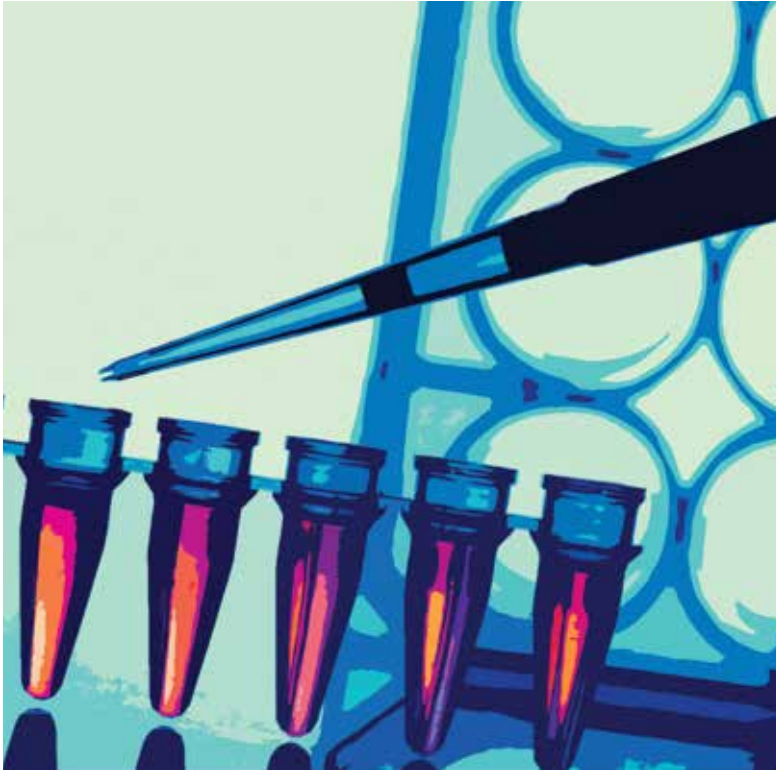


# Liquid Biopsy On Course To Transform Cancer Management



Liquid biopsies look set to revolutionize the management of cancer patients. A significant application could come in immuno-oncology, given the rapid uptake of IO drugs and the need to monitor changes in a patient's immune profile over time to guide therapy selection.

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## BY PETER CHARLISH

Liquid biopsy techniques greatly facilitate the genetic analysis of tumors, which is key to the effective use of new immunotherapies for personalized medicine in cancer patients.

Three types of liquid biopsy technology are employed in cancer management: circulating tumor cell analysis, circulating cancer cell DNA analysis and analysis of extracellular vesicles. In terms of market penetration, circulating tumor cell analysis is the most advanced.

So what? Liquid biopsy techniques are playing an increasing role in drug development, but it will be a hurdle to move them into clinical practice. Younger physicians are likely to be early adopters, whereas older physicians will likely cling to more traditional techniques such as tissue biopsy.

**A**lthough the survival rates for many types of cancer have improved significantly in recent years, cancer was still responsible for 8.8 million deaths worldwide in 2015, or nearly one in six of all deaths, making it the second biggest cause of death globally after coronary heart disease.

In the past few years, scientific advances such as tumor genome sequencing have helped to identify the genetic basis of many types of cancer. Indeed, genomic sequencing of biopsy material from human tumors has become an essential part of cancer diagnosis and treatment. Knowing which mutations are present in a patient's tumor can provide important information about which chemotherapeutic agent or targeted immunotherapy may or may not be effective in treatment.

However, such knowledge comes at a cost: obtaining a tumor biopsy can be a highly invasive procedure and is often challenging in difficult-to-reach sites such as the brain or lung, or may even not be possible at all. It can be painful for the patient, and carries a risk of infection and bleeding. To complicate the problem, biopsies must be obtained repeatedly if the response of a tumor to medication is to be monitored over time. And in cases where the primary tumor has been removed surgically, it may not be possible to sample sites of metastatic disease.

Consequently, alternatives to tissue biopsy have been sought. Ideally, a biopsy technique should be minimally invasive, be suitable for repeated use and relatively easy to perform, and the biomarker of interest should be easily and reproducibly quantifiable in the sample. The technique referred to as "liquid biopsy," which simply means that the sample analyzed is a biological fluid such as blood or urine rather than tissue, largely meets these conditions and has been increasingly adopted by oncologists in recent years.

In addition, liquid biopsy assays have the potential to facilitate personalized medicine, where the goal is to treat the right patient with the right drug, at the right dose, at the right time. (See Exhibit 1.) In oncology, such treatment decisions are based on the molecular abnormality profile of a tumor.

The liquid biopsy sector is growing fast. A report published earlier this year by market research firm MarketsandMarkets predicted that the liquid biopsy market will grow from \$0.58 billion in 2016 to \$1.66 billion in 2021, a CAGR of 23.4%, driven mainly by technological advances, growing awareness about liquid biopsy and a greater availability of funding for liquid biopsy research. Other estimates put the size of the potential market even higher.

In the oncology sector, liquid biopsy is largely, although not exclusively, confined to blood-based testing, and three different categories of marker are used in liquid biopsy assessment of cancer patients: circulating tumor cells (CTCs), circulating tumor DNA (ctDNA) and exosomes, or extracellular vesicles (vesicles secreted by neoplastic cells).

Of these, the most highly developed technique is the analysis of CTCs. Gene Walther, CEO of Vortex Biosciences Inc., one of the companies active in the CTC space, says that analysis of CTC DNA has several advantages over other approaches, one of the main ones being that genetic characteristics are not always phenotypic, so simply analyzing ctDNA does not tell the whole story. A number of clinical trials are underway that use liquid biopsy to monitor the effect of therapy, but four times as many of those are based on CTCs as on ctDNA.

**Circulating Tumor Cells**

CTCs are cancer cells that detach from a primary tumor and travel through the bloodstream or lymphatic system to other parts of the body. Their presence, which can often be detected quite early in the course of a cancer, is a fundamental prerequisite to metastasis, and their identification offers great potential not only in the diagnosis of cancer patients but also in assessing their progression and likely outcome.

Until recently, however, the potential

**Exhibit 1  
Potential Benefits Of Liquid Biopsy**

CHARACTERISTIC	BENEFIT
Liquid biopsy screening allows earlier diagnosis	More curable, early-stage cancers
Liquid biopsy lessens need for radiation-based imaging before/during/after treatment	Less imaging/ radiation exposure
Genetic profiles indicate optimum combination of therapies from all treatment options (targeted, immuno-, radio- and chemotherapy)	Personalized medicine includes all treatment options
Liquid biopsy facilitates development of targeted therapies with fewer side effects	Era of targeted therapies and immunotherapies

SOURCE: JP Morgan Securities LLC

of CTCs as an aid to cancer management was limited by their low abundance: detecting and counting them truly is like searching for a needle in a haystack. Fortunately, that has not stopped companies and academic groups from trying to develop CTC capture systems that can harvest enough of the target cells for genomic analysis.

The first CTC-based test to reach the US market was Janssen Diagnostics LLC/ Johnson & Johnson’s CellSearch CTC Test, which was originally cleared by the FDA for *in vitro* diagnostic use as long ago as 2004. The system uses ferrofluid nanoparticles coated with antibodies that target epithelial cell adhesion molecules to permit the magnetic separation of CTCs from whole blood. The system, which is also CE-marked for clinical use in the EU, is employed to inform clinical decisions in patients with metastatic breast, prostate or colorectal cancer at any stage during the disease.

In December 2016, Menarini Silicon Biosystems Inc., which is a member of the Menarini Group, announced that it had agreed to purchase all the assets and relevant business relating to the CellSearch CTC system from Janssen Diagnostics. The deal would enable the Bologna, Italy-based company to provide CTC detection on an FDA-cleared platform, thereby providing oncologists and researchers with access to “unparalleled molecular characterization capabilities.” Menarini Silicon Biosystems’ CEO, Giuseppe Geor-

gini, commented that “the combined capabilities of CellSearch together with our DEPArray system will enhance our potential to enable meaningful advances in the field of personalized medicine.” As well as CTCs isolated by CellSearch, the DEPArray system can be used for the molecular characterization of formalin-fixed, paraffin-embedded specimens and fine-needle aspiration samples.

Now, 13 years after the introduction of CellSearch, more than a dozen other companies are at various stages in the development of CTC systems that can be used in the management of cancer patients, as shown in Exhibit 2.

Among the companies whose products have already received regulatory approval for clinical use is UK-based ANGLE PLC, whose lead product, the Parsortix cell separation system, uses a proprietary microfluidic system in the form of a disposable cassette the size of a microscope slide to capture and then harvest CTCs from a blood sample. The technique is based on the fact that CTCs are larger and less deformable than other blood components. (Also see “ANGLE Targets A Rich CTC Niche In Liquid Biopsy” - *In Vivo*, March, 2016.)

In the Parsortix system, CTCs become trapped on a stepped channel that crisscrosses the cassette: they can either be stained within the cassette to allow identification and enumeration or, because the Parsortix system does not use an antibody to capture the CTCs, they can be

**Exhibit 2  
Companies Active In The CTC Sector**

COMPANY	SYSTEM	SEPARATION METHOD	COMMENTS
Actorius Innovations and Research (India)	OncoDiscover	N/A	N/A
AdnaGen (Germany)	AdnaTest	Immunomagnetic	CE-marked
ANGLE PLC (UK)	Parsortix	Microfluidic	CE-marked
Biocept (US)	Target Selector	Immunologic	Also detects ctDNA
Celsee Diagnostics (US)	PREP	Microfluidic	For research use
Clearbridge BioMedics (Singapore)	ClearCell FX	Microfluidic	For research use
Datar Genetics (India)	Cancertrack	N/A	N/A
Epic Sciences (US)	Epic AR-V7 Test	Optical	N/A
GILUPI Nanomedizin (Germany)	CellCollector	Immunologic	CE-marked
Menarini Silicon Biosystems (Italy)	DEPArray	Dielectrophoresis	N/A
Miltenyi Biotec (UK)	MACS	Magnetic	CE-marked
pluriSelect (Germany)	pluriBead	Immunologic	N/A
Rarecells (France)	ISET	Filtration	CE-marked
ScreenCell (France)	ScreenCell	Filtration	CE-marked
simfo (Germany)	Maintrac	Immunologic	N/A
STEMCELL Technologies (UK)	EasySep	Immunomagnetic	N/A
Vortex Biosciences (US)	VTX-1	Microfluidic	CE-marked

SOURCE: In Vivo Research

collected and submitted to downstream genetic analysis such as quantitative PCR sequencing.

ANGLE is currently carrying out two clinical studies (one in Europe, the other in the US) aimed at assessing the usefulness of Parsortix in triaging women with ovarian masses before surgery to determine whether the mass is benign or malignant. In January 2017, the company released interim data from the first 50 patients enrolled in each trial (patient enrollment is now complete at 200

women in each trial) that suggested that it is possible to differentiate accurately between women with ovarian cancer and those with benign tumors. More detailed results from the two trials are expected by the end of June, Angle's chief executive, Andrew Newland, told *In Vivo*.

In addition, ANGLE says it has successfully completed fundamental aspects of a US analytical study in metastatic breast cancer, with **University of Texas' MD Anderson Cancer Center** acting as lead. The company has engaged with insti-

tutional review boards at six US cancer centers to provide patient samples and to process them with Parsortix for subsequent analysis. The studies are on track for completion by the end of calendar year 2017. Other ongoing investigations into use of Parsortix include a study of head and neck squamous cell carcinoma in association with the **University of Athens and Attikon University Hospital** in Greece, and a prostate cancer study in collaboration with **Barts Cancer Institute, Queen Mary, University of London**.

The Parsortix system is CE-marked for use as an *in vitro* diagnostic device in the EU and is available for research purposes in the US; it does not yet have FDA approval for clinical applications. The installed base of Parsortix instruments stood at more than 145 at the end of April 2017, up from around 85 one year previously. Total revenues for the year were up by more than 40%, and sales are expected to be around £0.5 million for the year.

If the early findings of the ovarian cancer studies are confirmed, they should greatly help the company get approval for the system from the FDA. However, as analysts at Edison Investment Research pointed out in a recent note, the CTC analysis sector is evolving rapidly, and ANGLE's greatest challenge will be to communicate with and convince potential users of the benefits of Parsortix compared with competitive products. "Key to widespread adoption of Parsortix in the clinical markets, and therefore substantial sales, is the continued positive evaluations of the system and demonstration of its clinical utility by KOLs and the data from the initiated prospective clinical studies," they say. The same is probably true of other companies' technologies.

While ANGLE, like several competitor companies, uses a microfluidic technique to isolate CTCs, others have adopted a different route. GILUPI Nanomedizin, based in Potsdam, Germany, is one of several companies that has taken an immunologic approach. In the GILUPI system, known as *CellCollector*, a thin stainless steel infusion catheter is inserted into a vein in the patient's arm and remains in place for about 30 minutes. The catheter has a gold coating bearing a three-dimensional polymer functionalized to attract cells that express epithelial cell surface markers such as the epithelial cell adhesion molecule EpCAM. Functionalization is achieved through specific antibodies against EpCAM and other cell surface antigens. After 30 minutes the catheter is withdrawn for downstream processing.

According to GILUPI, the *CellCollector* system overcomes the restrictions of a limited blood sample: whereas most diagnostic approaches focus on maximizing the efficient exploitation of a blood sample, the *CellCollector* is designed to

collect the targeted cells *in vivo* from the peripheral bloodstream.

In January 2017, GILUPI released clinical data from a prostate cancer study that demonstrated the isolation of CTCs from the blood of non-metastatic, high-risk prostate cancer patients using the *CellCollector* system. The aim of the study was to increase the sensitivity of CTC detection through the combination of three complementary assays, including the *CellCollector* system: it not only revealed a high incidence of CTCs in these patients, but also showed that use of the *CellCollector* system could circumvent the usual restriction of having to analyze large blood volumes from cancer patients. The study also confirmed a reduced number of EpCAM-positive CTCs in the circulation following radical prostatectomy.

### Electric Field

Menarini Silicon Biosystems' DEPAArray system is based around a completely different separation technique to other systems. It is based on the ability of a non-uniform electric field to exert forces on neutral, polarizable particles, such as cells, that are suspended in a liquid, a phenomenon known as dielectrophoresis (DEP). The technique can be used to trap cells in DEP "cages" by creating an electric field above a subset of electrodes in an array that is in counter phase with the electric field of adjacent electrodes. When a DEP cage is moved by a change in the electric field pattern, the trapped cell moves with it. The DEPAArray System combines this technology with high-quality, image-based cell selection to create the ability to identify and recover specific individual cells of interest from complex, heterogeneous samples.

Last year, a study published in *Nature Medicine* showed that genetic patterns found in CTCs isolated from lung cancer patients using the DEPAArray System correlated with clinical outcomes. Historically, lung cancer has been relatively poorly studied compared with other cancer types because of the challenges in collecting tumor biopsies for genetic analysis.

A more recent market entrant is Vortex Biosciences, which has its headquarters in Menlo Park, CA. In February this year the company launched its *VTX-1* Liquid Biopsy System at the *Molecular*

*Medicine Tri-Conference Annual Meeting* in San Francisco, following the award of CE-marking in November 2016. The system is initially being distributed into the research use only market in the US, although Vortex is currently talking with potential distributors with a view to launching the system globally early in 2018, CEO Walther told *In Vivo*.

The *VTX-1* system selectively captures CTCs from whole blood using microvortices in the proprietary *Vortex Chip*. The chip comprises a narrow channel followed by a series of expansion regions termed reservoirs. Flow through the narrow entry channel is relatively rapid, which gives rise to inertial forces that direct larger cells into trapping vortices in the reservoirs, where they remain until the flow is interrupted. The size of the cells trapped is dependent on several factors including the cross-sectional area of the reservoirs. Suitable design of the reservoirs thus allows control over the size of cells collected.

The system requires no sample preparation: the blood draw is added to a disposable cartridge that is inserted into the instrument. Thereafter the process is fully automated and allows the collection of CTCs within roughly an hour; they are then ready for downstream enumeration and processing such as genomic or proteomic analysis or *in vitro* culture, the latter being particularly relevant to drug development and cancer research. Walther says the *VTX-1* system can collect CTCs with fewer than 100 contaminating white blood cells per milliliter, thanks to the unidirectional flow through the *Vortex Chip*. This is significant because cells other than CTCs can contaminate the DNA extracted for subsequent analysis.

Vortex is a core subsidiary of AIM-listed NetScientific PLC, a transatlantic biomedical and health care technology group that sources, develops and manages early/mid-stage health care technology companies with a focus on diagnostics, digital health and therapeutics: NetScientific currently owns 95% of Vortex, having invested just over £13 million in the business. However, Vortex is now seeking its own Series A funding, which Walther says he hopes will be completed later this year.

One other recent notable event in the



CTC area was the setting up in November last year of a research partnership between Clearbridge BioMedics and the John Wayne Cancer Institute at the St John's Health Center, Providence Health Systems in Santa Monica, CA, with the establishment of a Circulating Tumor Cell Center of Research Excellence (CTC CoRE). The initial focus of the center is melanoma, followed by epithelial cancers. It is envisaged that the center will evolve into a CLIA service for testing blood samples to support diagnosis, treatment monitoring and the development of personalized treatments.

Clearbridge had previously set up a CTC CoRE in collaboration with the National Cancer Centre Singapore and Singapore General Hospital in 2014. Commenting on the JWCI partnership, Michael Paumen, PhD, Clearbridge's CEO, said that the company "will continue working towards validation of clinical utility of ClearCell FX System, to help oncologists have access to better tools to diagnose, treat and manage cancer."

### Circulating Tumor DNA

Circulating tumor DNA (ctDNA) is cell-free DNA that is released by tumor cells into the circulatory system. While cell-free DNA can be detected in a number of conditions, ctDNA is distinguished by the presence of specific somatic mutations that appear to correlate with mutations in the DNA of the original tumor.

The mechanism by which ctDNA enters the circulation is not fully understood: it may be secreted by viable tumor cells, it may arise as a consequence of tumor cell death under the effect of chemotherapy, or it may be released by phagocytes that have engulfed tumor cells. Whatever its origin, it has a very short half-life in blood – around one or two hours.

Compared with tissue biopsy, ctDNA offers several advantages, many of which it shares with CTCs. Its unique benefit is that it affords a better reflection of tissue heterogeneity in tumors. This is because gene expression often varies in different parts of the same tumor, so taking a biopsy from a single site may give an incomplete picture, but circulating ctDNA gives a snapshot of the tumor genome overall.

One company active in the ctDNA area is the Belgian start-up OncoDNA SA. Last

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year it launched *OncoTRACE*, a next-generation sequencing (NGS) test capable of monitoring up to 15 gene variants in ctDNA obtained from a patient's blood. The variants are initially identified using the company's *OncoDEEP* theragnostic product, which combines DNA, RNA and protein analysis of biopsy samples. Jean-Paul Detiffe, CEO and founder of OncoDNA, commented that the test can identify drug resistance or potential recurrence earlier than is possible with imaging technologies.

OncoDNA was founded in 2012 to focus on precision medicine in oncology. In 2014, the company announced its involvement in AURORA, a multi-year analysis program designed to improve the quality of life in patients with metastatic or locally relapsed breast cancer: OncoDNA is using the *OncoDEEP* platform to perform cancer-related targeted gene sequencing in up to 1,300 patients treated in about 60 hospitals in 15 European countries. In 2015 OncoDNA achieved a turnover of just over €1 million, (\$1.1 million) and last year raised €7.7 million (\$8.5 million) in private equity – shareholders include Bio.be, Sambrinvest and private investors.

Another company developing ctDNA testing is *Inivata Ltd.*, whose proprietary technology is based on research carried out at the Rosenfeld Laboratory at **Cancer Research UK (CRUK)** and licensed from CRUK and the **University of Cambridge**.

The company currently has facilities in Cambridge, and a CLIA-accredited laboratory in Research Triangle Park, NC. A Series A funding round in early 2016 raised around \$50 million/£32.5 million, with investors including Touchstone Innovations, Cancer Research Technology and Johnson & Johnson Innovation. Clive Morris, MD, Inivata's chief medical officer, told *In Vivo* that the company is currently starting a Series B funding round.

Inivata's *InVision* technology platform is a tagged-amplicon sequencing (*TAm-Seq*) technique that permits the amplification and deep sequencing of genomic regions from individual copies of fragmented DNA. A standard blood draw from a patient is spun down and DNA is extracted from the plasma using a commercially available kit. Inivata, via its laboratory in Cambridge or its CLIA facility in the US, then quantifies the presence of specific cancer-related gene mutations, a process it refers to as "comprehensive genomic profiling." Analysis of the data using proprietary algorithms is then used to generate a detailed report.

Morris said the data included in the report can provide clinically actionable information at every stage of the treatment cycle, from earlier diagnosis, molecular stratification, monitoring of treatment response (including emergence of drug resistance), detecting disease relapse and developing a personalized treatment plan.

So far, Inivata has been concentrating its activities mainly on lung cancer. In March 2017, the company announced the results of a two-year study, carried out in partnership with the Gustave Roussy cancer center in France, into the use of the *InVision* platform to guide treatment in 48 patients with advanced non-small cell lung cancer (NSCLC) who had developed resistance to tyrosine kinase inhibition. T790M mutations, a known mechanism of acquired resistance to tyrosine kinase inhibitors, were detected in half the patients studied, a proportion consistent with the detection rate seen in tissue biopsies. Morris told *In Vivo* that the patients in the study were unable to undergo traditional tissue biopsy for reasons such as lack of available tissue or the localization of the tumor.

Last October, the company announced

the launch of a US clinical validation study (INI-001) of the InVision technology in the management of NSCLC. The prospective study is being conducted at more than 30 centers across the US, and is expected to recruit several hundred patients. Its goal is to compare the performance of Inivata's targeted molecular profiling liquid biopsy platform with tissue-based molecular profiling in patients with advanced NSCLC. It will be carried out by the CRO Vector Oncology, based in Memphis, TN.

The month after Inivata announced the INI-001 study, two other companies active in the ctDNA sector, **Trovogene Inc.** and the privately held Canadian company **Boreal Genomics Inc.**, entered a long-term supply and distribution agreement that had the effect of merging their respective technologies to co-develop urine and blood ctDNA assay kits. The stated aim of the partnership is to address an unmet need in the liquid biopsy testing market by providing a simple, routine and low-cost ctDNA assay for NGS platforms run by cancer centers, research institutions and other laboratories. Both companies are contributing proprietary technology to help develop a series of cancer-specific multigene panels scheduled for launch this year. Trovogene would distribute such kits globally to run on Boreal's *OnTarget* system.

Trovogene, based in San Diego, develops technology for detecting DNA in urine and blood for use in pharmaceutical development, clinical research and medical testing across a variety of clinical disciplines, including oncology and virology. It commercializes its technology by providing testing services from its CLIA/CAP-accredited laboratory in San Diego, as well as by distributing research use only kits and systems to clinical research laboratories, cancer centers and pharmaceutical companies. For its part, Boreal is developing techniques for removing DNA damage and sequencing errors in liquid biopsy applications based on a proprietary linked-library preparation technology.

### Extracellular Vesicles

Once considered merely waste products of cellular activity, extracellular vesicles (EVs) are small lipid vesicles that contain specific proteins, mRNAs, long non-coding

RNAs and microRNAs (miRNAs). They are believed to have a role in intercellular communications. Some of the nucleic acid species they contain are believed to be involved in processes associated with cancer cell development, such as tumor initiation, drug resistance, immune surveillance, angiogenesis, invasion and metastasis, and they may function as biomarkers.

One company active in this sector is privately held **Exosome Diagnostics Inc.**, based in Cambridge, MA, which in February this year entered an agreement with **Merck KGAA** to help the German company's drug development efforts in oncology and other areas. The deal includes access to Exosome's *Shahky* exosomal protein capture and quantitative analysis instrument, whose high signal-to-noise capabilities "make it a powerful technology for discovering, assessing and validating clinical biomarkers," according to Exosome.

Although it can be used as part of a drug discovery program, *Shahky* was designed and developed as a medical device. At the beginning of 2017, Exosome announced that the instrument had been placed within a major (unidentified) hospital in Boston, making it the world's first point-of-care liquid biopsy instrument, the company said. System placement and consumables are expected to drive significant incremental revenues in 2017.

In addition to *Shahky*, Exosome has developed a nucleic acid isolation technology platform known as *ExoLution*. In November 2016, Exosome announced that a partnership with **Takeda Pharmaceutical Co. Ltd.** had produced data demonstrating the power of exosomal RNA for long RNA sequencing. The clinical RNA biomarker area is dominated by mRNA, but historically long RNA has been difficult to isolate and measure. The *ExoLution* technology is expected to enable new insights into patients' genetic makeup.

Exosome also has an agreement with **Amgen Inc.** to evaluate the potential of its liquid biopsy technology platform to advance drug development.

Considerable interest in EVs has also come from academic labs. For example, researchers at the Cancer Institute at **Pennsylvania State University College of Medicine** recently published a technique

for capturing EVs from plasma using lipid nanoprobe and a biotin-avidin-based magnetic separation system. This allows the DNA in the EV to be subsequently analyzed. Meanwhile, researchers at Ulsan National Institute of Science and Technology in Korea have developed an integrated centrifugal microfluidic platform, known as *Exodisc*, for isolating EVs from urine.

### Conclusions

The billion-dollar liquid biopsy market can be broken down into three segments: treating patients who are already in remission, to scan for recurrence; tailoring therapy to individual patients via genetic tumor analysis; and to screen healthy populations as a way of early cancer detection. And this does not include the research-only sector.

Clearly, several issues remain to be addressed before liquid biopsy can realize its full potential, including:

- validation of biomarkers and the technology used to measure them;
- optimization of isolation and analytical systems;
- obtaining regulatory approval;
- education of clinicians about the benefits of liquid biopsy, including carrying out studies in association with key opinion leaders; and
- establishing reimbursement status, including generating outcomes and cost-effectiveness data.

The main hurdle is data, says Vortex's Walther: it will take time to generate the data to confirm the correlation between tissue biopsy and liquid biopsy. A large amount of work in this area is currently underway. Once those data are available, the second hurdle will be to convince physicians of the validity of liquid biopsy. Walther believes the younger generation of physicians are likely to be early adopters, whereas older physicians, more set in their ways, will likely cling to more traditional techniques such as tissue biopsy.

However, with liquid biopsy technology already at an advanced stage of development, progress over the coming months and years is likely to be even more rapid. ▶

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#### Comments:

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