DELFI approach as ‘pretest’ in early cancer detection

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December 2021—A cost-effective liquid biopsy focused on analyzing genomewide fragmentation profiles in cell-free DNA has been shown in proof-of-concept studies to detect early-stage lung and other cancers. And the goal is to move the needle for widespread adoption and accessibility, says Victor E. Velculescu, MD, PhD, co-director of cancer genetics and epigenetics and associate director for precision medicine, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine.

In a prospective study of 365 individuals at high risk for lung cancer, Dr. Velculescu and colleagues used a machine-learning model to detect tumor-derived cfDNA through genomewide analyses of cfDNA fragmentation patterns. The noninvasive DELFI (DNA evaluation of fragments for early interception) cancer detection model was validated with an independent cohort of 385 non-cancer individuals and 46 lung cancer patients (Mathios D, et al. *Nat Commun.* 2021;12[1]:5060).

“Between the discovery and validation cohorts, we found that the approach can detect lung cancer through a simple blood test,” Dr. Velculescu, who is also professor of oncology, pathology, and medicine at Johns Hopkins, said in a recent CAP TODAY interview. “It detects it in a high-performance way—in this initial proof-of-concept study—across all stages and subtypes.”

Dr. Velculescu

Dr. Velculescu describes it as a simple test: “We take the blood, we purify the DNA from it, we add on adaptors and sequence it. It’s three steps, as opposed to many types of liquid biopsy approaches that have a dozen, two dozen, steps.” He sees it as scalable and able to be performed at labs worldwide and affordable from a public health perspective.

Dr. Velculescu and colleagues examined patient blood samples obtained from 365 individuals at Bispebjerg Hospital in Copenhagen (LUCAS cohort) from September 2012 to March 2013. The majority of the subjects in the cohort were symptomatic individuals at high risk for lung cancer (age 50–80 and smoking history >20 pack–years). The cohort included 323 subjects (90 percent) with pulmonary, non-pulmonary, or constitutional symptoms, with the majority having smoking-related symptoms. The remainder were asymptomatic at enrollment, with an incidental chest image finding by X-ray or CT that was suspicious for lung malignancy.

The study’s authors isolated 2–4 mL of plasma from each patient in the LUCAS cohort and examined the extracted DNA using the DELFI approach. “Overall we detected more than 90 percent of these lung cancers across these different stages and subtypes, which is very encouraging for a test at this stage of development,” Dr. Velculescu says.

He described the DELFI approach and its use for cancer detection across seven cancer types in a 2020 AMP annual meeting virtual session, shortly after its publication in *Nature* (Cristiano S, et al. *Nature.* 2019;570[7761]:385–389). “This is a methodology that you can utilize broadly in both a high-risk and general population,” he says. The goal is to not only detect the cancer, he says, but also to identify the tissue of origin so individuals “don’t have to go through a complicated diagnostic odyssey.”

Using blood-based fragmentation profiles, the DELFI score—the probability that a person analyzed in this way has cancer—can more accurately identify those likely to have cancer. “The approach should be thought of as a pretest,” he says, used to send someone for imaging or a diagnostic follow-up test to identify the cancer, find out where it is, and determine the next intervention.

In his AMP presentation last year, Dr. Velculescu discussed what he called an earlier “monumental effort” within Johns Hopkins and internationally that led to the discovery that among individuals with breast, ovarian, lung, and colorectal cancers, a majority could be identified as having detectable alterations in the blood (Phallen J, et al. *Sci Transl Med.* 2017;9[403]:eaan2415).

In the study, the plasma of 44 healthy individuals and of 194 individuals with breast, colorectal, lung, or ovarian cancer provided “the first systematic analysis of sequence alterations in cell-free DNA for direct detection of early-stage tumors,” Dr. Velculescu said. The alterations were detectable at different stages, and the levels of circulating tumor DNA typically increased across different stages, with lower levels at earlier stages and higher levels at later stages.

Even multiple mutations in the blood could potentially be identified when looking at a targeted panel, sequencing deeply, and trying to identify cancer-associated alterations in the circulation, he said. “Although this study served as a precursor for our early detection efforts and was the first one to do it in this way, it had a number of weaknesses and highlights the kind of difficulties one has in utilizing mutations for early detection.”

The first is that these mutations typically make up only a small fraction of the circulating cell-free tumor DNA that’s present, Dr. Velculescu said. “In part, that’s because not all molecules have mutations and not all molecules that are tumor derived are even assessed. And these are orders of magnitude different in the circulation” (Cristiano S, et al. *Nature.* 2019;570[7761]:385–389).

With a small fraction of molecules evaluated, only a few mutations will be detected in a panel of any reasonable size, “whether it’s a few genes that are highly mutated in cancer or even when looking at the top 50 or 100 genes that are mutated in cancer. One typically just gets a handful of mutations.”

The effect is that the limit of detection, simply because of the number of cfDNA molecules present, will be limited by the number of types of observations that can be obtained. “That may be inadequate if you have a low number of mutations,” he said, adding that the obvious goal would be to increase the number of potentially observed changes to increase the limit of detection.

There’s also a confounding difficulty in which mutations occur not only in cfDNA but also in white blood cells. An analysis of samples from patients in the CRITICS trial showed that in patients with gastric cancer, mutations identified in cfDNA came from both white blood cells and cancer. “Interestingly, those that were present in both typically were at similar levels,” Dr. Velculescu said, suggesting there is a population of WBCs that is rapidly turning over and releasing these types of alterations. However, “there’s also a population in the cell-free DNA that is not at all present in the white blood cells and more likely the tumor-derived alterations” (Leal A, et al. *Nat Commun.* 2020;11[1]:525).

“One can see from this type of effort that mutations in cell-free DNA can be truly confounded by clonal hematopoiesis, by the kinds of changes that occur in white blood cells,” Dr. Velculescu said. At first glance, then, it may be difficult to discern whether mutations are tumor or WBC derived.

Leal, et al., identified 21 alterations in *p53*, for example, and all but six alterations occurred in the white blood cells, Dr. Velculescu said. “Many of these occurred in hotspots in *p53* and other pathogenically predicted changes, making it almost impossible to distinguish a priori which would have been tumor derived and which would have been derived from white blood cells.”

For these reasons, he said, the Johns Hopkins group and others began considering other types of alterations to evaluate early detection changes in individuals who may have cancer. One possibility is tumor fragmentation.

A number of observers noted cfDNA size in the blood is typically small, Dr. Velculescu said. A natural fragmentation occurs in the process of generating cfDNA and leaves only the protected DNA—around 167 bases—to be ultimately detectable in the blood (Cristiano S, et al. *Nature.* 2019;570[7761]:385–389).

"It turns out that individuals with cancer have slight shifts in that cell-free DNA," Dr. Velculescu said. Although the overall difference is not significant enough to help identify individuals with cancer, "it got us thinking as to whether these fragmentation changes could perhaps be useful in another way."

In looking through a targeted approach at the mutations they observed as altered in human cancer in the blood, they were able to see that in some cases the mutated DNA was smaller while in other cases it was larger, Dr. Velculescu said. "For example, in the case with a *PIK3CA* mutation, the DNA that includes the mutant molecules—those that are tumor derived—are smaller than those that are wild type." However, the cfDNA derived from tumors with a *CDKN2A* mutation were larger than those that were wild type, "suggesting that different regions of the genome might be fragmented in different ways. And that may be a way to take advantage of this difference to identify those individuals with cancer."

Though mutations can be confounded by clonal hematopoiesis, when they went back and looked at the cfDNA fragments with alterations in *p53*, all those that were tumor derived were shifted in size, while those that were mutant derived but from white blood cells were unchanged in size. This demonstrated that "fragmentation changes are likely to be tumor specific as opposed to a result of changes occurring during clonal hematopoiesis or other changes in white blood cells," he said.

In looking at random variants occurring in the genome, whether germline or from other sources but not tumor derived, there is little difference between the mutated and wild-type fragments. "This concept of using fragment sizes, but thinking about them throughout the genome as a profile," ultimately led to the study published in 2019 of genomewide cfDNA fragmentation in patients with cancer, by Cristiano, et al., and to the development of the DELFI approach, he said.

A person's cfDNA is derived largely from white blood cells, "and when you do whole genome sequencing of that DNA, essentially scooping up all the DNA that's in the blood and looking at size and location, you can obtain a fragmentation profile," Dr. Velculescu said.

In individuals with cancer, the fragmentation profiles are typically different, he said, because the tumor-derived cells have changes in the way their chromatin has been organized. "It's no longer packaged in the same way in an ordered fashion. In fact, the differences in the size of the nucleosomes and the nucleosomal DNA that's wrapping the nucleosomes, as well as the distance between them, the open and closed regions of the genome, and other genomic characteristics can end up affecting this fragmentation profile."

Dr. Velculescu and his colleagues used machine-learning approaches to compare these different profiles and distinguish those with cancer from those who are healthy. And because the residual fragmentation profile still has information on the tissue of origin, he said, it can be used to look at the source of the tumor-derived DNA.

"When you think of mutations, you typically think you have one to hundreds of mutations in a targeted panel," he said. "When you're thinking of methylation, perhaps you can get thousands of these changes that one can evaluate in a targeted way. And when you're looking at all the fragmentation differences, there are potentially millions of differences to identify."

This can greatly expand the sensitivity of such an approach, he said. "It increases the number of shots on goal and can identify not only those individuals who have cancer but also the tissue of origin."

In a pilot analysis, his team isolated cfDNA from approximately 4 mL of plasma from 30 healthy individuals and eight patients with stage I to III resectable lung cancer and performed whole genome sequencing at approximately 9× coverage. The fragmentation profiles of the 30 healthy individuals had similar and consistent patterns, Dr. Velculescu said, while fragmentation profiles of the eight lung cancer patients had dramatic changes, "in many

cases occurring at multiple regions throughout the genome."

"We wondered whether one can evaluate this at lower coverage" of the genome than 9×, he said. His team subsampled whole genome sequencing data and determined that altered fragmentation profiles from cancer patients were identified as low as .5× coverage. The benefit is that this approach is now broadly applicable and inexpensive, he said.

The questions they had initially, when using this approach, were what is the source of the healthy cfDNA, why have a profile at all, and why isn't the DNA uniformly distributed throughout the genome. So they isolated the nuclei of WBCs, evaluated nucleosomal DNA from those individual cells, and sequenced them.

"When you sequence that nucleosomal DNA coming from the cells in healthy individuals, you can see a profile," he said, "and it turns out that profile is similar to that of healthy individuals and their cell-free DNA, demonstrating that the source of cell-free DNA in a healthy individual is nucleosomal DNA. And the profile we're seeing genomewide is the same profile you see from these cells."

What about those who have cancer? Cristiano, et al., wrote in their 2019 article, "In contrast to healthy cfDNA, patients with cancer had multiple distinct genomic differences with increases and decreases in fragment sizes at different regions." While chromosomal gains and losses present in the tumor of one individual with cancer were easily detectable in the cfDNA, Dr. Velculescu said, "the profile genomewide is what makes the difference. If you just looked at the overall distribution of sizes, for example, of these cell-free DNA patterns, and looked in the tumor versus the healthy individuals," the overall cfDNA fragment size differences are small and would not have been useful for distinguishing cancer-derived cfDNA from healthy cfDNA. "It's a genomewide pattern that is useful."

Dr. Velculescu and his team then expanded the study, performing WGS at 1× to 2× coverage of cfDNA from 215 healthy individuals and 208 patients with various (largely early stage) cancers: breast (54), colorectal (27), lung (12), ovarian (28), pancreatic (34), gastric (27), and bile duct (26). "Of course, one can use machine learning to take advantage of all this information," he said. The team implemented a gradient-tree boosting, machine-learning model to examine whether cfDNA can be categorized as having characteristics of a patient with cancer or a healthy individual.

"This allows us to consider this not necessarily a multianalyte test but a multi-feature test that can take this multitude of information and utilize it to develop the best algorithm to, in a high-confidence way, detect and distinguish those individuals with cancer from those who are healthy."

Ultimately, they were able to identify with high specificity between 60 and nearly 100 percent of individuals across the seven cancer types while detecting few abnormalities in those without cancer, Dr. Velculescu said.

"This led to an analysis of the sensitivity and specificity. An ROC curve for the overall cohort of cancer patients was 0.94 in the study, and turned out to be much higher than previously proposed methods," such as looking at mitochondrial DNA in the blood or chromosomal copy numbers, he said.

Dr. Velculescu's team was the first to propose a machine-learning approach for genomewide fragmentation patterns, he said, "and the performance of the DELFI approach was higher in this analysis than other approaches and shows highest performance across the different cancer types analyzed."

One can consider combining the fragmentation profile approach with other types of alterations, he said. For some set of patients analyzed, his team also performed targeted deep sequencing, looking at specific mutations in the cfDNA. Of 126 individuals analyzed with the two approaches, "we can detect about 82 percent with DELFI and about 66 percent with mutations." Looking at the DELFI score and mutations in combination led to the identification of about 91 percent of patients with cancer.

The study findings demonstrated that "genomewide fragmentation profiles are a universal feature of human cancer," Dr. Velculescu said. "They can be used to identify those individuals with cancer and are successful" in late- and early-stage disease.

Identifying the source of the cancer is a remaining challenge. The fragmentation profiles were different among the different cancer types, Dr. Velculescu said, and he and colleagues tested whether the information could be used to identify the tissue of origin. They found they can predict the tissue of origin accurately about 61 percent of the time; about 75 percent of the time one of the top two predictions would be accurate, he said.

“This is an important first step,” Dr. Velculescu said. “We anticipate that with larger numbers of tumors that are analyzed for each of these tumor types, we will be able to improve these performance metrics.”

The DELFI method can have applications other than screening, Dr. Velculescu said in the recent interview. Monitoring of therapy is one example. Another is early recurrence detection. In the Mathios, et al., study of lung cancer patients published this year, Dr. Velculescu and coauthors looked after resection at whether these individuals had recurrence. “And we were able to identify in a number of them that they had recurrence of disease months prior to when they were ultimately diagnosed.”

Prognosis is yet another application. Individuals who had a higher DELFI score had a worse outcome. This was true even after accounting in a multivariate analysis for such things as the stage, size, and type of cancer and the histology, Dr. Velculescu says. One reason may be that the tumor is more aggressive, “because this fragmentation is a measure of the disorganization, in a way, of the cancer genome.” The second reason may be that the DELFI approach is detecting occult metastases.

Next up, Dr. Velculescu says, is a 1,700-person first-of-a-kind national clinical trial—DELFI L101—sponsored by the Johns Hopkins University spin-out, Delfi Diagnostics, with U.S. participants who are healthy individuals, individuals with lung cancer, and individuals with other cancers. “Over time we envision the technology being applicable to other cancer types as well,” he says.

Cost-effectiveness is an important aspect of the DELFI approach, Dr. Velculescu says. “Sometimes technologies that are developed are quite expensive, and if we have tests that are too expensive, they end up being like VIP tests which then don’t help from a public health perspective. And early cancer screening and detection is a public health effort. So if one does such tests, and they’re only applicable to one percent of a population, then we’ve failed as a society to help those who need this most.”

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