Transcription of the question and answer period from the June 18, 2014, OMedLive event: Advancing Personalized Care for Patients with NSCLC—Tumor Genotyping and Biomarkers featuring Jyoti Patel, MD and Ajit Paintal, MD.

Jyoti Patel, MD: So a couple of questions. First of all I encourage everyone to please send in any questions that you have from this wonderful and comprehensive presentation. A question that I have and that we have been sort of facing recently, the patient who has a lung adenocarcinoma and is a never smoker, and so we had a certainly a high interest in expediting his molecular diagnostic workup.

We sent for in our lab a EGFR and RAS followed by reflex FISH and we are now facing a situation in which his insurance company is refusing to pay for his RAS testing, because there is no indication for this. We're certainly appealing this decision, but the rationale that you explained for RAS I think is a very important point from a societal and hospital point of view and why we would do RAS testing. Can you go over that again?

Ajit Paintal, MD: Yeah, I think RAS testing, you know, from a macro point of view, as you're kind of alluding to, makes a lot of sense, because again, you can set up your algorithm in a few different ways for EGFR and KRAS and ALK testing. And so in general, you're performing the same DNA extraction, because both ALK and EG or rather KRAS and EGFR testing are PCR based.

So performing one DNA extraction and to sort of use the same DNA that you've extracted for a KRAS test does not really add that much in the way of marginal cost, whereas ALK testing is again, much more labor intensive and it's a totally separate lab and there is a lot more sort of moving parts to it.

And again, the way you set your algorithm up, and again in the macro sets can probably serve to save money if you incorporate KRAS. So in our institution you perform EGFR and KRAS testing simultaneously. And then again, if we find one of those lesions we don't have to go to the trouble and expense of testing for ALK.

Other places may do it differently. You could test for KRAS upfront, and then maybe even forego testing for EGFR or vice versa. Test for EGFR and if that's there, you won't have to test for KRAS.

So there is a number of ways you can sort of set up that reflex testing. It differs institution to institution, but in general we feel like testing for KRAS is valuable, because it makes our job easier and we're able to provide testing at sort of lower cost, just because again, we can forego a large number of patients that would have to undergo ALK testing otherwise, because we excluded them on the basis of being KRAS mutated.

Jyoti Patel, MD: I think just as importantly from a therapeutic point of view, I would want to know if a patient harbored a RAS mutation and sometimes even never smokers do, so it would be wasting money and toxicity and time by treating it a lot in a ____.

Ajit Paintal, MD: Yeah, so it is, as you allude to, useful information clinically sort of as well, but again, I guess with the insurance companies again, is it's not strictly required by the NCCN or CAP IASLC guidelines at the current time.

Jyoti Patel, MD: So we have a lot of questions coming in.

Ajit Paintal, MD: Oh good.

Jyoti Patel, MD: So the first question, does alumina testing assess the entire exome of each gene or just the hot spots?

Ajit Paintal, MD: My understanding it's NextGen sequencing assay and I'm not sure about the other genes. In general any test that you perform at least for EGFR is going to have to pick up any mutation that's present at a frequency of one percent or more described. So I'm sure they would have to test for all those deletions in 19, as well as LA58R and the mutations that occur in exon 20 that are associated with resistance, but beyond that I'm not – I don't really think I can honestly answer that question.

Jyoti Patel, MD: Okay. So you alluded to this also during your presentation. One question we have is any guideline on the ratio of tumor to normal required for molecular testing?

Ajit Paintal, MD: Oh, it depends, you know, assay to assay. I think back in the days of Sanger sequencing you would need 25 percent tumor or more to get a valid result. With these current assays again, the Roche

cobas is the one that we're familiar with at our institution, but I think it would be similar for more of these – most of these more recent molecular assays or PCR based assays.

The requirement that's I think written into labeling for the test is that you need ten percent tumor or less – or more, sorry. You can probably get away with less, but that's what's written, ten percent tumor or more. Even if you have a small biopsy like a core of tissue, where you have less than ten percent tumor it is possible to sort of enrich your specimen.

You can sort of macro dissect out the benign elements from the slide, and then you can just use the part of the specimen that's then enriched for tumor content and use that for testing. So in general, the answer is ten percent, but even if you have less than ten percent, if it's in a core biopsy or a specimen of that nature you can typically perform enrichment.

And in an infusion specimen that's not really possible, because everything is just kind of mixed together and it's not like there is tumor here and benign here. Everything is just floating amongst one another, but for more solid biopsies you can perform macro dissection and enrich your specimen.

Jyoti Patel, MD: Any guidance on FISH testing?

Ajit Paintal, MD: Oh, FISH testing, that's a good question. So FISH testing, it's not really based on percentage. It's based on the number of viable tumor cells you have, so you need 50 tumor cells.

And so, one issue is that for the FISH lab, the people reading the FISH and just the way that the specimen is processing it can be somewhat difficult for them to distinguish because the cells are digested what the normal elements are versus what the tumor is. And again, you want to have 15 percent positive signal, so it's important that you're only counting tumor for purposes of this ALK FISH testing.

And so what you can do is that the tumor percentage isn't important, but if you can just even circle on an H&E slide that you send to the lab where the tumor is, they can then orient themselves on their slides and make sure that they're only counting tumor.

So for ALK FISH it's different, sort of more visual so it's not an absolute percentage. It's just that they have to have 50 cells and they have to know where they are, so and that's generally pretty feasible.

Jyoti Patel, MD: So we have a series of questions on cost testing and costs of testing and cost containment. So the first question is, would it be valuable to perform molecular testing on cytologic material to diagnose non-small cell lung cancer, and then decide who should be undergoing more invasive bronchoscopic biopsies?

Ajit Paintal, MD: Well I think that's maybe an issue that we've sort of dealt with in the early days of our testing to some degree, and in general the cost of this testing isn't that much, 1 or $2,000.00. Even for Foundation One I think the test goes for only a few thousand dollars, which compared to the cost of a second bronch or a second procedure down in IR is sort of a lot less.

So I think really you're much better off just getting what you need the first time versus A, subjecting the patient to the initial – to the additional sort of burden of undergoing a second procedure and again, these procedures tend to be more expensive than this testing that we would be ostensively trying to avoid.

Jyoti Patel, MD: We've absolutely adopted that. Just the delays with repeat testing and again, the putting patients through another invasive procedure has really changed my practice such that than asking for a biopsy. I always want an immediate one like I want enough to be able to do it and also to triage patients to appropriate clinical trials if eligible.

So, a little bit more granular and you alluded to this, but what's the cost of testing? So what does EGFR testing cost? What does RAS testing cost? ALK and then NextGen?

Ajit Paintal, MD: So I can only speak on what I know. We're an academic institution, so I'm somewhat insulated from this. I know that Foundation One testing what people tend to regard as being expensive, they charge about $6,000.00, but on average they collect $3,000.00.

So I'm assuming that this other single gene assays that we tend to perform routinely is going to be less than that. So I would want to say maybe a couple of thousand dollars – a few thousand dollars overall, but again, I'm sort of insulated from those considerations, unfortunately.

Jyoti Patel, MD: Mm-hmm, and then EGFR and RAS testing, they were hundreds of dollars.

Ajit Paintal, MD: Yeah.

Jyoti Patel, MD: Correct?

Ajit Paintal, MD: Yeah. It's getting cheaper every day.

Jyoti Patel, MD: Right. So that would even – testing for EGFR or RAS would be less than five days of rulatinum, for example, so low is the cost.

Ajit Paintal, MD: Yeah. In the grand scheme of how much it probably costs to treat these patients if you count the chemo and all of the procedures they undergo, this is kind of a drop in the bucket, so it's ____.

Jyoti Patel, MD: And the way it leads to less exposure to drugs that are going to be ineffective and more toxicity.

Ajit Paintal, MD: Yes.

Jyoti Patel, MD: Another question, so differences or innate differences between primary and metastatic disease, I know that sort of the paradigm that we had learned and a workup of lung cancers you always go for the metastatic site, because then you establish diagnosis and stage.

Ajit Paintal, MD: Yeah.

Jyoti Patel, MD: Clearly that has changed for me. If patients have disease that it's metastatic to bone, primarily because of testing, but other differences in terms of feasibility of testing if you're looking at different sites or if there is an area that may have more necrosis or more stromal inflammation, any guidance on that?

Ajit Paintal, MD: I think in general I think what you alluded to is right. We just go. If they feel like it's easier to get to a soft tissue or some sort of visceral lesion versus going after the primary lung tumor, that's sort of the principal that we are sort of taught, as well. You want to go for the lesion that's going to give you the high state and the diagnosis.

And I think you mentioned the one caveat there, which was you want to avoid bone biopsy. So but we have actually had success in dealing with boney biopsies. We can either aspirate the lesion so that we don't get bone. We can sort of pick tiny pieces of tumor out of the bone. We've gotten that to work from time to time.

Or even people report on decalcifying with EDTA versus hydrochloric acid and that tissue may be useful for molecular testing, but in general, whatever the bronch – the pulmonologist and the radiologist think is easiest to get to and we'll subject the patient to the least morbidity, that's kind of what we go after.

You know there were a couple of papers a few years ago, one from Pittsburg and one for Hopkins, where they documented change in driver mutation status from the primary versus the metastatic lesion. In my conversations with one of the authors of those papers they actually in retrospect thought that they were actually separate tumors that different tumors that had metastasized.

So I think really for purposes of driver mutation testing, you're fine just going after whatever is easy to get and whatever is going to give you the best specimen and again, the only caveat would be decalcified bone doesn't work very well for this testing.

I know you have mentioned that on protocol you will test patients for T790M once they have become resistant to EGFR inhibitors, but again, my understanding from you is that that's not really standard of care.

Jyoti Patel, MD: So, I guess the caveat would be that now routinely patients who develop acquired resistance we're re-biopsying to see certainly if there is a T790 mutation, but also to see if there if there is a histologic shift.

And in that instance I usually ask for the single region that's changing, so if the primary disease is controlled in the chest, then perhaps there is progressive liver disease. I would perhaps ask for a biopsy for that simply for that very small subset of patients that may have evolved to a small cell.

But more and more of our, certainly our clinical trials are requiring biopsies at the prior to initiating dragon at the time of progression to see if there are true PK change or pharmacodynamic changes within the tumor.

A few more questions. So now that surgery has evolved so much and that we're able to do minimally invasive surgeries and truly get adequate tissue perhaps in a patient who has had maybe a failed attempt at a very central lesion through an IO biopsy or a bronchoscopy, has that changed your paradigm actually getting a wedge of tissue now as you're diagnostic _____?

Ajit Paintal, MD: I would say, yeah I would say if you can get a wedge or a resection that's almost an embarrassment of riches compared to what we're used to dealing with. Again, you remember that slide with just the grains of salt, so getting a wedge of tissue is that's great, because you'll have tons of material to work with for a routine EGFR and ALK.

Any other testing that you may decide that you want to perform will almost always have enough tissue to perform it on. So when you can get a bigger resection specimen or a lymph node dissection from mediastinoscopy or a lung resection, I mean those are always great, because you have tons to deal with.

But again, we're used to dealing with small amounts of tissue, because most of these patients are never going to get that far to get resected.

Jyoti Patel, MD: We're seeing, well we have seen a rapid number of drug trials that have demonstrated significant benefit in some sets of oncogenically defined lung cancers and we know that just in the past several years this field has changed so much.

What, as we look at other markers, so as we look at the BRAF mutations, the ROS 1 translocations, what do – how do you think

the IASLC and CAP guidelines will evolve? I mean what will sort of the onus be on home institutions to be able to do all of these in a timely manner?

Ajit Paintal, MD: So is I think as more FDA approved therapies have come out with different molecular targets, it's kind of at some point not going to be feasible to just keep adding more and more one off companion diagnostics to your list of what you're going to do.

And so that companion diagnostic paradigm may shift and in the near future, but in general once you start looking for more and more of these different driver mutations it's really going to make more sense to do multiplex testing and not just again, you know, you got your ALK for FISH or your FISH for ALK. You got your EGFR PCR and your KRAS PCR. It's just going to be multiplex testing seems to be where things are headed. Again and even the near to mid-term and again, at larger institutions.

Jyoti Patel, MD: Maybe even this year, right?

Ajit Paintal, MD: Maybe, who knows? Who knows?

Jyoti Patel, MD: But it certainly seems that that's the most time effective, tissue effective.

Ajit Paintal, MD: Yes. Yes, absolutely.

Jyoti Patel, MD: And will likely be the most cost effective if we're doing these one off one percent and two percent testing for drugs that will be available for our patients.

Ajit Paintal, MD: Yeah. I mean I would be surprised if multiplex testing was not the standard of care in a few years from now. That really seems to be where things are headed.

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