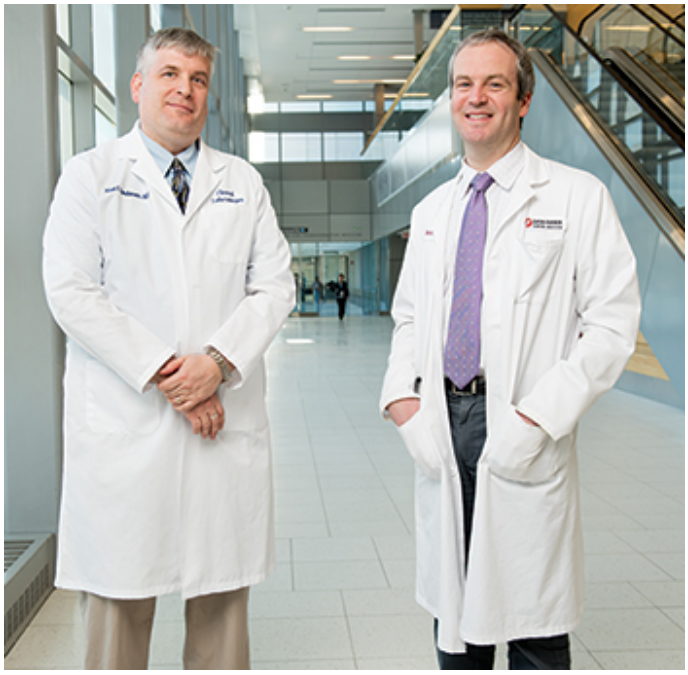
In cancer sequencing, a new lingua franca

Karen Titus

February 2017—NGS has taken its NBS, or next big step: a newly published joint consensus guideline on how to interpret and report sequence variants in cancer (Li MM, et al. *J Mol Diagn.* 2017;19[1]:4–23). With these 20 pages of best practices for making next-generation sequencing a regular part of cancer diagnostics, the field is moving, essentially, from frontier town to gated community.



Dr. Neal Lindeman (left) with Eliezer Van Allen, MD, of the Department of Medical Oncology, Dana-Farber Cancer Institute, and Department of Medicine, Brigham and Women's Hospital. The consensus reached by the CAP, AMP, and ASCO is aimed at "any laboratory that's involved in performing multiplex genetic analysis for cancer samples and cancer patients," Dr. Lindeman says.

Broadly speaking, it's part of the evolutionary process in diagnostic methods. "We've seen this in the molecular field—with BCR-ABL, for instance, a long time ago," says Neal Lindeman, MD, a guideline author and associate professor of pathology, Harvard Medical School, and director of molecular diagnostics, Brigham and Women's Hospital, Boston. For many years, he recalls, different laboratories used different housekeeping genes before a standardized approach emerged. This sort of creative license was long common in coagulation testing as well, he says. "And you see it still with some immunoassays in clinical chemistry."

The time is ripe to develop concrete guidance for NGS, says lead author Marilyn Li, MD, who chaired the working group. (The guideline was put together by representatives from the Association for Molecular Pathology, the American Society of Clinical Oncology, and the CAP.) As NGS has become more widespread, and as each laboratory makes in-house adjustments, the landscape has come to resemble the city of Babel, post-Tower, with different labs using different words to describe what they're doing. "That makes communication difficult not only among pathologists and oncologists, but even within the same group," she says. "You need a common language."

Just as critical, adds Dr. Lindeman, is that the language needs to be understood. “Standard nomenclature is very hard to read if you’re not an expert in the field,” says Dr. Lindeman, whose particular guideline assignment, so to speak, was overseeing the section on reporting. “My overriding principle, which is a little hard to argue with—although sometimes people do—is that the most important thing about a report is that it be easy to read and understand.”

“Sometimes in pathology, we communicate to each other,” he continues. “And we need to keep in mind that these reports are going out to our colleagues in medical and surgical oncology, and often cancer patients.” The audience isn’t looking for Shakespeare, in other words. Arthur Miller will work just fine.

Dr. Lindeman even offers a tongue-twister of his own to get the point across: The reports need to be “interpretable in the vernacular.”

“You can’t just put all of them (variants) in a report and let the physicians figure out the significance,” says Marilyn Li, MD.

The only guideline available prior to this was the American College of Medical Genetics and Genomics/AMP guideline for germline variant interpretation. As the authors began their work, they conducted two surveys, one to see how labs are currently interpreting results (67 responses) and one to see how they are reporting variants (44 responses).

Those results, as well as a review of the literature, various databases, and the existing germline document, made it clear: There was a real need for a somatic variant guideline. “Not only because we’re looking at different diseases, but also there are different focuses for each,” says Dr. Li, who is a professor of pathology and laboratory medicine, and a professor of pediatrics, Perelman School of Medicine, University of Pennsylvania.

The numbers can explode rapidly. Germline testing usually looks at one mutation, which might be heterozygous or homozygous. Cancer, of course, requires looking at multiple genes with multiple mutations. Add in multiple levels, and different types of variants—point mutations, copy number changes, structural changes that can lead to fusion genes, etc.—as well as two genomes (tumor and normal) and multiple tumor clones, and it should be evident why the working group thought a new guideline was in order.

If there was any lingering doubt, Dr. Li says it was banished from her mind when the guideline was presented at the Association for Molecular Pathology meeting last November, shortly before its publication. “It was on a Saturday. And early in the morning, at 7:00,” she recalls. The room, with a capacity of 600-plus people, was packed to overflowing.

The survey results gave the participants pause, she says, even if there were no real surprises, and launched animated discussions. “To me—and this was a no-brainer—if you find a relevant germline variant, you report that. But others say, ‘We’re doing cancer testing. Why should we worry about germline?’ So those were some of the issues we encountered, even while creating the guideline,” says Dr. Li, who is also director of cancer genomic diagnostics and vice chief, Division of Genomic Diagnostics, Children’s Hospital of Philadelphia.

Another notable finding, she says, was that some laboratories use a one percent cutoff for population databases, while other labs use much higher or lower percentages, with each generating a different set of variants. Laboratories also use different testing cutoffs, ranging from five to 10 percent, or sometimes even higher.

The reporting tiers resembled another variety show. Some laboratories opt for the simplified, two-tiered, “mutation or benign” approach, says Dr. Li. Other labs use three, four, or five. “It’s all over the place.” Some laboratories even provide subtiers.

Taking this all in, Dr. Lindeman says, “It seemed like every center had its own language, almost, in terms of what different variants mean in different contexts.”

Though all these differences point to the need for more standardization, Dr. Li is careful to note that the guideline does leave room for laboratory directors to use their own discretion and professional judgment. In somatic testing and for most tumors, for example, her laboratory’s variant allele frequency (mutation fraction) cutoff is five percent. But in cases where testing is done to follow residual disease, laboratories may want to set a lower cutoff rate, such as one percent or lower. For those that do that, she adds, “Then you have to validate your test accordingly. You have to sequence much deeper and may need additional strategies to eliminate false-positives and make sure you can rely on it to detect variants at that level.”

Once it knew the lay of the land, the group got to work.

The highlight of the guideline, for Dr. Li, is the tier-based categorization and reporting system. “We feel this will allow us to weigh all available evidence,” she says.

The guideline stratifies the variants into four levels based on clinical significance:

- Tier I: variants with strong clinical evidence for response or resistance to therapies that are FDA approved or included in professional guidelines for specific tumor types (e.g. *BRAF V600E* predicts response to vemurafenib in melanoma).
- Tier II: variants that are potentially clinically significant, but which may not yet be included in professional guidelines, or where an FDA-approved drug exists, but for a different tumor type. One example is the JAK inhibitor ruxolitinib, FDA approved for treating myelofibrosis, and which has shown potential in treating patients with acute lymphoblastic leukemia and mutations that activate the JAK-STAT pathway.
- Tier III: variants of unknown significance. This can include somatic variants in genes reported in the same or different cancer types with unknown clinical significance, and variants that have not been reported in any cancers. These variants should not have been seen in the general

population with a significant allele frequency.

- Tier IV: variants that are benign or likely benign. Laboratories are not encouraged to report these mutations, says Dr. Li, unless requested by the referring physician.

The guideline suggests that laboratories report variants in descending order of importance—start with DEFCON 1, in other words. That way, says Dr. Li, physicians will know quickly what's important and what they need to do next. It is, in some ways, a guided tour of variants. Especially for labs that do large panels, "You can't just put all of them in a report and let the physicians figure out the significance," Dr. Li says.

The working group found it wasn't unusual for laboratories to report a mutation followed by extensive information about the FDA-approved drug(s) and clinical trials. "A lot of physicians found this not useful or even misleading," Dr. Li says, adding that FDA-approved therapies or clinical trials described in the report should be carefully matched with patients' genomic alterations and tumor types based on available clinical evidence. Since treatment or other patient management decisions are based on many pieces of medical information beyond genetic alterations, the report should not recommend specific therapies or clinical trials, she says, although general statements about availability of relevant trials or citing results of published trials is acceptable.

Deciding on the optimal number of tiers was the most time-consuming part of developing the guideline, says Dr. Lindeman. That may not come as a surprise to pathologists, but, he says, the oncologists in the working group may have been puzzled by the outsized effort. "They have two categories," Dr. Lindeman explains: 1) treat. 2) don't.

His laboratory is among those that have been using a five-tier system. "Some of my colleagues are a little grumpy that we're going to change some things," he says. "But I think they're the right changes to make."

That will mean deciding whether to reclassify each of the 16,000 cases that have been signed out to date, a daunting task that seems better suited to clerks in a Dickens novel toiling away at the Inns of Chancery. "I really don't want to do it," Dr. Lindeman admits. "But then it will throw our database off for scientific inquiry if we don't. So we're going to have to figure out a solution."

The guideline also calls on laboratories to report significant negatives. In cancer sequencing, these can function like the absent character in a certain Beckett play—Godot never shows up, but he manages to drive the play's action (such as it is) anyway.

Dr. Lindeman says he was acutely aware of this need as he oversaw this section of the guideline. "One of the things you see a lot in this field are reports that list all the mutations that were discovered. And the reader—an oncologist, surgeon, patient—is left to conclude that if something is not mentioned, it must not be there."

There is a third possibility, he continues: The assay didn't test that region, either through design or because that particular region didn't work well in the assay run. So when an alteration is not found, he says, there's a need for the report to make "some qualification that the assay was capable of detecting things in that area, and that it's truly missing."

The report should also make clear the frequency of sequences, or variant allele fraction, since it can have considerable impact on clinical care, he says.

With its emphasis on language, the guideline attempts to return genomics to a pre-Tower Babel. "Basically," Dr. Li says, "this guideline provides a common language among pathologists, oncologists, and other health care providers." Speaking in one tongue will help ensure patients benefit directly from genomic profiling. If test results are conveyed in ways others "can't understand, then it doesn't do the patient any good."

The guideline also makes a point of telling laboratories to use "colloquial nomenclature" in their reports.

One example is the use of the cancer gene formerly known as *MLL*, or mixed-lineage leukemia, now called *KMT2A*. Since other publications—including many older ones—use the original name, the guideline recommends using the standard name but also suggests noting the previous name, "so there will be no confusion," says Dr. Li.

Her own laboratory does this. "We even do it at the exon level," she says, citing the example of NPM1, where the prognosis is good in AML patients with an exon 11 (formerly exon 12) insertion mutation. She and her colleagues report the current name but parenthetically note the old name, "so people will know it's the same thing."

Even the best guideline will fall short unless pathologists and oncologists talk about what's in it, says Dr. Li.

It's not unusual, she says, for oncologists to request NGS without specifying which genes they're most interested in. "When laboratories look at a result, we don't want to be biased to a specific mutation, but we do check a patient's history." When there are conflicts between sequencing results and a pathology diagnosis, or with what the oncologist might be looking for, dialogue is the remedy. "When everything is typical, that's easy. When it's atypical, that's when we need multidisciplinary discussion." She recalls the example of a patient with an unusually tricky diagnosis; NGS eventually identified a fusion gene that led to a different diagnosis and treatment plan. The patient continues to do well, she says, but that success hinged on continual conversations between the geneticist, pathologist, and oncologist.

At Children's Hospital of Philadelphia, Dr. Li notes, the genomic data is considerable. All patients with cancer undergo genomic profiling at diagnosis or at relapse, including mutation and point mutation, copy number changes, and fusions. The comprehensive report isn't simply filed away into the medical record. The laboratory professionals meet weekly and monthly to discuss results with pathologists, oncologists, and radiologists. The regular meetings have an educational purpose as well as a clinical one. "Even for our oncologists, they don't always go to the lab and don't always know exactly what we do," she says.

The meetings also reinforce an important notion for pathologists: The information they provide, while critical, is only one part of patient care, Dr. Li says. "We may not know about a patient's cardiac or GI condition, which may influence treatment."

Though the four-tier system anchors the guideline, it's prefaced by nearly four pages of information about databases.

As Dr. Li puts it, "If you don't understand those databases, you may misuse them." Pathologists need to understand how they're aggregated and their limitations, to avoid overinterpretation of annotation variants as well as to avoid missing rare but important somatic variants.

If laboratories aren't using databases, Dr. Lindeman says, "they're tying one hand behind their back, if not both." (Insert joke about Irish step-dancing here.) The heavy emphasis early in the guideline was not an accident. "It may be that we hit people over the head a little bit, because we think it's really important," he says.

The reasons are obvious to Dr. Lindeman. At Brigham and Women's, "we would be in trouble if we didn't have our database of alterations and interpretations," he says. The database helps him and his colleagues learn from each case and apply that knowledge to subsequent cases. Ditto for other databases that share knowledge more broadly. His institution, he says, is large by academic standards. With their 16,000 samples, "We can rely on what we've seen before.

"But," he asks, "if you're a smaller center, and maybe you've sequenced only a couple hundred, where do you get that breadth of experience to know what you're looking at?" For any lab that has the personnel and resources to build its own database, "by all means do," says Dr. Lindeman. But even then, "I think it would be a good idea to tap into what's out there anyway."

The guideline also acknowledges the brisk dynamics of NGS. Whatever experts know now is likely to change and be crowded by new, sometimes conflicting, and often messy updates. Those who try to keep up may feel like

they’re swiping through medicine’s version of a Tinder account.

The guideline makes the sensible recommendation that laboratories figure out how to continually evaluate variants—then concedes this is a “monumental task.” Even as NGS becomes more affordable, and spreads to smaller labs, the upkeep, so to speak, might be difficult—like buying a Porsche, then being done in by the oil changes and other routine maintenance.

Smaller laboratories should consider reaching out to others with more experience, Dr. Li suggests. They should also participate in somatic variant NGS proficiency testing programs, as well as in ongoing QI and QC.

Dr. Lindeman offers observations in lieu of fail-safe answers. “It’s hard to keep up,” he says, conceding that even his own relatively large practice works hard to do its best. “We keep each other on our toes, and we go to meetings and read.” Perhaps an academic educational listserv would work, he muses, or a shared database. “But I don’t have an easy answer.” There simply may not be one, not with 20,000 genes. “People say, ‘Well, which ones are important?’ Well, every one is important,” he says. “They wouldn’t be there if they weren’t.”

Apostolia-Maria Tsimberidou, MD, PhD, a guideline coauthor and professor, Department of Investigational Cancer Therapeutics, University of Texas MD Anderson Cancer Center, Houston (where she also pioneered the personalized medicine department, in 2007), says she’s hopeful the guideline will help tame the data she and her fellow oncologists receive.

Dr. Tsimberidou

“There are many companies that do this testing, many institutions, many laboratories,” with vast differences in how results are interpreted and reported, says Dr. Tsimberidou, who is the current chair of ASCO’s clinical research committee.

For oncologists, the next step after receiving sequencing results is to ask about clinical implications. That question is as natural and inevitable as drawing one’s next breath. It’s also “the most crucial one,” she says, “because patients’ clinical outcomes depend on the efficacy of the treatments they receive using the sequencing results.”

Sometimes the next step is clear, but not always. The majority of molecular abnormalities that turn up in sequencing are not “targetable,” she notes. In practice, there’s a gap between identification of the driver molecular alteration(s) and available drugs that inhibit the function of these alterations. Nonetheless, she says, “Some investigators propose that even if preclinical data exist, we should match the molecular abnormality with a drug because on the package insert, preclinical data demonstrate that targeted agents inhibit certain alterations.” While this might seem reasonable in theory, she says, “we cannot extrapolate that this information is relevant in humans, unless clinical trials in patients with cancer have demonstrated that a targeted agent has antitumor activity.”

Experience has taught Dr. Tsimberidou that dictums are unhelpful, or worse. Every patient’s tumor has unique characteristics requiring treatment based on the personalized medicine approach. “What oncologists need in the report is an accurate description of the molecular abnormalities and their function.” When pathologists indicate treatment, however, that can add complexity, says Dr. Tsimberidou, who shares reports with her patients. Some reports list a recommended treatment based on an animal study or limited information about a patient’s tumor. In other reports, the recommended treatments are contraindicated based on other clinical information or coexisting

molecular alterations.

“We’ve been asked not to be too prescriptive,” Dr. Lindeman acknowledges. He’s seen it in others’ reports, where laboratories recommend a particular drug. “Sometimes patients don’t qualify for that drug,” he says. He cites the case of a report, from another lab, that recommended a treatment that was contraindicated because the patient was diabetic. “So now my colleague in oncology had to spend a considerable amount of time explaining to the patient that even though the report said they should get the drug, they can’t.”

On the other hand, it’s useful when pathologists disclose test limitations up front. “Then we can exercise our own personal experience about the clinical significance of the findings and discuss results with the lab,” Dr. Tsimberidou says.

Adds Dr. Lindeman, “I haven’t encountered too many oncologists who don’t want to know when something isn’t working.” But keep it brief. “We’re writing this report for people who are very busy and don’t have a lot of time. They would like to have a very quick and simple explanation.” For those who find themselves writing three pages, know that it’s going to be a wasted effort, he says, like turning out the lights in a burning building.

Laboratories might consider trying an old newspaper technique, using so-called inverted pyramid style to cram the key details on top. “The first page should convey everything the physician needs to know in order to treat,” says Dr. Lindeman. “If they have to go to page two for that, it’s a problem.” That doesn’t mean there can’t be a page two, but use it for noncritical information, such as additional technical details and regulatory requirements.

With an oncologist’s preference for action, Dr. Tsimberidou speaks in the same breath about the strengths of the guideline and the next steps she’d like to see taken.

“Molecular abnormalities are crucial,” she says. “But oncologists also have to take into account other mechanisms of carcinogenesis, including immune markers, epigenetics, and proteomics.”

The guideline covers none of these, of course. “So we look to the four tiers we now have,” she continues, “and we ask, what is the level of evidence for acting on a mutation?” But even with the clearly defined tiers of evidence, “We still have much to sort through. Variants of unknown significance are informative but perplexing,” she says. If such a variant is the only molecular alteration available, patients are eligible for clinical trials with targeted agents; there are no other available treatments. “We discuss these data with the patient, and we may offer experimental treatment.”

“Looking at one molecular abnormality alone is, I think, inadequate,” she adds. The guideline drives home a related point, however. “We need to prospectively collect these data, assess what is clinically significant and what is not, and report them,” particularly to improve treatment in such subsets of patients.

Continuing to look ahead, Dr. Tsimberidou sees the guideline as a snapshot of sorts, a picture of what to do now while developing a long-term plan. “There are so many other challenges in the implementation of personalized medicine,” she says. She points to a reported case of a patient with multiple myeloma who had a subclone that was not monitored and was not eliminated with treatment; the subclone, it turned out, expanded, causing disease progression, and the patient died. “It’s important to have sequential molecular profiling,” she says. Likewise, she adds, it’s important to consider how molecular abnormalities can differ between original tumor and metastatic sites.

Dr. Tsimberidou recognizes the costs involved in doing additional sequencing. And she understands that for this to happen, cell-free DNA testing will have to advance.

Even then, oncologists will be limited by the portfolio of drugs available and their costs. And when the appropriate drug is available, she says, oftentimes its use is delayed for months while she negotiates approval with insurers. It’s a sobering ritual. “You hope the patient, who has no other treatment options, will still be alive to receive it.”

She pauses for a breath. There’s so much more to say. But the guideline, she says, “Is a step forward.”
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