NGS in more labs? IFCC group aims to ease the way

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May 2021—When it comes to next-generation sequencing, don't count out community hospital labs, especially as black-box solutions come on the market.

That's the hope of members of an International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) working group that aims to help clinical labs develop in-house NGS programs.

Large-scale genomic testing won't be necessary or practical at the community hospital level. But hospital-based genomic testing programs should set out to meet the NCCN guideline targets and provide testing for which a wide range of sample input and quality can be accepted, says Robyn Sussman, PhD, a member of the IFCC working group and molecular development assistant director, Penn Precision and Computational Diagnostics, University of Pennsylvania Perelman School of Medicine.

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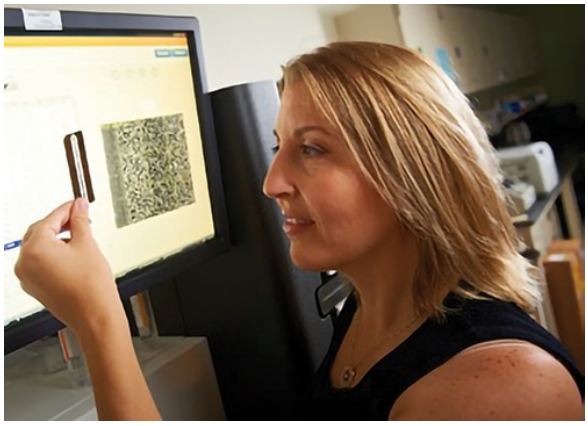
NGS can be costly, as can targeted therapies. But it's upfront spending to find the patients with targetable variants, and that's going to save money later, says Jennifer Morrissette, PhD, working group chair and clinical director, Penn Center for Personalized Diagnostics, and associate professor of clinical pathology and laboratory medicine, University of Pennsylvania Perelman School of Medicine. "When you think about a patient who has an advanced cancer, even though NGS testing may appear to be expensive when compared with other laboratory tests, and reaping the benefits of targeted therapy will potentially cost more, it's also likely going to decrease hospitalizations, ICU stays, and other expensive treatments that may be less efficacious."

"NGS is not necessarily one thing," Dr. Sussman says. "It's like a bowl of different fruit. There are expensive, exotic tests that tertiary care centers may bring on." But there also are solutions less costly to bring on upfront, and less expensive to run long term. "There are simple things," she adds, "that everybody can do."

Education is one of the goals of the IFCC working group, which came together in 2019. The group's first order of business was to conduct a survey of laboratories' current testing strategies, needs, and barriers, Dr. Sussman says.

The survey's first iteration, distributed to laboratories through the IFCC and its subsidiaries, had an unusual response rate, Dr. Morrissette says. "It was mostly Eastern European and a few European laboratories that responded," suggesting a distribution problem. The group is working now on a second version of the survey, and a Spanish translation, and with the IFCC subsidiaries to distribute it to their laboratories. In the United States that is the AACC.



Dr. Jennifer Morrissette, IFCC working group chair, says the goal of the workshops the group is seeking to run in partnership with sequencing companies is to demystify clinical genomics.

“We’re trying to figure out how we can best help people,” she says, “and without making that connection we are working a little blind.”

The working group also is seeking to partner with sequencing companies to run workshops at IFCC-sponsored meetings to demonstrate how to perform rapid genomic testing and provide hands-on experience. The goal, Dr. Morrissette says, is to demystify clinical genomics. The group is also developing an educational website with practical technical information and lectures. “It doesn’t all have to be NGS based,” Dr. Sussman says. “There are easier strategies to get answers.”

“Some point-of-care tests will be simpler to implement than something that requires multiple instruments and a pre- and post-phase, but the rules for validation are the same. So the education component is critical because if you have a single person at a community hospital interested in bringing on genomic point-of-care testing,” they may feel overwhelmed by the technical minutiae. “‘What samples do I source to run, how many runs do I need to do, and when can I decide that this is clinically validated?’ That’s where the educational materials we’re hoping to put out will come in handy.”

Know-how is just one of the requirements. Another is instrumentation with the servers and informatics built in, Dr. Sussman says. For example, at the Center for Personalized Diagnostics, she and colleagues have developed multiple custom panels using Illumina sequencers with bioinformatic pipelines that are specific to those panels developed in-house. “Community hospitals are not going to be able to hire an informatician,” she says. “They may have restrictions on using cloud-based informatics pipelines” to which they can upload data. And “the lower the threshold for technical requirements the better, on the wet bench side.”

It won’t be long before such solutions head to the FDA, Dr. Sussman says. “You can buy a paired sequencer and server that is FDA cleared, and then you have an easy pathway for reimbursement, and you only have to go through the verification process of the assay,” not a full validation, she says. “So you also save money in the development phase.” And progress has been made with reimbursement, she says. “So much of the country is now under MolDx that I think much of the homework has been done. It’s not perfect. It’s certainly still a headache, but nobody has to start from scratch, and many community hospitals probably can be paired with somebody within their Medicare administrative contractor to learn how to get the reimbursement going.”

Non-NGS genomic solutions also can be considered for some applications, though they’re not comprehensive, Dr. Sussman says, citing as examples the Cepheid and Idylla platforms. “You get some answers, and you don’t have to put a lot of work in.” Cepheid’s platform is already used in low- and middle-income countries for infectious disease testing. “So it would be using a similar platform for oncology,” Dr. Morrissette says. Idylla can provide rapid results for specific genomic types of abnormalities, she says, though it may be more cost-prohibitive in those countries.

“But it may be a great solution for community hospitals in the United States and Canada because the development for some of these assays is not as difficult as it would be for large-scale genomic testing.”

Thermo Fisher Scientific’s Ion Torrent Genexus System is geared toward meeting the NGS testing needs of laboratories of all sizes, including those without NGS experience. It requires minimal amounts of tissue; has an automated workflow that includes nucleic acid purification, sequencing, and reporting; and delivers results in a single day—the same speed as PCR. The platform is “potentially hands-off,” Dr. Sussman says. “You just need a molecular technologist; you don’t need a dedicated R&D staff to work up the validation.”

“The Genexus is trying to straddle both worlds,” Dr. Morrissette says. “It’s a decent-sized panel that will detect copy number changes and fusions, in addition to the bread and butter of single nucleotide variants and indels.” The Genexus Oncomine Precision Assay, a pan-cancer panel, covers 50 genes and 78 variants and is compatible with formalin-fixed, paraffin-embedded tissue and liquid biopsy samples. Thermo Fisher is in the process of submitting the platform to the FDA as an in vitro diagnostic product and intends to submit the Oncomine assay for premarket approval, says Andy Felton, vice president, clinical next-generation sequencing, Thermo Fisher.

Most black-box solutions, Dr. Sussman says, aren’t suited to tertiary care and academic medical centers. “We want to look under the hood and understand exactly what’s happening. Our demands may be too great.” But they are considering the Genexus for their lab because it may prove to be a solution for a stalled development effort on a ctDNA assay to guide rapid therapy selection for lung tumors.

Penn Precision and Computational Diagnostics has a clinician request process for tests not currently offered, Dr. Sussman says. “Sometimes we’ll say, ‘Yes, but it’s going to take us two years.’” The rapid ctDNA assay was one such request. The development team began several years ago by evaluating a variety of available platforms for ctDNA analysis, she says. “It seemed at the time that having a very low advertised sensitivity was important. I think it’s safe to say now that some of the advertised sensitivities for the sequencing platforms probably don’t hold up,” based on the experiences of other labs. “So that plus cost and reliability got us to focus on the Bio-Rad ddPCR platform.”

The team designed an assay to detect exon 19 deletions and T790M and L858R mutations in *EGFR*, as well as agnostic assays for *BRAF* V600 and *KRAS* G12. “But by the time we were ready to begin thinking about moving this into the clinical lab for validation, it became clear that for lung cancer we were missing some targets”—*EGFR* exon 20 insertions and C797S, *KRAS* G13X, *ALK* and *ROS1* fusions and resistance mutations, and *RET* and *NTRK* 1/2/3 fusions. “So we had to go back to the drawing board,” she says.

“The target moved during the time we were working on the assay, unfortunately. But that happens.”

Reimbursement is another issue. “If we want to perform NGS on the tissue, we can’t be reimbursed for the same genes being tested on ctDNA,” Dr. Sussman says. To build an affordable assay, the team determined, it would have to run on shared, dual-use equipment. “Because the hospital will not purchase something specifically for a use that will not be reimbursed.”

This is where they see a role for the Genexus. “It can have a dual-use purpose,” Dr. Morrissette says. First, Thermo Fisher is seeking FDA approval for a tissue and cfTNA assay for the instrument. “They advertise that it requires 20 ng of circulating cell-free total nucleic acid,” Dr. Sussman says, “and that’s so it can detect fusions off of the RNA and DNA hotspots.” Second, with the Genexus they may be able to provide results critical to treatment decision-making faster than is possible with the center’s large-scale panel.

“For example, you’ve got a patient with AML, and the question is, do they have a handful of mutations that will alter how clinicians would perform induction chemotherapy,” Dr. Morrissette says. Newly diagnosed AML patients currently receive *FLT3* testing, cytogenetics, and FISH, “but they don’t get the broad panel before that three-day ‘I have to start chemotherapy’ window. With the Genexus, you would be able to give a broader but not complete picture of what is in that cancer.”

Dr. Sussman says ctDNA sequencing “seems like the next frontier.”

“But we’re still right at the cusp of it becoming clinically useful. It’s clinically actionable now, but the reimbursement landscape is difficult.” And the cell-free DNA assays are “in many cases not a replacement for tissue testing,” which may make investing in the capital equipment a tough sell, she says.

Still, Dr. Morrissette is optimistic. “There are situations where we would like broad enough testing to be able to identify all of the immediately actionable targets. And at the same time if we have ctDNA for those patients with smaller or no biopsies, or the tissue is necrotic, we would be able to salvage those patient [samples], as well as be able to follow all of our solid tumor patients—or at least a subset of our solid tumor patients—through the course of their disease.”

“I’ve always said my goal is to micromanage the genomics of all of our cancer patients,” she says. “I would love to know everything that’s going on with their cancers. And this would bring us one step closer to that.”

The IFCC working group members hope to bring some of the benefits of in-house NGS testing to more labs, one of which is greater ease in managing the specimen, Dr. Morrissette says. “Pathology departments know more about the patient than an outside laboratory. The laboratory, pathologists, and clinicians can determine the appropriate scope of testing—which are the ideal tissues for testing and which testing will be most meaningful for patient care.”

For example, a Penn thoracic oncologist called Dr. Morrissette about *MET* amplification as a resistance mechanism for *EGFR*-targeted therapy. “There are papers out now that say about a third of *EGFR* resistance is secondary to *MET* amplification,” she says. “So he asked whether I thought NGS was the ideal way to identify *MET* amplification. I explained that although you can see copy number changes for *MET* by NGS,” FISH is the better modality for determining if there is low-level copy number amplification in a subset of cells. With FISH, she told him, “you can analyze on a cell-by-cell basis and potentially identify small clusters of resistance within the sample.”

While send-out testing is a good option in some cases, Dr. Sussman says, reference laboratories often have stringent sample requirements. “If that is your only option, you may end up with a large number of patients who are unable to get any type of molecular testing.”

Dr. Sussman

The Penn Center for Personalized Diagnostics currently runs a 152-gene solid tumor panel, a 116-gene hematological malignancies panel, and a 55-gene RNA fusion transcript panel. But it also runs small focused panels, Dr. Morrissette says, that can accommodate low input and poor-quality samples. “And a lot of the samples we get are poor quality. It’s not because they are mishandled; it’s just that some cancers are really necrotic. And some sampling methods can access only very small tissue samples.”

On-site NGS can also increase testing rates for actionable biomarkers in patients with diseases like non-small cell lung cancer. “One of the reasons is because it’s easy to incorporate genomic testing into clinical algorithms,” Dr. Morrissette says.

“Having laboratory expertise in-house to consult with the oncologist can be important,” Dr. Sussman says, including to help clinicians interpret reference lab reports. “We’ve seen misinterpretation with outside lab reports and with reports in our labs, and we have taken the approach of trying to use that as a quality improvement opportunity to make our reports as easily interpretable as possible for the clinicians.”

Ideally, finding a targetable biomarker should yield a discrete result in the EMR that triggers an alert for clinicians. “But that’s difficult to design in the EMR,” Dr. Morrissette says, “because you are sequencing many, many genes, and even within a gene you can have different variants with different actionability.” For example, “If you find a *BRAF* V600E in a melanoma, that’s directly actionable. But if you find a *BRAF* V600E in AML, they’re not going to put the patient on a clinical trial because they’re going for curative intent. So you can have different interpretations of the same variant depending on the clinical context,” which makes it tricky to design a straightforward alert.

In some cases in which ctDNA was sent out in parallel with Penn’s own internal tissue-based assay, ctDNA results were negative and the results on the internal assay were positive. “We’ve found multiple cases in which *EML4-ALK* gene rearrangements and *EGFR* resistance mutations associated with non-small cell lung cancers were not detected by circulating tumor DNA,” Dr. Morrissette says. “I think there have been misunderstandings about the technology and the biology of these cancers—where the nucleic acids are coming from and how to detect them on the part of the oncologist.” The findings can also be the opposite, she notes, since with ctDNA the assay is theoretically sampling all the metastatic sites, while tissue testing detects variants present in that piece of tissue.

“The onus is on the laboratory and the pathologist to explain why one test may be more appropriate than another, or where the positive predictive value of a certain test may differ, even though they’re both under the umbrella of next-generation sequencing,” she says. “Genomic testing is complex, and there’s always room for more clinician education.”

Charna Albert is CAP TODAY associate contributing editor.