Molecular lung cancer testing: from guideline to practice

Karen Lusky

August 2018—Testing turnaround times can affect whether non-small cell lung cancer patients receive an EGFR or ALK tyrosine kinase inhibitor when indicated. At disease progression on an EGFR TKI, integrating circulating tumor DNA and tissue-based testing may lessen some of the limitations of each form of testing.

That and more was part of a webinar hosted by CAP TODAY with support from AstraZeneca and presented in May by Michelle Shiller, DO, MSPT, a molecular pathologist with Pathologists Bio-Medical Laboratories who is co-medical director of cancer genetics and the Division of Molecular Medicine and Pathology at Baylor University Medical Center, Dallas. Dr. Shiller pointed to the updates in the CAP/IASLC/AMP molecular testing guideline for the selection of lung cancer patients for treatment with targeted TKIs, used cases created to illustrate the updates, and spoke recently with CAP TODAY about how her laboratory achieves its turnaround times.

The 2013 CAP/IASLC/AMP molecular testing guideline recommended testing any histology other than adenocarcinoma when clinical features indicate a high likelihood of an oncogenic driver and in the setting of more limited lung cancer specimens. The 2018 guideline doesn't "talk as much about the quantity of the specimen," Dr. Shiller said, but it does suggest (in an expert consensus opinion) proceeding with testing when the clinical features indicate a high probability of an oncogenic driver.

That opinion has data to support it, Dr. Shiller said. Pooled data show that people of primarily Asian descent have a 50 percent prevalence of *EGFR* mutations in lung adenocarcinoma. The prevalence is about half that in those of primarily non-Asian descent. "With respect to squamous histology, you do see *EGFR* mutation detection in equal incidence [five percent] independent of ethnic origin," she said. For "adenosquamous, you see a very high prevalence of *EGFR* mutation detection in individuals of Asian descent [67 percent], and we still see it detected in those of non-Asian descent [13 percent]." *EGFR* mutations are sometimes also seen in large cell lung carcinoma (Lindeman NI, et al. *Arch Pathol Lab Med.* 2013;137[6]:828–860).

Dr. Shiller

Dr. Shiller's first case illustrates that "even though we may not have adenocarcinoma histology on the slide, squamous testing is still a candidate." The case is that of a 52-year-old female who presented to her primary care provider with shortness of breath and worsening cough. She is referred for a CT scan that identifies a 2-cm mass in the left lower lobe of her lung. The interventional radiologist performs a CT-guided core needle biopsy, and the pathology diagnosis is squamous cell carcinoma. The patient is a never-smoker, approximately age 50, so the pathologist recommends testing "based on these highly suggestive clinical criteria."

The National Comprehensive Cancer Network also recommends considering testing for *EGFR* mutations and *ALK* rearrangements for squamous cell histology if patients are never-smokers, if the biopsy specimen is small, or if mixed histology was reported, Dr. Shiller said. Age under 50 years is another highly suggestive clinical criteria.

The 2018 guideline says nearly every type of cytology or small biopsy specimen is suitable for mutation testing, with some exceptions. "The accumulated data confirmed the feasibility of EGFR mutational analysis with cytologic specimens," she said, noting that the guideline calls for separate validation studies. "There is also no statistical

difference between smears or cell blocks when testing for *EGFR* mutation status."

In a case illustrating that samples with low cellularity can be considered for testing, a 72-year-old male with COPD who quit smoking 12 years prior had become increasingly short of breath. His pulmonologist ordered a chest CT scan that displayed a 3-cm mass in the left upper lobe of the lung and several lytic lesions in the ribs and spine. "The patient is not a candidate for an open biopsy and resection due to a history of pulmonary stenosis," Dr. Shiller said. "So the interventional radiologist performed a CT-guided FNA, and due to the low cellularity of the sample, smears were prepared." The pathology demonstrated mucinous adenocarcinoma. "Some laboratories have higher-sensitivity assays than others," Dr. Shiller said, "so if it [the specimen] is low cellularity, you want to send it to a lab that can detect in low-cellularity specimens." Moreover, mucin can be an inhibitor in molecular assays.

Dr. Shiller pointed to a movement toward recommending more sensitive tests. "Part of that is due to a shift in the technology, transitioning from less sensitive Sanger sequencing to wider implementation of more sensitive next-generation sequencing." In 2013, the recommendation was that laboratories use *EGFR* test methods (or have them available at a reference lab) able to detect mutations in specimens with at least 50 percent cancer cell content, though use of more sensitive tests was encouraged. "Next-generation sequencing was coming of age at that time," Dr. Shiller notes. The 2018 guideline says the assays should be able to detect molecular alterations in specimens with as little as 20 percent cancer cells, "largely due to the development of more sensitive techniques," she says.

Dr. Shiller noted the varying sensitivities of mutation detection techniques, citing a 2014 study (Diaz LA Jr., et al. *J Clin Oncol.* 2014;32[6]:579–586). "Sanger sequencing, which we have to be thankful for because it was a wonderful starting point in the world of sequencing DNA . . . is the least sensitive of the methods at around greater than 10 percent and optimally applied to tumor tissue," she said. Pyrosequencing's sensitivity is about 10 percent, "again tumor tissue being the best candidate for that." Qualitative PCR is at about five percent for both tumor tissue and circulating tumor DNA.

NGS sensitivity is at two percent applied to tumor tissue. Quantitative PCR is at one percent and for tumor tissue. "And then ARMS, or amplification refractory mutation system, at 0.1 percent and applied optimally to tumors." BEAMing, PAP, digital PCR, and TAm-seq have a sensitivity of under 0.01 percent and are "optimally applied for cell-free or circulating assays, as well as for rare variants in tumor tissue," Dr. Shiller said.

The four FDA-approved companion diagnostics for *EGFR* testing are Therascreen EGFR RGQ PCR, Cobas EGFR Mutation Test v2, Oncomine Dx Target Test (doesn't include T790M), and FoundationOne CDx.

A CAP survey of companion diagnostic use found off-label practices more than 60 percent of the time, which included unapproved specimen and tumor types, accepting specimens with low tumor content, and not quantifying DNA prior to the assay, Dr. Shiller said (Kim AS, et al. *JAMA Oncol.* 2018;4[6]:838–841).

In the 2013 and 2018 guidelines, *EGFR* T790M testing is recommended with assay sensitivity for *EGFR* T790M detection to as little as five percent allele frequency (incidence) in patients who progress on EGFR tyrosine kinase inhibitor therapy. The expert panel also acknowledged that "cell-free DNA may be the best sample source," Dr. Shiller said, depending on location, feasibility, and other factors. "However, if it's negative, it's still recommended to reflex to tissue, if possible." It helps to remember, she said, that the T790M status of individual samples may not represent the T790M status of the overall tumor owing to intra- and intertumoral heterogeneity. Moreover, at progression, the resistant population is usually lower in quantity, which may translate into less ctDNA in the bloodstream and "a potential reason why we could miss the mutation."

The T790M mutation occurs as the acquired mechanism of resistance in two-thirds of patients who have an initial *EGFR* driver. So if 100 patients are eligible for biopsy at disease progression on an EGFR TKI, at most 63 will be positive for T790M on average. Plasma testing alone should identify 35 of those 63 patients, or about 56 percent. "So 44 percent of patients would not be identified due to false-negative results, invalid results, mutant DNA below the detection limit, or insufficient circulating tumor DNA content," Dr. Shiller said.

Forty-two of the 63 patients, or about 67 percent, should be identified by tissue-based testing alone. That means

“approximately 33 percent of patients are not identified due to similar reasons as before, or also due to an insufficient sample, patient refusal, or due to the fact that the location of that lesion is not feasible for biopsy.” When plasma and tissue testing are combined, she said, “you should be able to capture 47 of those 63 patients, which is 75 percent.”

Dr. Shiller presented a case to serve as a reminder to reflex to tissue if plasma testing is negative. Before elective surgery, a 69-year-old active Asian female, never-smoker, underwent a chest x-ray and an abdominal CT that showed a primary mass in the left upper lobe of the lung. A CT-guided FNA biopsy found a well-differentiated adenocarcinoma. Subsequent *EGFR* mutation analysis was positive for a sensitizing exon 19 deletion. The oncologist prescribed an EGFR TKI.

About seven months later, the patient had a cough, and a CT scan identified numerous pulmonary nodules in both of her lungs. “An oncologist requested a plasma-based test, which was managed in-house and negative for the T790M mutation.” A reflex testing protocol in place allowed the pathologist to request reflex tissue testing when the biopsy was acquired due to the negative plasma results.

Tissue and plasma-based tests have limitations, “and integrating both into clinical practice may overcome some of the challenges,” Dr. Shiller said.

The recommended turnaround time for molecular testing continues to be within 10 working days from when the molecular laboratory receives the specimen. The expert consensus opinion in the 2018 guideline is that laboratories should have processes to ensure that specimens that have a histopathological diagnosis are sent to the molecular pathology lab within three days of receiving requests.

The following case shows how easily the days add up. A 63-year-old female nonsmoker had a malignant pleural effusion and several lung nodules and rib metastases. A biopsy confirmed NSCLC adenocarcinoma. “In this case, the sample was acquired on a Thursday and the diagnosis rendered on a Friday. Three working days passed and the specimen was sent by this very responsible lab for biomarker testing,” Dr. Shiller said.

Within 10 working days of receiving the specimen, the lab reported the final results to the physician. “So yes, the turnaround time was achieved. However, because of the timing of when the specimen came in and weekends in between, a total of 21 calendar days passed from the time the patient had the procedure to when the molecular results were available.”

The time between when the patient first saw the person who recommended the procedure and receipt of these data was unknown. “So the window could actually even be longer,” Dr. Shiller pointed out.

Questions to ask: “How or does your lab track the three-day recommended window, and what are you logging and/or tracking and time stamping?” “What do you do when a tissue sample comes in on a Friday, and how do you keep things moving over the weekend?” (See “Turnaround time: the PBM process.”)

A study presented at the 2017 ASCO Quality Care Symposium found that when the *EGFR* results were released to clinicians before they initiated first-line therapy, *EGFR*-positive patients received EGFR TKI therapy (versus other therapy) 80 percent of the time. However, when the results were reported after first-line therapy was initiated, 43 percent of the patients got the EGFR TKI.

“A similar pattern was seen with *ALK*,” Dr. Shiller said. “When the *ALK* results were known prior to first-line therapy initiation, 77 percent of the time they did receive the relevant targeted therapy. However, when the results were reported after first-line therapy was initiated, 42 percent of the time they received the targeted therapy” (Ruggiero JE, et al. *J Clin Oncol.* 2017;35[8 suppl]:abstract 212).

Immunohistochemical testing for ALK is now viewed as equivalent to FISH. Two commercially available monoclonal antibodies, D5F3 and 5A4, can be used for ALK testing. “They have demonstrated acceptable sensitivities and specificities, ranging from 95 to 100 percent when compared with *ALK* FISH results,” Dr. Shiller said. The monoclonal antibody for anaplastic large cell lymphoma, CD246, is not recommended for ALK testing in NSCLC.

The 2013 guideline did not have a recommendation for *ROS1* testing, but the 2018 guideline says *ROS1* testing must be performed on all patients who have advanced-stage lung adenocarcinoma, irrespective of clinical characteristics. "The frequency of *ROS1* rearrangement is one to two percent," Dr. Shiller said, and *ROS1* gene arrangements produce fusion proteins that are powerful oncogenic drivers (Gainor JF, et al. *Oncologist.* 2013;18[7]:865–875).

"The expert consensus opinion is that ROS1 IHC may be used as a screening test [in advanced-stage patients]; however, if it's positive, it should be confirmed by a molecular or cytogenetic method." Oncomine Dx, which has *EGFR* and *ROS1* (and is FDA approved), is an example of one of the available tests. "FISH is the gold standard, and it is performed with a break-apart probe with a splitting of the signal by at least one probe diameter in greater than or equal to 15 percent of tumor cells."

Real-time-PCR RNA sequencing and NGS DNA sequencing are acceptable. "Targeted real-time PCR may be challenging due to locus infidelity with *ROS1* breakpoints. And capture-based sequencing strategies for RNA and DNA are preferred, if properly validated," she said.

"The expert panel seemed to advocate panel testing, and I think some of that is part of the maturation of next-generation sequencing," Dr. Shiller said. "So other markers to consider, particularly when *EGFR, ALK*, and *ROS1* are negative, include *RET, BRAF, MET, HER2*, and *KRAS* testing. They are not indicated or recommended as routine single-gene tests outside the context of a clinical trial for patients with lung cancer, but they are appropriate to include as part of a larger panel."

KRAS mutations are present in about 20 percent to 30 percent of NSCLC, and they are characterized by point mutations. The *RET* alteration occurs between 1.2 and two percent of the time. It is a gene rearrangement, and there is no gold standard. It can be tested by NGS, real-time PCR, IHC, and FISH. "But immunohistochemistry and FISH can be challenging," Dr. Shiller said.

MET occurs in NSCLC about two percent of the time, and testing should include exon 14 skipping mutations and MET amplification. "It can be a mechanism of resistance or a de novo event, and there is no gold standard in terms of how to detect it."

HER2 is present two percent of the time and is usually an exon 20 insertion, "so it's not necessarily *HER2* amplification." There is no gold standard for testing. "You can use multiplex assays or next-generation sequencing."

BRAF has a frequency in NSCLC of 0.5 to 4.9 percent. "Most of the time in other sites of origin, we think of the V600E mutation as being the relevant site or hot spot. However, in the lung, greater than 50 percent of the time the alterations in *BRAF* do not involve V600E," she said. "There is no gold standard," and many tests are available that detect *BRAF* V600E alterations.

Dr. Johnson

A combination of two drugs (dabrafenib and trametinib) was approved by the FDA last year for patients whose metastatic NSCLC has a *BRAF* V600E mutation. *RET* drugs may be next. In a CAP TODAY interview, Bruce Johnson, MD, chief clinical research officer at Dana-Farber Cancer Institute, said it's likely that drugs will be approved for *RET* rearrangements based on a presentation at this year's ASCO meeting showing "response rates in excess of 70 percent." (These drugs are more specific in their mechanism of action, Dr. Shiller tells CAP TODAY, with greater efficacy and improved adverse event profile, especially for particular *RET* alterations.) It also looks like the

progression-free survival is going to exceed 10 months, Dr. Johnson says. “So that will be the fifth indication.” Alex Drilon, MD, of Memorial Sloan Kettering Cancer Center, was lead author of the presentation.

Turnaround time: the PBM process

Pathologists Bio-Medical Laboratories “hits its turnaround times” for molecular testing, says Dr. Michelle Shiller, by time stamping specimens and relying on an institutionwide standing order for reflex testing with many of the sites for which testing is provided.

“We time stamp every step of the way, from the moment the specimen was received until it leaves the originating facility,” and when the specimen arrives in the molecular laboratory. A daily dashboard displays the status of the cases. “All of these measures help to maintain awareness,” she tells CAP TODAY.

The laboratory also uses time-saving tactics. For cases that have a clinical indication of lung nodule, they cut 15 unstained slides at the outset and H&E stain the first slide and the 15th slide, without knowing whether the tissue specimens are benign or malignant. That leaves “13 slides in the middle to work with for IHC and FISH, or if we are really low on tumor, we can also use those for the sequencing assays,” Dr. Shiller explains. “This not only helps to preserve tissue,” but since the slides are already cut, “there is no additional delay waiting for unstained slides to be prepared, unless you are running out of tissue from having to run other tests.”

Dr. Shiller finds that a lot of pathology groups wait for the clinician to send an order, in part because of concerns for reimbursement, as well as concerns about Stark law or kickback law violations (a legitimate concern, she notes), since the downstream testing also occurs within pathology laboratories. Her institution has a standing order from the clinicians to the pathologists for all cases diagnosed as primary lung adenocarcinoma to automatically reflex to the pertinent testing. “Once the diagnosis is made, the testing just happens immediately,” she says. These orders are also in place for the relevant primary lung squamous cell carcinoma (PD-L1).

She has heard that some insurers are beginning to oppose this approach. “If that’s the case, I think there is a need to educate the insurance companies because some patients are so sick that their illness will not tolerate these types of delays.”

In the institutions that don’t have a tumor site committee to recommend the reflex testing, the clinician could provide a written order to the pathologists who read the clinician’s cases, she suggests. The order could then be correlated with the specimen once it is received and the diagnosis made, “as long as the clinician is amenable to that.”

Dr. Shiller says PBM performs NGS testing for *AKT1, BRAF, EGFR, HER2, KRAS, MEK, NRAS, PIK3CA,* and *PTEN*. PBM also offers *ALK, ROS1, RET*, and *MET* by FISH. “We are not having reimbursement issues in terms of getting the panel testing reimbursed,” she says. “If you continue to do one gene at a time, it exhausts time and tissue and resources.”*—Karen Lusky*

Dr. Shiller shared a case to illustrate the value of extended-panel testing. A 59-year-old man had a CT of the chest and abdomen that identified a 6-cm lung mass and diffuse liver nodules. The pathology revealed adenocarcinoma. The oncologist requested a next-generation-sequencing–based multigene panel, and it is negative for *EGFR* mutations and *ALK* or *ROS1* rearrangements. “Considering the guidelines and the negative testing, the oncologist orders an extended panel, which is positive for *MET* exon 14 skipping mutation, qualifying the patient now for a clinical trial.”

“Every scenario is different, from tissue availability to what is going on with the patient and so on, but the first test took five days to get a result. The second test 18 days, so the total cycle to get the results of the exon 14 skipping mutation qualifying the patient for a clinical trial was 23 days.”

In her view, pathologists can be of great value to clinicians by assisting them in understanding the strengths and other considerations of using extended-panel testing versus multiple single-gene tests. How probable is it that the

patient's tumor has an initial oncogenic driver—*EGFR, ROS1*, or *ALK*, for example? Is there enough tissue? "We are the ones who have that answer and can help to guide them along with that," she said. "And what are the implications of the total turnaround time for the patient? In other words, how sick are they, and do we have enough time to wait 23 days for a final result?"

"We can educate our colleagues regarding the strengths and considerations of particular individual tests, including weighing the individual variables for each patient, to facilitate ordering the right test on the right patient at the right time, with the most relevant results," Dr. Shiller says.

The 2018 expert panel prefers multiplexed genetic sequencing over multiple single-gene testing when testing beyond *EGFR, ALK*, and *ROS1*. "And part of this is a tissue stewardship and turnaround time consideration," Dr. Shiller said, noting that studies have demonstrated 100 percent concordance between single-gene assays and NGS.

Dr. Johnson says a study presented at the 2018 ASCO meeting by Nathan A. Pennell, MD, PhD, of Cleveland Clinic, showed that doing an NGS panel was faster and less costly than performing sequential or single-gene tests. "Rather than saying how much each individual test is indicated, we would advocate for doing a large panel for testing, so that you don't have to have discussions about whether you should or shouldn't do a *RET* rearrangement, if a drug for this indication is likely to be approved in the next six months to a year," says Dr. Johnson, who is also a professor of medicine at Harvard Medical School.

For the past five years, Dana-Farber Cancer Institute has been performing a large panel of several hundred genes. "All of the genes you think could be actionable during the lifetime of the patient are available for patient management," Dr. Johnson says, "not only for the FDA-approved agents but also the genomic changes for which there are promising therapies."

Dr. Shiller presented a final case to highlight that all molecular test results must be incorporated in treatment strategies. A 79-year-old man is diagnosed with lung adenocarcinoma. A tissue specimen is sent for in-house testing, which is immunohistochemistry for PD-L1 using the SP263 clone and EGFR IHC mutation screening using SP111 and SP125 antibodies. Results available after five working days show PD-L1 expression of 25 percent and no *EGFR* mutation. "What would you do next?" Dr. Shiller asked.

The information is insufficient to make a decision about therapy, she said. "EGFR testing by IHC is not considered adequate. It needs to be done by next-generation sequencing, so we still have some testing to do." Molecular testing performed at an external laboratory for *EGFR, ALK, ROS1*, and *BRAF* uncovered an *EGFR* mutation with an exon 19 deletion.

Concomitant oncogenic driver mutations

Can a lung cancer have more than one oncogenic driver?

"The technical answer is yes," Dr. Michelle Shiller tells CAP TODAY. "We have historically thought of all of the oncogenic drivers as being mutually exclusive, which is what we see as a majority, hands down, but we are finding out that's not always true."

You can have concomitant *ROS1* and *EGFR*, according to the findings of a study published in the *Journal of Thoracic Oncology*, she says. "The *n* was 1,345, and 25 were *ROS1* positive." In the article, titled "High prevalence of concomitant oncogene mutations in prospectively identified patients with *ROS1*-positive metastatic lung cancer," the authors write, "Of 25 cases with ROS1 positivity at IHC analysis, six involved tumors harboring *EGFR* mutations, including one with an *EGFR* mutation plus a *PIK3CA* mutation, two with a *KRAS* mutation, and one with a *BRAF* V600E mutation" (Wiesweg M, et al. *J Thorac Oncol.* 2017;12[1]:54–64). Five of the six treated for *EGFR* positivity responded, Dr. Shiller says.

As for treatment, Harvard oncologist Dr. Bruce Johnson says there's data from a phase one study on combining

erlotinib, an EGFR inhibitor, with crizotinib, which is the drug that's approved for *ROS1* (Ou SI, et al. *J Thorac Oncol.* 2017;12[1]:145–151). "If you have those oncogenic drivers, you can try to give both drugs at the same time. If there hasn't been a phase one, so you don't know the doses, it becomes a challenge," he says. In that case, "we commonly treat with one and then add the second drug in later with a small dose escalation." *—Karen Lusky*

The physician should wait for all molecular results before selecting therapy. PD-L1 results are often available well before all of the other results. "All of the indications for PD-L1 therapy assume *EGFR*– and *ALK*-negative status. So not releasing PD-L1 results until everything is available, with the exception of the case of a very sick patient, is one way to make sure they are acted on in the right order."

Dr. Shiller says her laboratory releases all test results at the same time. "The only time we fall outside of that is when the clinicians communicate, 'For this patient, any result we can get sooner than later is great,' and so I literally will call the clinician and report the results individually." This is the exception rather than the rule in the lab, she adds. "I think it's important to have that nimbleness since people's lives are on the line."

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