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Liquid Biopsy In Oncology: An Increasingly Crowded Landscape

by Peter Charlish

Liquid biopsies – carrying out diagnostic tests on liquid samples such as blood or urine rather than on tissue biopsy material – look set to revolutionize the management of cancer patients. This area has recently captured the interest of a growing number of diagnostic players, both big and small, and spawned the development of different approaches to liquid biopsy. This article marks out who's who in this increasingly busy landscape and the key technologies that are showing promise, and looks at the obstacles that companies are facing.

The ability to diagnose a tumor and monitor its progression and response to treatment without the need to obtain a tissue biopsy has been a long-standing goal of cancer management. This capability is now in hand thanks to the technology known as liquid biopsy, currently one of the most exciting sectors of the *in vitro* diagnostics market.

The term 'liquid biopsy' is used to refer to a noninvasive diagnostic test that can identify various types of cancer or other conditions by detecting biomarkers in fluid samples such as blood or urine, obviating the need to obtain a tissue biopsy. This approach is being pioneered by several companies and is expected to bring multiple benefits, not least the fact that such tests are relatively quick and easy to perform and are far less invasive than tissue biopsy.

Although the main target of efforts to develop liquid biopsy products up to now has been cancer, the technology also has other potential applications, such as prenatal diagnosis of chromosome abnormalities via a blood sample or monitoring chronic kidney disease via urinary proteome analysis.

Four Distinct Cancer Applications

In a recent report on liquid biopsy, analysts at financial services company JP Morgan noted that

although the technology is still in the early stages of development and adoption, it has the potential to be a powerful tool in guiding physicians to the most appropriate course of therapy for any given patient.

The analysts envisage four distinct applications for the technology. Closest to realization is probably its use in theranostics, as a companion diagnostic to guide targeted therapeutics. By 2020, this market could be worth $2bn a year, they say. Within two to three years from now, liquid biopsy technology will also come to be used for predicting the likely course and outcome of disease for individual patients, the analysts believe, a market that could be worth an additional $4-7bn by 2020. (*See Figure 1*.)

A little further in the future, the JP Morgan analysts believe that liquid biopsy will increasingly be used for monitoring therapy, particularly for tracking drug-resistant mutations and quantifying the response to treatment. This segment could be worth an additional $5bn by the end of the decade. However, the largest potential market for liquid biopsy would be its use as a screening tool, for testing the general asymptomatic population for particular types of cancer. Although this application is not likely to take off before 2020, it could eventually be worth as much as $9bn annually, they say.

Figure 1

Total liquid biopsy market, 2020

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JP Morgan forecasts of the liquid biopsy market for cancer, 2020

Source: JP Morgan

Drivers And Challenges

While there are a number of factors driving the uptake of liquid biopsy, the JP Morgan analysts also identify a several challenges. Among the drivers is the improved patient experience from a noninvasive approach to molecular profiling of a tumor compared with tissue biopsy, especially for patients who require serial biopsies, for example to monitor the development of drug-resistant mutations. In addition, the liquid biopsy approach is potentially less expensive than a tissue biopsy, as it does not require the same level of highly trained professional to obtain the sample, and offers a quicker turnaround time.

On the other hand, the key challenge to uptake of liquid biopsy is the cost of actual sequencing: at present, it is only possible to obtain an adequate depth of sequencing at a cost that is too

expensive for routine clinical use. However, just as the cost of whole genome sequencing has fallen dramatically, so will the cost of sequencing DNA from liquid biopsy samples.

An additional hurdle that needs to be overcome is a lack of physician and insurer awareness of liquid biopsy. It is still a relatively new technology, and health professionals are only just beginning to appreciate the benefits that liquid biopsy may bring. Similarly, until the technology is more widely accepted, there are likely to be reimbursement issues associated with its use.

A number of companies are already marketing liquid biopsy products for use in cancer management, and several others have revealed plans to enter the market. However, those that have already put a toe in the water tend to be in the early stages of product ramp-up or have had limited experience in commercializing such products so far, the JP Morgan analysts state.

Companies in this sector break down into several broad categories. There are those with systems that capture circulating tumor cells (CTCs) in the bloodstream for use as a biomarker in liquid biopsy; those that offer sequencing platforms *per se* for analyzing the CTCs or circulating tumor DNA (ctDNA) biomarkers; those that offer next-generation sequencing (NGS) instrumentation; and those that operate reference laboratories offering liquid-biopsy testing.

CTCs And ctDNA

Of the different types of marker used in liquid biopsy assessment of cancer patients, the oldest and most highly developed technique is the analysis of CTCs – 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 the disease, is a fundamental prerequisite to metastasis, and their enumeration thus offers great potential for diagnosing cancer patients but also in assessing prognosis. Furthermore, the molecular characterization of CTCs can provide considerable information about things like the sensitivity of the primary tumor to treatment and its potential for developing resistance to anticancer agents.

Until recently, however, the potential of CTCs as an aid to cancer management was limited by the low abundance of the cells; detecting and counting them has been compared with finding a needle in a haystack. That has not stopped companies from trying to develop CTC capture systems that can harvest enough of the target cells as well as maintain the integrity of the cells. *Janssen Diagnostics LLC*'s *CellSearch* CTC test was originally

Potential benefits of CTC counting and characterization

- May lead to more accurate prognosis
- May assist in measuring response to anticancer therapy

cleared by US FDA for *in vitro* diagnostic use in 2004 and, 12 years later, several firms are at different stages of taking their CTC system to market. (*See Table 1*).

Table 1

- May help in selecting patients for adjuvant chemotherapy
- May help detect disease recurrence
- May act as surrogate indicator of therapeutic effect
- May assist in the detection of drug-resistant tumors
- May help in identifying new therapeutic targets

Source: MG Krebs et al., Therapeutic Advances in Medical Oncology, 2010, 2(6), 351-365

Company	System	Separation method
ANGLE	Parsortix	Microfluidic
CellSearch	CELLSEARCH System	Magnetic
Clearbridge Biomedics	ClearCell FV	Microfluidic
Epic Sciences	Epic AR-V7 Test	Optical
GILUPI Nanomedizin	CellCollector	Immunologic
Miltenyi Biotec	MACS	Magnetic
pluriSelect	pluriBead	Immunologic
ScreenCell	ScreenCell	Filtration
SIMFO	Maintrac	Immunologic
STEMCELL Technologies	EasySep	Immunomagnetic

One such company is UK-based ANGLE plc. Its lead product is the *Parsortix* cell separation system, which uses a patented microfluidic system in the form of a disposable microscope slide-sized cassette to capture and then harvest CTCs from blood. The separation process is predicated on the larger size and less deformable nature of CTCs compared with other blood components.

In the Parsortix system, CTCs are trapped on a step that crisscrosses the cassette, where they can be fixed and stained to allow identification and enumeration. And because Parsortix uses a label-free approach that does don't require an antibody to bind to the CTC, the captured cells can be used for downstream genetic analyses such as quantitative PCR sequencing.

Parsortix is CE-marked for use as an *in vitro* diagnostic device in the EU, but the company, so far,

is commercializing it for research use only and is conducting several programs to build evidence of Parsortix's utility as a clinical diagnostic tool. Angle has partnered with several research and medical institutions to investigate the use of its system as a diagnostic tool for several cancers, ovarian cancer being its most advanced program, followed by breast and prostate cancer. (Also see "*Angle Strikes Out Into New Cancer Dx Markets* " - Medtech Insight, 25 May, 2016.). The company is also working towards gaining US clearance for Parsortix.

Many liquid biopsy approaches target circulating tumor DNA, rather than whole CTCs, as the biomarker. ctDNA is cell-free DNA that is shed from tumor cells into the circulatory system. Cell-free DNA occurs in a number of other conditions, including renal failure and myocardial infarction, but ctDNA is distinguished by the presence of specific somatic mutations that appear to correlate with mutations in tumor DNA. 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 only has a half-life in blood of perhaps one or two hours: it is typically between 180 and 200 base pairs in length.

Like CTCs, ctDNA has several advantages over tissue biopsy as a source of cancer biomarkers, including quick and easy collection, low risk of complications such as pain or infection, and a better reflection of tissue heterogeneity in tumors – gene expression often varies in different parts of the same tumor, so while taking a biopsy from a single site may give an incomplete picture, circulating ctDNA gives a snapshot of the tumor genome overall.

Sequencing Platforms

Another company in the liquid biopsy space is *Biocept Inc.*, which develops cell-free tests using proprietary technology to capture and analyze ctDNA in both CTC and plasma. It offers NGS-based tests for assessing targeted mutations in non-small cell lung cancer (NSCLC) and gastric and breast cancers as well as a test based on NGS to monitor breast cancer patients. It also offers tests for colon and prostate cancer and melanoma.

In May 2016, Biocept launched its *Liquid Biopsy Immuno-Oncology PD-L1 Test*, which uses the company's *Target Selector* platform with CTCs from a patient's blood sample to detect and monitor PD-L1

Exosomes et al.

Exosomes, or extracellular vesicles (EVs), are also used as biomarkers in some liquid biopsy approaches. EVs are small membrane vesicles that contain specific proteins, mRNAs, long non-coding RNAs, and microRNAs (miRNAs), and play an important role in intercellular communications. Some of these nucleic acid species 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

protein expression throughout the course of a patient's cancer therapy. Patients with cancers that express the PD-L1 protein are more likely to respond to immunooncology therapeutics such as [Merck & Co. Inc.](#)'s *Keytruda* (pembrolizumab), which was recently approved for use in patients with advanced NSCLC who test positive for PD-L1 expression using Dako North America's *PD-L1 IHC 22C3 pharmDx* immunohistochemical assay, based on testing of formalin-fixed, paraffin-embedded NSCLC tissue.

may function as biomarkers.

Launch of its PD-L1 test came just days after Biocept introduced a test to detect *RET* oncogene fusions in blood samples from patients with lung cancer. Positive identification of patients with the *RET* gene can help guide treatment with tyrosine kinase inhibitors.

Michael Nall, Biocept's president and CEO, commented that the new Liquid Biopsy PD-L1 test is one of the few, if not the only, commercial, CLIA-validated, blood-based test for detecting PD-L1 expression. He said that the test provides a new option for physicians to qualify patients for approved immuno-oncology therapies and for companies developing such products (Ono, Novartis and Pfizer are among the companies developing anticancer agents that act in a similar way to Keytruda).

Biocept works closely with pharma companies. In March of this year it announced a collaboration with an unspecified biopharmaceutical company on a clinical trial analyzing biomarkers using both CTCs and ctDNA from cerebrospinal fluid. The trial is being conducted in patients with NSCLC whose disease has spread to the brain or meninges, a condition known as leptomeningeal disease. In May, Biocept said it had entered a multi-project Master Services Agreement whereby it is developing research and clinical trial assays for a large pharma organization.

Biocept plans to introduce further CLIA-validated tests in the near term. Nall told *Medtech Insight* that the company has already commercially launched 10 other assays that have undergone rigorous analytical and clinical validation in its CLIA laboratory. "The goal is to offer medical oncologists and the patients they care for the same NCCN [National Comprehensive Cancer Network] guideline-based biomarkers used for clinical decision making via liquid biopsy that are traditionally performed on a tissue biopsy. In addition, we offer many other markers for both Pharma and academic research."

Another early mover in the liquid biopsy area is [Trovagene Inc.](#) which, like Biocept, is headquartered in San Diego, California. Trovagene is one of the few market participants focused on developing urine-based liquid biopsy tests, although it does also produce blood-based tests. The company points to the advantages of urine over blood: samples can be collected conveniently in the patient's own home, there is a virtually unlimited sample supply, and

information about treatment can be obtained faster and more often. Indeed, Trovagene has registered the trade mark "Yellow is the new red".

Trovagene's platform technology is a polymerase chain reaction-based technique known as *Precision Cancer Monitoring* (PCM), and is the basis of its CLIA and CAP-accredited Trovera line of urine- and blood-based liquid biopsy tests for quantifying oncogenic mutations of genes such as *EGFR* (for lung cancer), *KRAS* (for lung, ovarian, pancreatic and colorectal cancers) and *BRAF* (for melanoma, Erdheim-Chester disease and Langerhans cell histiocytosis, two rare conditions related to leukemia).

Trovagene's technology includes a novel enrichment technique optimized for small DNA fragments in urine, and has been validated in a number of studies published over the past two years or so. "We have demonstrated the analytic performance of our test as well as the clinical performance," Mark Erlander, Trovagene's chief scientific officer told *Medtech Insight*. Most recently, at the 2016 American Society of Clinical Oncology (ASCO) meeting in Chicago, Illinois, clinical results demonstrating highly sensitive detection of *EGFR* T790M mutations in the urine of patients with NSCLC were presented. "Trovagene's urinary ctDNA test was able to identify the *EGFR* resistance mutation in cases not detected in tissue," said Karen Reckamp, one of the lead investigators. "The data suggest that urinary and plasma *EGFR* analyses complement tissue biopsies in *EGFR* tyrosine kinase inhibitor (TKI) resistant NSCLC."

Although *EGFR*-TKIs such as erlotinib (*Astellas Pharma Inc.* 's *Tarceva*), afatinib (Boehringer Ingelheim GMBH's *Gilotrif*) and gefitinib (*AstraZeneca PLC* 's *Iressa*) have demonstrated response rates as high as 80% in patients with NSCLC, most tumors subsequently develop resistance related to the T790M mutation. Last November the FDA approved osimertinib (AstraZeneca's *Tagrisso*) for the treatment of NSCLC patients with this mutation: several other drug candidates are in development for this indication.

Like Biocept, Trovagene has worked with a number of pharma companies in this area, including a partnership with Clovis Oncology as part of the diagnostic arm of a third generation tyrosine kinase inhibitor development program. In addition, the company is engaged in numerous clinical collaborations with leading cancer centers and academic institutions. Its technology is backed by a "robust" intellectual property portfolio, Dr Erlander said.

Rather than look for specific changes in genes or protein expression, San Jose, California-based *Chronix Biomedical Inc.* has developed technology that takes a more holistic approach by measuring chromosomal gains or losses in cell-free DNA and calculating the genomic copy number instability index (CNI). The CNI technique is based on sequencing studies conducted by the company showing that, among 500 cancer patients and a similar number of matched controls, the main difference was not, as expected, at the level of individual single nucleotide polymorphisms but in regions on the genome where the number of DNA fragments differed

between the two groups. In other words, if there are more or fewer DNA fragments in one area of the test genome compared to the same area of a matched control genome, this represents genomic instability caused by bad local DNA repair, which is indicative of cancer.

The company's *Delta Dots* test is designed to calculate the CNI score as an indicator of how well the patient is responding to cancer therapy. Earlier in April, at the American Association for Cancer Research meeting in New Orleans, Louisiana, data from a 24-patient, blinded proof-of-concept study showed that Delta Dots was able to correctly stratify patients as either responders or non-responders to chemotherapy in 92% of cases, compared with the industry standard for measuring cancer treatment response (RECIST). (Also see " *Chronix's new liquid biopsy approach show promise as early predictor of cancer therapies* " - Medtech Insight, 18 Apr, 2016.). Chronix is also investigating Delta Dots for measuring response in two other types of cancer therapy: immunotherapy – the company had presented early data for this indication at ASCO in June (Also see "*ASCO Round-Up: Chronix Delta Dots, HTG EdgeSeq, and Guardant360 Liquid Biopsy*" - Medtech Insight, 9 Jun, 2016.) – and radiotherapy. Evidence from Chronix's studies of Delta Dots have suggested that the test can predict the patient's response to therapy as early as two weeks, which mean physicians can change the patient's treatment earlier if required to a regime that is more effective.

As well as the Delta Dots test, Chronix offers the *Second Opinion* supplemental test for breast and prostate cancer. However, rather than replace mammography or PSA testing, Second Opinion is intended to provide additional information that can be used with the CNI score to assist doctors' decision-making.

The company is also seeking to develop a "pan-cancer test", which would be able to screen asymptomatic patients for cancer. This is based on the principle that "if you have gains or losses in your blood, you have cancer somewhere," Chronix CEO Howard Urnovitz had told *Medtech Insight* in an interview earlier this year.

More Established Players

The liquid biopsy area has also attracted more established diagnostics companies such as Hilden, Germany-based *Qiagen NV* , a global leader in technologies for the extraction and isolation of nucleic acids from biological samples. In 2015, Qiagen introduced the world's first regulated companion diagnostic for lung cancer based on liquid biopsy, the CE-marked *therascreenEGFR* RGQ Plasma PCR kit, which was developed in collaboration with AstraZeneca and paired with AZ's Iressa. The test enables *EGFR* mutation profiling in patients for whom surgical biopsy is not an option, thereby permitting physicians to select patients who could benefit from Iressa treatment. Also in 2015, Qiagen launched a fully automated protocol for isolation of ctDNA from human plasma on the *QIAsymphony* platform for research applications.

In March 2015 Qiagen acquired technology from the German company *AdnaGen AG* that enables

enrichment and molecular analysis of CTCs from blood samples. At the same time, Qiagen entered a partnership with *Tokai Pharmaceuticals Inc.* to co-develop a companion diagnostic using the CTC technology to guide the use of Tokai's androgen receptor modulator/lyase inhibitor galeterone, which is in Phase III trials for treatment of castration-resistant prostate cancer.

In addition, Qiagen is collaborating with *Exosome Diagnostics Inc.* to develop products for the extraction of exosomes from blood and other body fluids. A kit for the isolation of RNA from plasma or serum to analyze mutations and gene expression profiles was introduced in 2014 for use in cancer research. The companies are also working on a non-invasive test for the analysis of key biomarkers in NSCLC and other malignancies: this test could potentially be developed into a companion diagnostic for several new cancer drugs.

Michael Kazinski, senior director and head of Qiagen's liquid biopsy franchise for life sciences, told *MedTech Insight* he sees point-of-care testing as a likely application for the future of liquid biopsy technology. "Acceptance will rely on easy-to-perform assays and on standardization of sample collection and stabilization procedures," he said. "Further, automation will be of increasing importance for cost and standardization reasons." Kazinski also expects a clearer picture to emerge on which analytical domain (CTC vs ctDNA) is the most appropriate to use for the various applications, and a stronger utilization of NGS-based assays.

Sysmex Inostics GMBH , the molecular diagnostics subsidiary of Sysmex Corp, is another established diagnostics outfit that has been drawn to liquid biopsies. It is collaborating with *Merck Serono SA* on the introduction of the *OncoBEAM RAS* digital polymerase chain reaction assay, which detects ctDNA in blood or plasma and which is designed to provide information on the patient's *RAS* mutation status. Tissue-based testing for *RAS* mutations normally takes several weeks, but the Sysmex test is expected to provide results within days. Approximately 50% of patients with metastatic colorectal cancer have tumors with *RAS* mutations, and anti-epidermal growth factor receptor (anti-EGFR) monoclonal antibody therapies, such as Merck's Erbitux (cetuximab), may improve outcomes in metastatic colorectal cancer with the wild-type *RAS*. The test is already available in Italy.

The potential of liquid biopsy in the management of cancer has also been recognized by *Illumina Inc.* , a major player in the DNA sequencing and array-based technology sectors. At the beginning of this year it formed a majority-owned new company, Grail, whose stated mission is to enable cancer screening from a simple blood test. The aim is to use Illumina sequencing technology to develop a pan-cancer screening test by directly measuring ctDNA in blood.

Grail was initially funded by more than $100m in Series A financing from Illumina and ARCH Venture Partners, with participating investments from Bezos Expeditions, Bill Gates and Sutter Hill Ventures. The company says its unique relationship with Illumina provides the ability to

sequence economically and at the depths necessary to create a screening test with the needed sensitivity and a hoped-for level of specificity hitherto unavailable for cancer screening.

Table 2 summarizes the established and emerging companies in liquid biopsy for oncology.

Table 2

Company	Technology	Application(s)
Abbott	UroVysion Bladder Cancer kit detects aneuploidy for chromosomes 3, 7 and 17 and loss of the 9p21 locus via fluorescence in situ hybridization in urine sample	Bladder cancer
Aspira Labs (a Vermillion company)	OVA1 test measures five ovarian cancer biomarkers	Screen for ovarian cancer in patients with adnexal mass
Beckman Coulter	phi (prostate health index) based on three PSA markers	Prostate cancer
Biocept	Isolation and analysis of CTCs/ctDNA using real-time PCR	Currently gastric, breast lung and other cancers
Caris Life Sciences	Carisome TOP platform	Isolation of circulating microvesicles
Chronix Biomedical	Copy number instability index	Identifying responders/non-responders
Cynvenio	ClearID genetically analyzes both CTC and ctDNA	Breast cancer
Exosome Diagnostics	Sequencing of exosomal RNA or DNA	Prostate cancer, lung cancer and solid tumors
Foundation Medicine	NGS-based oncology panels	Solid tumors; hematologic malignancies
Genomic Health	ctDNA in urine	Bladder cancer
Guardant Health	NGS-based tests using Digital Sequencing filtering method	Lung, breast, colorectal and other cancers
Hologic	Progensa prostate cancer gene 3 (PCA3)/PSA RNA assay	Prostate cancer
Inivata	InVision amplifies panel of 34	Various cancers

	selected cancer-related genes	
Matritech	NMP22 Bladderchek measures nuclear matrix protein in urine	Bladder cancer
MDxHealth	Real-time quantitative PCR	Urologic cancers
Myriad Genetics	BRACAnalysisCDx	Companion diagnostic to help identify advanced ovarian cancer patient s who may benefit from treatment with Lynparza (olaparib)
Onocyte	Analysis of proprietary cancer markers using proprietary algorithm	Lung and breast cancer
OPKO Health	Prostate-specific kallikrein assay in blood	Risk of aggressive prostate cancer
Pacific Edge	Cxbladder measures five specific mRNA biomarkers in urine	Bladder cancer
Personal Genome Diagnostics	MYPG	Comprehensive analysis of cancer genomes
Qiagen	therascreen EGFR RGQ Plasma PCR kit	Companion diagnostic test for AstraZeneca's Iressa
RainDance	RainDrop digital PCR instruments and consumables; sample prep for NGS	Minimal residual disease in AML patients
Resolution Bioscience	NGS-based tests to sequence mutations in most commonly found genes	Lung and other cancers
Roche (thru acquisition of CAPP Medical)	DNA sequencing	Cancer (unspecified)
Trovagene	Digital droplet polymerase chain reaction (ddPCR)	Companion diagnostics to detect mutations on tumor-related genes (*BRAF*, *KRAS*, *EGFR*) to assist in choice of cancer therapy and to detect 13 high-risk HPV types from urine

Veracyte	Percepta Bronchial Genomic Classifier	Lung cancer

Reference Labs

Big testing labs too have been attracted to liquid biopsy. For example, *Laboratory Corp. of America Holdings* (LabCorp), one of the largest independent clinical laboratory company in the US, offers not only a menu of several hundred routine tests to physicians, hospitals, governmental agencies and pharmaceutical companies, but also esoteric testing in a number of areas such as cardiovascular disease, infectious disease and oncology. In the latter area the company offers advanced comprehensive tumor tissue analysis through its Dianon Pathology and Integrated Oncology specialty testing laboratories. It also provides access to the latest pharmacogenetic tests: in June 2016 LabCorp announced the availability of the *Rochecobas EGFR Mutation Test v2*, the first blood-based test approved for clinical use in the US to detect certain *EGFR* gene mutations in NSCLC patients (it was approved by the FDA on June 1, 2016).

Other reference labs are also jumping on the bandwagon. *NeoGenomics Inc.*, which operates a network of CLIA-certified clinical laboratories that specialize in cancer genetics testing, expanded its liquid biopsy offering at the end of last year with the launch of two new tests for monitoring solid tumors and predicting drug resistance, bringing the total number of liquid biopsy tests available to 15. The *NeoLAB Solid Tumor Monitor* is an NGS-based test that analyzes ctDNA to quantify and track genomic abnormalities in patients with documented metastatic cancer who carry specific abnormalities already confirmed by tissue biopsy testing. Results obtained with the test can be used to capture the heterogeneity in the cancer, monitor the emergence of new resistant clones, and predict relapse.

US Policy-Makers Prioritize Liquid Biopsies

By Ferdous Al-Faruque

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Liquid biopsies have been a priority topic for policy development by US FDA and the broader Obama administration. During the past year, FDA has kicked itself into high gear to provide test-makers with more insight into its thinking and, this summer, the agency approved the first liquid biopsy test.

Read the full article here

The *NeoLAB BTK Inhibitor Acquired Resistance* test is designed to predict resistance to Bruton's tyrosine kinase (BTK) inhibitors such as *AbbVie Inc.* 's *Imbruvica* (ibrutinib). Resistance to BTK inhibitors is associated with mutations in the *BTK* and *PLCG2* genes. The test can detect mutations in these two genes prior to tissue or cell-based testing, and can be used to monitor patients treated with BTK inhibitors, especially in chronic lymphocytic leukemia, mantle cell lymphoma and diffuse large B-cell lymphoma. NeoGenomics claims mutations in *BTK* and *PLCG2*

can be detected up to 12 months before the appearance of overt clinical resistance to therapy.

Other liquid biopsy products available from NeoGenomics include a series of NGS-based assays for the detection and measurement of biomarkers in the peripheral blood plasma of patients with known or suspected hematologic cancers, and a blood- and urine-based test designed to be used as an adjunct to PSA testing to diagnose the presence of prostate cancer and to distinguish high-grade from low-grade cancer to help inform whether a biopsy is needed. "The overall market for oncology testing is growing and is contributing to our company growth," says NeoGenomics' chairman and CEO, Douglas M VanOort. The company increased testing volumes in its base business by 25% last year, and is looking to better that performance this year following the acquisition of Clarient from GE in December 2015.

Beyond Cancer - NIPT

Another company offering cancer diagnostics based on liquid biopsies is San Carlos, California-based Natera Inc. What sets Natera apart from many companies offering liquid biopsy oncology tests is that it is also involved in the non-invasive prenatal testing (NIPT) sector. Of particular note is its *Panorama* prenatal screening test, the only commercially available NIPT that utilizes single nucleotide polymorphisms (SNPs) to analyze chromosomal abnormalities. This allows the test to distinguish between fetal (placental) and maternal cell-free DNA, thereby delivering higher sensitivities than other tests, the company says.

The Panorama test is based on a blood sample from the mother and a comprehensive panel of chromosomal aneuploidies and other chromosomal variations. It provides what the company claims are the most accurate results of any screening test, as early as nine weeks into the pregnancy, which can help healthcare providers and patients decide whether more invasive testing, such as amniocentesis and chorionic villus sampling, is appropriate.

Other companies working on technology for NIPT include LabCorp, Illumina, and Quest Diagnostics Inc. (*See Table 3.*) This is an attractive segment to operate. With around five million pregnancies each year in the US – leading to approximately four million live births – and assuming peak penetration of about 70% of the potential market, and a unit cost of around $400, the total market in the US could approach $1.5bn.

According to analysts at financial services firm Cowen Group, the NIPT market breaks down into two segments: high-risk pregnancies and average-risk pregnancies. The high-risk segment is already more than 50% penetrated, they say, and growth this year is therefore likely to be in single figures. On the other hand, penetration of the average-risk segment is still below 10%, and this therefore potentially offers better growth opportunities. However, the analysts say, growth will depend on NIPT being reimbursed for this population segment and becoming recognized in formal guidelines (such as those of the American College of Obstetricians and Gynecologists) for the management of average-risk pregnancies.

Qiagen's Kazinski comments that, although oncology is the most promising area of application for liquid biopsy technology, NIPT is currently the most advanced. "In the future we also see application potential in transplant rejection and other nonmalignant diseases, he said.

Table 3

Company	Test	Details
Ariosa Diagnostics (now part of Roche)	Harmony Prenatal Test	Cell-free (cf) DNA analysis of fragments from specific chromosomes to identify trisomies 21, 18 and 13
BGI	Nifty	cfDNA analysis across entire genome to identify trisomies 21, 18 and 13 plus additional chromosome aneuploidies and chromosomal deletion syndromes
Illumina	Verify Prenatal Test (aka Genesis Serenity (Genesis Genetics))	cfDNA analysis identifies trisomies 21, 18 and 13, monosomy X, XXX, XXY and XYY
Premaitha Health	IONA	Placental cfDNA analysis to identify trisomies 21, 18 and 13
Natera	Panorama Prenatal Screen	Uses SNPs and proprietary informatics to identify chromosomal aneuploidies and other chromosomal variations
Quest	QNatal Advanced	Detects trisomies 21, 18 and 13, monosomy X, XXX, XXy and XYY
Sequenom		
	MaterniT GENOME	Identifies any trisomy, monosomy, sex chromosome abnormalities, partial chromosome abnormalities and fetal sex
	MaterniT 21 Plus	Identifies trisomies 21, 18 and 13, monosomy X, XXY, XXX and XYY

	VisibiliT	Identifies trisomies 21 and 18 and fetal sex
	SensiGene	Detects Rhesus incompatibility

Lastly, it is worth mentioning that liquid biopsy technologies may turn out to have a role in the management of conditions such as kidney disease as well. While urinary proteome analysis potentially holds much information about the condition of the kidney, there is something of a question mark over how easily proteomic information can be obtained, and whether it could be accepted into routine use. Most of the research in this area centers on chronic kidney disease (CKD), the most prevalent form of kidney disease, and a large multicenter trial aimed at preventing CKD through urinary proteomics-directed intervention (the PRIORITY trial) has recently been launched. As such, efforts to develop liquid biopsy in nephrology are in their infancy.

For the moment, then, the focus is on oncology. “Over the next five years, liquid biopsy tests have the potential to evolve into the standard of care for physicians in patient cancer detection and monitoring,” says Trovagene’s Erlander. “The ability to detect ctDNA, whether in urine or blood, early enough may lead to earlier intervention and earlier treatment.” Liquid biopsy is still an emerging technology, and one, moreover, that faces barriers to its wider acceptance, such as reimbursement issues, but the benefits it can offer seem likely to ensure that the sector is set for steady growth.