Markers, methods remake the NSCLC map

Karen Titus

February 2021—Absorbing new biomarkers into lung cancer workups makes for a complicated diplomacy. How best to balance so many rivals?

Does it make the most sense for laboratories to try to do everything at once, a full-court press involving next-generation sequencing panels? Or is it more practical to add a new marker only as a new targeted therapy receives approval? Where do RNA-based assays fit in? What about IHC? When do you make the switch? Or do you? And how best to handle cell-free DNA tests (which seem to be the rogue states in all this)?

How do you weight external factors, such as reimbursement, existing equipment and capital expenditures, and physician expertise?

Driving this all are medical breakthroughs. As with all forms of statecraft, the latest incident can change everything.

Dr. Lynette Sholl of Brigham and Women's Hospital: While there has been a broad push to give targeted therapies, "appropriate testing has not been incentivized," she says.

For lung cancer, the most recent advance comes from the ADAURA trial (Wu Y-L, et al. *N Engl J Med.* 2020;383[18]:1711–1723), which showed a significant benefit of using osimertinib to treat stage IB to IIIA *EGFR*-mutation positive non-small-cell lung cancer. While EGFR tyrosine kinase inhibitors have long been used to treat metastatic disease, this offers a hopeful approach to treating localized resectable disease.

"The ADAURA trial is extremely impressive," says Mary Beth Beasley, MD, professor of pathology, Icahn School of Medicine at Mount Sinai, and head of thoracic pathology, Mount Sinai Medical Center, New York. It's exciting, too, particularly for practitioners like her who've spent decades waiting for gains that could offer genuine hope to patients. "When I started, there was often very little we could do for patients. It was so sad."

Now that's changing. "It was baby steps at first, and now it's kind of exploded," says Dr. Beasley.

Other meliorations include the drug capmatinib, which appears to be efficacious in treating advanced NSCLC tumors with *MET* exon 14 skipping mutations (Wolf J, et al. *N Engl J Med.* 2020;383[10]:944–957). And tumors with *RET* gene fusions appear to respond well to two newly approved agents: the *RET* inhibitor selpercatinib (Drilon A, et al. *N Engl J Med.* 2020;383[9]:813–824), as well as pralsetinib. *EGFR* insertion mutations on exon 20 wait in the

wings.

But, says Dr. Beasley, “I think the ADAURA trial in particular is going to be the impetus for a potential shift, from testing just advanced stage cancers to showing a benefit in testing in earlier stage cancers.” It might also be a tipping point for how labs consider their approach to NSCLC—both the *when* and the *how* of testing.

Dr. Beasley’s own institution illustrates the challenges labs face as they try to stay current. The ADAURA trial is unlikely to change much at academic centers like hers. “We end up testing for everything,” Dr. Beasley says. “But that’s definitely not the case at all institutions or laboratories.”

Even labs at the forefront have to work to keep up. Dr. Beasley is a coauthor of the CAP/IASLC/AMP molecular testing guideline for lung cancer. “And of course, by the time we get that out, something new has already come up,” she says. No treaty, no matter how well crafted, is ever final.

For the last seven years or so, Lynette Sholl, MD, and her colleagues at Brigham and Women’s Hospital have been using a several-hundred-gene NGS panel for routine testing. Even when they didn’t know something was important, “We’ve been including those targets on our panel pretty much the entire time.” One goal was to identify variants so patients could enroll in clinical trials at Dana-Farber Cancer Institute. But doing so has also made it easier to stay abreast of clinically relevant biomarkers, says Dr. Sholl, who is associate professor of pathology, Harvard Medical School, and chief of the pulmonary pathology service and associate director of the Center for Advanced Molecular Diagnostics, Brigham and Women’s.

Awareness and action are only a start. While the lab has been able to detect *RET* fusions and *MET* exon 14 mutations, Dr. Sholl says, “We often didn’t know what we were looking for.”

“That’s one of the challenges of DNA-based testing,” she continues. “There’s a tremendous amount of diversity in the types of mutations that ultimately can lead to *MET* exon 14 skipping alterations.”

Dr. Sholl speaks from experience. She and her colleagues have retrospectively reviewed their data from time to time. In one study, published in 2016, they manually reviewed DNA sequences from patients who didn’t have other driver alterations “and identified *MET* exon 14 skipping mutations as 15- or 20-base pair deletions sitting fully in an intron, not touching an exon at all,” she says (Awad MM, et al. *J Clin Oncol.* 2016;34[7]:721–730). “Honestly, historically we would just ignore that kind of thing.” That was before they realized the deletion was sitting right over the polypyrimidine tract, which means it has critical implications for enabling the splicing to occur properly, she explains.

“We got a lot of insight into basic mechanisms of splicing that get altered in these tumors, and they weren’t on our radar for many years,” Dr. Sholl says. “We know to look for that now,” but many DNA-based tests may not be optimized to pick up the broad range of changes.

Sometimes pathologists aren’t “optimized,” either. “Because the diversity of alterations is so great, we rely on a preexisting knowledge base to help provide accurate annotations for variants on a panel,” Dr. Sholl says. For example, *MET* splice mutation tumors often have sarcomatoid morphology. “It may actually come into your system as a specimen that’s not necessarily recognized a priori as a primary lung cancer.” It may be a metastatic sarcomatoid carcinoma, with an uncertain primary site and an uncommon variant. “You can imagine the combination of events that transpires to miss the importance of one of these unusual *MET* splicing mutations.”

That can include overestimating the power of NGS. Not everyone fully appreciates the limitations of NGS, says Laura Tafe, MD, associate professor of pathology and laboratory medicine, Dartmouth-Hitchcock Medical Center and the Geisel School of Medicine, Dartmouth College, and assistant director of the laboratory for clinical genomics and advanced technology. “I’m sure the understanding of NGS’ capabilities is still mixed. I start out almost every single one of my talks talking about the different types of variants NGS can detect—and that not all assays are created equally.” Pathologists and their clinical colleagues need to be aware of—and even look for—the limitations and weaknesses of a particular assay, she says.

Dr. Sholl and her colleagues sometimes turn to a partner lab across town to perform RNA-based anchored multiplex PCR testing as needed. This approach is gaining a foothold in clinical labs, Dr. Sholl reports. An RNA-based assay can help pick up both *MET* splicing mutations as well as other fusion events—to confirm, for example, a suspected splicing variant, or in cases involving, say, a pan wild-type lung cancer with an unknown driver.

Some labs will run DNA- and RNA-based approaches in parallel. It's a good way to immediately confirm that the DNA-variant of interest is functionally relevant, Dr. Sholl says. It also allows labs to achieve the challenging task of validating low-level fusion transcripts. (See "Study: Combined DNA-RNA testing improves detection of *MET* mutations," CAP TODAY, March 2020.)

Though her lab's *MET* adventures rely heavily on those two modalities, Dr. Sholl notes the growing interest in *MET* amplification by FISH or next-gen assays, as well as MET protein overexpression by IHC.

This part of the story is particularly complicated, she says. A number of studies (largely crizotinib-based) have shown that response to MET-targeted inhibitors correlated with the level of *MET* amplification. Patients with high levels—a five-to-one ratio of *MET* to the centromere, say—were much more likely to respond to treatment. The historic data may not have consistently looked for an underlying splice mutation, however, and Dr. Sholl suspects a subset of patients had one.

Newer studies involving capmatinib also examined amplification-positive, splice-negative patients, she says, demonstrating different response rates around a cutpoint of 10 gene copies. Capmatinib showed efficacy only in those patients whose tumors had at least 10 copies; however, the analysis did not meet prespecified levels defining significance (Wolf J, et al. *N Engl J Med.* 2020;383[10]:944–957). Until more data emerge, labs will wrestle with defining what level of amplification predicts response.

The other issue is that *MET* amplification, particularly at lower levels, can often be seen as a co-alteration, Dr. Sholl says, which upends the understanding of standalone driver alterations such as *EGFR, MET, RET,* etc. "That's the Achilles' heel of your tumor." When patients potentially have another driver, whether it's recognized at the time or not, "that's going to potentially influence the outcomes to targeted therapies."

IHC tells its own twisty tale. "The data is pretty confusing right now," Dr. Sholl says. "We have historically seen that IHC is not a good predictor of response to some of the earlier MET inhibitors such as onartuzumab. There were some disappointing trial results using IHC as a biomarker initially" (Spigel DR, et al. *J Clin Oncol.* 2017;35[4]:412–420).

The question then became, could IHC be used as a surrogate for splicing mutations? Initial data suggested that IHC's poor sensitivity for *MET* exon 14 skipping mutations limited its use. Recently, a Memorial Sloan Kettering study of patients receiving anti-*MET* therapies showed overall clinical outcomes were best in patients who had a splice mutation and also MET IHC overexpression (Guo R, et al. *Clin Cancer Res.* 2021;27[3]:799–806).

Dr. Sholl says this lines up with the correlations she and her colleagues reported five years ago. The strong responders could be a unique subset of patients who have that high-level addiction to MET signaling, she says. While IHC in and of itself is not going to be an adequate screening tool, she says, it might help identify which patients are likely to benefit the most from targeted therapies.

She gets requests for MET IHC but is reluctant to perform it outside the context of a clinical trial enrollment. "We don't know what it means in most contexts."

As with children (so the joke goes), *MET* is unique but not special. With every new biomarker, "It's the same story, writ a different way, every single time," Dr. Sholl says.

RET, for example, is included on the next-gen panel in Dr. Sholl's lab, which added it about five years ago as efforts increased nationally to bring profiling into routine practice at academic labs to qualify patients for RET inhibitors, among other targeted therapies. The lab was participating in the Lung Cancer Research Foundation's Lung Cancer Mutation Consortium protocol, which involved testing patients prospectively by FISH as well as NGS. In her

experience, *RET* FISH assays are fairly easy to interpret.

Explains Dr. Sholl: DNA-based NGS offers quite-high sensitivity. Most breakpoints within the *RET* introns are fairly well defined. But as with *MET*, RNA-based assays that look for *RET* transcripts are a powerful complement. And an RNA-based approach offers an advantage over FISH: It can identify a fusion transcript and determine the fusion partner bound to *RET*, which is important confirmatory evidence, whereas FISH stops short of identifying functionality.

Numerous studies have looked at IHC as a screening tool for RET. The majority, says Dr. Sholl, have been disappointing, showing poor sensitivity and specificity in identifying fusions with RET overexpression.

She says comprehensive testing is crucial. "I talk to people who, in their practice, never see these [newer] alterations. It's always surprising to me, because *MET* alterations are three percent of lung cancer. That's a lot. That's like *ALK*, and people are used to seeing *ALK* all the time."

She suspects labs have simply become more comfortable with *ALK* as the testing trajectory moved from FISH to IHC and eventually to widespread screening.

MET could follow, although, as noted, it lacks a good IHC option. Another complicating factor, Dr. Sholl notes, is that generating useful sequencing information for *MET* requires an infrastructure "that captures everything." A number of commercially available assays do this, and more labs are bringing on RNA-based targeted panels. "That will be very helpful, with the caveat that everything needs to be interpreted in context," she says. The RNA-based approaches run the risk of false-positives (in terms of low-level physiologic splicing) and false-negatives (in terms of poor quality RNA, assay failures, etc.).

"Persistence is probably the most important thing," Dr. Sholl says. "If you have patients who have pan wild-type tumors, you need to keep pushing until you're able to define the underlying drivers."

MET affects a heterogeneous group of patients, she continues. While *ALK* and *ROS1* mutations are unusual in tumors of patients who smoke, with *MET*, "it's 50-50. You really just have to be looking for it in every context." Age is another somewhat iffy clue. "Our experience is that there's a bias toward age that's higher than what we see for our average lung cancer patient—but not always," says Dr. Sholl, who reports seeing 40-year-olds whose tumors have *MET* splice mutations.

The aforementioned phenotypic heterogeneity makes the puzzle harder to solve as well. In addition to sarcomatoid carcinomas, "We're also seeing these in squamous cell carcinomas," Dr. Sholl says. If she sees an SCC from a patient who is a light smoker, "The first thing I think of right now, based on my own biased experience, is the possibility of an underlying *MET* splice mutation. I've seen it enough times."

EGFR is an old kid on the block, compared with *MET* and *RET*, but now worth another look.

When Dr. Sholl and colleagues saw the results of the ADAURA trial presented at ASCO in June, they were ready to implement reflexive *EGFR* testing for patients with stage IB through IIIA tumors.

"This is a great use case for reflexive testing," Dr. Sholl says. The trial included only patients whose tumors had L858R and ex19del mutations. "You can rationalize the use of focused assays for *EGFR*"—which are fairly inexpensive, she adds—"just looking at those particular alterations."

She and her colleagues focus on all patients who get their tumors resected at Brigham and Women's—*EGFR* testing is initiated when the pathology is signed out. "We choose the best block and send it right off to the lab."

KRAS is another old-timer getting a makeover. It's the most commonly seen lung cancer mutation, Dr. Beasley says. Although not recommended now, early on, given the relatively high frequency of occurrence, testing for *KRAS* alone was considered cost-effective. While *KRAS* itself was not amenable to targeted therapy, the presence of a *KRAS* mutation typically excluded the presence of *EGFR* mutations, indicating a patient was unlikely to respond to

EGFR TKIs, the chief treatment at the time.

That has been turned on its head more recently. Now people are interested in the *KRAS* status because the presence of a G12C mutation enables patients to go on potentially promising targeted therapies. Labs thus need to perform tests that tell exactly what the mutation is. Black box genotyping approaches that discern a *KRAS* mutation but don't specify which one are no longer appropriate, Dr. Beasley says.

KRAS mutations are complex, and the number of co-mutations could be high. Given that the tumor mutational burden is often high, it will be interesting to follow ongoing *KRAS* G12C trials to see if co-mutation types and mutational burden inform response rates to targeted therapies, Dr. Sholl says. Her medical oncologist colleagues tell her that toxicities associated with G12C inhibitors might make the drugs slightly less appealing than EGFR (or other) TKIs. With the availability of good immunotherapy and combined chemo-immunotherapy, "It's a more complex thought process to figure out the right thing for patients in the frontline setting," she says, although the answer will ultimately rest with whether G12C inhibitors are approved.

If no biomarker (or child) is special, "No single assay is perfect," Dr. Sholl says. "Having multiple lines of evidence can be helpful, especially if you're potentially dealing with novel alteration. You can't close the door on any of our techniques that we've been working on for years."

Nevertheless, for labs that have been using the serial dating approach to lung cancer biomarkers (but keeping them all), might the time be ripe to update their approach?

"To get comprehensive testing, you need multimodality or at least a DNA and RNA combined next-gen sequencing approach," says Dr. Tafe.

Dr. Sholl concedes her bias is toward comprehensive sequencing, having used it for years. It does pick up mutations in other areas of genes that may be meaningless—at least for now—which invites more questions. It's also, to some extent, a clinical discovery tool. Because it's impossible to anticipate all the varied mechanisms that can activate different oncogenic pathways, "having an agnostic, unbiased test can show you things you didn't know to expect. That's important, now that we understand just how heterogeneous the mechanisms of activation are for many of the oncogenes. We may discover alterations that are actually more common than we realized—we just haven't gone about looking for them the right way."

NGS could also help boost biomarker testing rates, which, experts agree, need to be boosted. (See "In NSCLC, biomarker testing rates fall short," CAP TODAY, June 2020.)

But technique isn't everything. *RET* testing is critical, for example, but it doesn't require NGS. As Dr. Sholl notes, "We've had the techniques to detect these fusions for many, many years.

"But that doesn't mean we get paid to do it," she adds.

This is a sticking point. No test can sustain a solo act. Even the best biomarker needs a posse: an FDA-approved targeted drug and an NCCN stamp of approval. Without them, labs will be reluctant to perform it clinically.

The reflexive *EGFR* testing at Dr. Sholl's lab delineates the problem. Patients getting lobectomy or some kind of anatomic lung resection and mediastinal staging will be discharged within a few days, while the lab is still working up the pathology. Medicare rules are such that the lab will not get paid to do molecular testing on samples obtained in the inpatient setting when performed within the first two weeks of the patient leaving the hospital. "We essentially recognized that we were going to eat the costs of this. But because it was a low-cost endeavor, and because it looked like the right thing to do to get patients onto osimertinib, we just rolled it out."

Dr. Sholl asked her oncologist colleagues how long they could wait for *EGFR* mutation status. It appeared two months might be acceptable. That might allow labs to rationalize not doing the test reflexively and instead wait for the clinician to order the test when they see the patient in clinic postsurgically. But dancing past Medicare's 14-day rule has its own downsides: The clinician may neglect to order the test, and labs will need to go back and pull

tissues a second time.

Reflexive testing is less cumbersome, “but you have to have a hospital that’s willing to accept the risk of not getting paid,” Dr. Sholl says. At her institution, that meant “a detailed conversation with our finance people and our compliance office to make sure it was all aboveboard and appropriate.” It helped that the ADAURA results were so compelling that when oncologists knew they had a patient with an *EGFR* mutation and a IB–IIIA tumor, “It was very clear what they were going to do.” If the drug hadn’t received such quick approval, they would likely have considered osimertinib use off-label. “So we felt justified in the approach we took.”

Dr. Beasley and her colleagues at Mount Sinai “have worked very hard to find a solution” to the ordering piece, to avoid duplicate testing and related billing issues, as well as delays in testing. “What we finally ended up doing was having a standing blanket order for all the tumors that might need testing.”

At Dartmouth-Hitchcock, pathologists will automatically order the molecular and PD-L1 testing upfront in NSCLC cases. “That gets the ball rolling as soon as we have a diagnosis,” Dr. Tafe says.

Labs could more easily provide needed comprehensive testing, says Dr. Sholl, “if we didn’t get nickeled and dimed at every turn.” Following up initial *EGFR* and *ALK* tests using NGS is cheaper and easier than running six or seven single-gene assays to fill in the rest of the targets. But payers won’t reimburse for a second *EGFR* or *ALK* test that’s part of the panel, nor will they consistently pay for a panel code. “What are we supposed to do?” Dr. Sholl asks.

Comprehensive testing is better for patient care and saves money in the long run. “I don’t want to throw insurers under the bus, although sometimes I do,” Dr. Sholl laughs. While there’s been a broad push to give targeted therapies, “appropriate testing has not been incentivized.” Biomarker testing, she says, is “the forgotten stepchild of oncology.”

The nudge toward NGS is likely to persist. “As we add on more and more biomarkers, a sequential strategy is simply not viable,” Dr. Sholl says. “Just tagging on yet another variant to a long list of complex variants that we need to pick up is not, operationally, a good way to go. You’ve got to flip the switch over to more of a panel-based approach to get all these things.”

“You need to be doing a multigene panel,” Dr. Beasley agrees, “and NGS in particular, because the number of potentially targetable mutations is just going to keep changing and growing.” When a new marker emerges, “I just verify it’s part of our panel—it usually is.”

“People need to realize that these more infrequent mutations, like *RET* and *MET*, are evolving to the point where they have targeted therapies,” she continues. “So they need to be doing a bigger panel. And NGS is the best way to do that.”

NGS is useful in another regard, Dr. Beasley says. “We see more and more lung cancer patients with multiple tumors.” NGS can help sort out whether they belong to the same tumor, or if they’re multiple synchronous primaries, which is important to know for treatment strategy and prognosis.

Dr. Tafe

And because lung cancer has been the poster child for doing more tests with small biopsies, NGS helps with efficiency, Dr. Tafe says. “Doing more and more targets used a lot of tissue when we were doing single-gene assays.”

But it's not as if once a drug is approved, "you snap your fingers and you get the right platform in your lab the next day," Dr. Sholl says. "We end up playing catch-up."

There's the negotiation for capital equipment upgrade, for starters. And even if the hospital green-lights the expense, there's the validation of an entirely new panel. It also requires personnel with the experience to run it. "That's not always available in every practice," says Dr. Beasley.

Even the larger academic labs have their personnel problems. Dartmouth-Hitchcock uses a third-party vendor for the bioinformatics piece. "You don't have to have a team of 20 bioinformaticians in order to do this type of testing," Dr. Tafe says. "It's not our dream to always be that way, but it has filled in some gaps, where we haven't had the manpower to cover those areas of expertise to build out our own pipelines."

If a lab recognizes its internal assays are no longer sufficient to perform comprehensive workups, send-outs are an option. But that can be expensive.

They can also take longer, says Dr. Sholl. "The reality is, these big, hybrid-capture assays that a lot of us have adopted over the last seven or eight years just take a long time. It's complex chemistry. It can take a day just to go through the bioinformatics pipeline." A 500-gene assay, from start to finish, takes a minimum of one week, "and often it's closer to two weeks. And it can be longer than that, depending on logistical hurdles of getting the specimen into the lab."

That's why Dr. Sholl expects to see labs continue using quick, very focused assays to ensure basic testing. *EGFR* assays are robust, and ALK and ROS1 can be tested quickly with IHC.

Sometimes the need for speed is paramount. At Dartmouth-Hitchcock, "We keep a few single-gene assays live and available for some circumstances—where there's high clinical suspicion in advanced stage disease, and that one-week difference would make a difference in the patient's care," says Dr. Tafe.

But complications can arise quickly as well. Dr. Sholl describes this typical scenario: A patient pre-sents with an aggressive sarcomatoid lung cancer with widely disseminated metastases. The tumor is negative for *EGFR* mutation, and ALK and ROS1 IHC are negative as well; PD-L1 is 90 percent. "If you read the letter of the law for immunotherapy approvals, this person is ideal" for such treatment, Dr. Sholl says. If full-panel sequencing results will take two weeks, "What are you going to do?" Often patients will be started on chemo or chemo-immunotherapy before full genotyping results are available.

On the other hand, some results come almost too quickly, often through the back door. Cell-free DNA tests are now pervasive, Dr. Sholl says. Their value is real; so are the headaches they cause. Results typically are sent directly to the clinicians. These private transactions mean the data isn't flowing to the lab. Dr. Sholl has had cases where a sample is sent out for cell-free testing "literally before we get a diagnostic biopsy. We spend five days trying to figure out what a poorly differentiated tumor is," while the clinician already knows there's an *ALK* rearrangement, for example.

Even when the information makes it into the medical record—and there's no guarantee it will—it's an odyssey trying to find the information. Says Dr. Beasley, "We usually don't know the results." That can, furthermore, create issues if patients change oncologists or seek a second opinion. "Having all of these different, outside tests is being recognized as a problem." It's a pending point of discussion with clinicians at Mount Sinai—even they are struggling to keep up with so many free-floating results. "But we haven't found a solution yet."

As the list of biomarkers swells, pathologists might also be feeling overwhelmed. "In all fairness, this is hard to keep up with if you're not solely dedicated to lung," Dr. Beasley says. Mount Sinai is very subspecialized—her own practice is almost exclusively focused on thoracic.

Also worth remembering, says Dr. Beasley, is that lung cancer doesn't account for a substantial percentage of most pathology practices. CME won't necessarily help. "You're going to be focusing on things you do the most," such as prostate, GI, breast. A similar story unfolds on the clinician side. "As you get away from subspecialized

practices, you get people who are treating all comers for cancer, as opposed to just focusing on one cancer. It's just so difficult to keep up with this literature, because everything's coming at you so quickly."

Dr. Sholl is the first to admit, "I live and breathe this stuff, but not everybody does."

Even Dr. Beasley says she relies on oncologists to keep her updated with news from ASCO and ESMO; she in turn will tell them about updates in the pathology literature that haven't yet trickled over to the oncology side, and she will ask whether they want to consider new testing. "It's a give and take." But oncologist-pathologist communication is critical to "make sure everything's covered for your lung cancer patients."

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