Next-gen sequencing settling in, making its mark

William Check, PhD

November 2015—Resource heavy, reimbursement challenged. Next-generation sequencing has its difficulties, but its value to patient care is without question. For many laboratories today, it's a test sent out, but as for so many other tests, it won't always be.

Dr. Pfeifer

"As techniques evolve, platforms become more user-friendly, and solid bioinformatic pipelines become available, it will not be too long before labs at large hospitals or large health systems are faced with the question of whether the economics are at the point to bring testing in-house," says John Pfeifer, MD, PhD, vice chair for clinical affairs in pathology and immunology and professor of pathology and immunology and of obstetrics and gynecology at Washington University School of Medicine.
Until then, a handful of early adopters share their experiences and a few tips.

Dr. Fernandes

At Weill Cornell College of Medicine, the laboratory of Helen Fernandes, PhD, moved from single-gene tests to NGS panels for oncology specimens in September 2014. (One single-gene test, for EGFR, was retained for its rapid turnaround time.) In addition to helping guide therapy, she and colleagues are detecting mutations in specimens that help enroll patients into clinical trials. They are using a 50-gene panel for routine clinical care, one that took four months to validate and four to five months to earn final approval from the New York State Department of Health.

They use the AmpliSeq panel on the Ion Torrent for solid tumors. For hematologic neoplasms, the laboratory is validating a hematology panel on the Illumina MiSeq, which, Dr. Fernandes says, "is an easier platform to use and less labor-intensive."

She describes two cases in which the technology had a positive impact. First, in a patient with cholangiocarcinoma, NGS identified a specific variant in the IDH1 gene that was a therapeutic target in ongoing clinical trials. In a second case, NGS findings facilitated the classification of thyroid carcinoma. "The pathologist sent two tumor nodules for molecular analysis. NGS detected a BRAF mutation in one thyroid nodule, which classified it as papillary, and an NRAS mutation in the other nodule, which qualified it as a follicular variant." NGS showed that the two nodules harbored different driver mutations and supported the histopathological findings.

The technology lends itself to several observations, Dr. Fernandes says. "Over the last few months we have identified about 20 specimens from patients where we tested two different lung adenocarcinoma biopsies from the

same lobe or different lobe. Interestingly, tumors in different lobes of the same lung or contralateral lungs had distinct genomic profiles. That complements the histopathological findings and has the potential to identify independent primaries."

Dr. Fernandes' laboratory has performed a second type of comparison with NGS: between an FNA (cytology) specimen and the corresponding surgical resection from the same patient. Of the 17 or 18 lung cancer cases they have studied so far, the same genomic variant was identified in both specimen types in 90 percent of the cases. "Cytologic specimens [cell blocks] are adequate for NGS and give you the same information as the surgical resection specimen 90 percent of the time," she says.

In collaboration with Mark Rubin, MD, director of the Institute for Precision Medicine and the team at Cornell, Dr. Fernandes has assisted in the validation of a whole exome sequencing test for cancer that captures 80 to 90 percent of the exome. They submitted it eight months ago and are awaiting approval from the New York State Department of Health. "It is run on the Illumina HiSeq because it needs deeper coverage and higher throughput," she says.

The molecular pathology laboratory at Weill Cornell is currently validating a commercial NGS test that looks at DNA and RNA from more than 140 genes simultaneously. To illustrate the utility of this type of panel, Dr. Fernandes points to lung cancer's two types of important mutations—DNA variants in EGFR and gene fusions in ALK, ROS, and RET genes. "Right now, when we get a sample from a patient with lung adenocarcinoma, we interrogate the DNA variants on our 50-gene panel and send tissue to the cytogenetic laboratory for testing of gene fusions. Using a single NGS assay that can detect and identify DNA variants and RNA fusions simultaneously provides for a comprehensive testing approach."

These advanced tests will be far more costly, Dr. Fernandes says, adding, "A major concern is getting reimbursed for NGS testing. We really need to get reimbursed for the 50-gene panel." She expects it to happen soon.

Dr. Lazar

At the University of Texas MD Anderson Cancer Center, simultaneous testing of multiple genes with NGS has taken over molecular testing in all cancers, including hematologic neoplasms. In April 2012, the molecular laboratories switched all mutation genotyping to NGS. Since then, NGS volume has been about 500 samples per month.

"We realized that we needed information on multiple genes at once," Alexander Lazar, MD, PhD, says. Targeted therapy in melanoma, for example, requires knowledge of at least three genes—BRAF, NRAS, and KIT. "It is relatively straightforward to test for the first two with Sanger sequencing or pyrosequencing," says Dr. Lazar, who is an associate professor in the Department of Pathology. However, it is much more challenging and expensive to determine the genetic status of KIT by those methods. "There is a wide variety of relevant exons and codons in the KIT gene," Dr. Lazar says. "We used to do fancy triaging. Only if the first two markers were negative did we go to KIT. Now with NGS it is easy to do all three at once, which improves turnaround time and patient care."

Prior to the broader mutation testing for melanoma, he and colleagues had the impression that mutations in the p53 gene were rare. "Now, with NGS, we see p53 mutations in the 15 to 20 percent range." These patients can be entered into various clinical trials once standard treatment options are exhausted.

The main therapeutically relevant variants are in the BRAF gene. To be eligible for BRAF inhibitors, patients must have the specific mutations, usually V600E or V600K. Patients without the indicated mutations will not respond to BRAF inhibitors, and may actually progress faster if treated with these pharmaceuticals, Dr. Lazar says. “In the earlier generation of NGS tests for BRAF, we saw a much broader family or range of mutations than we had appreciated.” For example, mutations were present in exons 15 and 11.

In addition to targeted inhibitors against BRAF and KIT, immunotherapies—multiple immune checkpoint inhibitors—were also recently FDA approved for patients with metastatic melanoma. “What they do is to unleash the immune system against specific melanoma cells.” Melanoma is immunogenic, he notes, but it can evade the immune system. Immunotherapy neutralizes the escape mechanism.

“Immune checkpoint therapy provides durable control for some patients,” Dr. Lazar says. “We are searching for ways to define the subset of patients that respond and how to increase rates of response.” The total mutational load correlates with response to these agents. According to current theory, most of the many, often ultraviolet radiation-linked mutations may not be drivers but in some genes may create neoantigens. “It would be very difficult to evaluate total mutational load without NGS,” he says.

In practice the MD Anderson group has found that clinical molecular testing in oncology is critical and complex. To simplify this testing process, they have devised preapproved biomarker order sets for each type of solid and hematologic cancer, consisting of from two to 10 genes (for example, for thoracic cancers, EGFR, KRAS, and BRAF; for melanoma, BRAF, NRAS, KIT). “These were determined by an internal physician panel and are used to clinically manage patients with each kind of tumor,” Dr. Lazar says. The oncogene testing panel includes all of these biomarkers. Changes in genes not on an order set are not masked from clinicians. “Everything gets reported,” he says. “However, the genes clinicians think are clinically important are in the first category of the report and are used to help justify payment from third parties.”

In addition to the 50-gene panel used for standard clinical testing, the laboratory also has a 400-plus gene expanded panel. “We don’t apply this until the patient is tested with smaller panels without adequate results,” Dr. Lazar says. He calls the decision of which panel to start with an “evolving” situation. “Large panels provide us with information we don’t know how to use yet,” he says. “So we are moving to an intermediate size, about 150 genes.”

The key question is not the total number of genes in a panel, he says, but “whether the panel covers all the genes relevant to the tumor you are looking at and all the kinds of variants you want to see.” These include not only single base changes but copy number variants and loss of tumor suppression genes.

Dr. Baudhuin

Thanks to NGS, it’s now easier than ever to provide comprehensive genetic analysis to guide therapeutic decisions for patients with cancer, but it’s important to use resources wisely, says Linnea Baudhuin, PhD. She is an associate professor of laboratory medicine and pathology at Mayo Medical School and co-director of the clinical genome sequencing and personalized genomics laboratory at Mayo Clinic in Rochester, Minn.

“Our physicians will initially say they want a large somatic gene panel, with 500, 100, or more genes, until they get their initial results back and they have all these variants of uncertain significance [VUS],” Dr. Baudhuin says. “Then they’ll say, ‘We don’t want all these genes. We just want the four or five genes strongly associated with this type of cancer.’” Reimbursement also factors in to wanting fewer genes and a more focused, clinically relevant panel.

The most important factor in determining how many genes to include is a strong evidence base. “There is kind of a race out there to have the most genes to catch the eye of the physicians,” she says, a phenomenon she calls “marketability.” Dr. Baudhuin advises laboratorians to balance marketability with high clinical utility “or you could end up with a lot of background noise, or VUS, that could impact the quality of your report.” Clinical utility can be determined through literature and databases such as OMIM and the Human Gene Mutation Database. “This is not a quick process. It takes us months to design a gene panel before any actual development can begin.” During this time, there is a lot of “back and forth” at Mayo among genetic counselors, laboratory directors, and clinicians.

“The larger the panel, the more time-consuming to the lab. A smaller lab would be wise to develop smaller panels to save on resources yet still provide clinically meaningful results,” she says. A laboratory can look at some of the strong offerings on the market and model its own panel on those.

But when designing the reagent, include all you can, even hundreds of genes, she advises. By “reagent,” Dr. Baudhuin means the capture reagent, nucleic acid probes that pull out the genes of interest. “You can make the capture reagent pretty big and still be able to multiplex a lot of samples,” she says. The laboratory will capture all the genes but analyze and report only those that were ordered. Furthermore, with this approach, multiple orderable tests can be created off a single reagent.

“If you have enough real estate, include probes/primers for genes that are of potential clinical utility but didn’t make the cut for ultimate inclusion in the orderable test. You can mask them. Later, if they are found important, you can unmask them. Otherwise you have to go through development and validation again. This is one of the easiest ways to upgrade your test.” Overall, she advises, aim for a good balance between maximum gene number, multiplexing, and optimal depth of coverage.

Another suggestion: “Don’t spend a lot of time on genes that have pseudogenes or homologous regions. There are limited workaround solutions for those with NGS.”
Sample size, she says, is not a sequencing instrument issue, but a sample prep chemistry issue. “If you use a PCR-based sample prep, such as Thermo Fisher, you can use lower input DNA, such as 10 ng, and run it on the Illumina and/or Ion Torrent. This is compared to a capture-based prep, which requires more DNA, for example 30 ng.”

For their somatic applications, they use Illumina and Ion Torrent, both with Thermo Fisher PCR-based prep, in order to have an orthogonal confirmation approach.

The molecular diagnostics laboratory at Virginia Commonwealth University is using the Thermo Fisher Ion AmpliSeq Hotspot panel for 50 genes, says Andrea Ferreira-Gonzalez, PhD. “This assay requires a very small amount of DNA so it can work well with very small samples,” she says, “partly because of its chemistry and partly because of the Ion Torrent platform. So we can offer large-scale genotyping on fine-needle aspirates or pleural effusions.”

Thermo Fisher and Illumina offer chemistry and instruments to support NGS, and this makes it easier for more laboratories to jump in. “Even the bioinformatic component is more resolved and starting to be within reach of labs that are not part of big cancer genome centers,” says Dr. Ferreira-Gonzalez, professor and chair of the Division of Molecular Diagnostics and director of the molecular diagnostics laboratory.

“With that said,” she cautions, “even with those pipelines developed, it still requires some knowledge from laboratorians or end users on understanding the limitations of the chemistry and informatic pipeline. Laboratories have to have a good understanding of inspecting raw data as it comes out of the instrument and as it goes through the pipeline. You can’t take it at face value.” Another complication: “We continue to see variants that need to be assessed and evaluated for pathogenicity.” Resources for this evaluation include the peer-reviewed literature and OMIM, My Cancer Genome, ClinVar, and others.

“We still need the pathologist,” Dr. Ferreira-Gonzalez says. “We still need a laboratorian, a trained professional to make judgments on annotation and confirmation.” In her laboratory, three people work on each case. A technical

specialist does a quality control check on the data, after which the raw data go to a genomic analyst who does a review and begins to provide annotations. Then the case goes to the medical director for a review of the conclusions. “Sometimes the lab director and pathologist need to consult and decide how to annotate variants,” she says.

“As we continue to move into less-invasive procedures, with NGS we don’t require a lot of specimen to be able to acquire a large amount of genotyping information in a single run.” She notes the contrast with just a few years ago, when single-gene testing or even single-mutation testing required a large amount of tissue.

For many laboratories, it may be some time before the economics point to bringing NGS in-house. Others may never do so. For those laboratories, Washington University’s Dr. Pfeifer offers guidelines for choosing a reference laboratory.

Next-generation sequencing means a lot of different things, Dr. Pfeifer says. “It can be a panel focused on hotspots for prediction of response to cancer therapy, a panel that has hundreds of genes, even whole exome sequencing. So first talk to your oncologists to find out what they are going to use the information for. They may only want actionable variants to guide treatment with FDA-approved drugs. Or they may want a broader range or larger number of genes to enter patients into clinical trials.”

Turnaround time will be influenced by the number of genes sequenced and could range from three to five days to two to three weeks. “What breadth of testing do your oncologists want related to the time frame of getting back results?”

The better laboratories provide a physician contact, Dr. Pfeifer says. “When a client pathologist calls, some labs provide a client services rep or technical supervisor. What is really necessary [for NGS] is a physician with molecular genetics background, a pathologist or geneticist or lab director, someone who actually signs out.”

With regard to price, “You get what you pay for.” He finds that a quality laboratory readily provides a detailed description of the technical components of its testing and provides its validation documentation. Also, he says, “It is reasonable to ask how long the lab has been doing this testing and the number of cases they do per week or month. And ask about the qualifications of the lab director and the people who are signing out the tests.”
Of the heavy marketing by various labs and pharma, he says: “We have a role to ensure a reasonable and efficient use of health care resources. Just because a marketing person has talked to your oncologists and suggested ordering a specific test doesn’t mean you can’t make sure that test is appropriate and necessary and useful.”

He continues to be surprised by the level of intimidation among pathologists.

“Everyone recognizes that most pathologists in clinical practice didn’t get high-level genetics or genomics education in medical school or residency. This is a new field. Pathologists need to overcome the intimidation factor. At some level we need to engage with our clinical colleagues. Even if we don’t give the meaning of specific mutations, we can still suggest what testing is appropriate relative to clinical expectations.”

Changes in reimbursement provide motivation for pathologists to get involved in NGS, he adds. “If oncologists are speaking with other oncologists or with geneticists, then health care administrators will move resources to those people to support that activity. Pathologists will be left out of the conversation. We risk being marginalized not only in terms of professional activities but also reimbursement for service and value added.”

How to learn about NGS? Courses at national meetings and webinars. And he recommends Clinical Genomics, a book that he and Shashikant Kulkarni, PhD, edited that came out earlier this year. Another of his recommendations: Genomic Applications in Pathology, co-edited by Iris Schrijver, MD, and George Netto, MD. “These were not written for aficionados but for pathologists,” Dr. Pfeifer says, “to try to minimize the intimidation many feel.”

[hr]

William Check is a writer in Ft. Lauderdale, Fla.