TRANSFORMING THE CANCER LANDSCAPE

CTDNA IS MAKING BIG ADVANCES WITHIN THE CANCER LANDSCAPE, SO WE GATHERED TOGETHER A PANEL OF EXPERTS TO DISCUSS ITS FULL POTENTIAL

1. Where in the clinic do you see ctDNA (and RNA) having relevance?

Gary Pestano: Currently, ctDNA is being used in the field of oncology as a supplement to tissue testing when testing for molecular variants is required. In the absence of a tissue result, blood can be drawn, plasma can be isolated and circulating tumour DNA can be assessed for example, for the presence of specific somatic mutant variants. ctDNA is primarily being used as a supplementary test in the absence of a tissue result. Reports have shown that nearly 80% of cancer patients do not have genetic mutation results available at initial oncology consultation; and that up to 25% of patients begin treatment prior to receiving their results. Although the time to result varies with the tests, and technologies used, results have been delivered in as little as 72 hours after the receipt of the blood sample in the testing laboratory.

Christopher Abbosh: We look for these genetic faults by analysing tumour tissue acquired by biopsy procedures. Yet in some patients biopsies are dangerous or difficult to perform, for example if the cancer has spread to the bones. Performing a liquid biopsy in these patients can circumvent these issues. Circulating tumour DNA analyses could have broader relevance in the clinic in the future. For example, (i) Detecting ctDNA in the post-operative blood of patients who have undergone surgery to remove a cancer may allow clinicians to decide who to give chemotherapy to following surgery. This could spare patients who have been cured by surgery, the risks and side effects associated with having chemotherapy. (ii)Tracking the fraction of the entire DNA detectable in blood that is coming from a patient's tumour may act as an indicator of total disease burden. Therefore, clinicians may be able to infer whether their treatment for a cancer is working based on tracking the quantity of circulating tumour DNA in a patient's circulation over time. (iii) Detecting circulating tumour DNA in the blood of healthy patients may allow doctors to screen for cancer in the future.

Muhammed Murtaza: In patients with metastatic cancer, choosing a therapy often depends on the tumour genotype. In these cases, ctDNA can complement tissue analysis to guide treatment selection. We can identify somatic mutations by analysing cell-free DNA in plasma from cancer patients. This can potentially overcome the limitations of a single and often archival tumour biopsy and provide a more representative view of molecular heterogeneity. Depending on cancer type and treatment options, we can genotype ctDNA using PCR assays for one or more actionable mutations or using next generation sequencing of cancer gene panels and whole exomes.

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Rick Lanman: ctDNA is widely used in the clinic today to detect genomic alterations that are sensitive to targeted therapies. This is important because we can precisely target mutations in tumours with response rates two to three times better than with cytotoxic chemotherapy or immunotherapy. Our comprehensive liquid biopsy, the Guardant360 assay, has been ordered more than 40,000 times by more than 4,000 oncologists worldwide to help guide treatment decisions with up-to-date genomic information without requiring a repeat invasive biopsy. Tissue is useful when it's available for sequencing, but research has shown that most lung cancer patients do not undergo the recommended testing due to the challenges of acquiring and working with tissue. Furthermore, cancer is a heterogeneous and dynamic disease. A tissue needle biopsy may capture only a small slice of a tumour, while ctDNA may provide a summary of alterations across metastatic lesions.

Landon Olap: Currently, I've seen ctDNA having the highest impact in defining the tumour molecular profile and subsequent treatment selection. Since ctDNA is shed from the tumour into bodily fluids, it is a much more accessible source of tumour genetic information compared to tissue biopsies. Going forward, ctDNA has the potential to be highly relevant in all points of clinical oncology management; from screening and diagnosis to genotyping and treatment selection to minimum residual disease monitoring and disease surveillance after treatment.

George Karlin-Neumann: For a whole host of solid tumours, including lung, melanoma, breast, colorectal, prostate, and pancreatic and other cancers – ctDNA and/or cfRNA have been detected in blood and their levels have generally shown to reflect the tumour mass in the body. →

They can thus act as a surrogate for assessing the shrinking or growth of a tumour. This can enable initial diagnosis through blood via plasma genotyping, either when tumour tissue is not available, as in ~25% of advanced lung cancers, or where it is desirable to avoid risk of complications from tissue biopsy such as possible lung collapse. We are still learning to what extent clinical information from tissue biopsy can be substituted by liquid biopsy but the FDA has approved its first liquid biopsy test for initial diagnosis and recurrence of lung cancer.

Another area of promise for liquid biopsy is in identifying patients who are likely to be resistant to a particular therapy, such as anti-hormone therapy in breast and prostate cancer due to existing mutations in the hormone receptor genes (ESR1 and AR, respectively), thus directing them to more fruitful treatments or newer generations of drugs which may overcome this resistance.

2. The use of ctDNA has been described as a non-invasive, or minimally-invasive, biomarker that can provide diagnostic and prognostic information. Could you explain what is meant by this?

CA: Circulating tumour DNA is described as a minimally invasive biomarker since it can be isolated from a simple blood test. This contrasts with tissue based biomarkers which can require biopsy procedures and are therefore invasive. Circulating tumour DNA can provide diagnostic information since genetic faults that are described as actionable (i.e. capable of influencing clinical management decisions) can be identified through profiling DNA present in plasma.

Circulating tumour DNA may also be able to provide prognostic information regarding relapse of cancer following surgery. Patients with early-stage non-small cell lung cancer are often offered surgery to remove their tumour and try and cure them from the disease. Unfortunately, in a proportion of patients (up to 40%) the lung cancer returns after surgery. At this point it is very difficult to cure.

MM: When we quantify somatic mutations in plasma DNA, we can measure changes in ctDNA levels that correlate with changes in tumour burden during treatment. Several studies show that ctDNA levels decrease in patients who respond to treatment or after surgery. When patients develop recurrence or progress on treatment, ctDNA levels increase, often months before progression is obvious on imaging. In addition, some studies show ctDNA levels before treatment and at the end of treatment can predict progression-free survival and overall survival.

RL: Our customers use ctDNA to inform treatment decisions for advanced cancer patients. Targeted therapy drugs, such as osimertinib and alectinib, work best when a patient's tumour harbours specific genomic alterations. Because acquiring a ctDNA sample is as simple as a blood draw, oncologists often use a liquid biopsy when acquiring a new tissue specimen is infeasible or would put the patient at risk. In contrast, even small needle biopsies to acquire tissue for genotyping are invasive and may be accompanied by severe complications depending on the organ biopsied.

LO: ctDNA is considered a non-invasive biomarker because it is shed from the tumour of interest and is present in the blood, urine, and other bodily fluids. These patient sample types are much simpler and less invasive to collect compared to traditional tissue biopsy methods. Needle or surgical biopsies have substantial procedural risks that can be further increased for tumours in difficult locations (i.e pancreatic or brain). Blood draws, on the other hand, pose minimal risks and are often already standard procedures built into routine medical visits.

GKN: Molecular biologists have recognised for decades that when both diseased and healthy cells die, they slough their contents – including their DNA – into the bloodstream, and that the genetic variation present in these cells might be discernible in circulating cell-free DNA (cfDNA) found in blood plasma. This idea gave rise to the hypothesis that it might be possible to monitor the status of solid tissues through a minimally invasive blood draw – thus, a "liquid biopsy."

GP: There are now several published examples of ctDNA tests providing potentially diagnostic information. A recent publication cites several studies where measurement of circulating nucleic acids in plasma has shown utility in the diagnostic detection of metastatic cancer; and as a prognostic as levels are modulated with tumoUr burden as disease progresses or as early measure of treatment response. More recently, work from our laboratory was presented at ASCO that highlighted this latter potential clinical utility in monitoring progression free survival for patients that had cleared cfDNA in response to treatment with the EGFR T790M targeted therapy TAGRISSO.

3. What are the specific benefits of the process of obtaining ctDNA through a liquid biopsy over a traditional tumour biopsy?

MM: At the time of first presentation, a traditional tumour biopsy will remain the mainstay of cancer diagnostics. This is because a complete pathological workup for a new cancer patient includes tissue architecture, cell morphology, immunohistochemistry and in situ hybridisation, none of which can be performed on fragments of plasma DNA isolated from a blood sample. However, for tumour genotyping, liquid biopsies can capture tumour DNA shed from multiple tumour sites, something a single tissue biopsy cannot achieve by definition. In advanced cancer, intratumour heterogeneity is well recognised and metastatic sites can harbour subclonal mutations. In contrast to a tissue biopsy, liquid biopsies can provide a more representative view of the tumour genotype. Obtaining a repeat tissue biopsy is challenging and often impractical. Obtaining a blood sample for ctDNA analysis may be a better option.

RL: There are several benefits to a liquid biopsy versus a tissue biopsy. For one, a liquid biopsy is minimally invasive and simple to obtain. By contrast, tissue biopsies can be expensive, painful, and risky, particularly for lung cancers, and liver and bone metastases. Moreover, in lung cancer, traditional biopsies frequently fail to obtain sufficient tissue to complete the recommended testing. Liquid biopsies can also provide real-time genomic information about a patient, because tumour genomics evolve over time, particularly under treatment pressure. National guidelines call for repeat biopsies in breast and lung cancers; these can largely be avoided with a comprehensive liquid biopsy test.

LO: Related to the non-invasive nature of liquid biopsies, one major benefit is the increased accessibility to cancer testing that liquid biopsies afford. When liquid biopsies are collected in a stabiliSing collection device, such as Streck's Cell-Free DNA BCT®, samples can be easily collected in local doctors' offices and sent to testing facilities via standard shipping.

Another major benefit liquid biopsy provides is a more representative picture of tumour heterogeneity. A tissue biopsy

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is taken from a single cell cluster within a single tumour, whereas ctDNA is shed into the blood from all tumour cells and multiple tumours if present.

GKN: • Potential unavailability of tumour tissue; safety of patient (e.g. avoiding collapsing a lung while obtaining a lung biopsy)

• Tumour heterogeneity which may be missed upon sampling of the tumour for DNA characterisation

• Ability to repeat at intervals to monitor response to therapy. A liquid biopsy can detect many types of genetic variations, ranging from single nucleotide mutations to amplifications (or deletions) of entire genes. If a physician is trying to determine the optimal treatment for his or her patient, particularly after this patient's tumour has been profiled, it may be more expeditious and economically sustainable to use a more focused approach such as Droplet Digital™ PCR (ddPCR™).

GP: Tissue biopsies are established methods of diagnosing cancer and more recently (last two decades) has also become established as the medium of choice when performing molecular testing. ctDNA is a relative newcomer in the field of testing and has only recently (last three to four years) become accepted as a viable test format for providing molecular information that may aid in the diagnosis of patients with cancer. Tissue biopsies additionally provide context information that may be of utility in the work up of specific patient cases and may provide diagnostic information at the very earliest stages of cancer.

Certain qualities that have made clinical testing using blood useful include the rapid turn-around time for molecular results; the non-invasiveness of performing a blood draw, the potentially quantitative nature of the molecular results, the ability to assess molecular heterogeneity in the circulatory system. Blood based testing also facilitates early treatment decisions as well as opens up the potential for monitoring patients for prognosis as disease progresses, or after treatment intervention. There is clearly a role today for ctDNA: from initial diagnosis, disease and treatment prognostication as well as monitoring. More cost effective technology and reagents that can deliver accurate and rapid results from a simple blood draw (or eventually finger sticks) could significantly help transform the way cancer is diagnosed around the world.

CA: Liquid biopsies can be acquired by simple blood tests which are usually tolerated well by patients and present little risk to a patient's well-being. In contrast, sampling tissue can be more difficult depending on where the tumour is in the body. For example, patients will often require camera tests to guide biopsy procedures of organs such as the lung (bronchoscopy) or the stomach (gastroscopy). Although the information gathered by tissue analysis is often vital to helping doctors construct a treatment plan for a patient, these procedures can often be uncomfortable and present risks to patients.

Using ctDNA to identify patients following surgery who still have residual cancer can help us re-design these studies to only include patients with a very high risk of cancer relapse following their surgery. This should reduce the number of patients required for adjuvant studies making them more economical and feasible in a personalised setting. Since the adjuvant setting is the only setting where chemotherapy drugs can cure patients I hope that circulating tumour DNA will stimulate new approaches here.

4. Although exciting, there are considerations that need attention when thinking of ctDNA-based testing, including detection of ctDNA in earlier stages of cancer, test access due to sample collection methods, assay sensitivity, concordance with the gold standard and throughput... Why is this and what can be done to overcome these problems?

RL: The fragments of mutated tumour DNA found in a typical blood sample taken from an advanced cancer patient generally make up a small fraction of the cell-free DNA in that sample. →

Most of the cell-free DNA is from white blood cells and other normal cells, with a minute amount shed into the bloodstream by the tumour. It's understating it to say it's like looking for a needle in a haystack. The challenge is magnified for earlier stage cancers because smaller tumours generally shed far less DNA into the bloodstream. As we learn more about the genomic landscape of cancer, we have been able to increase both the sensitivity and specificity of our technology to the point where new applications such as early detection are becoming feasible, especially in persons at higher risk for cancer such as those who had a resected early stage cancer with a significant risk of recurrence.

LO: ctDNA based testing is still a new technology and the research community has yet to define standardised methods. A lack of standardisation around the pre-analytical processing of liquid biopsy samples is particularly problematic. When standard blood tubes are used to collect samples, variations in storage and transportation methods will lead to sample degradation and have negative impacts on ctDNA test performance and accessibility. However, the Cell-Free DNA BCT® is well-documented to counteract these variables by stabilising the sample immediately in the collection tube and enabling it to withstand such pre-analytical stressors. By maintaining sample integrity, the Cell-Free DNA BCT® ensures consistency of patient material and reliability of ctDNA based test results.

GKN: Weak biological signals need to be detected and discriminated from benign changes that are present in apparently healthy individuals (e.g. widespread TP53 mutations and CHIP). Appropriate pre-analytical issues need to be addressed to verify that the analyte to be tested is preserved until testing can be performed, and that cell lysis of blood cells in the blood sample does not distort the detection or quantification of ctDNA. Concordance with the gold standard is often performed on archival tumour samples that have been collected months or years prior to the blood samples to which they are being compared; it is important for these to be contemporaneously obtained samples for maximal concordance – but even still, tissue may have stronger signal for mutations that are below the limit of detection in the blood, while conversely, imperfect sampling of the tumour may result in mutations being missed but detected in the blood.

GP: There are multiple technologies and tests being applied in the clinic for the measurement of ctDNA. Some of these approaches include NGS, ddPCR, qPCR, BEAMing, MASS Array, and mmPCR. All of these have advantages and disadvantages in the field of blood based testing and importantly continue to improve. One recent key improvement in early detection for example used an NGS approach. Other tests use ambient shipping methods and pre-packaged specimen collection kits to circumvent the need for cold shipment and to increase test stability and access by physicians and patients.

Analytic assay sensitivity and specificity using these mostly PCR-based methods seems to not be a big differentiator among blood tests as all tests have similar levels of performance in terms of analytic sensitivity and specificity within their test process. However, availability of high quality tissue specimens representing the various stages of cancer for which matched plasma is available is a significant

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DeNovix Inc., Business Director, Kevin Kelly said, "It is a real honor to receive this award that reflects the experience of thousands of scientists around the world who use our products. Gaining the experience of their global peers that use our products in their daily research is an invaluable way for scientists to make informed buying decisions."

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hurdle for clinical validation of blood test developers. In the ideal circumstance there would be a tissue biopsy and a blood sample collected simultaneously. However, this is rarely available from biobanks (even in academia) and increasingly requires a prospective study with these objectives. Another challenge that the development of tests for rare variants (or early stages of cancer) is difficult attain readily. In all of these latter, prospective collection studies could provide a solution. We have found that collaborations between test developers and physicians and pharma can be of utility.

CA: The field of liquid biopsies is still in its infancy. Although there are many studies that are producing important data to help us understand how this technology could be used in a clinical setting, important questions remain. There is much interest in the use of circulating tumour DNA to screen for cancer. However, we still need to establish whether all cancer types, especially primary cancers, release circulating tumour DNA at the same rate. We also need to understand the relationship between the volume of a tumour and how much circulating tumour DNA is detectable in blood. This is because the quantity of circulating tumour DNA in blood may limit how sensitive our tests can be in early stage cancer using current technological platforms.

MM: After obtaining a blood sample, if plasma isolation is delayed, peripheral blood cells lyse and shed large amounts of background DNA, making it more challenging to detect and accurately quantify ctDNA. There are multiple new blood collection tubes now available that use proprietary preservatives to stabiliSe blood cells, enabling routine collection and shipping. In early results, these preserve ctDNA quantities but whether preservatives induce background noise and sequencing errors that can complicate detection of low-abundance mutations is still an unresolved question.

There are many assays described for ctDNA analysis with varying accuracy and direct comparisons are difficult. Standardised reference material (from organisations such as NIST in the USA) can help meaningfully compare different assays. With many potential applications of ctDNA in cancer diagnostics, such efforts probably need to focus first on non-invasive genotyping for actionable cancer mutations.

5. What technological advances are you most looking forward to?

LO: This may not be one of the flashiest technologies, but I am looking forward to advancements in sample preparation technologies (i.e. circulating nucleic acid isolations). Current isolation methods can be time consuming and inefficient—needing large volumes of plasma to meet ctDNA input requirements for testing. This places a real burden on physicians and patients and creates a bottleneck for wide clinical implementation of ctDNA testing. As new isolation technologies arise, we will be able to get more information from less sample making liquid biopsies even less invasive but more beneficial for patients.

GKN: On the technological side, we expect more evolved instrumentation that enables larger numbers of targets to be assayed per ddPCR reaction (perhaps half a dozen to a dozen), and that integrates steps from sample processing through detection and analysis and concluding with report generation. This will be built around a better-informed view of sample types and preparations that offer the highest biological signal for the most informative biomarkers.

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Also, we anticipate a better understanding of the nature of the biological signals of cancer and where and when they can best be accessed (e.g. ctDNA, ctRNA, exosomes, tumour-educated platelets) leading to greater clinical sensitivity and specificity for diagnosing and treating this family of diseases.

GP: Technologies that will aid the uptake of ctDNA will include more sensitive, faster and cost effective solutions. These could be addressed in multiple parallel approaches: 1. Nucleic acid recovery process improvements: better fixatives that can preserve nucleic acids (DNA and RNA) so that prospective collections are not always required or that samples can have better integrity in transit and on the bench and finally that more nucleic acid can be extracted from the same sample volume; in-process reagents that improve recovery efficiencies of nucleic acids as the test process is conducted; more accurate QC tests that could be used to fail test samples that have deteriorated or suffered in process losses early on in test workflow; and 2. Instrumentation that has more sensitive detector/dye combinations; ability to develop multiplexed (variant discriminating) assays especially for some of the non-NGS (rapid) technologies; higher through-put of samples into the workflows as utility increases and test volumes continue to rise; improvement in speed and auto-calling of results by software tools that also for the moment also facilitate user interrogation (QC); and 3. Cost effective solutions will drive adoption. Smaller footprint, low overhead/maintenance technologies can help drive the uptake of circulating nucleic acid testing into laboratories and into the field globally.

CA: I am excited about novel technological approaches to circulating tumour DNA analyses that aim to improve the sensitivity by which we can detect ctDNA molecules. These approaches involve improvement in pre-sequencing handling of plasma samples and controlling for sequencing errors that can create noise in data. This noise makes the identification of low frequency genetic alterations in plasma difficult. I would also like to begin to use these technologies in interventional clinical trials to understand if making clinical decisions based on circulating tumour DNA findings can help improve outcomes for patients with cancer. Finally, I am also looking forward to learning more about approaches designed to understand epigenetic alterations present in cell free DNA and circulating RNAs which could improve our ability to screen for cancer.

MM: One challenge in quantitative ctDNA analysis for treatment monitoring is the limit on sensitivity and on precision due to sub-sampling. This arises from a combination of limited blood volume practically obtainable at a time and a variable and often small contribution of tumour DNA in plasma. One way to get around this challenge is to assay multiple mutations simultaneously. I am looking forward to technical advances in simultaneous analysis of multiple mutations that can help improve accuracy during treatment monitoring.

RL: For advanced cancer, technological improvements will help improve ctDNA analysis, but the biggest benefit will come when the wave of targeted oncology drugs, currently in development, reach the market. We simply find more mutations than we have targeted therapies for today. I also believe liquid biopsies can speed up this process by accelerating the clinical trials that will get these drugs approved. ■