Need for speed in solid tumor molecular testing

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April 2024—As the call for fast turnaround of genetic testing results in tumor profiling grows louder, the need for rapid, reliable test methods becomes more pressing.

Meanwhile, with new genetic biomarkers emerging at a rapid pace, "everything has tipped the balance toward comprehensive next-generation sequencing analysis," said Maria E. Arcila, MD, attending pathologist, molecular diagnostics and hematopathology services, Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center. In the midst of this complexity, "the ability to provide rapid and simple results is lagging behind," said Dr. Arcila, in addressing rapid molecular testing in solid tumors at the Association for Molecular Pathology meeting last year.

"The delivery of rapid and comprehensive molecular results remains a challenge and is a gap for all of us in clinical laboratories," she said.

The windows for patient care are often narrow, Dr. Arcila noted, and every laboratory has "the pile of urgent cases that very soon become too late if the appropriate technology is not implemented. It's disheartening to hear from our clinicians when results are urgently needed and we cannot provide those results."

While recent advances in technology are addressing the needs for comprehensive assessment, she said, "rapid results get caught on complex testing, complex workflows, and often technical requirements for test batching."

Dr. Arcila called lung adenocarcinoma "the poster child exemplifying the complexity of delivering both rapid and comprehensive results in solid tumors," and the answer must come from more "elegant solutions," she said, particularly given the need to provide results within 14 days of receipt of tissue despite operational inefficiencies that may make it difficult. In the context of lung cancer, she notes, "one must deal with very limited tumor material, while there is a need to assess a broad range of alterations, including point mutations, indels, and fusions." Comprehensive NGS panels that can address this effectively could take many weeks. Sequential single-gene or low-multiplex assays may be faster, but they come at the risk of incomplete analysis and depleting the available tissue. The need for batching further complicates rapid results delivery: "It is commonly required for NGS technology or to facilitate workflows and cost-containment," she notes.

Despite the existing guidelines for molecular testing in non-small cell lung carcinoma, in real-world practice many patients go untested or are tested but results cannot be provided in a clinically actionable time. In 2019, for instance, Gierman, et al., reported a review of 1,203 patients with advanced disease in a community oncology setting. This study found that less than 50 percent of patients with advanced NSCLC were tested for basic molecular markers and, of those tested, only 45 percent had documented evidence of treatment with the corresponding targeted therapies (Gierman HJ,

et al. *J Clin Oncol.* 2019;37[15 suppl]:1585).

Things haven't changed significantly since then, Dr. Arcila said.

In 2022, Sadik, et al., reported on their multisource U.S. commercial and Medicare claims and laboratory database analysis of patients with advanced NSCLC who could have, but did not, benefit from a personalized treatment because of various clinical practice gaps (Sadik H, et al. *JCO Precis Oncol*. 2022;6:e2200246). For every 1,000 patients in the study cohort, the authors reported, 497 are lost to precision oncology because of factors associated with getting biomarker test results. Of the 503 of 1,000 who did receive results from a biomarker test, 29 percent didn't receive appropriate targeted treatment.

When choosing a technology to implement in a laboratory, numerous factors have to be considered, Dr. Arcila said, citing a list: the patient population and sample journeys, geographic location, institutional and laboratory workflows, the local expertise on the technology, and how it all fits into the laboratory in terms of its technical requirements, needs for batching, stop points, and bioinformatics support.

For rapid results, local testing is the No. 1 need, followed by instruments that are uncomplicated, compact, stable, durable, and ideally closed systems. For broad implementation, rapid testing technology has to be easy to implement, she said, and should be cost-effective and not labor-intensive. "Not everybody can implement Illumina or Genexus systems in their laboratory," she said, and because instruments must be highly precise, repair and maintenance schedules require redundant backup systems to be in place to maintain clinical laboratory operations, adding to the overall cost. Rapid systems should also minimize manual processing steps and tube transfers, to eliminate possibilities for error and contamination, and should allow sample number flexibility so that one sample or 10 samples can be run, depending on case volume fluctuations.

"Batching requirements are a major issue in our laboratories," Dr. Arcila noted, "because systems and workflows are generally designed for running multiple samples at a time. When rapid testing is needed, you may be forced to run one or a few samples, compromising the standard laboratory workflows and the cost-effectiveness."

Rapid testing platforms must also allow robust performance across different sample types, she said, with minimal tissue requirements and the ability to multiplex several biomarkers. "By design, assays should allow easy updates to the content of the panels, to incorporate additional biomarkers that constantly emerge as clinically relevant." The analysis process should be streamlined, ideally through remote access, for rapid review and interpretation and timely delivery of results.

At MSK, every lung adenocarcinoma is tested by the 505-gene MSK-IMPACT, a panel that requires complex workflows and extensive bioinformatic support. Despite the many benefits of such a comprehensive panel, Dr. Arcila said, providing rapid results is challenging.

"We deal with this by instituting reflex rapid, multiplex, and often non-NGS solutions that can take care of the most common genetic targets. Still, this further increases the overall complexity of testing very small samples and requires extensive optimization of every step to ensure maximal performance."

Dr. Arcila

For a non-NGS method, the MSK molecular diagnostics laboratory uses the cartridge-based approach from Idylla (Biocartis, Mechelen, Belgium). “This is a multiplex qPCR assay that allows testing of multiple mutations for single samples within a closed system without prior DNA extraction,” she said. All sample processing (liquefaction, cell lysis, extraction), amplification, mutation detection, and data analysis occur within the cartridge, and results are ready within two hours, with just a few minutes of hands-on time, Dr. Arcila said.

For lung adenocarcinoma, multiplex testing with the Idylla *EGFR* cartridge (51 mutations) allows for rapid screening of the most common targetable alterations. The Idylla *KRAS* cartridge (21 mutations) can be used as part of a sequential algorithm as *KRAS* mutations are mutually exclusive with other common drivers.

Idylla’s new assay, the GeneFusion cartridge, “is an elegant solution to screen for common fusions,” she said, referring to *ALK, ROS1, RET, NTRK1/2/3* rearrangements, and *MET* exon 14 skipping. “The assay is RNA based and tests by targeting the gene partners with fusion-specific primers, but it also incorporates a more generic fusion detection approach, by expression imbalance between the three-prime and five-prime end of specific genes, as a surrogate for the presence of a fusion,” she explained. Because RNA can be abundant, one tissue section is often sufficient to perform the test.

Dr. Arcila and colleagues validated the GeneFusion assay for body fluids, brushes, washes, stained smear slides, and formalin-fixed, paraffin-embedded tissue (Chu Y-H, et al. *J Mol Diagn.* 2022,24[6]:642–654). They wrote: “The assay enables rapid screening for clinically actionable kinase alterations with quicker turnaround and lower tissue requirements compared with immunohistochemistry and molecular methods, while also circumventing the infrastructure dependencies associated with next-generation sequencing and fluorescence in *situ* hybridization.”

For the DNA-based assays (*EGFR* and *KRAS*), Dr. Arcila said, “we can also use aliquots of the NGS libraries from MSK-IMPACT to test on the Idylla cartridges while we’re waiting for NGS results.”

One of the drawbacks of the Idylla platform: “If you’re not extracting DNA or RNA, assessing the input and sufficiency of the material prior to testing may be challenging for new users, and it requires familiarity with the platform,” she said.

The ultimate assessment of suitability is made at the time the results are viewed. “As with other qPCR data, the larger the starting material—in this case the section of tissue—and proportion of tumor, the fewer cycles you need to be able to amplify the template and to reach the quantification thresholds [Cq]. Very limited material requires many more cycles to reach the Cq,” Dr. Arcila said. Interpreting these thresholds, the variability imparted by the different types of tissue loaded directly, and the potential artifacts are critical for a robust validation, she explained, adding, “Once this happens, it becomes a very usable platform.”

The key to multitarget testing with rapid assays is in the multiplex capabilities, Dr. Arcila said, and

multiplexing with qPCR is extremely difficult. SpeeDx (New South Wales, Australia) is one company with a solution, she said. “Utilizing two proprietary technologies, PlexZyme and PlexPrime, they can enable very high and reliable qPCR compared to other methods. In their primer-based amplification, they insert mismatches to the target parent sequence to make the PCR products for each target sufficiently different so you have more specificity for detection.” They combine this with the MNA enzymes, which are double enzymes that can use universal reporter probes. By combining the different probes and mismatches, “you can multiplex numerous targets in similar regions.” Some Idylla cartridges, for instance, are based on this technology, enabling detection of 51 and 21 different mutations in *EGFR* and *KRAS*, respectively.

More recently, a more flexible cartridge design began to be offered for the Idylla platform: a generic cartridge with liquid reagents for sample preparation, and dried reagents “where you can use vials with different designs with similar results,” she said. Her laboratory has been using it for the Idylla IDH1-2 mutation assay, with rapid results.

Dr. Arcila shared the case of a 65-year-old male who never smoked and had a 5.8-cm left upper lobe mass with extensive intrathoracic and cervical lymphadenopathy. By using pelleted cells from a pericardial fluid on one slide, they were able to determine this patient had a *ROS1* fusion by expression imbalance and fusion-specific primers.

Dr. Arcila and colleagues also reported their experience with NSCLC cytology samples using Idylla’s ultra-rapid *EGFR* assay followed by NGS, primarily with MSK-IMPACT (Arcila ME, et al. *JTO Clin Res Rep.* 2020;1[3]:100077). They found that testing with the Idylla platform “enables rapid and accurate determination of the *EGFR* status without compromising subsequent NGS testing.” Prioritizing the assessment of *EGFR* mutation status by a rapid assay, the authors say, “would represent a critical step in guiding initial treatment decisions.” In general, nine percent of the cases tested by NGS had *EGFR* mutations not covered by the Idylla assay.

They evaluated the multitest approach in a second study of 1,240 biopsy samples and using the same Idylla *EGFR* assay as a screening method before NGS (Momeni-Boroujeni A, et al. *J Mol Diagn.* 2021;23[3]:310–322). Here, too, they found that use of the Idylla platform streamlined the assessment of the most common mutations in NSCLC while still allowing for comprehensive NGS.

With very targeted primer-based assays, Dr. Arcila said, the issue is covering all relevant insertions and deletions in lung cancer because they can be highly variable and difficult to multiplex. “As a rapid assay, we use fragment analysis to be able to rapidly screen these and make sure we’re not missing some of the rare alterations.”

For a rapid alternative to the Idylla platform, ChromaCode (Carlsbad, Calif.) offers high-definition PCR to highly multiplex qPCR and digital PCR applications. The HDPCR NSCLC panel is available off the shelf and includes the major targets for NSCLC with a minimum DNA input of 15 nanograms across two wells (about 7.5 ng per well). There is a third well for RNA targeting fusions, using five nanograms.

Similar to Idylla, the ChromaCode assay is designed to cover the most common indels, but does cover both *ERBB2* and *EGFR* exon 20, “so you have to complement with additional non-NGS assays for more comprehensive assessment or wait for your next-generation sequencing platform results,” Dr. Arcila said. DNA and RNA extraction are needed for this platform; it takes about six hours (but depends on the method), and the extracted sample-to-result workflow can be completed in four hours. “You can have results for numerous key targets in the same day,” Dr. Arcila said, noting that this would not be possible

if multiple serial qPCR had to be done. “You would rapidly deplete the tissue and it would take several days to complete.”

For testing by next-generation sequencing, Pillar Biosciences (Natick, Mass.) has a rapid platform with several NGS testing solutions, one of which is its pan-cancer OncoReveal solid tumor panel.

Pillar has more than 20 NGS testing kits in IVD or RUO formats, designed to scale on low-to-mid throughput NGS systems, Dr. Arcila said. Pillar recently partnered with Illumina to enhance its testing solutions, but the assays are agnostic and can be used with other sequencing platforms.

“The key to this technology is using the proprietary VersaTile, SLIMamp, and PiVAT technologies,” Dr. Arcila said. The VersaTile primer design is AI-enabled, automated, and ultra-high plex. Pillar’s SLIMamp enrichment chemistry involves a one-tube library preparation that eliminates the need for tube changes. “They have a standardized, single-day workflow, low-cost sequencing reagents, a very low assay failure rate, and it’s fully automatable and works well on damaged FFPE samples,” she said. Pillar’s PiVAT data analysis platform is the bioinformatics solution that incorporates what she describes as a highly effective error reduction algorithm to reduce sequencing errors, mapping errors, and other artifacts.

What sets this platform apart from others, she said, is that the technology inhibits the amplification of unwanted regions of the genome. To amplify large contiguous regions of the genome with PCR, primer targets have to be overlapped, “so you do not end up with uncovered areas. Usually those overlap areas are the ones that get the highest amplification and coverage because of the design.” Pillar’s stem loop structures inhibit the amplification of these areas, “so that you’re preferentially amplifying the regions you really want, so it gives you a very even coverage.”

Pillar is a two-day approach, Dr. Arcila said. Library preparation takes place during the first half of day one, followed by sequencing between days one and two, and analysis on day two. “Your analysis is about two hours.” The whole process can be completed within two to three days after extraction with this assay. With no transfers, “everything happens in a single tube, so you reduce the number of steps, which is key to decreasing errors, while also reducing time and associated labor costs compared with other conventional methods.”

The Genexus platform (Thermo Fisher, Waltham, Mass.) is a rapid NGS technology that provides rapid results with less complex workflows. With the Ion Torrent Genexus, Thermo Fisher has addressed major bottlenecks in comprehensive testing, Dr. Arcila tells CAP TODAY. It’s a two-instrument NGS system platform that automates and integrates the main steps of highly complex NGS workflows (sample purification, quantification, library preparation, sequencing, bioinformatics analysis and reporting). This facilitates NGS implementation in small laboratories, limiting the opportunities for human error and reducing variability of results.

Kojo S. J. Elenitoba-Johnson, MD, of MSK presented in the AMP session on the use of this system for hematologic malignancies and reported last year on the use of the Oncomine Myeloid Assay GX panel (Sande CM, et al. *J Mol Diagn*. 2023;25[2]87–93). In the context of lung cancer, the Oncomine Precision assay, which contains 50 genes, is designed to detect the most relevant mutations and fusions, with results available in two to three days.

Oxford Nanopore's sequencing technology is the only one that offers a real-time analysis, Dr. Arcila said.

The formats are fully scalable, from pocket-size devices to population scale. "You can analyze native DNA or RNA and sequence any length of fragment and analyze not only the short cell-free DNA but also the ultra-long reads required for certain applications." Sequencing from FFPE is not yet optimized but in the works for potential future use.

"This is an end-to-end platform that allows a lot of flexibility and control at every stage of the sequencing," she said.

It is a PCR-free application "where you're taking the native DNA or RNA and threading it through a nanopore. This incorporates a motor enzyme that threads that DNA or RNA, and you're looking at about 400 base pairs per second that go through that pore," she explained. "You're capturing the electrical signal of the individual molecule as they go through, and it is the electro current variations that are ultimately translated into base calls."

Oxford Nanopore's sequencing devices range from the single-sample-capacity and portable ones such as the Flongle and the MinION, to high-throughput benchtop sequencers including the GridION and PromethION. "You can choose between simplex or duplex base calling models," with the latter providing the highest quality and accuracy. "Simplex models allow greater output while duplex reduces the output and has more computational requirements," Dr. Arcila said. Results are available within two to three days.

With molecular markers driving much of patient care, she said, "there is an equal need for rapid and reliable methods of analysis."

And with the emphasis also on comprehensive assessment, "we often need to re-evaluate and change our approach to testing to be able to deliver results in clinically actionable times."

As the opportunities for rapid testing through non-NGS and NGS assays increase, Dr. Arcila said, the creation of technology that's easy to implement in local laboratories, with less dependency on extensive infrastructure, "has to be our push."

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