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Liquid Biopsies Show Sensitivity in Monitoring Pancreatic Cancer

Liquid biopsies are sensitive enough to detect shifts in mutational load in response to treatment of patients with advanced pancreatic cancer, according to an abstract presented by Chronix Biomedical (San Jose, Calif.) at the American Society of Clinical Oncology's 2015 Gastrointestinal Cancers Symposium (San Francisco; Jan. 15-17).

The pilot study extends the company's previous studies on head/neck cancers and colorectal carcinomas and demonstrates that droplet digital polymerase chain reaction assays are "sufficiently sensitive" to monitor tumor mutations in patients with advanced pancreatic ductal adenocarcinomas. The liquid biopsy assay quantified cell-free plasma DNA for KRAS and TP53 mutations in five patients. Within the small sample set, varying degrees of response (measured by clinical, biochemical, and radiological means) were seen ranging between complete response, stable disease, partial response, and progression. The divergent behavior of the two mutations (indicative of a partial response to therapy) illustrates, the authors say, the necessity of testing a "robust" set of markers in liquid biopsy.

While it remains to be seen in larger studies with longer follow-up on the clinical impact of liquid biopsy monitoring, industry watchers are confident that there will be marked progress towards commercialization and clinical adoption of liquid biopsy technology in 2015. For more information on the momentum towards clinical commercialization and adoption of liquid biopsies, please see *DTET*'s special focus section on page 8.

MALDI-TOF MS 'Revolutionizing' Diagnosis of Mold Infections

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) can better identify mold species, compared to traditional microscopic-based techniques, according to a study published in the December issue of *Clinical Microbiology and Infection*. The combination of continued adoption of MS platforms and the extension of MALDI-TOF MS technology into diagnosis of mold infections will further challenge traditional techniques, as has been seen with microbiological identification of bacterial infections.

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MALDI-TOF MS 'Revolutionizing' Diagnosis of Mold Infections, *Continued from bottom of p.1*

As with most infections in hospitalized patients, prognosis with mold infections is largely dependent on rapid and accurate diagnosis and the resulting initiation of appropriate therapy. Current diagnosis of mold infections involves error-prone, species identification based on microscopic examination by highly skilled mycologists, followed by DNA sequencing, when necessary.

In the present study, the French researchers developed an extensive, in-house reference library (2,832 reference spectra with 708 strains from 347 species), which they incorporated into a routine laboratory analyses for mold identification. The accuracy of MALDI-TOF MS (Bruker) was compared with both traditional morphology-based identification and DNA sequencing both during a 4-month period in 2013 and using preserved samples from the same 4-month period in 2011.

"The increased proportion of successful MALDI-TOF MS-based identifications has enabled us to devote more resources to identifying the remaining unidentified specimens by increasing the number of analyzed DNA targets for DNA sequence-based identification. ... The clinical significance of these emerging fungal species, which have rarely been reported in Europe, must be examined further."

—Renaud Piarroux, Ph.D., Aix-Marseille University

The researchers found that implementation of MALDI-TOF MS resulted in a "dramatic" improvement in identification of mold species over the 4-month assessment period. Mold isolates were cultured in 262 of the 6,531 samples received from January through April 2014. Species-level identification using morphological features was achieved for 205 isolates (78.2 percent), compared to identification in 257 isolates (98.1 percent) using MALDI-TOF MS (243 after one run and an additional 14 after two runs). The five isolates not identified with MALDI-TOF MS or morphology could only be identified with DNA sequencing. The misidentification rate fell from 9.8 percent using morphology to 1.2 percent using MALDI-TOF MS.

When comparing pre- and post- MALDI-TOF MS initiation, species-level identification improved from 64.6 percent in 2011 (a rate the authors call "far from satisfactory") to 100 percent in 2013. Reassessment of 247 of the 2011-period isolates using MALDI-TOF MS yielded an increase in species diversity from 16 to 42 species.

In addition to the improved accuracy of species identification, the authors say that employment of MALDI-TOF MS for mold infections is "rapid and rather simple to perform," taking approximately 95 minutes for analysis for a series of 24 isolates. An added benefit, the authors say, is the ability to identify young colonies, even before the appearance of characteristics necessary for morphological identification.

"The current study demonstrates that use of an appropriate reference spectral library plays a major role in enhancing mold identification," writes co-author Renaud Piarroux, Ph.D., from Aix-Marseille University. "The increased proportion of successful MALDI-TOF MS-based identifications has enabled us to devote more resources to identifying the remaining unidentified specimens by increasing the number of analyzed DNA targets for DNA sequence-based identification. ... The clinical significance of these emerging fungal species, which have rarely been reported in Europe, must be examined further."

Takeaway: The increasing employment of MALDI-TOF MS technology in routine laboratory practice could transform diagnosis of mold infections, potentially rapidly replacing the need for traditional microscope-based techniques. G2

Personalized Cancer Screening Could Combat Overscreening

With President Barak Obama's call for expanded investment in precision medicine, the promise of personalized medicine has once again made national headlines, yet, much of the attention has been focused on personalized treatment of disease. In an era of cost containment, experts say that employing personalized screening strategies can increase the benefits yielded from screening while improving stewardship of health care resources.

"Maximizing benefits while minimizing the harms of screening requires moving from a '1-size-fits-all' guideline paradigm to more personalized strategies," says Tracy Onega, Ph.D., from Dartmouth University (Lebanon, N.H.) in a paper on personalized breast cancer screening strategies, published in *Cancer* in October 2014. "There is indeed a tension between personalized medicine and population-wide guidelines. ... A refined conceptual model for breast cancer screening is needed to align women's risks and preferences with screening regimens."

"Maximizing benefits while minimizing the harms of screening requires moving from a '1-size-fits-all' guideline paradigm to more personalized strategies."

—Tracy Onega, Ph.D., Dartmouth University

With improvements in the sensitivity of screening technology, it is acknowledged that the more we search for cancer, the more we will find. Yet, emerging evidence shows that for many manifestations of the disease, these additional cancer cases are often cases of overdiagnosis and would not negatively impact the patient had they been left undetected. Further, detection provokes costly workups and unnecessary invasive biopsies and treatment.

One often cited example is the "epidemic" of thyroid cancer diagnosis. Thanks to a free/low-cost screening program in South Korea the incidence of the disease has risen 15-fold over the past 20 years, but death rates have remained consistently low. Effective screening programs, experts say, should lead to a decrease in cancer deaths through initiation of treatment. But, in South Korea as in the United States, treatment for these additional thyroid cancer cases has not altered the death rate. While molecular diagnostics are improving in their ability to assess the pathogenic risk posed by certain mutations for some cancers, like prostate cancer, it remains imperfect at differentiating aggressive from slow-growing cancers.

Experts say that personalizing recommendations for cancer screening can be implemented that incorporate individual cancer risk as well as expected benefit to be derived from screening based on the patient's age, health status, and anticipated life expectancy.

There is substantial emerging evidence that overscreening with PSA tests, colonoscopies and mammograms in elderly patients or those with terminal illnesses remains a costly problem. Researchers from the University of North Carolina at Chapel Hill found that between 31 percent and 55 percent of study participants in patients aged 65 years or older with less than nine years to live were still receiving screenings for four common cancers (prostate, breast, cervical and colon cancer), according to a study published Aug. 18, 2014 in *JAMA Internal Medicine*.

Current screening guidelines are often age-based and fail to address the issue of current health status.

"Even though validated simulation models are available for a variety of screen-detectable cancers, the capability of these models to provide personalized recommendations for screening has not been fully exploited," writes coauthor Sameer D. Saini, M.D., from University of Michigan, Ann Arbor in a Dec. 3, 2014 Viewpoint published in the *Journal of the American Medical Association (JAMA)*.

"Although no physician has the intention to overtreat or overdiagnose cancer, screening and patient awareness have increased the chance of identifying a spectrum of cancers, some of which are not life threatening," writes Laura Esserman, M.D., from University of California, San Francisco, in a July 2013 *JAMA* Viewpoint. "The goal going forward is to personalize screening strategies, and focus screening policies on the conditions that are most likely to result in aggressive illness and death."

Such a shift in strategy will ultimately require patient education, shifts in physicians' habits, and some system-wide changes to quality measures. Experts recommend improved communication to ease patients' and physicians' discomfort at curtailing routine screening in low-risk individuals or in those unlikely to achieve benefit, including candid communication of the risk-benefit of screening and life expectancy. To facilitate these discussions physicians need accessible, personalized estimates of benefit (ideally integrated in electronic health record systems). System-wide, clinically sensitive, personalized measures of quality will need to be developed that may recognize effective care is not employing screening.

The flip side of personalized screening is that in certain high-risk populations, like those at risk for Lynch Syndrome, personalized strategies can triage those needing higher intensity screening. Key to implementing risk-based screening strategies rather than those guided strictly by age is the use of genomic data. Experts are hopeful that risk-stratified personalized screening has the potential to detect all relevant cancer, including in younger but higher-risk patients, while achieving greater cost-effectiveness by minimizing overdiagnosis, and overtreatment in low-risk patients.

Takeaway: While the preponderance of attention for precision medicine has been on targeting treatments more effectively, applying personalized strategies to cancer screening can better prioritize health care resources to those most likely to yield benefits from such testing. Personalized screening strategies based on risk can identify patients most likely to benefit from recommended or even aggressive screening, as well as those likely to only face harm from screening. G2

Inside The Diagnostics Industry

Standardizing Testing May Aid Sequencing-Based Test Reimbursement

Kathryn A. Phillips, Ph.D.
Founder & director, TRANSPERS

Q&A with Kathryn A. Phillips, Ph.D.

The translation of personalized medicine into clinical practice remains in its infancy. Many large challenges remain, including development of a standardized metric to evaluate the clinical utility and cost-effectiveness of molecular-based tests; ethical considerations surrounding return of results in a field where genomic interpretation is rapidly evolving; and establishing a reimbursement framework for large sequencing-based tests.

Rather than explore sequencing from a technical angle, this quarter *DTET* examined the transition of sequencing-based tests into clinical practice from a policy perspective. *DTET* recently spoke with Kathryn A. Phillips, Ph.D., founder and director of the Center for Translational and Policy Research on Personalized Medicine (TRANSPERS) at University of California, San Francisco, which focuses on developing evidence-based information to guide the use of personalized medicine. Phillips discusses TRANSPERS' work on increasing transparency of reimbursement policies, developing approaches to evaluating the downstream economic impact of sequencing, and understanding variance in test adoption.

TRANSPERS' Evidence and Reimbursement Policy Advisory Council brings together a variety of key stakeholders. How far apart are industry and payers from establishing requirements for evidence of clinical utility?

It is really critical each party understands where the other party is coming from. My sense is that often that doesn't occur. Industry doesn't understand why payers won't pay and thinks they need to be more specific in requirements. Payers think that industry just does not provide the data they need and that test developers want them to cover anything new and exciting, whether it has been proven or not. Of course, in reality, the truth is somewhere in the middle.

We are seeing these issues come up even more with sequencing technology, partly because it has received so much hype. There has been a lot of attention being paid to the good impact sequencing can have on health care. We are also seeing more patient and provider demand for it. We see this technological imperative taking place and there is no doubt that over time we will use panels and sequencing and in many cases it will be a great advance over single gene tests.

On the other hand, it is very murky how to evaluate these technologies given their complexity and that they raise all kinds of issues for payers, such as the distinction between what is being done for research purposes versus clinical purposes. Sequencing is often used for research as well as clinical purposes, but payers don't usually pay for research. It is not their mandate. We have to figure out what part of the results are going to be returned to patients; what to do with information that could potentially lead to inappropriate or even harmful care or variants of unknown significance where we just don't know what they mean; and the tension between patients who often think

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they want to know everything, and the need to protect the public interest. We do not want to give people information that is going to cause harm, or risk, or cost without the relevant benefits.

Is the health care system ready to adopt sequencing into clinical practice?

Personalized medicine is here. It is moving forward and is not going away. The trend is an upward trajectory in terms of use and adoption. But it will be selective as to how and when. I think single gene tests will still have a place. The move to panels is taking place in many areas. That trend is moving forward. In terms of size of panels—the number of genes included–it will vary by clinical context. Every situation is unique and of course that makes it complicated. You can't have a one-size fits all policy.

I think whole-exome and whole-genome sequencing is something that has some specific uses now, but this idea we are going to sequence everyone at birth, I think, is a ways off in terms of being a feasible proposition to consider.

"The move to panels is taking place in many areas. . . . In terms of size of panels—the number of genes included–it will vary by clinical context. Every situation is unique and of course that makes it complicated."

—Kathryn A. Phillips, Ph.D.

How big of an issue is test cost in adoption of sequencing-based tests?

Sequencing illustrates what's happening in our health care system as a whole, and it brings certain issues to the forefront. Right now it is murky as to who is going to pay for what and when.

For example, BRCA testing is moving to panels. Right now insurers generally do not have positive coverage policies to pay for the BRCA panels. That doesn't mean they won't cover them, but there is not clear guidance out there on which panels they will cover and when.

How do we best evaluate the economic impact of sequencing-based testing?

There is going to need to be a variety of approaches. For example, one could look just at what is the budget impact of using a panel versus a single gene test. That is more of a straightforward evaluation. Here is the clinical pathway and here are the costs associated with getting to a diagnosis.

What is trickier is when you have to look at the downstream implications. That is what people tend to forget. Just because you do a test, give back results, and get a diagnosis out of it, it doesn't end there. There is a pathway of events that occur after you return results and that is where we don't have much data yet to support testing for these genes where we think they have a relationship with a condition, but it is not well demonstrated yet. Over time that will become clearer. But right now we are looking at things like where is the tipping point. If you do three single gene tests sequentially versus a panel with six markers, you can identify the tipping point if you assume the panel is providing more information. But what if the additional information actually costs money and does not have benefits? Do you still come out ahead with the panel? Over time we will

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increasingly reach the point where you might as well do the panel because when you look at costs you are better off.

Adoption of molecular testing is non-uniform. How do physicians evaluate the benefit of one test over another?

We recently looked at the evidence and indeed there is a lot of variation in terms of what they order, when they order, how they order. One key is going to be standardizing how these tests are ordered. That has got to be done by professional groups. It is not realistic to expect physicians to keep up with the field. A lot of companies are stepping into this void and providing standardized panels, or offering interpretation, or offering reports that tell a physician what to do. Right now it is really pretty chaotic. That will shake out over time and we will move towards a more standardized, guideline-driven approach, instead of the Wild, Wild West we have right now. Hopefully the move toward regulating laboratory-developed tests will provide some standardization and some consistency as well.

"We have been funded by the National Institutes of Health to develop this registry to better understand what policies are out there; whether there are gaps in coverage; how the policies compare; do they have a positive or negative coverage decision; what evidence is cited in those policies; and how can one predict if there is going to be a positive or negative coverage decision."

—Kathryn A. Phillips, Ph.D.

From a policy perspective there have been calls for greater transparency related to reimbursement of molecular testing. Tell us how the Genetic Testing Reimbursement Registry will aid in these efforts.

Right now there is no place you can go that synthesizes different payer coverage policies in a standardized way and that is not part of a for-profit, proprietary database. We have been funded by the National Institutes of Health to develop this registry to better understand what policies are out there; whether there are gaps in coverage; how the policies compare; do they have a positive or negative coverage decision; what evidence is cited in those policies; and how can one predict if there is going to be a positive or negative coverage decision. This is ultimately what industry is interested in. What factors lead to a coverage decision? Using this, they can develop better evidence focusing on the factors that will have the greatest weight. We are now just starting this project, but we are hoping that it will provide more transparency and understanding of coverage policy.

What advice would you give to stakeholders in order to improve trust and transparency during this transition period?

Industry needs to be sure that they are providing the data that demonstrates the value of their products; not just the technical benefits, but how it meets a clinical need. Payers are really under the gun to figure out how to evaluate these new technologies in a consistent way. Some payers are starting to establish frameworks. But a lot of these tests have been under the radar and payers have made ad hoc reimbursement decisions, as opposed to putting out policies, and I don't think that can continue as these tests emerge into clinical care.

SPECIAL FOCUS: Liquid Biopsies

Liquid Biopsies for Oncology to Gain Clinical Momentum in 2015

Liquid biopsy technology holds enormous potential to transform the medical management of oncology patients by providing noninvasive, real-time insights of disease status. Also known as a "molecular stethoscope," liquid biopsies are integral to personalizing cancer care. 2015 is predicted to be the year when liquid biopsy tests become commercially available and gain traction in clinical oncology care.

"The applicability of a non-invasive cancer diagnostic and monitoring tool is immense," writes Doug Schenkel, managing director of the medical technology research group at Cowen and Company, in the equity research note *Ahead of the Curve Series*, published in November 2014. "Although the current field is somewhat nascent, liquid biopsies have a multi-billion dollar market potential that could eventually exceed $10 billion."

Liquid biopsy technology is expected to ultimately permeate the entire continuum of cancer care - from early detection, treatment selection and treatment monitoring through recurrence surveillance. (Though there is scientific evidence the technology is applicable to other clinical areas including transplant monitoring, the most meaningful initial adoption is expected in oncology care.)

> *"Cell death happens in minutes to hours, so you would expect the change in ctDNA to be a quick effect, and it is."*
>
> *—Paul B. Chapman, M.D.*

Early forays into personalized cancer care focus on tailoring treatment based on the molecular makeup of tumors. But, this molecular assessment provides only a single snapshot and is based on analysis of tumor biopsy tissue. Such samples are derived from invasive procedures and may not be feasible in all patients either due to anatomical location of the tumor or due to advanced metastasis. Additionally, tumor samples may not reflect the true molecular heterogeneity both within the tumor and in distant metastases.

Liquid biopsy affords many advantages over tumor sample analysis. Given its noninvasive nature, serial testing with liquid biopsies offers clinicians an ongoing opportunity to track disease status throughout treatment. This continuous monitoring enables clinicians to catch emerging mutations or disease progression and ensure treatment selection remains relevant much sooner than a radiological examination can. On the positive side, liquid biopsies can provide quick reassurance to patients and clinicians that treatment is working.

"The beauty of circulating tumor DNA (ctDNA) monitoring is the speed," Paul B. Chapman, M.D., chair of the Medical Advisory Panel at the Melanoma Research Alliance (Washington, D.C.), told Medscape. "If you are looking for a change in a tumor, based on CT scan, you are talking about not only killing billions of tumor cells but also waiting for the resulting cell debris to be cleared by the body before the change shows up on imaging. That can take weeks. But cell death happens in minutes to hours, so you would expect the change in ctDNA to be a quick effect, and it is."

CTCs versus ctDNA

Circulating tumor cells (CTCs) were the lead target marker of liquid biopsies because they provide both genetic and cellular level data. However, these cells are relatively rare and require sensitive collection and enrichment methods. Experts say fewer than 10 CTCs are

SPECIAL FOCUS: Liquid Biopsies

found among 1 million white blood cells and 1 billion red blood cells. CTCs have been detected in patients with breast, prostate, lung, and colon cancer. Importantly, CTCs have shown clinical value with many studies correlating higher CTC counts with a negative cancer prognosis.

However, cell-free tumor nucleic acids (ctDNA and circulating cell-free tumor RNA), are emerging as effective alternatives to CTCs due to their easier collection and analysis. 2014 represented a "tipping point" industry watchers say, in which the focus, in terms of published papers and commercial plans shifted from CTCs towards ctDNA. Most ctDNA strands contain roughly 180 base pairs and can be detected in higher levels than CTCs (more than a 50-fold increase, experts say). Yet, ctDNA is believed to constitute as little as one ten thousandth of total circulating DNA. But, improved sensitivity of analysis techniques (next generation sequencing, and digital polymerase chain reaction) are overcoming that barrier, as can be seen with noninvasive prenatal testing (NIPT), which analyzes circulating fetal DNA from the mother's blood.

"[T]he diversity of approaches has obfuscated the path to clinical adoption; therefore, standardization is necessary to ensure a clear direction and future success."

— Doug Schenkel, Cowen and Company

Despite the rapid clinical adoption of NIPT, liquid biopsies for cancer are at a more nascent clinical state in part because ctDNA is harder to detect than fetal DNA and until recently, clinical use of sequencing for DNA analysis was not feasible.

"Although the outlook is promising, the liquid biopsy field has significant technical and clinical hurdles to overcome," cautions Schenkel. "Technical challenges include sensitivity and specificity issues due to current isolation, enumeration, and enrichment methods. Furthermore, the diversity of approaches has obfuscated the path to clinical adoption; therefore, standardization is necessary to ensure a clear direction and future success."

Commercialization Prospects

Cowen and Company predicts that liquid biopsy represents a $10 billion market opportunity by the end of the decade ($4 billion of that in the United States). Cowen's estimate does not include the additional market potential for adjacent tests, instruments, and applications, so the full industry impact of liquid biopsy technology may be even greater. The true market size is also, of course, dependent upon test pricing and frequency. The $10 billion estimate is based on roughly a $500 price tag per test with testing once per year (for diagnosis of all new colorectal, lung, breast and prostate cancers and if all patients living with these cancers were tested annually). The market could again be higher if testing was performed serially more frequently and if liquid biopsy can penetrate the asymptomatic screening market.

While the "holy grail" of liquid biopsy would be to apply the technology as an early-detection screening test, the more imminent commercial application is in patient monitoring of diagnosed cases. *DTET*'s survey of the commercial landscape verifies that early entrants to the commercial liquid biopsy market are primarily developing applications for monitoring cancer treatment. Within the oncology market, it is estimated that liquid biopsies for monitoring will achieve routine clinical practice within five years, while the full potential of other liquid biopsy applications will take longer.

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Both test service providers and toolmakers stand to benefit from the clinical adoption of liquid biopsy technology. Among the commercial single cell analysis tools that could be utilized in liquid biopsy testing are Pacific Biosciences' (Menlo Park, Calif.) single molecule real-time sequencing (SMRT) products; Oxford Nanopore's (United Kingdom) nanopore-based readers to directly identify and sequence individual DNA bases; Fluidigm's (San Francisco) microfluidics-based C1 single cell sample preparation tool; and Nanostring's (Seattle) nCounter, which digitally detects single molecules using multiplex profiling.

As the dominant platform in the sequencing market, **Illumina** (San Diego) also of course stands to benefit on the equipment side from broader application of sequencing technology. But, Illumina is increasingly applying the technology itself. At the recent JP Morgan Healthcare Conference (San Francisco; Jan. 12-15), Illumina's CEO Jay Flatley stated that Illumina's goal is to sell a research-use-only liquid biopsy product by the end of this year, while the company works toward U.S. Food and Drug Administration approval.

Within the oncology market, it is estimated that liquid biopsies for monitoring will achieve routine clinical practice within five years, while the full potential of other liquid biopsy applications will take longer.

Another sign of Illumina's interest in the liquid biopsy market is its December 2014 agreement with Sequenom to pool their patent portfolios and settle all outstanding legal disputes. The pooled patents are reported to include intellectual property around the use of ctDNA. **Sequenom** (San Diego) has recently reported that while performing NIPT testing, the company has identified over 20 significant incidental findings of confirmed maternal tumors, including breast cancer, colon cancer, and lymphomas. CEO Bill Welch said at JP Morgan that the company's lead oncology product will focus on advanced cancer patients with solid tumors (stage III-IV) and metastatic patients with (re)-biopsy of tumor. Welch said that the early access program for a research use test is expected in the second half of the year.

Welch and Flatley were not the only large diagnostics players to speak of liquid biopsy test development plans at JP Morgan. **Genomic Health** (Redwood City, Calif.) is currently conducting larger-scale clinical trials to support the launch of its first liquid biopsy test in 2016, CEO Kim Popovits said at the conference. The company unveiled results from two feasibility studies in December — one assessing the application of liquid biopsy to detect bladder cancer from urine (2014 Society of Urologic Oncology) and breast cancer in blood (2014 Annual San Antonio Breast Cancer Symposium). The company said that the results represent "important progress" in the development of a proprietary liquid biopsy platform.

In mid-January **Qiagen** (Germany) received the first-ever CE mark of a lung cancer companion diagnostic based on liquid biopsies. The novel liquid biopsy-based companion diagnostic utilizes circulating nucleic acids obtained from plasma samples in patients with non-small cell lung cancer to assess EGFR mutations to determine eligibility for the drug IRESSA. The test was co-developed by Qiagen and the drug's maker AstraZeneca.

While the potential of liquid biopsy has attracted interest from major diagnostics players, smaller startups are not conceding the market. Many small companies will be entering the commercial realm in 2015 as well.

SPECIAL FOCUS: Liquid Biopsies

Thomas McLain, CEO of **Exosome Diagnostics** (Cambridge, Mass.), tells *DTET* that as liquid biopsy technology makes the transition into clinical care, his company is initially entering the market by selecting liquid biopsy tests for lung cancer markers such as the ALK and EGFR mutations (T790M) that are already covered by payers. He says that simultaneously introducing a new platform and new markers can complicate adoption efforts.

"We are demonstrating that exosomes are diagnostically significant, so we are picking tests of accepted markers, so that clinicians can appreciate the performance difference," says McLain. The company's platform relies upon exosome analysis. Exosomes contain both RNA and DNA, which the company says can more comprehensively detect mutational shifts than ctDNA alone. McLain explains that ctDNA is reflective of therapeutic-related cell death, while ctRNA reflects the living process and may indicate metastasis.

Also in 2015, the company plans to launch a solid tumor panel as a laboratory developed test that will target actionable 400 mutations (10 genes) in the most significant pathways in lung, colon, and breast cancers. The panel will focus on the most actionable mutations for current treatment and clinical trial eligibility.

In early January, **Personal Genome Diagnostics** (Baltimore) launched its PlasmaSelect–R liquid biopsy test for cancer researchers. The test detects sequence alterations and translocations in 63 well-characterized genes that include all important known biologic and therapeutic cancer targets, the company says. The company, a spinout from Johns Hopkins University, plans to launch a clinical use version of the test in the second half of the year.

"You will be able to overcome reimbursement barriers if you have a test that answers a clinical question that can't be answered another way. We spun out PGS to create high-value clinical tests that are 'must haves,'" Luis Diaz, M.D., the company's cofounder tells *DTET*. "Liquid biopsy must answer clinical questions — how will it help the patient live longer? How will it help oncologists decrease toxicity? How will it decrease the cost of patient care?"

Those in the field believe adoption of liquid biopsy tests will be quicker than other genomic cancer risk assessment or prognostic tests because liquid biopsy reflects the real-time cancer dynamics — information that has been previously inaccessible.

Chronix Biomedical (San Jose, Calif. and Germany) will begin offering liquid biopsies to assess minimal residual disease this year. The first of the company's anticipated launches will be a test for prostate cancer intended to supplement prostate serum antigen (PSA) testing. The $600 test, initially to be available in Germany, will guide clinician's decision-making in whether or not a man with marginally elevated PSA, in fact needs a biopsy. The hope, Chronix CEO Howard Urnovitz tells *DTET*, is that the test can help realize billions of dollars in savings by reducing unnecessary biopsies. In addition to the PSA test, the company is looking for partners to help bring to market its liquid biopsies for breast cancer, organ transplantation, and head/neck cancer.

Takeaway: 2015 is expected to be the year that liquid biopsy technology penetrates the clinical oncology market. Uptake for the monitoring application is expected to be quick, with predictions that the liquid biopsy will become part of routine care within five years. Expanded applications of the technology to cancer screening and other clinical areas are expected to take longer.

Neutrophils May Predict Development of Sepsis

The movement of white blood cells may predict which burn patients are at risk for sepsis, days before diagnosis is currently possible, according to a study published in *PLOS ONE* on Dec. 9, 2014. Using a novel microfluidic device, the researchers discovered that neutrophils from burn patients who went on to develop sepsis spontaneously migrate in the absence of chemical attractants, while neutrophils from healthy patients and burn patients without sepsis don't move. The researchers hope that with further validation and refinement, a microfluidic test that assesses neutrophil movement can be used to predict sepsis and monitor effectiveness of antibiotic therapy. While the current study was conducted on burn patients, the researchers believe evaluation of neutrophil movement may be useful in other patients at risk for sepsis.

"The most common blood test ordered to evaluate a patient's ability to fight infection is absolute neutrophil count, based on the assumption that — like well-trained soldiers — neutrophils are always fast, disciplined and effective in pursuing their targets, meaning that the size of the neutrophil 'army' is all that matters," said co-author Daniel Irimia, M.D., Ph.D., in a statement. "Our work challenges that assumption and shows that, even when the number of neutrophils is unchanged, the army can fall into disarray and become ineffective."

Neutrophils comprise the majority of white blood cells involved in immune responses. They travel to infection sites in response to chemical signals. The authors say that earlier efforts to characterize neutrophil chemotaxis in sepsis (such as the transwell assay and early microfluidic assays) were limited by the instability of the chemical gradients and the lack of single cell resolution.

"Our work challenges that assumption and shows that, even when the number of neutrophils is unchanged, the army can fall into disarray and become ineffective."

—Daniel Irimia, M.D., Ph.D., Massachusetts General Hospital

The researchers designed microfluidic devices to quantify the neutrophil migration phenotype with high precision. The narrow channels included straight sections to measure the speed and persistence of the cells' motion, as well as branches and obstacles to test the cells' ability to make directional decisions. Isolated neutrophils were loaded into the device and assessed in three distinct conditions: serum-free Hank's Balanced Salt Solution and gradients of formyl-methionyl-leucyl-phenylalanine and leukotriene B4 to test a total of 18 independent parameters. The device quantified neutrophil migration from 74 blood samples (taken from 13 patients with major burns and three healthy subjects) for more than four weeks.

The researchers found that neutrophils from healthy individuals moved "quickly and efficiently" toward a chemical attractant, while cells from burn patients (regardless of subsequent sepsis) showed "limited, slower, and poorly organized movement." Additionally, the researchers found "spontaneous migration" of neutrophils in the absence of a chemo-attractant in burn patients with sepsis. This spontaneous migration was witnessed before sepsis diagnosis and disappeared after sepsis was effectively treated. The spontaneous neutrophil migration phenotype was "rare" in patients with major burns in the absence of sepsis and was not seen in healthy individuals.

"Overall, sensitivity and specificity values for our assay are only slightly better than other markers for sepsis in burn patients. However, it is important to note that, enabled by technology, we could observe the changes in neutrophil migration phenotype, up to two days before sepsis," explains Irimia.

The analysis of neutrophil migration phenotype can be completed in less than 4 hours after the blood samples become available. Irimia says the next major step in the research is to test, this year, the applicability of the findings to prediction of sepsis in other patients with critical conditions. Additionally, he tells *DTET* that future iterations of the microfluidic device, which doesn't require a full laboratory, will address streamlining the blood sample processing protocols before neutrophils are loaded, making the device more user friendly, automating the image analysis.

Takeaway: Microfluidic evaluation of neutrophil migration may play a role in earlier diagnosis of sepsis and monitoring of the effectiveness of antibiotic treatment. While the current study was conducted in samples from major burn patients, the researchers believe neutrophil migration may be applicable to other critically ill patients as well.

Urine Test Can Tailor Kids' Asthma Therapy Dosage

A new urine test can help personalize anti-inflammatory treatment for pediatric asthma patients, according to an abstract presented at the British Thoracic Society Winter Meeting (England; Dec. 3–5, 2014). While further validation is needed, the authors say that a urine prostaglandin can predict future risk of asthma exacerbation better than other asthma assessment measures including sputum eosinophils, the childhood asthma control test (C-ACT), or spirometry.

Prostaglandin metabolites are released as part of an inflammation reaction by immune cells in the airway lining, following exposure to an asthma trigger. Noninvasive measurement of this airway inflammation can improve treatment decisions, including dosing of anti-inflammatory therapies.

Urine samples were prospectively compared between non-asthmatic children (n=48) and asthmatic children (n=25; aged 7 years to 15 years) on days when they showed no symptoms. Additionally, the researchers assessed asthma exacerbations (measured as the number of days of receipt of unscheduled medical attention or missed school due to asthma symptoms). Urine prostaglandin metabolites (PGD, PGE and PGJ) were measured using high-performance liquid chromatography–mass spectrometry (HPLC-MS). Sampling was repeated at three months.

Compared to controls, urine metabolites PGD2, PGE2 and PGJ2 were increased in asthma patients. Low levels of the protective prostaglandin metabolite 15-dPGJ2 significantly predicted subsequent asthma exacerbation within three months (positive predictive value, 75 percent; negative predictive value, 90 percent). Sputum eosinophil measurement, spirometry, and C-ACT did not predict subsequent exacerbations.

"When children see their general practitioner for their annual review, we hope that this test can help indicate the level of steroid medication they actually need," said lead author Rossa Brugha from Queen Mary University of London (United Kingdom) in a statement. "If implemented it will help the child to manage their asthma more effectively and hopefully reduce the number of asthma attacks."

Brugha tells *DTET* that validation is needed in a larger prospective trial and that the group is awaiting funding to complete that. Additionally, he says that they hope to make the test easier to perform.

Takeaway: A noninvasive urine test may aid clinicians in personalizing dosage of anti-inflammatory treatments for asthmatic children.

RNA in Sputum May Improve CT's Diagnosis of Lung Cancer

Micro RNA (miRNA) markers found in sputum may improve the accuracy of lung cancer diagnosis in conjunction with low-dose CT (LDCT) screening, according to a study published Jan. 15 in *Clinical Cancer Research*. The growing use of LDCT as a lung cancer-screening tool among smokers has been accompanied by a "tremendous" rise in the number of lung nodules detected, many of which are benign. The authors say clinical use of a future iteration of this noninvasive RNA-panel test may "dramatically" decrease the need for follow-up imaging and biopsies.

"The higher positive predictive value (PPV; 84 percent) of the biomarkers as compared with only two percent PPV of LDCT indicates that the biomarkers would result in much less overdiagnosis," writes senior author Feng Jiang, M.D., Ph.D., from University of Maryland, Baltimore. "The positive cases detected by the biomarkers in CT-found solitary pulmonary nodules (SNPs) are malignant SPNs, and should have instant surgical treatment. Furthermore, the negative cases discovered by the biomarkers in CT-found SPNs are benign growths, and will not be followed up."

It is estimated that one-fourth of all identified nodules are indeterminate based on CT, but that the vast majority (greater than 96 percent) ultimately prove to be false positives. But in making that determination, patients are subjected to ongoing, costly imaging and related radiation exposure, as well as invasive biopsies.

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—Feng Jiang, M.D., Ph.D.
University of Maryland

The researchers initially evaluated expression of 13 sputum miRNAs previously identified as signatures of lung cancer using quantitative reverse transcriptase polymerase chain reaction. The training set consisted of 122 patients (60 with malignant SPNs and 62 with benign SPNs). While all 13 miRNAs differed significantly between patients with lung cancer and those with benign nodules, miRs-21, 31, and 210 were selected as the best markers.

The 3-marker panel was then validated in an internal testing set of 136 patients (67 malignant and 69 benign) and in an external testing cohort of 155 patients (76 malignant and 79 benign). The three miRNA markers could diagnose early-stage

lung cancer among SPNs with 81 percent to 83 percent sensitivity and 86 percent to 88 percent specificity, yielding a PPV of 84 percent and a negative predictive value of 81 percent. The authors concede, though, that the accuracy of this panel is "not high enough" for clinical use, so ongoing efforts are aimed at identifying additional miRNA markers to expand the panel. The three miRNAs could not differentiate between stage I and stage II disease and the markers were not affected by age, gender, or ethnicity.

Takeaway: miRNAs offer hope as a noninvasive means to improve the diagnostic accuracy of LDCT lung cancer screening and ultimately reduce the need for follow-up imaging and invasive biopsies to determine indeterminate nodules' pathogenicity. G2

Panel Could Eliminate Need for Surgical Diagnosis of Endometriosis

Patterns of genetic activity can be used to diagnose and stage endometriosis, according to a study published in the December 2014 issue of *Endocrinology*. This could represent a significant breakthrough in providing earlier, less invasive diagnosis of endometriosis, which is estimated to affect 10 percent of all reproductive-age women.

Endometriosis is an inflammatory disorder that affects 50 percent of women with pelvic pain and/or infertility (estimated to be more than 100 million women worldwide). Definitive diagnosis of the condition is typically prolonged for up to a decade because it requires surgery and presents with nonspecific symptoms.

"A prompt, low-risk, low-cost diagnostic with high accuracy is needed to shorten time to diagnosis, minimize disease progression and ovarian cancer risk, optimize timing and strategies for pain and infertility therapies, and monitor disease recurrence," writes coauthor Linda Giudice, M.D., Ph.D., from University of California, San Francisco.

The researchers identified classifiers using genomic data from 148 archived endometrial samples from women (aged 20 to 50 years old) with (n = 77) or without endometriosis (normal controls, n = 34; or with other common uterine/pelvic pathologies, n = 37) across the menstrual cycle. The performance of the classifiers was then evaluated in independent sample sets. Ninety-two differentially expressed genes were analyzed using microarray technology and machine learning algorithms were used to develop a grouping system to analyze the gene activity of endometrium tissue samples.

The best performing classifiers, the researchers report, identified endometriosis with 90 percent to 100 percent accuracy and were menstrual cycle phase-specific or independent. The classifiers could additionally distinguish between samples from endometriosis patients and those patients with other uterine disorders, could differentiate between endometriosis stages, and could identify endometriosis at different points in the menstrual cycle.

Takeaway: A microarray panel consisting of protein and gene markers may lead to less invasive and earlier diagnosis of endometriosis. G2

G2 INSIDER

Physicians ID Gaps in Infectious Disease Testing

Despite the deployment of new technologies, physicians report unmet diagnostic needs in the field of infectious disease, according to a study published in the January issue of *Diagnostic Microbiology and Infectious Disease*. Surveyed physicians specifically say there is a need for improved tests to identify drug-resistant organisms, while acknowledging that emerging high-complexity testing must be judiciously used.

Even with advancements in diagnostic methods (next-generation sequencing and matrix-assisted laser desorption/ionization time of flight mass spectrometry) notable gaps remain in test development for rapid, point-of-care tests, those using direct from specimen analysis, and the ability to maintain high levels of accuracy across a wide range of disease syndromes.

Researchers surveyed infectious disease physicians who were members of the Emerging Infections Network to evaluate perceptions of "unmet" needs. Respondents ranked unmet needs by syndrome (central nervous system infection, community-acquired pneumonia, febrile neutropenia, infectious diarrhea, culture-negative endocarditis) and six pathogens (drug resistant gram-negative bacilli, methicillin-resistant Staphylococcus aureus, drug-resistant Mycobacterium tuberculosis, molds, influenza, and HIV resistance).

Based on 700 responses, the most important pathogen-specific unmet diagnostic need was the prompt identification of drug-resistant aerobic gram-negative bacilli (mean score, 4.33 out of 5). Culture-negative endocarditis was the clinical syndrome ranked most highly as in need of improved diagnostics, followed closely by infectious diarrhea. Topping physicians' wish lists for a new test not currently available were a pathogen-based test for respiratory infection (lower and upper respiratory tract); one that could distinguish viral from bacterial infection; and a test for antibiotic resistant organisms, including aerobic-gram negative bacilli and staphylococci.

"In several cases, tests ranked highly as 'unmet' needs (for example, rapid resistance testing for staphylococci, testing panel for infectious diarrhea) were actually commercially available or close to receiving [U.S.] Food and Drug Administration approval at the time the survey was given," writes lead author Anne Blaschke from University of Utah, Salt Lake City. "Lack of availability may be due to the complexity of the testing strategies, the economics of the laboratory, or the absence of outcome data that could be used to support adoption of new tests."

Recognizing the increased cost and complexity associated with new diagnostic technologies, roughly two-thirds of respondents felt that some testing is becoming too difficult for non-infectious disease physicians, and 79 percent report the need for stewardship for particularly complicated or expensive tests. Multiplex molecular respiratory panels, broad-range PCR testing, and antigen-based tests for fungal infection were selected as tests that should be restricted or require prior approval. G2

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