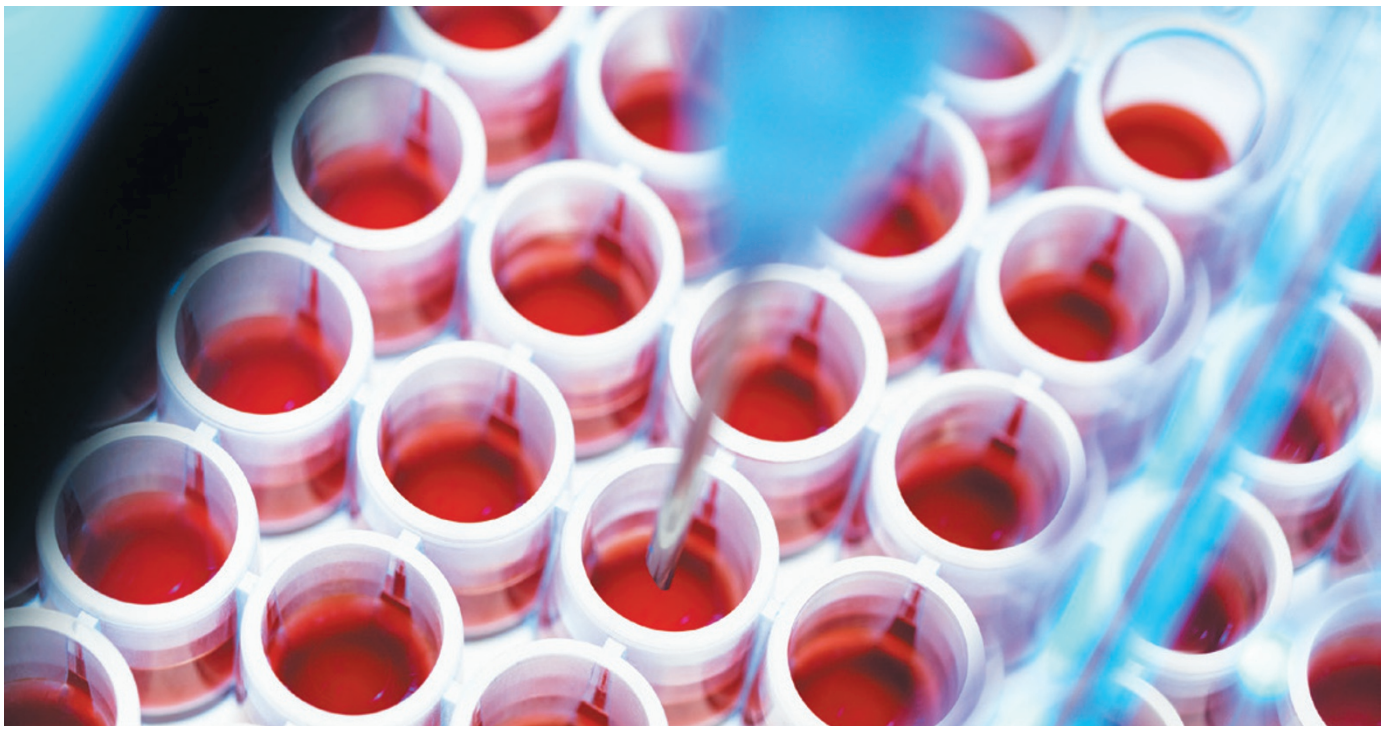
TECHNOLOGY FEATURE

THE TUMOUR TRAIL LEFT IN BLOOD

Liquid biopsies can detect cancer signs from a blood sample, without the need for invasive procedures. But further work is needed before they can become reliable diagnostic tools.

CULTURA RM/ALAMY STOCK PHOTO



Tumour DNA extracted from blood samples could be used to profile cancers, avoiding the need for surgical biopsies.

BY KELLY RAE CHI

A lung biopsy is an invasive and uncomfortable procedure — especially for an 80-year-old grandmother. But by profiling his elderly patient's tumours in this way, lung oncologist Geoffrey Oxnard could target them with a matched drug. After treatment, his patient's tumours seemed to disappear.

Then, some time later, the 80-year-old returned to Oxnard's clinic riddled with pain. Tests showed that the cancer had returned, and hunting down a genetic cause of this resistance would require another invasive lung biopsy.

But Oxnard, who is at the Dana-Farber Cancer Institute in Boston, Massachusetts, offered the woman an alternative: "Let's just check your blood." He performed what's known as a liquid biopsy, using nothing more than a blood sample. Within a day, he spotted minuscule amounts of tumour DNA that revealed a mutation that causes resistance to treatment. Luckily, a drug that targets the mutation was being tested in clinical trials. With the genetic profile in hand, Oxnard managed to enrol his patient into the study, and her tumours went into remission again.

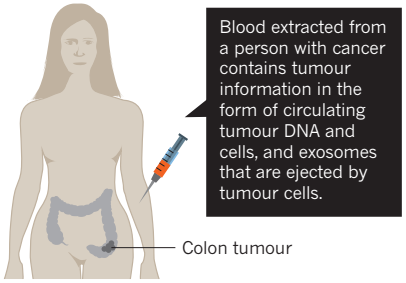
The discovery that parts of tumour cells, or even whole cells, break away from the original tumour and enter the bloodstream led to the idea of liquid biopsies. With this approach, cancers can be genetically characterized by analysing tumour DNA taken from a blood sample, thus bypassing the need to extract solid tumour tissue. Now, the rise of rapid genome-sequencing techniques has made it practical to translate this concept to the clinic. Three main approaches are being pursued: analysing circulating tumour DNA[1], examining whole tumour cells in the bloodstream[2] and capturing small vesicles called exosomes that are ejected by tumours[3] (see 'Scalpel-free biopsies'). And scientists have found that blood platelets might be able to offer up cancer clues, too (see 'Platelets ingest tumour data').

The allure of liquid biopsies is that they are quick, convenient and minimally painful, and they allow clinicians to closely monitor how tumours respond to therapies and to forecast cancer recurrences. In the long term, clinicians might even be able to use liquid biopsies to catch tumours at the earliest stages, before a person shows any symptoms. The genomic information in DNA circulating in the bloodstream could provide a snapshot of cancer genes in the body and may even point to where the cancer originated.

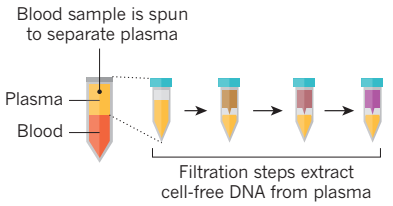
Investors are excited, and funds are pouring into start-ups focused on liquid biopsies. Sequencing firm Illumina of San Diego, ▶

SCALPEL-FREE BIOPSIES

Three different non-invasive techniques allow scientists to monitor tumours by performing 'liquid biopsies' on vials of blood.



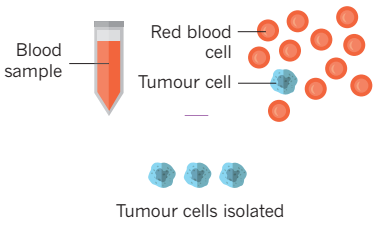
Circulating tumour DNA



DNA fragments from malignant cells (red) are separated from normal DNA (blue) and analysed by next-generation sequencing or digital polymerization chain reaction (dPCR).

Circulating tumour cells

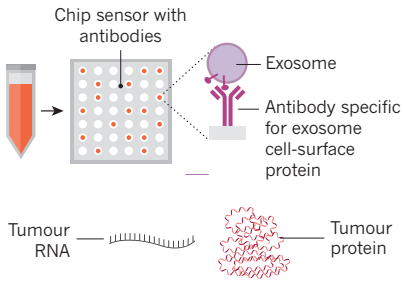
Circulating tumour cells are isolated from blood by cell-separation systems.



Cells are broken up to obtain tumour DNA that can be analysed by whole-genome sequencing.

Exosomes

Tumour exosomes are extracted from blood samples using different assays.



The material inside the captured exosomes — RNA and/or proteins — is then analysed.

California, for example, launched a spin-off company in January called Grail that will develop a plasma-based genetic screen for the early detection of multiple cancers.

But extensive testing is required before liquid biopsies can supersede surgical biopsies in the clinic. And there are still concerns from regulators about the sensitivity and accuracy of these procedures — for example, Oxnard's patient was required to have another, conventional biopsy to confirm the results of the liquid biopsy before she was allowed to enrol in the clinical trial.

Nevertheless, researchers say that it is no longer a question of whether liquid biopsies will one day replace surgical biopsies, but when and in what form. First, however, costs need to go down and sensitivity needs to rise.

FREE-FLOATING DNA

Bits of DNA are constantly flooding into the bloodstream. This genetic flotsam is present even in healthy people and could come from anywhere in the body. Dennis Lo, a chemical pathologist at the Chinese University of Hong Kong, realized that if the placenta of pregnant mothers released fetal DNA, then tumours may also shed DNA. Lo pioneered non-invasive prenatal screening for identifying chromosomal abnormalities in unborn babies, a test now in widespread use. But these screens have also yielded unexpected information — a few mothers-to-be found out that they had cancer.

The advent of more-accurate next-generation sequencing has enabled liquid biopsies on free-floating tumour DNA, because it can distinguish such sequences from normal DNA. In addition, digital polymerase chain reaction (dPCR) allows researchers to detect or quantify specific stretches of tumour DNA even at levels as low as 0.1% of total DNA in the blood.

"These are very sensitive tests," says dermatologist David Polsky of the New York University Langone Medical Center, who has shown that droplet-based dPCR can be used to monitor metastatic melanoma after treatment by tracking circulating DNA[4]. "It's because they're so impressive in the lab that clinicians are excited about them," he says.

Digital PCR, especially the droplet-based versions, has become so easy and cheap that many liquid-biopsy assays have the potential to become widely adopted. But the drawback of dPCR is that clinicians must know what aberrant DNA sequence they are looking for. As a result, sequencing could be preferable for some clinical indications because it allows clinicians to search for mutations without any pre-conceived notion of what genetic changes might be driving the cancer.

At the moment, liquid biopsies are mostly confined to basic- and clinical-research settings, although some blood tests are creeping onto the commercial market. Pathway Genomics, a medical-diagnostic company in San Diego, California, came under fire from the US Food and Drug Administration (FDA) in September 2015 for marketing its blood-based test directly to consumers. The regulator said that the CancerIntercept Detect screening test, which costs US$699 and is aimed at people who are at high-risk of developing cancer but are otherwise healthy, was not approved for direct marketing to consumers and had not been adequately clinically validated. The company countered that it had physician involvement and did not follow a direct-to-consumer model.

Lo is now finishing a 20,000-person screen for nasopharyngeal cancer, a rare type of head and neck cancer that is nevertheless common among men in southern China. In a 2013 study, Lo's team screened 1,300 healthy individuals and found 3 with early-stage cancer (stage 1). Lo will soon report the full results of his study. "It's a big deal that we are able to get them at stage 1," Lo says, because 95% who are treated at that stage survive. Those three people were promptly treated and are still healthy, he adds.

It helps that nasopharyngeal cancer is particularly easy to spot. The cancer is caused by Epstein–Barr virus, which leaves behind a specific genomic footprint that a simple, dPCR-based assay costing roughly $25 per test can pick up. But most other cancers have more undefined patterns of mutation. And although scientists can now access a growing catalogue of tumour signatures thanks to large-scale cancer-genome sequencing projects, there are many more to find, says Lo.

To track the source of the DNA fragments, scientists are starting to take size into account. Of the DNA bits floating in the bloodstream, those derived from normal cells are roughly 100–200 base pairs long, and are still wound around proteins called histones. Histones package DNA into the nuclei of different cells in different ways, so the length of the DNA may indicate the organ from which it was derived. Tumour DNA (like fetal DNA) is shorter than normal blood DNA fragments across multiple types of cancer, and large concentrations of very short fragments have been linked to metastasis (the migration of cancer through the body). The presence of tissue-specific transcription factors and other markers can also reveal clues about the tumour's provenance[5]. With all of this genetic information, clinicians might be able to make an informed guess as to where to look for a tumour, Lo says.

SPOTTING RELAPSE

Tracking a patient's circulating DNA also opens up the game-changing possibility of detecting metastasis at the very early stages, something that would otherwise require repeated invasive procedures. In the past few years, scientists have been studying circulating DNA for signs of cancer recurrence in women who were treated and presumed cancer-free. This is an important step towards early detection of metastatic relapse, says Muneesh Tewari, an oncologist and researcher at the University of Michigan Health System in Ann Arbor.

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Platelets ingest tumour data

Blood platelets are better known for doling out clotting factors after a scrape than for their eating prowess. So when he first set up his laboratory at the VU University Medical Center in Amsterdam, Tom Würdinger was astonished to see platelets swallowing vesicles loaded with tumour RNA.

If platelets, which are easily isolated and counted in everyday blood tests, can take in transcripts from tumours, they might well provide a diagnostic treasure trove, Würdinger reasoned. He and Jonas Nilsson, then a postdoctoral researcher in the lab, formed the company thromboDx and analysed the RNA in platelets from people with cancer; they found that the RNA profiles looked markedly different in cancer, even in the 39 people who had early-stage cancers.

Würdinger's team has since tested the technology on nearly 1,000 people with 10 different types of cancer. More lab studies need to be done, along with trials assessing clinical utility. Still, Würdinger says that in liquid-biopsy development, "we cannot ignore platelets any more, because the results are so powerful". He adds that platelet tests may work well in combination with tests on other biomarkers such as circulating DNA. Indeed, sequencing company Illumina of San Diego, California, acquired thromboDx in February as part of its move into liquid biopsies. K.R.C.

In a 2015 study[6], a blood test in 20 people with breast cancer revealed signs of metastasis 3 years before it could be diagnosed with standard clinical tools. The biomarker was chromosomal rearrangements in circulating DNA. These rearrangements seem to occur early in cancer development, and scientists can use them to design primers for running dPCR tests.

"We thought that [circulating tumour] DNA should be a good biomarker for recurrence of breast cancer, but it had not really been shown before. When we saw that, it was very exciting," says Lao Saal of Lund University in Sweden, who led the study. "To think that you could pick it up three years beforehand, one could speculate that perhaps that could make a clinical difference."

"These are very sensitive tests. It's because they're so impressive in the lab that clinicians are excited about them."

Another study[7] of 55 people with breast cancer identified relapse about 8 months before symptoms appeared, also using dPCR to detect mutations. "Twenty per cent of women with breast cancer will go on to die of their cancer," says Nicholas Turner of the Institute of Cancer Research in London, whose team published the results. "We need to get an awful lot better at identifying the 20% who aren't cured by their current treatment."

Both Saal and Turner compare DNA from patients' primary tumours with circulating DNA collected after treatment. Saal has formed a company, SAGA Diagnostics in Lund, Sweden, that expects to partner with cancer specialists to validate the assay in the clinic. Saal is also working to improve the sensitivity of dPCR for identifying other point mutations to diagnose and monitor disease. Turner's group is starting a clinical trial to assess whether its assay can predict a person's response to a new class of immunotherapeutic drugs, the 'checkpoint inhibitors', which kick start the body's immune system.

Although liquid biopsies are beginning to gain a foothold in some clinics, it's still unclear what impact the search for pre-defined genetic alterations will have on patients' lives, Tewari says. Tumour resistance can emerge from mutations that are not covered by today's tests, so next-generation sequencing approaches will probably be needed to monitor all possible cancer mutations, Turner says.

BREAKAWAY CELLS

Whereas free-floating tumour DNA was discovered fairly recently, scientists first reported finding tumour cells in the blood in 1869. More than 40 different devices for isolating tumour cells are described in the literature (although only one, CellSearch by Veridex in Raritan, New Jersey, is approved by the FDA). For a long time, scientists pursued the notion that the number of tumour cells in blood might be used to assess the aggressiveness of a patient's cancer.

However, results at the bedside have been disappointing. In 2014, for example, the National Cancer Institute's Southwest Oncology Group published a study[8] showing that it was possible, by capturing and counting tumour cells in the blood of people with metastatic breast cancer, to identify a subset of those individuals with a more aggressive cancer. But although these women received another round of chemotherapy, this did not improve their outcomes. From this, people concluded that counting tumour cells had no practical value as a predictor, says Stefanie Jeffrey, a clinical oncologist at Stanford University in California. But, she adds, this conclusion was too swift: rather, it was the extra treatment that did not work. Nevertheless, the American Society of Clinical Oncology does not recommend counting cells to help guide treatment.

Rather than just counting tumour cells, a better option is to sequence the DNA or RNA from them to provide more insight into the gene alterations driving the patient's cancer. And because isolation techniques are improving, greater numbers of viable cells can be harvested from a patient — enough to allow researchers to culture the cells or implant them into mice to study their functional attributes. Any insights they gain can then be used to guide the patient's treatment. Klaus Pantel, director of the Institute of Tumor Biology at the University Medical Center Hamburg-Eppendorf in Germany, foresees the potential of this technique for predicting a tumour's response to targeted treatment — for example, researchers might spot PD-L1 on the cell surface, a protein that helps tumours to avoid the immune system and that can be targeted with immunotherapeutic drugs.

EXOSOME EXAM

A third liquid-biopsy approach targets exosomes. These are tiny vesicles that are shed by all living cells, including tumours, and just like their parent cells, they contain DNA, RNA and proteins. Much about them remains unknown, but a cancer test based on exosomes has already been commercialized. In January, a blood test developed by Exosome Diagnostics of Cambridge, Massachusetts, for detecting lung cancer by analysing tumour-exosome RNA was certified for laboratory use under the Clinical Laboratory Improvement Amendments (CLIA) quality programme in the United States. Exosomes can be harvested from a patient's blood, and potentially from other bodily fluids such as urine, which are even more convenient and easy to access than blood. However, the isolation of tumour-derived exosomes, which are variable in size, and their separation from normal exosomes, remains particularly challenging when compared with isolating free-floating DNA or tumour cells, so it will take some time before the technique is mature enough to detect other forms of cancer.

Each type of measurement gives a different window into the biology and course of disease, Tewari says. Much refinement of the techniques remains to be done, but clinicians are still excited about what liquid biopsies can do today. "They have a powerful role in helping patients get to the right treatment," says Oxnard. ■

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