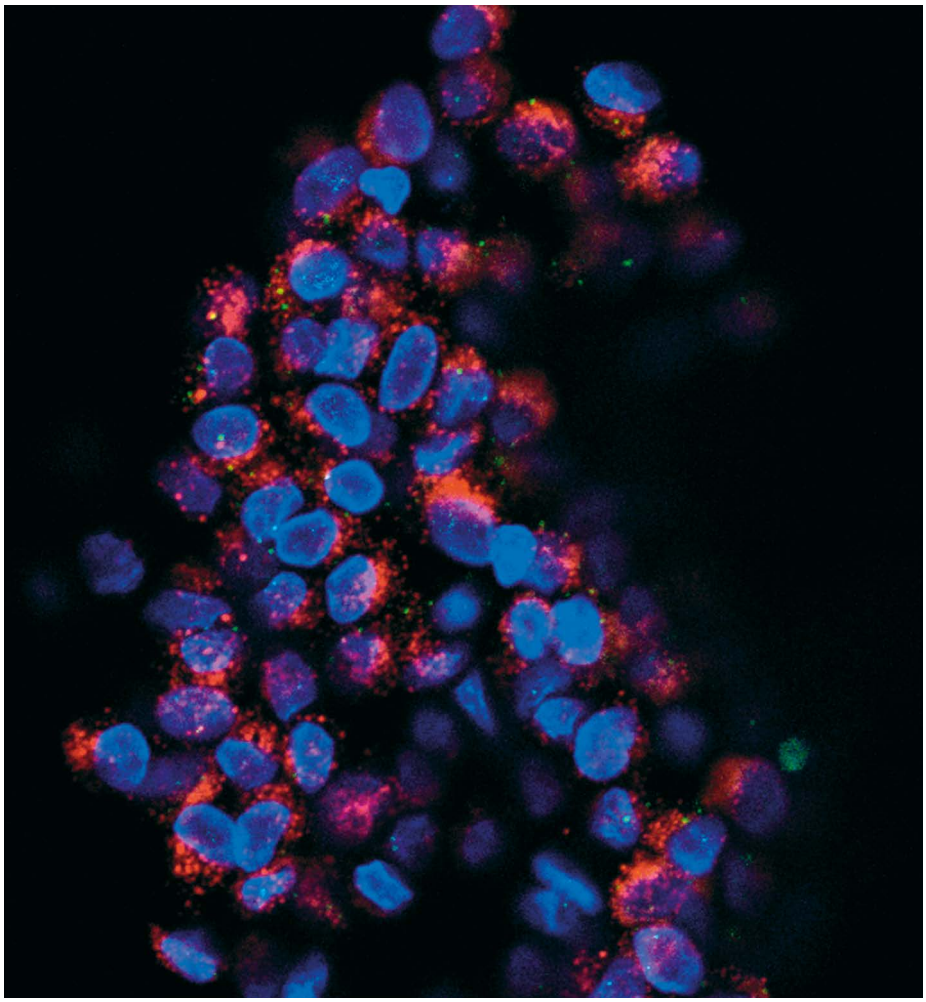


Light micrograph of circulating breast-cancer cells (red) in blood.

USC NORRIS COMPREHENSIVE CANCER CENTER/NATL CANCER INST./SPL

Taking cancer out of circulation

Tests that capture tumour signatures in blood could help to deliver better treatment, or detect cancer before symptoms appear. **By Michael Eisenstein**

The treatment was never going to work. Ryan Corcoran, an oncologist at Massachusetts General Hospital, Boston, didn't realize this when he began treating his patient's colorectal cancer in 2014. His team picked a therapy on the basis of genetic testing of a portion of the person's cancer, and initially the treatment performed well. But before long, it faltered amid overwhelming tumour resistance.

It was only when the person had a liquid biopsy, a test based on analysis of stray tumour DNA in the blood, that Corcoran learnt why the treatment the team had selected was doomed to failure. "There was a mutation known to cause resistance to that drug, which wasn't present in the tissue biopsy but was fairly abundant in the blood sample," he says. "If we had just biopsied a different lesion, we might've picked an entirely different therapy." The experience demonstrated the benefits of searching the blood for answers. Now, blood-based biopsies are commonplace at his hospital, and are increasingly used in cancer centres around the world.

For some cancers, such as advanced non-small-cell lung carcinoma, physicians in Europe and the United States already rely on liquid biopsies to spot the genetic markers that can forecast response or resistance to certain drugs. But oncologists still think there is vast untapped potential in the tests. Catherine Alix-Panabières, a cancer biologist at the University Medical Centre of Montpellier in France, says that liquid biopsies can give a more comprehensive profile of both primary tumours and metastases than can samples harvested directly from the cancer. This is particularly true in tricky tissues such as the brain, where taking a biopsy is extremely difficult, but also for more commonplace tumours of the prostate or breast. "If you don't put the needle in just the right place, you may miss some important tumour cells," Alix-Panabières says. Blood sampling could also help clinicians to monitor tumours over the course of treatment, to get advance warning of recurrence or even to predict cancer in seemingly healthy people, well before the onset of symptoms.

"We are just scratching the surface of what we will ultimately be able to do," says Maximilian Diehn, a radiation oncologist at Stanford University in California. But to get there, researchers still need to show that promising proof-of-concept results can be translated into interventions that provide better clinical outcomes.

Malignant messengers

Liquid biopsy describes a broad range of screening techniques used on samples that can be collected in a relatively non-invasive way, rather than directly from the tumour. The biopsies often involve blood, but not always – some researchers are exploring the possibility of testing urine, stool and saliva samples, which might offer better windows onto malignancies in some tissues.

There are several distinct indicators of cancer that researchers can look for in liquid biopsies. The earliest efforts focused on the detection and analysis of circulating tumour cells (CTCs). These are either detached components of the main tumour or indicators of ongoing metastatic spread, and therefore valuable signals of aggressive disease. "We do not detect them in all patients with early disease," says François-Clément Bidard, a medical oncologist at the Curie Institute in Paris. "But

when we do detect them, most of the time it is associated with a worse outcome."

CTCs are typically scarce – even with aggressive disease, they might be present at levels lower than a single cell per millilitre of blood. Furthermore, some tumours are more likely to shed cells into circulation than others. Alix-Panabières notes that CTCs are more easily detected in diseases such as prostate and breast cancer than in colorectal or pancreatic cancer, in which stray cells tend to get trapped in the liver.

The good news is that when CTCs are present in the blood, they generally stand out clearly. Most CTCs are epithelial – a type of cell that forms the surfaces of many organs in the body, and is rarely found in blood unless the cells have been released by a tumour. This means that tests designed to catch epithelial cells are generally effective CTC assays.

One of the most well-established of these assays is CellSearch, which has been approved by the US Food and Drug Administration as a test for metastatic prostate, breast and colorectal cancer. The assay uses beads coated with antibodies that bind to known epithelial cell proteins to capture CTCs. Different antibodies are also used to filter out healthy blood cells and identify tumour-specific proteins. "The system is really robust and reproducible for different types of metastatic and localized cancers," says Alix-Panabières. A retrospective analysis of 1,944 patients with metastatic breast cancer demonstrated the strong prognostic value of this assay[1]. People with fewer than five CTCs detected per 7.5-ml blood sample at the onset of treatment were nearly three times as likely to survive as those with more cells present in their sample.

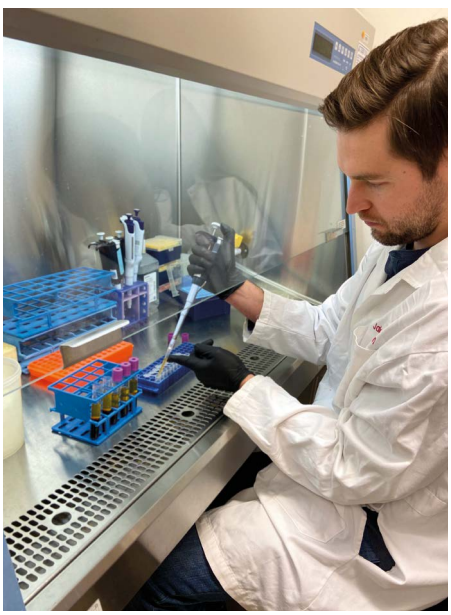
Oncologists still lack robust evidence that using CTC data to guide treatment improves survival. Bidard has coordinated one of the few trials to examine this, known as STIC CTC METABREAST, in which treatment plans for people with advanced breast cancer were based on CellSearch results[2]. "Our data suggest that patients with high CTC counts should be treated aggressively, and have a better outcome if they receive chemotherapy as front-line treatment," says Bidard.

CTCs also offer a unique opportunity to profile metastatic cancer without having to hunt down individual growths. "You can analyse whole tumour cells and get information about the genes, RNA, protein and secreted factors," says Alix-Panabières. Researchers can even culture these cells for short periods to monitor their growth behaviour and response to drugs, she adds. Her team has developed a platform called EPIDROP, which allows researchers to capture and profile individual viable CTCs. In 2019, the team received a series of European grants to support clinical testing of the technology as a tool for guiding treatment.

Picking up the pieces

Advances in genomic-analysis technology have made it possible to analyse vanishingly small amounts of DNA in biological samples. Many clinicians now use liquid-biopsy tests that can profile mutations and other abnormalities in circulating tumour DNA. Relatively little is known about the provenance of this DNA, but it is thought to be released by dying cancer cells and seems to be remarkably representative of tumour genomic diversity. "There's clear evidence that circulating tumour DNA can be contributed to by multiple tumour deposits present in patients," says Diehn.

Many of these assays are based on the polymerase chain reaction (PCR), a biochemical technique that can selectively replicate specific DNA sequences. PCR amplifies the information present in just a few molecules, making it much easier to detect and quantify mutations in known cancer-related genes. "PCR is for when you have a clean hypothesis and you know what mutations you're looking for," says Geoffrey Oxnard, a medical oncologist at the Dana-Farber Cancer Institute in Boston, Massachusetts. This is especially useful when physicians are considering drugs that target specific driver mutations (those that give cancer cells a growth advantage), such as the various drugs that target the epidermal growth factor receptor (EGFR) in non-small-cell lung carcinoma. Some EGFR mutations make tumours especially vulnerable to treatment, while others render those drugs ineffective.



MARGARITA GALLARDO/STANFORD MEDICINE

A researcher prepares samples for the CAPP-seq liquid-biopsy test.

PCR-based liquid biopsies typically only target a small number of genes at a time. But the falling cost of genome sequencing is allowing much more extensive testing, spanning dozens or even hundreds of cancer-associated genes in one go. The sequencing test used by Corcoran's group can currently detect mutations in roughly 70 genes at a time. "It's a good discovery tool," he says. But these tests are still more expensive and more time-consuming than PCR, and can yield results that are hard to interpret in the context of making a clinical decision – for example, revealing mutations that are ambiguous in terms of their contribution to cancer.

"If you don't put the needle in just the right place, you may miss some important tumour cells."

Despite their faults, both PCR and sequencing are now routinely used in the clinic – either in dedicated on-site programmes, such as the one run by Corcoran and his colleagues, or through commercial services that allow physicians to outsource liquid biopsies. "Companies provide a simple kit that comes with blood tubes and a protective shipping package," says Corcoran, "and results typically come back in about a week." These assays based on circulating tumour DNA are proving surprisingly powerful – Corcoran and his team showed that thorough liquid biopsies of circulating tumour DNA could match or even outperform conventional biopsies in mutational profiling of colorectal, gastro-oesophageal and biliary tumours[3]. Sequencing and PCR captured every drug susceptibility and resistance mutation found in tissue specimens, as well as many other important mutations. In 78% of the people tested, circulating tumour DNA revealed mutations associated with drug resistance that were absent from tissue biopsies.

Circulating tumour DNA is not a perfect diagnostic tool. Not all tumours shed DNA, Oxnard points out, and it can be a challenge to distinguish mutations in cancer-derived DNA from the randomly mutated DNA of some non-cancerous blood cells. "Liquid biopsies cut out the physician who makes sure we get a good tumour specimen, and the pathologist who tells us whether it's adequate," he says. "We have used a shortcut that sometimes leaves us with a marginal-quality specimen." As such, he still sees these tests as an adjunct to conventional biopsy, rather than a replacement. But a

clinician armed with genomic data from a tissue biopsy can take advantage of the repeat sampling allowed by liquid biopsies to track how a tumour's mutation profile shifts in response to treatment, and adapt therapy accordingly.

Striking early

In the aftermath of first-line treatment, it is difficult for clinicians to be sure that they have eradicated a malignancy or merely reduced it to a handful of rugged survivors that might eventually drive recurrence. "Radiation causes scarring in the areas where we aim it," says Diehn. "That's visible on the scans even when the patient is cured, and it can be very difficult to distinguish that from residual tumour." Liquid biopsies could help to capture signatures of such minimal residual disease, because physicians would already know from the initial biopsy what any cancerous remnants would look like.

Diehn and his colleagues have developed a liquid-biopsy test based on circulating tumour DNA. Known as cancer personalized profiling by deep sequencing (CAPP-seq), it has repeatedly demonstrated the potential to detect minimal residual disease[4]. Using a sequencing-based approach, CAPP-seq targets distinctive regions of the tumour genome on the basis of information obtained from biopsies collected before treatment. This genetic fingerprint can be readily distinguished from non-tumour DNA. Diehn and his team have tested CAPP-seq in a variety of tumours, including oesophageal cancer, in which the team's assay could catch signs of recurrence nearly three months before cancers became radiologically visible[5].

"Patients who still have detectable circulating tumour DNA are basically guaranteed to recur, and those patients could be good candidates for escalation of therapy," says Diehn. These tests have not yet been shown to be clinically useful, however, and Corcoran notes that the current limited sensitivity of biopsies based on circulating tumour DNA will probably produce high levels of false negatives. But the potential is clear. In 2019, the DNA-testing company Natera in San Carlos, California, launched an assay for detecting minimal residual disease in various tumours that leverages its expertise in monitoring trace levels of circulating genetic material.

"We have used a shortcut that sometimes leaves us with a marginal-quality specimen."

Even more exciting is the possibility of intercepting cancer in asymptomatic people with a routine blood test. Nickolas Papadopoulos, an oncology researcher at Johns Hopkins Medicine in Baltimore, Maryland, points to the sharp reductions in deaths from cervical and colorectal cancer that screening programmes have brought about. "The data suggest that when you find cancers early, more patients not only survive but you can also consider them cured after treatment," he says. CTCs are generally too scarce to detect in very early-stage disease, but a number of research groups are making headway with spotting circulating tumour DNA early.

Genetic-diagnostics company Grail in Menlo Park, California, presented striking data from one such liquid-biopsy study at the European Society for Medical Oncology Congress in September last year. The company tested multiple assays that analyse circulating tumour DNA in blood samples from a cohort of 3,600 people with and without cancer[6]. Grail looked at both sequence data and patterns of a DNA chemical modification called methylation, which is known to strongly affect gene expression. Oxnard, who led the study, notes that the methylation data proved particularly powerful at revealing tumour-specific DNA and the tissue from which it originated — achieving a 76% detection rate for stage 2 disease in hard-to-treat malignancies such as pancreatic cancer (see page S12), with a false-positive rate of less than 1%. "This test is now being prepared for clinical use," says Oxnard.

Combination approaches hold a lot of promise. For example, Papadopoulos and his colleagues developed CancerSEEK, a blood-based assay that looks at tumour DNA and protein biomarkers in parallel. They showed that they could achieve early-detection rates of 69–98% for 5 different malignancies in samples from a cohort of more than 1,000 people, with a false-positive rate of less than 1% (see 'Seeking out tumour types')[7]. CancerSEEK is now being commercialized by Thrive Earlier Detection in Cambridge, Massachusetts, a company founded by the test's inventors.

Papadopoulos says that data from a follow-up study of 10,000 women aged 65–75 is due to be published this year. "This was a good way to kick the tyres of the technology, because this is an age group where you're going to see co-morbidities like potential inflammatory disease that could affect the specificity of your test," he says. In Europe, Alix-Panabières is part of a multinational effort called PROLIPSY, which is exploring whether a combination of CTCs, circulating tumour DNA and tumour-secreted RNA bundles known as exosomes might assist early detection and prognostic profiling of prostate cancer.

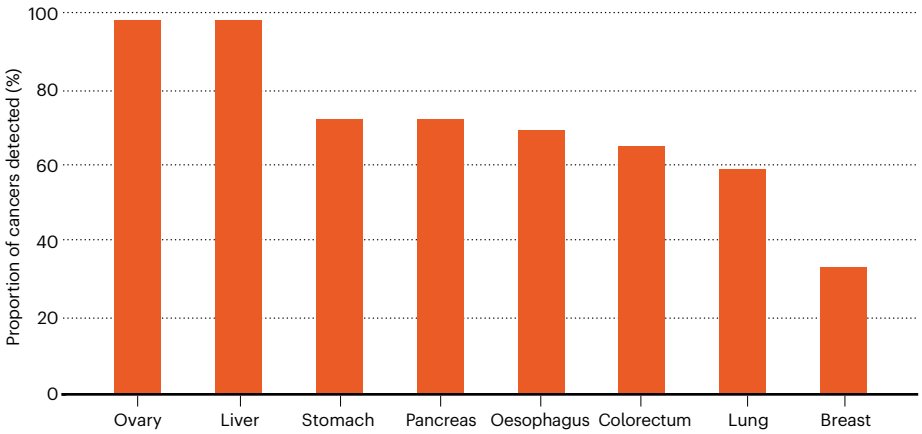
Early diagnosis in people without symptoms creates a host of other challenges — for example, even a relatively low 1% false-positive rate can mean unnecessary stress for some people. It also increases costs — a problem that could see the use of liquid biopsies limited to well-funded medical systems. "If this is a test you give something like every six months and it's expensive, in the end it's not going to have an impact on cancer mortality worldwide," says Bidard. But an accessible and effective test could rewrite the cancer playbook, potentially turning lethal malignancies into curable conditions.

Michael Eisenstein is a science writer in Philadelphia, Pennsylvania.

1. Bidard, F. C. *et al. Lancet Oncol.* **15**, 406–414 (2014).
2. Pierga, J.-Y. *et al. Cancer Res.* **73**, Abstract 2410 (2013).
3. Parikh, A. R. *et al. Nature Med.* **25**, 1415–1421 (2019).
4. Newman, A. M. *et al. Nature Med.* **20**, 548–554 (2014).
5. Azad, T. D. *et al. Gastroenterol.* **158**, 494–505 (2020).
6. Oxnard, G. R. *et al. Ann. Oncol.* **30**, v851–v934 (2019).
7. Cohen, J. D. *et al. Science* **359**, 926–930 (2018).

SEEKING OUT TUMOUR TYPES

A blood-based assay called CancerSEEK that looks for tumour DNA and protein biomarkers is especially accurate at detecting ovarian and liver cancers.



SOURCE: REF 7.