Lung guideline goals: more tests, treatment

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March 2018—Among the many never-ending chores that humans undertake—paying bills, filing taxes, flossing—writing medical guidelines can seem like an especially perpetual task. Just ask the architects of an updated document on molecular testing for lung cancer, issued by the CAP, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology.

How empowering to produce a hefty, 18-statement-strong work (plus three specific changes to the previous guideline), aimed at selecting patients for treatment with targeted tyrosine kinase inhibitors (Lindeman NI, Cagle PT, Aisner DL, et al. *Arch Pathol Lab Med.* Published online ahead of print Jan. 22, 2018. doi:10.5858/arpa.2017-0388-CP).

For lung cancer molecular biomarker testing, cell blocks were recommended over smear preparations in the 2013 guideline. The new guideline says any cytology sample with adequate cellularity and preservation can be tested. “We’re going to see significant patient benefit,” Dr. Dara Aisner (right) says of the change.

And how fatiguing to realize, even as the guideline neared completion, that compelling new research was already knocking at the door.

“One of the challenges in a systematic review process is that the information comes faster than you can establish the systematic review process,” says coauthor Dara Aisner, MD, PhD, associate professor, Department of Pathology, and director, Colorado Molecular Correlates Laboratory, University of Colorado School of Medicine, Aurora.

That’s not to say the guideline is deficient. Rather, the guideline reflects molecular testing in all its complexity and importance, says coauthor Philip Cagle, MD. “Just because something is newly described” doesn’t mean it’s earned

guideline status, no matter how promising. “This is intended to assist physicians, their patients, and their families in selecting the best and most up-to-date treatment with these inhibitors. We don’t want to endorse something that may not pan out,” says Dr. Cagle, director of pulmonary pathology and professor, Department of Pathology and Genomic Medicine, Houston Methodist Hospital, and professor of pathology and laboratory medicine, Weill Cornell Medical College, New York.

It also serves as a welcome update to the previous guideline, published in 2013 (and a massive undertaking in its own right). The latest iteration doesn’t so much change the tenor of discussions as it addresses advances and technical knowledge that have emerged in recent years, says lead author Neal Lindeman, MD.

But he already spies areas where updates are likely to be needed. There is no rest for the weary. As the Almighty told ancient followers stamping their feet impatiently at the edge of the Promised Land, arrival brings both a blessing and a curse.

Among the new arrivals in this latest guideline is *ROS1* testing, which joins *EGFR* and *ALK* as routine testing in lung adenocarcinoma cases. Testing for all three mutations must be performed on all patients, regardless of clinical characteristics.

“That’s probably the No. 1 change,” says Dr. Lindeman, associate professor of pathology, Harvard Medical School, and director, Center for Advanced Molecular Diagnostics, Brigham and Women’s Hospital.

A second highlight, he says, is the recognition that immunohistochemistry can be used as an alternative to FISH for testing *ALK*. The previous guideline recommended FISH; at the time, the *ALK* FISH break-apart assay was the only one supported by prospective studies.

Equally noteworthy is the guideline’s endorsement of multiplex-based approaches to testing. Though it’s not a requirement, the winds are noticeably shifting in that direction.

Dr. Cagle

“Over the last five years next-generation sequencing has emerged as a bona fide clinical laboratory technique,” Dr. Lindeman says. When the first guideline appeared, it was an investigational procedure, and the guideline’s authors (which included Drs. Lindeman and Cagle) recommended single-gene assays, done sequentially or in parallel. “But now we’re encouraging the use of next-generation sequencing panels, because it’s quicker, it spares the sample, and you’re able to get—if you design it the right way—all the alterations you need in one test.”

On a related note, the guideline writers also recommend a second set of genes that are considered “should-test” biomarkers—some refer to them as “nice-to-haves”—in terms of clinical utility: *MET, BRAF, ERBB2 (HER2), KRAS*, and *RET*. While these are not “must-test” biomarkers, they’re important in clinical care, Dr. Lindeman says, particularly when *EGFR, ALK*, and *ROS1* are negative and patients are being considered for an investigational agent.

The guideline covers another relatively new area: testing in the setting of acquired resistance. While the *EGFR* T790M mutation is hardly new knowledge, there was no treatment for it when the previous guideline came out. “There wasn’t a reason to test for it other than curiosity,” Dr. Lindeman says. Now, however, patients with *EGFR* mutations who regress after treatment with a targeted tyrosine kinase inhibitor will need T790M testing to see if

they qualify for third-generation targeted therapies, such as osimertinib.

Dr. Aisner suggests pathologists will be especially interested in the change that brings more clarity to testing cytopathology specimens. In 2013, the guideline urged that testing be done on cell blocks. Since then, Dr. Aisner says, strong evidence has emerged that says other cytopathology preparations, including smears, are just as good, if not better.

"This is a particularly big deal," says Dr. Aisner, noting that the combination of molecular testing and cytopathology specimens has been in evolution for some time, particularly in lung cancer. In the past few years, "It's become clear that it's not only feasible but very adoptable" to use non-cell block samples.

For many patients, the only diagnostic sampling comes from a cytopathology specimen, so this change should boost access to testing. "We're going to see significant patient benefit," Dr. Aisner says.

She notes, furthermore, that sites that already use cytology specimens are mostly academic molecular pathology labs. Since the bulk of specimen processing in molecular testing happens outside of these centers, she says, the hope is that more centers will be encouraged to do testing, now that they no longer have to rely on cell blocks.

Dr. Lindeman says physicians had been asking for this change. The previous guideline didn't forbid using cytology preparations, he says, "but some people interpreted it that way."

At her lab, says Dr. Aisner, she and her colleagues have used non-cell block preparations for years. "They're very effective. Consistent with other publications, I find the quality of the nucleic acids we get from them to be quite high, mostly because they haven't been subjected to formalin fixation and paraffin embedding."

The next step, she suggests, will be to figure out how to incorporate this change earlier in the testing process, with the goal of reducing turnaround times for molecular results. Why not, she asks, have cytopathologists send an additional smear to the molecular lab at the time of an on-site diagnostic procedure? Instead of delaying molecular testing until a cytopathology case is finalized, "this would be an active step." In many cases, she says, cytopathology material is often seen as an afterthought when the biopsy material appears to be of lesser quality. In such situations, absent any reflex procedures, no testing will occur without an oncologist's order. "We might be better with an active process that starts with the cytopathologist."

The guideline breaks new ground by placing genes into three testing categories: must, should, and investigational. Prior to this, genes were viewed as either necessary or investigational.

How big of an adjustment will this be?

"I worry about that confusing people," Dr. Lindeman says. "I worry about payers drawing one conclusion and practitioners drawing another."

For labs, adapting will largely hinge on the use of next-generation sequencing. Labs can do 1) a comprehensive panel with all the genes in the first two categories—*EGFR, ALK, ROS1, BRAF, MET, ERBB2 (HER2), KRAS*, and *RET*—or 2) targeted testing for those first three, followed by the others as appropriate.

Dr. Aisner says the guideline gives laboratories "a gentle nudge" toward panel-based testing. The benefits of next-generation sequencing are considerable. Not only can labs tackle the "musts" and the "shoulds" simultaneously, but they can do so using what is typically a very small tissue sample. "Seventy percent of lung cancers are going to present at late stage," Dr. Cagle says. "The only sample we're going to get will be a small biopsy or cytology specimen or both."

The guideline's readership is intended to be broad, however, and the authors recognize that not every lab has the same resources. "That was an issue for some of our colleagues in other countries," Dr. Cagle says. "So we backed

off from requiring next-gen sequencing, which we really do prefer if that's available." By having must-tests and should-tests, those without NGS can still sequentially test for the critical mutations.

The premise, Dr. Lindeman says, is that advanced lung cancer patients who don't have other options based on *EGFR, ALK,* or *ROS1* status should have access to an investigational therapy. Each has its own marker; individually, each marker is rare. "We wanted to give these patients an opportunity to be selected for these interventions, even though the evidence for them isn't as strong as for the other three alterations."

Beyond that, he says, "Our hope is that the field will progress toward panel sequencing because we think there are compelling reasons regardless," including efficiency, sparing patients biopsies, and quicker turnaround times. NGS also means genes can be added less expensively. "But we just aren't in the position to tell labs they have to move to next-gen sequencing because the evidence to say single-gene testing is inappropriate is just not there." *Yet*, one can almost see in the thought bubble above his head.

Cost and reimbursement are the wild cards here, and Dr. Lindeman makes no pretense of having a firm answer related to either one. But for labs that already offer a panel, it's reasonable to simply do that up front. For centers that do single genes, start with *EGFR, ALK,* and *ROS1*, he says, and then send out for the panel if the "musts" are negative.

KRAS might strike some as an interesting choice for the "should" category, given that no targeted therapy is currently available. It's not recommended as a standalone assay. The role of a positive *KRAS* here is to exclude the "should" gene testing for patients who are negative by sequential testing for *EGFR, ALK*, and *ROS1*, rather than to use the positive *KRAS* result to direct patients to a clinical trial.

The guideline also allows labs to use immunohistochemistry as a screening test for *ROS1*. IHC interpretation can be challenging, however; in up to a third of tumors with no underlying rearrangement, the authors note, expression can be seen in a patchy pattern, typically at weak intensity. "There is a false-positive problem with the *ROS* IHC," Dr. Lindeman says. "So we are recommending that even labs that do immunohistochemistry have a plan in place to confirm with FISH, even if it means sending it out at that point."

As already noted, IHC is recommended as an equivalent alternative to FISH for *ALK*. Treatment decisions can be based on clearly positive IHC results, the authors say, demonstrated "by strong granular cytoplasmic staining with/without membrane accentuation, or negative."

As labs consider adding the intermediate tier of molecular tests, they'll need to keep other technical challenges in mind as well.

RET FISH is tricky, says Dr. Lindeman, and labs that choose to do this testing will have to tread cautiously when it comes to identification and validation, given the narrow spacing between the split probe signals found in common fusion types. "The split is not as dramatic as what you see with some assays," he says.

In the relapse setting, assays for *EGFR* T790M need to be able to detect the mutations in as little as five percent of *EGFR* alleles. Labs that test patients who progress on EGFR inhibitors and plan to use the same method they used for the diagnosis will need to hit that lower threshold, says Dr. Lindeman, "which is about two-and-a-half percent allele fraction, or about fourfold more sensitive than what we're recommending for the initial test. So that's a technical challenge, too."

Circulating plasma cell-free DNA testing offers challenges of its own. Though evidence does not support its use for making a primary diagnosis, the guideline explores its use in identifying *EGFR* mutations when tissue samples can't be used for molecular testing. Labs can also use this method to identify *EGFR* T790M mutations in patients with progression or secondary clinical resistance to *EGFR*-targeted tyrosine kinase inhibitors.

Labs doing cell-free DNA testing, especially those that are less familiar with the process, need to understand that they'll have to process samples quickly, within a few hours. "It's not a sample that can sit around for a day or two," Dr. Lindeman cautions.

Some might find cell-free DNA discussions to be an unexpected presence in the guideline, given the breathless hype that has surrounded the field in general. "But we felt that there was data for cell-free DNA in specific applications in specific circumstances," Dr. Lindeman says.

He makes clear that the guideline does not advocate use of next-gen sequencing panels on cell-free DNA (an approach that's received plenty of attention of late). "We're only recommending that the initial workup of a tissue sample be replaced by cell-free DNA if a tissue sample can't be obtained"—and again, only for *EGFR*.

The bigger need, Dr. Lindeman continues, relates to patients who relapse, particularly when they do so with multiple lesions. In these cases, plasma would be a more viable option. This is reliable if the results are positive for *EGFR* single-gene analyte testing using cell-free DNA. But if results are negative, tissue needs to be obtained, since the test has a significant false-negative rate—sensitivity is only about 70 percent.

If cell-free DNA puts in a surprising appearance in this guideline, *BRAF* might be considered a surprising absence in the must-test category.

"There was a lot of discussion about this," Dr. Lindeman says. Typical for a guideline, timing was everything. In June 2017, the FDA approved a combination of two TKIs to treat *BRAF* V600E-positive nonsquamous non-small cell lung cancer. At the same time, the agency approved a companion diagnostic.

He worries that not requiring *BRAF* mutation testing in the guideline will look silly, and he anticipates pathologists will be asked by colleagues about this gap. In fact, he says, during the comment period, the authors received numerous questions about the *BRAF* mutations, all of them from thoracic oncologists.

The guideline addresses the issue directly, calling it "the most controversial of all the recommendations of the working panel." The authors acknowledge that sufficient evidence will emerge and that they expect *BRAF* testing to become standard in the future. But in the meantime, there's good evidence only from single-arm studies. Says Dr. Lindeman, "It would be great if there were a prospective controlled study that we could compare it to. The response rates looked great. But we didn't have a comparison cohort to compare it to, other than historical."

Guidelines that rely on published evidence and formal, systematic reviews are not a good fit with the news cycle, essentially—Twitter is one source of information, a quarterly publication another. Guidelines cannot be a wheel of perpetual motion. "At a certain point," Dr. Lindeman says, "we just had to say, 'We're going to stop here and publish where we are.' We didn't want to make this a massive opinion exercise." Opinions have their advantages, certainly—speedy deliverance being one of them—"but I don't think that's the right way to do it."

Dr. Cagle is already anticipating the next wave of questions. "I expect we'll hear about it from the public," he says with a laugh. He notes, however, that the matter becomes moot for labs that do NGS.

"We acknowledged there's a large number of other biomarkers that are under investigation," Dr. Cagle continues. "Some of these will probably pan out, and maybe some will not. We just don't know at this time, and there's not enough evidence to make any recommendation."

The expanded use of immunotherapies to treat advanced NSCLC also created dilemmas for the authors. As they note, patients with high levels of PD-L1 expression and the absence of sensitizing *EGFR* mutations or *ALK* rearrangements can be treated by immunomodulatory therapies as a first-line treatment. So why not recommend PD-L1 testing?

As it turns out, PD-L1 follows the *BRAF* trail to some extent, though the off-roading starts sooner.

For starters, the guideline is built around genomics, and PD-L1 is essentially an immunohistochemistry matter, albeit with genomic elements. “We had many philosophical discussions about the meaning of molecular alterations,” Dr. Lindeman recalls.

As the group dug in deeper, says Dr. Aisner, “I was surprised by how complicated the issues were.”

In the end, the group realized they could make no useful recommendation. Dr. Lindeman reports that “the literature was all over the place” with respect to markers, antibodies, and DNA methods. A separate guideline devoted to PD-L1 will address the topic in depth.

Dr. Lindeman says researchers are only starting to tell the PD-L1 story. “It’s going to be more complex than simply PD-1 and PD-L1 interaction,” he predicts, and will include inhibitors to other signaling molecules involved in immunologic tolerance, as well as figuring out the best mechanism to predict response to them. “Is it really going to be immunohistochemical, or will there be a DNA- or RNA-based solution that works better in the end?” he asks. What’s most clear right now, he adds, is that the field will not lie dormant.

It’s also a safe bet that when the PD-L1 guideline is written, it, too, will have a tangled relationship with promising research, and, like a Liz Taylor marriage, one guideline will not suffice.

Dr. Cagle explains it this way: “We want as many patients as possible to have the chance at therapy. Having said that, we don’t want to endorse something that seems promising at the present time, but a year from now turns out not to be.”

The *ROS1* story is a good example. When the 2013 guideline was written, it was already well known that patients with a ROS1 mutation might respond to crizotinib (which was already approved for patients with *ALK* mutations). Oncologists were ordering *ROS1* testing when *EGFR* and *ALK* results were negative, and using the drug off-label when *ROS1* results were positive. The evidence was based on case reports and small studies, however, and the guideline did not recommend routine *ROS1* testing. The evidence is now sufficient to support a recommendation in the new guideline, a path Dr. Cagle suspects other mutations will follow in future revisions.

“We have to remember that practice is both ahead of the evidence, but also behind it,” he says. “Some laboratories do not have next-gen sequencing, which makes all of this easier. So the guideline is ahead of the curve and behind the curve. What we’re shooting for is the best evidence.”

But the definition of “best evidence” is not as stable as it once was. As the guideline notes, the concepts of precision oncology, with its focus on the individual patient, must be balanced with reliance on large interventional studies.

In the past, says Dr. Cagle, “We’ve been very strict as to how we grade evidence in the literature. But we don’t want to prevent anyone from getting their chance to get treated. And we may have more and more of these situations where a mutation is rare enough that you’re only going to have individual patients for small series receiving the drug.”

“There aren’t rules for this. How much evidence do you need before you say there’s enough?” Dr. Lindeman asks. “There’s not a formula you can plug in.” When targets are applied to one or two percent of lung cancer patients, for example, there simply aren’t that many people. “A study of 50 folks might be as much as you’re going to get. Is that compelling enough to make an international practice guideline? The field has to face this.”

As the evidence for large-scale trials is less likely to be accumulated, there will be many alterations that are supported by small studies. “But when they work, they work,” Dr. Lindeman says. “And for patients who have these alterations, they deserve the treatment that will be effective.” To make that happen, while avoiding useless treatment, “The only way to sort that out is to test for them.”

And, eventually, to write another guideline.

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