Diacarta Positioning Liquid Biopsy Assay to Gauge Chemotherapy Response in Multiple Cancers

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NEW YORK – On the heels of newly published data, Diacarta plans to invest further in blood-based testing to determine the utility of its assays to predict drug response in patients with smoking-related cancers.

The San Francisco-based firm has launched a new validation trial that aims to evaluate the specificity of its in-development QuantiDNA pan-cancer chemotherapy assay, and plans to perform more cancer-related testing once the demand for COVID-19 testing in the US has calmed down.

"The major problem is that there is no great quantitative liquid biopsy test [for chemotherapy]," Paul Okunieff, cofounder of Diacarta and professor of radiation oncology at the University of Florida, explained. "It'd be cheaper for the patient, if after the first dose of chemistry, we could tell it was working, but [the tests] are not egalitarian and do not work for everyone's tumors."

Diacarta initially received a $2 million Small Business Innovation Research (SBIR) contract from the National Cancer Institute in 2018 to develop the QuantiDNA Radiotherapy Toxicity Measurement (RadTox) Assay, which is based on the firm's proprietary SuperbDNA technology. The firm currently offers the RadTox assay, which received a CE mark in 2019, as a laboratory-developed test out of its San Francisco-based CLIA-certified, CAP-accredited lab.

As part of the SBIR grant, Diacarta partnered with the University of Florida Health Cancer Center to examine blood samples collected from about 500 patients undergoing radiotherapy for prostate cancer.

However, the NCI agreed to extend the original study beyond the $2 million SBIR grant to determine whether the firm's SuperbDNA technology could be applied to predict chemotherapy response.

"We are closing the Phase II grant, and studies are going smoothly with strong support from [the] NCI," Ramanathan Vairavan, senior VP of commercial operations at DiaCarta, explained in an email. "We have encouraging data to apply for the Phase IIb grant, since we met all the Phase II milestones and got NCI concurrence to make the clinical study as a clinical trial with the [US] Food and Drug Administration."

Okunieff said that the SuperbDNA method, which the firm hopes to use as part of a new assay, can potentially determine the efficacy of chemotherapy and predict metastasis early in the patient's treatment journey.

"We've looked at over 100,000 samples [using SuperbDNA] over the past few years, with a proportion of patients with radiation, some with chemotherapy, [and] some with both," Diacarta CEO Aiguo Zhang said. "These therapies can be applied with the test for different cutoffs or algorithms."

Okunieff explained that the QuantiDNA assays use about 5 ml of blood from a patient. Rather than performing a PCR amplification step, Diacarta processes the blood sample through the SuperbDNA hybridization platform. He described the platform as an "ELISA-like method but with a branched DNA hybridization sandwich" — instead of an antibody — to quantify the concentration of a patient's cell-free DNA.

As part of the SuperbDNA workflow, the platform's capture probes — pre-coated on the bottom of plates, beads, or disks — bind to specific target DNA or RNA sequences, Zhang said. Once a patients' blood sample is added, the DNA sequence is captured, and the rest of the sample is washed away.

A second set of probes, including "preamplifier" and "amplifier probes" labeled with alkaline phosphates or biotin are then added to hybridize with the target molecule to create a "sandwich," Okunieff added.

After performing a second wash to remove unhybridized probes, Diacarta technicians add a substrate that allows chemiluminescent or fluorescence-based quantitative measurement of DNA concentration.

"You have your bead, and the [cell-free] DNA sticks to it because you have a complimentary piece of DNA on it," Okunieff said. "Then another piece of complimentary DNA fits on the other side that has an amplifier on it. Only DNA will stick to the bead, since if another [analyte] sticks to the bead, it will fail to stick to the complimentary side."

Running on the SuperbDNA platform, the QuantiDNA assays build a baseline profile using the patient's initial blood sample. One to two days following first-line treatment, a second sample is collected to measure the treatment-induced levels of circulating DNA.

"When you take the ratio, you have an agnostic test that's also quantitative," Okunieff added. "If the results went down by a factor of five, then the [size] of the tumor [shrank] by five times, assuming the same level of cell death."

Zhang said that the SuperbDNA platform runs on the Magpix microarray instrument from Luminex (recently acquired by Diasorin). He highlighted that the QuantiDNA assays can generate a patient-specific report within three hours.

Validation study

In a validation study published last month in *Nature Scientific Reports,* Okunieff, Zhang, and their colleagues quantified peripheral cell-free DNA (cfDNA) in 154 NSCLC patients — including 128 lung adenocarcinoma (LUAD) and 26 lung squamous-cell carcinoma (LUSC) — using the SuperbDNA platform before and after the first cycle of chemotherapy.

The group analyzed correlations between cfDNA and tumor burden, clinical characteristics, progression-free survival (PFS) and disease-free survival, objective response ratio, and therapy regimens.

Zhang and his team found that baseline cfDNA, instead of post-chemotherapeutic cfDNA, positively correlated with tumor burden in the NSCLC patients. In addition, cfDNA kinetics — including the ratio of post-chemotherapeutic cfDNA to baseline cfDNA — significantly separated responsive individuals from non-responsive patients.

The researchers also saw that cfDNA ratios were negatively correlated with PFS in LUAD patients, but not patients with LUSC.

"In theory, [the patient's] baseline [tumor cfDNA] levels would be low, but sometimes, the baseline was even high before treatment," Zhang said. "We don't know how to explain that, but this is good information for the doctor, so when [they] decide treatment … they're more cautious and might make more meaningful decisions."

When patients were stratified by therapy regimen, the predictive value of cfDNA was significant in individuals with chemotherapy and VEGF inhibitor targeted therapy.

While the authors believe that measuring the levels of plasma DNA during therapy may serve as a prognostic marker for NSCLC patients, Zhang acknowledged that his team will need to validate the method in larger patient cohorts with longer-term follow-ups to establish cfDNA's value.

Okunieff noted his team has also found promising results for QuantiDNA's use in gastric, breast, and other types of cancer.

In Diacarta's new validation trial, which Zhang labeled "Project X," the firm's US lab will work with its lab in China and European partners to analyze blood samples from patients suffering from late-stage smoking-related cancers on the unnamed QuantiDNA chemotherapy assay and predict their response to the treatment. While the trial will attempt to demonstrate the chemotherapy test's use in several cancers, Zhang noted that his team will focus on three to four tumor types to achieve international regulatory approval in the next two years.

At the same time, it remains unclear whether an assay like Diacarta's chemotherapy test would be clinically useful in the cancer space. Fred Hirsch, executive director of the Tisch Cancer Institute's Center for Thoracic Oncology at Mount Sinai, pointed out that most oncologists typically choose molecular targeted therapy or immunotherapy as preferred treatments for patients with lung cancer. In contrast, he noted that chemotherapy has faced problems with toxicity and a lack of efficacy.

Hirsch believes that research into treatment for lung and other smoking-related cancers should focus on areas including the identification of new drug targets, spotting patients who will actually respond to the variety of drug treatments, and understanding the mechanisms behind a patient's tumor resistance to the drugs.

Commercial plans

Diacarta expects to offer a LDT version of the QuantiDNA chemotherapy prediction assay following the completion of the international trial, Zhang said.

Zhang acknowledged that Diacarta's lab is "currently swamped" with COVID-19 diagnostic services, testing up to 2,000 samples a day with its US Food and Drug Administration Emergency Use Authorized QuantiVirus SARS-CoV-2 Multiplex Test Kit. However, he anticipates that the firm will resume offering cancer-related RUO services this year as the demand for COVID-19 testing dwindles.

In addition to the RadTox and Quantivirus assays, Diacarta offers a suite of testing services that uses its QClamp technology and xenonucleic acid (XNA) to "clamp" or silence amplification of non-mutant DNA in patient samples. The tests include Diacarta's CE-marked single-gene QClamp qPCR assays and its OptiSeq XNA next-generation sequencing panels.

Diacarta also offers a CE-marked PCR-based screening kit called ColoScape, which uses QClamp on DNA from formalin-fixed, paraffin embedded tumor, stool, or plasma samples to detect 20 DNA mutations associated with colorectal cancer.

Diacarta has raised a total of $43 million in funding since it was launched. While the firm had reported a $45 million Series B round in 2018, Zhang explained that one investor "had financial issues and did not make it," leading to a reduced total of $35 million in the round.

Zhang noted that his team has received about 80 patents from the US Patent and Trademark Office related to the SuperbDNA technology and suite of QuantiDNA assays.

Zhang believes that Diacarta's assays stand out from potential competing liquid biopsy methods that use "deep sequencing" and are thus "very costly and time-consuming." In contrast, he argued that the QuantiDNA assays are "quick and quantitative" for clinical users such as radiation oncologists.

"If you want to see if the treatment is too toxic, and if there's not much tumor DNA, then the cfDNA is coming from the normal tissue," Zhang added. "[QuantiDNA] is not specific for an individual patient's tumor, or their individual tumor site, or the gene the mutation is on. But it also doesn't require deep gene sequencing or purification."

In addition, DiaCarta this week indicated potential future directions for its XNA and SuperbDNA technologies by announcing the hire of Honjun Yang as its senior VP of companion diagnostics. He will be responsible for incorporating the XNA technology into

companion diagnostics, the company said in a statement. Yang previously served as executive personalized healthcare strategy leader at AstraZeneca.

Vairavan said that the firm will initially develop companion diagnostic assays for lung, colorectal, and breast cancers. He believes the platforms "are very attractive to drug companies" for biomarker screening and companion diagnostic assays.

"For example, the recent new development of drugs targeting … KRAS G12C and EGFR exon 20 insertions are perfect CDx programs with our XNA technology for [ultra-high] sensitivity and specificity," Vairavan said. He added that the firm will explore CDx use in chemotherapy, radiation therapy, cell therapy, and immunotherapy to monitor patient responses.