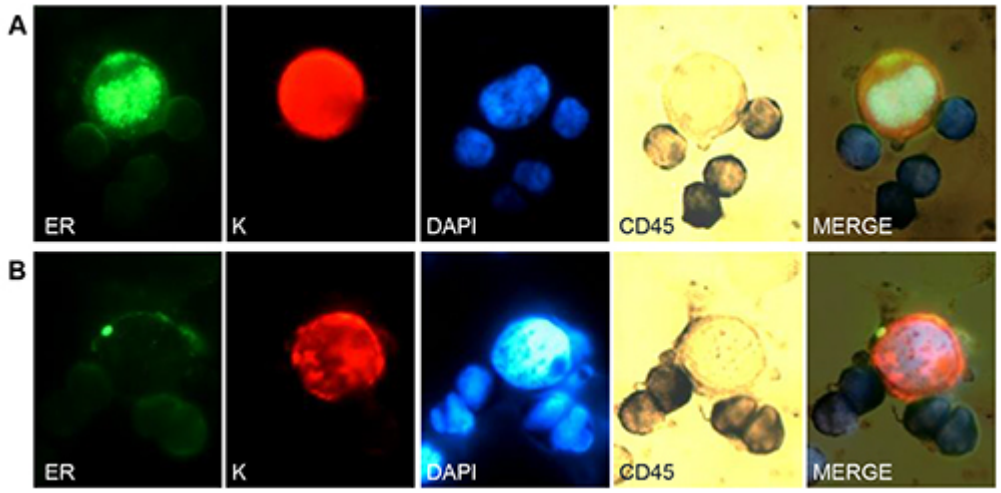
Patient-Friendly Cancer Testing and Monitoring Is “in the Blood”



Since the completion of the $3 billion Human Genome Project in 2003, genomic sequencing and analysis techniques have improved beyond all recognition. These advances have allowed companies to significantly reduce the cost of the technology. It is now possible to sequence a genome for less than $1,000.

Genomic techniques have improved so much that a simple blood test, a “liquid biopsy,” can detect a patient’s cancer and establish the cancer’s genetic makeup. Liquid biopsies can also help clinicians choose the most appropriate therapy, monitor its effectiveness, and determine whether it is encountering resistance.

Liquid biopsies in oncology initially focused on detecting and analyzing the numbers and types of circulating tumor cells (CTCs) in the blood, but more recently many researchers have focused on cell-free DNA (cfDNA) or circulating tumor DNA (ctDNA). cfDNA testing was first used in the United States in 2011 to diagnose Down’s syndrome, through analysis of fetal DNA present in the mother’s blood. Its use in oncology is more recent. In June 2016, the FDA approved the first liquid biopsy cancer test, the Cobas EGFR Mutation Test v2, for use in non-small cell lung cancer.

Because they are noninvasive, liquid biopsies are convenient and well tolerated and even greatly appreciated by patients. Unlike many traditional cancer biopsies, these tests ask nothing more of a patient than a blood sample. They also have the potential to reduce the amount of harmful radiation that patients are exposed to during investigation, treatment, and follow-up.

Many liquid biopsy tests are now in development, some of which are taking advantage of a new development: technology for examining exosome content. Exosomes are small, cell-derived vesicles that are released by tumor cells. They often carry nucleic acids and, as such, hold great potential as biomarkers for detecting and monitoring cancers.

Although there is a lot of evidence demonstrating the power of liquid biopsy techniques in oncology, much still needs to be done to bring it into clinical practice. Many of the tasks ahead will be discussed at Cell-Free DNA in Clinical Oncology, a Cambridge Healthtech Institute (CHI) conference that is scheduled to take place in Lisbon in 2017. This event will focus on the use of ctDNA and other biomarkers, both in research and clinical practice.

The Challenge of Early Detection

A key goal for clinicians and researchers working in this field is to use liquid biopsy testing to detect tumors at a very early stage. If successful, this would give patients the best possible chance of cure, while minimizing the amount of treatment needed, including the risk of undesirable side-effects.

With respect to the use of CTCs and ctDNA for early detection, Klaus Pantel, M.D., from the University Medical Center Hamburg-Eppendorf, Germany, tells GEN: “One common challenge is the low number and the low concentration of both CTCs and ctDNA. In the beginning, many researchers who worked with advanced stage cancer patients found that there is much more ctDNA than there are CTCs, but when you go to earlier stage cancer patients you have the same problem for both, in that you come to the detection limit of the assays.”

Lorena Diéguez, Ph.D., from the International Iberian Nanotechnology Laboratory in Portugal, is working on innovative methods to isolate cancer cells in the blood to allow early and noninvasive diagnosis of cancer.

“We are working very closely with clinicians in the development of point-of-care systems that base liquid biopsies on CTCs,” she explains. “We believe that so far, one of the limitations of liquid biopsy systems based on CTCs has been their cost and the fact that they rely on the expression of epithelial proteins in the CTC membrane. We are circumventing this issue by developing a low-cost antibody-independent system for CTC isolation based on microfluidics.”

In a medical specialty that is notorious for high-cost treatment, the development of cost-effective testing could have widespread implications, for example, by allowing lower-income countries better access to diagnostic investigations.

Discussing differences between using ctDNA and CTCs for diagnostic purposes, Dr. Diéguez points out that at present ctDNA testing is not at a

stage where it can be used in the absence of a tissue biopsy. ctDNA testing, she continues, is being used more to gain additional information at the point of diagnosis. She said that while CTCs are more difficult to collect than ctDNA, they can provide a lot more information.

Something that can be an issue with standard tumor biopsies, particularly if only a section of the tumor is removed for analysis, is that many tumors are made up of more than one cell type.

Peter Vandenberghe, Ph.D., University Hospitals Leuven, Belgium, comments on the role of ctDNA is this regard: “If there are molecular differences within the tumor, and if you neglect to sample all the areas within the primary tumor, then of course you might miss information. A very attractive feature of ctDNA is that it should, in principle, provide a representative mixture of all tumor sites.”

Another factor that needs to be considered regarding diagnosis is that some tumors shed much more DNA into the bloodstream than others. This means that it may not be possible to diagnose all cancers with this technology, as levels of ctDNA or tumor cells in the blood are simply too low. In glioblastoma, for example, ctDNA is not present at measurable levels in the peripheral blood due to the blood-brain barrier, according to Dr. Diéguez.

Monitoring Treatment Response and Personalizing Therapy

Measurement of ctDNA has shown great potential as a noninvasive method of monitoring cancer progression and the efficacy of therapy.

“A continuous monitoring of changing levels of ctDNA during initiation and maintenance of cancer therapy can be used to assess the patient´s response,” notes Ellen Heitzer, Ph.D., from Medical University, Graz, Austria. “More important, progression can be detected before it is clinically obvious.”

Dr. Heitzer explained that focusing on a single tumor mutation, such as one of those found in the *KRAS* gene, is the most common method that has been used to date, as this type of test can reach a high sensitivity level. In comparison, less targeted, genome-wide approaches need ctDNA levels to above a relatively high threshold (5–10% minimum) to produce useful results.

“One of the major problems is that every cancer has a unique fingerprint,” she complains. “There is no universal marker that can be used for these purposes.

“Although genome-wide approaches can comprehensively analyze a tumor’s genome, the lack of sensitivity…is a major drawback. Even in highly metastasized patients, there are clinical situations where ctDNA is present

below optimal levels for the detection of genome wide alterations. Approximately 20–30% of analyses will not yield any results."

Helen Winter, Ph.D., from the University of Oxford in the U.K., works on monitoring responses to radiation therapy in patients with metastatic liver cancer.

"We have seen changes in quantification and somatic mutation allele frequency in patients treated with selective internal radiation therapy," she explains. "Although the ability to detect radiation resistance earlier (or even before therapy) would be hugely beneficial to patients, we are planning to sequence the genome to look for evolving mutations after the high dose radiation that the liver malignancies receive."

"The hope," she adds," is that by having more information on who may respond, we will be able to truly tailor the therapies to patients that will be most likely to benefit."

Dr. Vandenberghe and his team are focusing on early response monitoring using ctDNA in people with Hodgkin's lymphoma.

"In Hodgkin's lymphoma, if you take a tumor biopsy, very limited numbers of malignant cells are present," he explains. "Most of the cells that you will find in this biopsy are reactive cells, which are nonmalignant.

"We found that we can retrieve such information from ctDNA relatively easily. This was quite a surprise because it's difficult to extract this information from the biopsy itself."

Dr. Vandenberghe and colleagues are currently observing a large series of patients with Hodgkin's lymphoma in a Phase II trial that is comparing standard treatment with an experimental drug. The scientists have tested participants at diagnosis and are also testing patients after treatment in each arm of the study to find out if there are changes in ctDNA.

Previous research has shown that tumor metabolism slows down more quickly than tumor tissue disappears. This can make response to treatment difficult to measure using imaging alone, remarks Dr. Vandenberghe:"CtDNA has a very short half-life, and as such, it gives you an idea of how much tumor is there in real time."

Reaching the Clinic

A key barrier to successful translation of liquid biopsy research into the clinic is a lack of test standardization, says Jens Habermann, M.D., Ph.D., from the University of Lübeck in Germany.

“The approaches in current use substantially vary from one study to another and compromise comparability,” he comments. “However, without comparability of study results, it will be impossible to implement the best suitable approach into clinical routine.”

Dr. Habermann is working to facilitate standardization of testing among the different biomarkers measured using liquid biopsy in oncology. “Preanalytic bias on sample quality has long been underestimated,” he explains. “The low rate of reproducibility of study results for clinical implementation has been alarming. Thus, professional centralized biobanks have evolved to ensure high quality of samples for research.”

He believes that a first step toward achieving better comparability of study results would be the creation of common guidelines describing best practice for use by researchers in this field. Nevertheless, he adds that “a close interaction of all disciplines…is necessary to succeed in paving the way for liquid biopsies to enter clinical routine.”

Dr. Pantel and his team use CTCs to predict whether a certain therapy will be effective and to monitor the efficacy of treatments, primarily in breast and prostate cancer. These investigators also use CTC-based techniques to help stratify patients for treatment with targeted therapies. “Patients that have *HER2*-negative primaries never get anti-*HER2* therapy,” he remarks. “We have now initiated a trial where we treat these patients if they have *HER2*-positive CTCs.”

Addressing the issue of liquid biopsy test sensitivity, Dr. Pantel comments that highly sensitive tests need excellent controls to avoid detection of false positives. He said that the tests have now become quite sophisticated and include barcodes to control for test artefacts, but that care is still needed in this regard.

Future Directions—Regulatory and Economic Hurdles

In general, liquid biopsy testing (CTCs and ctDNA) stands up reasonably well cost-wise against more standardized diagnostic and monitoring procedures such as CT scanning. As is often the case with new therapies and diagnostic tests, the new liquid biopsy products tend to be more expensive than the established products they are meant to accompany or replace, but it is likely that as demand for liquid biopsies rises, the economic burden will fall.

Regulatory approval of tests is key to encouraging broader use. The regulatory situation in Europe, Dr. Diéguez comments, could be influenced by developments in the United States, particularly FDA approvals for clinical use.

“Development of these systems normally arises from research laboratories and from established pharmaceutical and MedTech companies,” explains Dr. Diéguez. “The regulation for clinical use takes many years and needs various clinical trials in different countries by different laboratories, which is very costly. So normally the companies reach the market providing solutions for laboratory use only, while performing clinical validation.”

The different regulatory and health insurance systems in Europe also complicate the issue, with many countries not yet including liquid biopsy tests in their state reimbursement schemes.

“Europe is an assembly of different countries, and the individual countries have different rules,” says Dr. Pantel. He notes that in Germany, for example, ctDNA tests for EGFR were recently declared ineligible for reimbursement. “The pathologists in Germany made a very strong claim against liquid biopsy. At the same time, in other European countries, it was recommended for reimbursement.”

“In Germany, we have 10% of our patients with private health insurance,” he continues. “With this 10% of patients, you can do a lot of new diagnostics. Private health insurance is reimbursing many things that the governmental system is not reimbursing, so this gives you a very quick entry into a market.”

It seems that liquid biopsy is here to stay. With improved standardization and increasingly sensitive tests, as well as the introduction of new biomarkers such as exosomes into the field, it seems certain to become a major player in cancer diagnostics and monitoring in the future.