A few years in, a new picture for liquid biopsy

Charna Albert

July 2021—Liquid biopsy has entered a more confident era, with two FDA-approved next-generation sequencing assays for comprehensive tumor mutation profiling, evidence of its clinical utility, and broadened patient access.

“Looking back, it’s remarkable how we’ve evolved,” says Geoffrey R. Oxnard, MD, vice president and global medical lead of Foundation Medicine’s liquid biopsy portfolio. Three years ago, he coauthored a joint ASCO-CAP review on circulating tumor DNA analysis in patients with cancer (Merker JD, et al. *J Clin Oncol.* 2018;36[16]:1631–1641). The tone of the review, he says, was enthusiastic but cautious. There was “clear excitement about the potential of liquid biopsy but uncertainty if the data would come together.” Today, “we have a lot more confidence,” says Dr. Oxnard, a thoracic oncologist who is also in the section of hematology and medical oncology, Boston University School of Medicine.

This confidence stems, in part, from the FDA’s approval in 2020 of two liquid biopsy tests—FoundationOne Liquid CDx and Guardant360. The approvals speak to the rigor with which the assays were developed and their strong analytic validity, Dr. Oxnard says. “I recognize that not every liquid biopsy is FDA approved and many labs offer lab-developed tests. But the ability of some liquid biopsies to make it through FDA approval highlights how the technology has matured.”

Also making a difference are the data from the BFAST, TRITON2, and SOLAR-1 trials. In the 2018 ASCO-CAP review, Dr. Oxnard and his coauthors cautioned that data on clinical utility were lacking. Liquid biopsy was being used organically and it was meeting a need for clinicians, he says. “But was that need leading to good patient outcomes? That is the second piece we’re now seeing come to fruition.”

Greater patient access is piece No. 3. “Liquid biopsy is now a reality. With FDA approval, these tests are covered by Medicare and increasingly by other insurers. They’re covered across oncology. It’s moving past lung cancer, and it’s creating access to next-generation sequencing wherever the patient may be.” And the NCCN recently updated its prostate cancer guidelines, he notes, to say that ctDNA testing should be considered when tissue is not available. “What I’m hearing from community doctors is that a bone biopsy at recurrence just isn’t a scalable solution for prostate cancer patients and liquid is actually a compelling approach to try first.”

Access to genomic analysis is at the heart of a study Dr. Oxnard and others coauthored, which found that in patients with metastatic castration-resistant prostate cancer (mCRPC), comprehensive genomic profiling of ctDNA is a “compelling clinical complement to tissue CGP” (Tukachinsky H, et al. *Clin Cancer Res.* 2021;27[11]: 3094–3105). Using plasma from 3,334 patients with mCRPC, they evaluated the landscape of genomic alterations detected in ctDNA and assessed concordance with tissue-based comprehensive genomic profiling. In the concordance analysis, 72 of 837 had *BRCA1/2* mutations detected in tissue, 67 (93 percent) of which were also identified in ctDNA, including 100 percent of predicted germline variants. ctDNA identified more acquired resistance alterations than tissue.

“Let’s be forthright about liquid biopsy,” Dr. Oxnard says. “It can be a great specimen and it can be a bad specimen. Fundamentally, we don’t know how much tumor is in one. But it can be a great specimen.”

“It’s not always going to work out,” he says. “But in that patient with bone mets, you could have high shed, high signal. You could find the actionable variants you’re looking for. So let’s see this as an alternative option when tissue is not meeting our need.” Detection of *BRCA* mutations wasn’t perfect, he acknowledges. “But sensitivity was high, and we know that when we don’t find it in the blood, we do need to be ready to use tissue as a backup.” There are two ways to obtain answers for two kinds of patients, he adds: “When you’ve got a great [tissue] specimen, test that specimen, no question. And when you don’t have a good specimen, here’s the alternative to create access.”

Less clear, he says, is what's indicated for a tissue specimen of uncertain quality and tumor content. "Should you try tumor first? Should you try liquid first? There's still a lack of clarity there, and that's where we need to collaborate with our pathology colleagues."

Other advances of the past few years include the clinical trials like BFAST, the Blood-First Assay Screening Trial, which is the first prospective study to treat patients with advanced non-small cell lung cancer based on blood testing alone. ctDNA testing with FoundationOne Liquid identified *ALK* fusions at similar frequencies historically detected in tissue. Of 2,219 patients, blood-based NGS yielded results in 2,188 patients, and 119 had *ALK*-positive disease and 87 of those patients were enrolled and treated with alectinib, with an objective response rate of 87.4 percent (Gadgeel SM, et al. *Ann Oncol.* 2019;30[suppl 5]:v918). "When you treat patients based on liquid biopsy, you can get great results," Dr. Oxnard says.

In the phase three SOLAR-1 trial, which evaluated the alpha-specific P13K inhibitor alpelisib in combination with fulvestrant in men and postmenopausal women who have *PIK3CA*-mutated HR-positive/HER2-negative advanced breast cancer, a significant progression-free survival benefit was seen regardless of whether the *PIK3CA* mutation was identified by a tumor tissue test or ctDNA test (Andre F, et al. *N Engl J Med.* 2019;380[20]:1929–1940).

Dr. Oxnard

"We very much see use in the community," particularly in advanced lung cancer, Dr. Oxnard says of ctDNA testing. "What we are getting from academia is creativity in how to use it next." He cites as an example liquid biopsy-based detection of *BRCA* reversion mutations in *BRCA2*-associated pancreatic cancer (Kondo T, et al. *Pancreas*. 2020;49[10]:e101–e103). "That's a space that's scientifically interesting. The clinical story is still playing out."

Despite greater access to and use of liquid biopsy, he says, current testing rates for actionable biomarkers are too low. The MYLUNG (Molecularly Informed Lung Cancer Treatment in a Community Cancer Network) Consortium chart review study, reported in June at the ASCO annual meeting, found that most metastatic NSCLC patients received at least one biomarker test before first-line systemic therapy, but less than 50 percent were tested for the recommended *ALK, BRAF, EGFR, ROS1,* and PD-L1. Testing with NGS was done in less than 50 percent of patients though it increased over the periods examined. "We have such a cumulation of evidence for multigene testing. It's on us to better educate and communicate the reality of how to help patients," Dr. Oxnard says. "It's in our grasp but we need to deliver."

A good liquid biopsy is sometimes richer in DNA than a tumor biopsy, Dr. Oxnard says. "Certainly it can be richer in tumor DNA than a poor-quality tumor biopsy." FoundationOne Liquid CDx measures specimen quality using tumor fraction. When tumor fraction is elevated, he says, clinicians can trust and act not only on a positive result but also on a negative result. "If you have a great, rich liquid specimen, you don't need to worry that the negative is a false-negative." And "when it's a high-shed specimen, it's a more aggressive cancer—act on that negative result. When it's a low-shed specimen that can be a less aggressive cancer, that's when you want to follow through and get the tissue."

His worry is that clinicians are using liquid on its own more and more because it's convenient, and he wants them to understand, he says, that for a low-tumor-content specimen, reflexing to tissue may be especially valuable, whereas for a specimen of high tumor content, "the relative value of follow-through might not be as high."

"It's also appropriate to point out that we need to do better" at improving test sensitivity, he says. "What's great about the constraints of an FDA approval is there is an expectation of continuous improvement and an expectation

that as you evolve the chemistry and algorithms you can improve sensitivity."

An approach that fundamentally changes the sensitivity of liquid biopsy is starting with tumor tissue to inform the analysis and then "diving into liquid to find low-level signal with greater sensitivity," Dr. Oxnard says. Foundation Medicine is exploring this approach for measurable residual disease monitoring with its FoundationOne Tracker ctDNA monitoring assay. "It takes baseline tissue NGS, designs bespoke probes, and then searches with high sensitivity for that specific signal, to monitor that signal at low levels, and to look for residual disease after curative care." The research version of the assay was released in June.

Ignatiadis, et al., in an article published in May, said "the next frontier" for the clinical use of liquid biopsy is likely to be "the systemic treatment of patients with 'ctDNA relapse,'" which is their term for ctDNA detection prior to imaging-detected relapse (Ignatiadis M, et al. *Nat Rev Clin Oncol.* 2021;18[5]:297–312). But blood testing on its own, Dr. Oxnard says, isn't likely to supplant current methods for diagnosing recurrent cancer.

He recalls a patient case of recurrent lung cancer with a positive bone scan: "I sent a liquid biopsy and it showed the patient's *EGFR* mutation. And I asked my colleagues, 'Can I treat based on this blood test, or do I need to get a biopsy to confirm advanced recurrent lung cancer?'" His colleagues advised him to order the biopsy, arguing the patient was owed pathologic confirmation. "That was many years ago. Today, if we can sacrifice the biopsy, we do. But I do think there's something true about pathologic confirmation, especially with regard to incurable cancer." Using blood testing to instigate clinical workup and accelerate detection of recurrence is more likely. "We know there's something true about biopsy confirmation, and I don't see us losing that in the near term." Rather, he says, "liquid biopsy will make us more nimble, more patient centered, and more efficient in how we undergo diagnostic workup and get patients the therapies they need."

Population-level screening for multiple malignancies using genomewide analysis of cfDNA is another application. The Circulating Cell-Free Genome Atlas (CCGA) study (NCT02889978), supported by Grail, is an ongoing effort to determine if genomewide cfDNA sequencing in combination with machine learning can detect and localize multiple cancer types at sufficiently high sensitivity and specificity for general population screening.

This multicenter, longitudinal, case-controlled observational trial is split into three pre-planned substudies. Biospecimens were prospectively collected from participants with newly diagnosed untreated cancer and from healthy controls. CCGA1 focused on assay development. Three prototype sequencing assays were performed: paired cfDNA and white blood cell targeted sequencing of 507 genes for single nucleotide variants/indels; paired cfDNA and WBC whole genome sequencing for copy number variation; and cfDNA whole genome bisulfite sequencing (WGBS) for methylation. WGBS had the highest sensitivity and was taken forward for further assay and clinical development (Klein EA, et al. *J Clin Oncol.* 2018;36[15 suppl]:12021; doi:10.1200/JCO.2018.36.15_suppl.12021).

Dr. Liu

"The focus on methylation avoids the need for WBC sequencing to avoid the variable of clonal hematopoiesis of indeterminate potential that confounds targeted sequencing," says Minetta C. Liu, MD, CCGA co-investigator (with Dr. Oxnard and others) and research chair, Department of Medical Oncology, Mayo Clinic. Methylation signals are also organ specific, allowing for tissue localization of the cancer signal. "This is critical in guiding the clinical evaluation to diagnostic resolution," Dr. Liu says.

CCGA2 followed, she says, to develop, train, and validate a targeted methylation assay to classify cancer versus

non-cancer and identify the tissue signal origin for multi-cancer detection across all stages. A total of 6,689 participants (2,482 with cancers of more than 50 types and 4,207 without cancer) were divided into training and validation sets. Plasma cfDNA was collected and subjected to bisulfite sequencing, targeting a panel of more than 100,000 informative methylation regions. A classifier was developed using machine learning algorithms. The classifier's performance was consistent across the training and validation sets: specificity was 99.3 percent in the validation set (CI: 98.3 percent to 99.8 percent; 0.7 percent false-positive rate). And stage I to III sensitivity was 67.3 percent in a prespecified set of 12 cancer types and 43.9 percent across all cancer types. Sensitivity of detection increased with increasing stage. In the prespecified types (anus, bladder, colon/rectum, esophagus, head and neck, liver/bile duct, lung, lymphoma, ovary, pancreas, plasma cell neoplasm, stomach), sensitivity was 39 percent in stage I, 69 percent in stage II, 83 percent in stage III, and 92 percent in stage IV. Detection increased similarly across all 50 cancer types. Tissue of origin was predicted in 96 percent of samples with a cancer-like signal, and of those, tissue of origin localization was accurate in 93 percent (Liu MC, et al. *Ann Oncol.* 2020; 31[6]: 745–759). CCGA3 was designed for further refinement and validation of the classifier in a large population (Klein EA, et al. *Ann Oncol.* Online ahead of print June 23, 2021. doi:10.1016/j.annonc.2021.05.806).

The CCGA study recruited participants with a known cancer diagnosis in order to develop and validate the multi-cancer early detection test, now known as Galleri. "In parallel with CCGA, Grail has been conducting other prospective, longitudinal studies in asymptomatic intended use populations, where biospecimens and clinical data are collected to support population-based cancer screening," Dr. Liu says. These large-scale efforts have established the necessary clinical validation cohorts post-assay development, she says. These studies include STRIVE (NCT03085888), which enrolled approximately 100,000 female participants at the time of screening mammography from 35 U.S. clinical sites (including five Mayo Clinic sites), as well as SUMMIT (NCT03934866), which enrolled approximately 25,000 smokers and former smokers in the United Kingdom at high risk of lung cancer.

Longitudinal follow-up is a critical component of these trials, Dr. Liu says, especially for participants who have a cancer signal-detected test result but a subsequent unremarkable diagnostic workup. Those participants could develop cancer later, reflecting a lead-time bias. Participants without a detected signal also may develop cancer later. "These observations will provide critical insight into overall test performance," she says.

PATHFINDER (NCT04241796) is a prospective pilot implementation study of about 6,200 participants without a known diagnosis of malignancy, in which results of Grail's Galleri test are returned to participants and their clinicians. Participant-reported outcomes and perceptions of the test are ascertained, and diagnostic pathways are recorded toward resolution of a signal-detected test result. The first results were presented at the ASCO annual meeting in June, simultaneously with Grail's June 4 announcement that Galleri is available in the U.S. by prescription.

Of the 6,629 individuals age 50 or older enrolled in PATHFINDER, Grail's test accurately detected 29 cancers across 13 types: breast, colon and rectum, head and neck, liver and bile duct, lung, lymphoid leukemia, lymphoma, ovary, pancreas, plasma cell neoplasm, prostate, small intestine, and Waldenstrom macroglobulinemia. Of the new cancers detected, nearly 40 percent (9/23) were localized (stage I–II), and 13 of 23 were detected before distant metastases (stage I–III). The positive predictive value was 44.6 percent (95 percent CI: 33.2–56.7 percent), which is consistent with findings from the CCGA study. Final results are expected in the first half of 2022.

Multi-cancer early detection screening detects malignancies that have no current screening paradigm, such as pancreatic and ovarian cancer, Dr. Liu says. "This is critical, as over 70 percent of cancer-related deaths between ages 50 and 79 are attributed to malignancies without recommended standard screening options." Galleri and other multi-cancer early detection tests under development have demonstrated the ability to detect multiple cancer types across all stages of disease, she says. "It is not surprising that sensitivity of detection increases with increasing stage because circulating tumor DNA is a function of tumor burden." But not every tumor sheds DNA into the circulation at the same rate, Dr. Liu notes, "so there is a function of tumor type and underlying tumor biology that plays into sensitivity of detection." In CCGA2, cancers of every stage were detected, "but stage distribution is different across different cancers. For example, we remarkably detected a fair number of pancreatic

tumors at an early stage, when intervention is more likely to reduce cancer-related mortality." In the validation set, sensitivity of detection for pancreatic cancer was 63 percent in stage I and 83 percent in stage II.

Dr. Liu emphasizes that blood-based multi-cancer early detection tests should be used in conjunction with standard-of-care screening recommendations. "The goal is to enhance—not replace—single cancer screening paradigms," she says. "We also have to keep in mind that ctDNA has a lower limit of detection. We are only as good as the assays are, and if we don't detect a cancer signal, it doesn't mean that cancer is not there."

Practical issues are also under consideration, specifically with respect to incorporating multi-cancer early detection blood tests into physician practices. "We need to develop operational workflows related to ordering, interpreting, and managing the test results," Dr. Liu says. "We assume primary care providers will order the blood test, as they already order mammograms, low-dose chest CT scans, and the like. But who will direct the diagnostic workup for a cancer signal-detected result, or follow patients who have a cancer signal-detected result but no identified malignancy? We are in the midst of a paradigm shift in cancer screening that requires collaboration across medical specialties to incorporate multi-cancer early detection into our general practices."

"And those discussions are gratefully taking place."

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