite the pandemic, we have seven. You had a right, seven drug approvals for lung cancer. And this year, we are expecting one more that will be added to this pile. So, it is easier to conduct next gen sequencing so you don't have to be catching up with all these approvals.

Also, in patients who are as symptomatic, it requires a lot of talk. So, you know, sometimes the pulmonologist is the first phase for these patients. So, it's like I'm going to do the biopsy, and they're like, 'When am I going to know the results? When is this coming back? I want to start therapy tomorrow, take it out.' So, if patients are asymptomatic, we can wait for results. It can be hard to tell a patient that we have to wait weeks. But it's to find the key for the lock. And this analogy, you can steal it because it really works for my patients. That, and the wedding dress analogy, in which I tell them, 'Let's find out what is your biomarkers, it's like finding your wedding dress, you won't settle for any dress, right? We have to wait for the right one.' And I can tell you many patients years after they're like, 'Yeah, my wedding dress.' So, you know, explaining the need, and what is so important because there's a mix between you wanting to wait, and the patient wanting to start.

So, one of the things is potentially can be suboptimal. But over time, I have seen that new methodologies are evolving, and we're able in some cases, to obtain good samples. As I say with NGS, I have - there's data that comes from Cleveland Clinic that you may require a least amount of tissue for NGS and for squamous cell. And I want to stop for a second here. There also can be testing for NGS, there's not a complete black and white exclusion. These include younger patients of less than 50 years of age, never or light smokers, or if the specimen is very small, or you have a adenosquamous. It is important to understand that the PD-L1 will come back first so we cannot get too excited, like the patient I present it, you know, ooh 90%, woohoo, let's call pembro. That wouldn't have worked for that patient. We needed to wait to find out that she had the EGFR mutation.

The turnaround time changes based on in each situation. I always get a big range of patients that helps me, because if you tell them 14 days, they will call you on day 14 to say, 'Where are my results?' So, I always give week intervals and you know, re-explain the importance of this.

Something that has changed a lot of things is liquid biopsy or circulating tumor DNA. Circulating tumor DNA has been more validated in EGFR mutations. It was initially studied there, and slowly we're learning more in other patients. But this provides a plan B. But it's important to understand that liquid biopsies have a higher positive predictive value in patients that have disease outside of the chest. Liver metastases are the patients that tend to have higher positive predictive value. Some patients may have a lung lesion and a brain lesion. If it's in the brain, the positive predictive value comes down. But it has allowed us to have a plan B to collect samples to understand and also to understand resistance to drugs. So, we're not only using a diagnosis, we're also the time of progression. The problem with liquid biopsy is that if it's negative, it doesn't mean much, you still need your tissue. And some patients may not have enough tumor secreting DNA. So, it's a good way as a Plan B but it's not 100% because negative results mean you still need the tissue.

So, I have been asked recently due to the new approvals late last year and early last year for neoadjuvant and adjuvant therapy in lung cancer, when is the right time to test? And this comes because of the ADAURA trial. So, in 2020, EGFR drugs were approved for adjuvant therapy. This means for patients with stage Ib to IIIa resected. Before this, we mostly test the patients that have metastatic disease, but ADAURA, now we have a drug for patients that would go for three years after they're resected.

So, I created this table that summarizes NCCN guidelines of who and when people should get tested. So, all non-squamous resected, the stage Ib to IIIa, this is based on FDA-approved drugs today, they get - they should get EGFR tested because we have drug approved for the adjuvant setting. And stage III non-squamous, they get definitely chemoradiation just have no resected, they should get EGFR testing. Why? Because the PACIFIC study, the PACIFIC study that show consolidation, immunotherapy, new data and data that keeps coming up every year shows that patients with EGFR mutation, we know benefit from the adjuvant immunotherapy. So, they go on immunotherapy for a year without benefit. So, all these testing allow us to tailor not only targeted therapy but allow us to tailor who would benefit from immunotherapy. So, it's not only beyond you take this pill for this drug, it's what can I do for you after resection, right? So, metastatic, we talk about this non-squamous comprehensive NGS in all patients, regardless or risk factors. Squamous selected patients young, non-smoker, small samples, patients with mixed histology.

And I want to mention about the LAURA study, because that's ongoing, and those are patients with EGFR tests that would get consolidated targeted therapy. So, this is based on approvals, who should get tested. So, if you look at anybody in stage Ib or above

© 2023 ReachMD

should get some type of biomarker testing. So, we need to stop thinking this only the patients we advanced disease. These are the patients in early stage as well.

And there are more and more adjuvant trials for targeted therapy, ALK, ROS, MET, and RET, they're all recruiting. And so, I know they are going to be reported soon. So, this table was going to be updated for six months until we get new data.

There are disparities and biomarker testing, particularly for black men who are tests around 39% versus 50%. But let's take into account that this data shows that only 50% of white patients are getting tested for biomarkers. That is still suboptimal, because then patients are treated the wrong way. Patients are treated the wrong way because we don't have the appropriate testing. But what the study shows is there's a direct correlation between biomarker testing, that's the vehicle, for clinical trial participation. For patients that get resected and they don't get NGS, they don't have the option of going into a targeted therapy adjuvant trial. Or the patients that get resected and don't get biomarker testing are now going to get adjuvant atezolizumab. But we know that doesn't work in EGFR patients, they should go on EGFR therapy. So that's why there's a direct correlation between biomarker testing and clinical trial participation.

But updated data in 2022, showed that only 60 to 61% of patients with metastatic lung cancer are getting adequate biomarker testing. I'm now hoping to fix the problem, well, I know I was hoping to fix it, but I think we should be committed to biomarker testing in lung cancer as we are committed to DVT prophylaxis in the hospital. And these are the [57:48]. Even Dr. Hartley, who is a pathologist, knows how much we, you know, [57:56] and DVT prophylaxis in the hospital. So, let's use that model to make sure patients are getting appropriate testing.

Reimbursement. That's always good to motivate people to follow the guidelines and metrics. You know, we get these metrics about being the magnetic hospital, the name [58:19] with the hope to improve biomarker testing.

So, this is my last slide. What is the consensus? When should we test these patients? My belief is that patients should get biomarker testing at the time of diagnosis. Because we have a large number of neoadjuvant trials now, we have neoadjuvant therapy, that before it was not a thing. Like neoadjuvant therapy is less than six months in lung cancer, because all the previous studies before immunotherapy show that neoadjuvant therapy was actually harmful. But in this case, with inclusion of nivolumab, we are doing neoadjuvant therapy, changing the whole paradigm. So, we should test early so the patient can target to the right neo adjuvant trial or to the right therapy after resection. And this is question number two. Which of the following is true about biomarker testing in lung cancer?

Dr. Murgu:
And these people are answering. Dr. Duma, I have a couple of questions for you. And I know we're close to the hour here, but I think this would be important for the audience to think about as well. You mentioned that only 60% of people get biomarker testing. Do you personally think that's because of an awareness issue? Because of quality of the specimen that's available to the oncologist where tissue cannot be tested for molecular because there's not enough material or purity as we just heard? Or are there any other incentives? You know, is the community incentivized to use chemotherapy and immunotherapy because they want to start earlier or for whatever reason they are not wait for the NGS results? What is your personal opinion? And maybe if there is evidence behind your statement, I would like to hear that as well.

Dr. Duma:
So, the answer to you is all of the above. So as a multi-institutional multilevel, multifactorial issue for testing, one that we have seen is that immunotherapy has come like the, you know, like the Holy Grail, and everybody thinks that there's just a greatest and that, but there's no for every patient. So, I've seen a lot of excitement with these PD-L1s because they come faster. You have a PD-L1 100%, everybody's like woo, but it's like, no, not yet, not yet, let's wait, let's wait. So, there's some excitement about that. It also can be very troublesome in some rural parts of the United States for testing and it can take not two to three weeks, it can take a month, it can take a month and a half for small practices. I had a practice that I met with in North Dakota that takes around two months. And Mayo Clinic is close, but I train at Mayo, but you know, it's still the whole processing. That's a challenge.

Another challenge is also the lack of awareness about liquid biopsies. During COVID-19, I used a lot of liquid biopsies because they have a home phlebotomy service, which is a majority of the company so they go to the patient's house and they draw the blood, like - and the patient's just love it. So, I think it's a multilevel. It's not only about awareness, it's also about reimbursement. Because getting the patient in targeted therapy takes three to four times longer for a medical oncologist. Last week, and I have an army of people here, I have to argue with an insurance company or a drug that the patient had a mutation for. So, you know, sometimes, it's, to be honest, sometimes it's easier to prescribe chemotherapy, because the whole preauthorization, the co-pays with targeted therapy are quite challenging.

Dr. Murgu:
That's very useful. And thank you for that. And as a pulmonologist doing these procedures for our oncology colleagues, one of the

© 2023 ReachMD

challenges I personally have is the time of rebiopsy, or the time of tumor progression or recurrence in the field of irradiated tumor. Do we need an easier value for analyzing the tissue, again for molecular markers, and PD-L1 knowing that the original tumor did not have a targetable mutation?

Dr. Duma:
If the original tumor didn't have a target mutation, it's unlikely to you're going find something. You're going to find acquired mutations that don't have target, you're going find TPP3 MET amplifications HER2 amplifications. But if you are patient with a target mutation, and now I want to make sure people know is 50% of lung cancer patients now have a target mutation, as before, right, when I started fellowship, it was like, I think we were talking about 20% now it's 50%. So, there's one out of two patients that you are seeing. And at the time of progression, we really like rebiopsies, because we are now developing clinical trials for resistant mechanisms. So, patients can stay in target therapy longer before they go to the chemotherapy suite.

Dr. Murgu:
Okay, thank you.

Dr. Edell:
Excellent job. I'm really thrilled to be able to co-host this with Dr. Murgu. You can see our outstanding panelists. I want to thank each one of you for spending time with us tonight, your expertise and sharing with the audience. Again, I want to thank the American College of Chest Physicians, and our partners, the pharmaceutical companies listed below. And I would encourage you to attend the next two of the final two of the five series webinars that we're putting on that you see in front of you. Please consider joining us have a great evening. Please be safe and order those next gen sequence testing. Thank you all again very much have a good evening.

Announcer:
This activity was part of a seven-part series brought to you by the American College of Chest Physicians and supported by an independent educational grant from AstraZeneca Pharmaceuticals, an educational grant from Genentech, a member of the Roche group, and an independent medical education grants from Merck Sharp and Dohme Corporation. To receive your free CME credit or to view other activities in this series, go to reachmd.com/CME. This is CME on ReachMD. Be part of the knowledge

© 2023 ReachMD