WRITTEN IN BLOOD

DNA circulating in the bloodstream could guide cancer treatment — if researchers can work out how best to use it.

BY ED YONG

In 2012, Charles Swanton was forced to confront one of cancer's dirtiest tricks. When he and his team at the Cancer Research UK London Research Institute sequenced DNA from a handful of kidney tumours, they expected to find a lot of different mutations, but the breadth of genetic diversity within even a single tumour shocked them. Cells from one end differed from those at the other and only one-third of the mutations were shared throughout the whole mass. Secondary tumours that had spread and taken root elsewhere in the patients' bodies were different again[1].

The results confirmed that the standard prognostic procedure for cancer, the tissue biopsy, is woefully inadequate — like trying to gauge a nation's behaviour by surveying a single street. A biopsy could miss mutations just centimetres away that might radically change a person's chances for survival. And although biopsies can provide data about specific mutations that might make a tumour vulnerable to targeted therapies, that information is static and bound to become inaccurate as the cancer evolves.

Swanton and his team laid bare a diversity that seemed insurmountable. "I am still quite depressed about it, if I'm honest," he says. "And if we had higher-resolution assays, the complexity would be far worse."

But researchers have found ways to get a richer view of a patient's cancer, and even track it over time. When cancer cells rupture and die, they release their contents, including circulating tumour DNA (ctDNA): genome fragments that float freely through the bloodstream. Debris from normal cells is normally mopped up and destroyed by 'cleaning cells' such as macrophages, but tumours are so large and their cells multiply so quickly that the cleaners cannot cope completely.

By developing and refining techniques for measuring and sequencing tumour DNA in the bloodstream, scientists are turning vials of blood into 'liquid biopsies' — portraits of a cancer that are much more comprehensive than the keyhole peeps that conventional biopsies provide. Taken over time, such blood samples would show clinicians whether treatments are working and whether tumours are evolving resistance.

As ever, there are caveats. Levels of ctDNA vary a lot from person to person and can be hard to detect, especially for small tumours in their early stages. And most studies so far have dealt with only handfuls or dozens of patients, with just a few types of cancer. Although the results are promising, they must be validated in larger studies before it will be clear whether ctDNA truly offers an accurate view — and, more importantly, whether it can save or improve lives. "Just monitoring your tumour isn't good enough," says Luis Diaz, an oncologist at Johns Hopkins University in Baltimore, Maryland. "The challenge that we face is finding true utility."

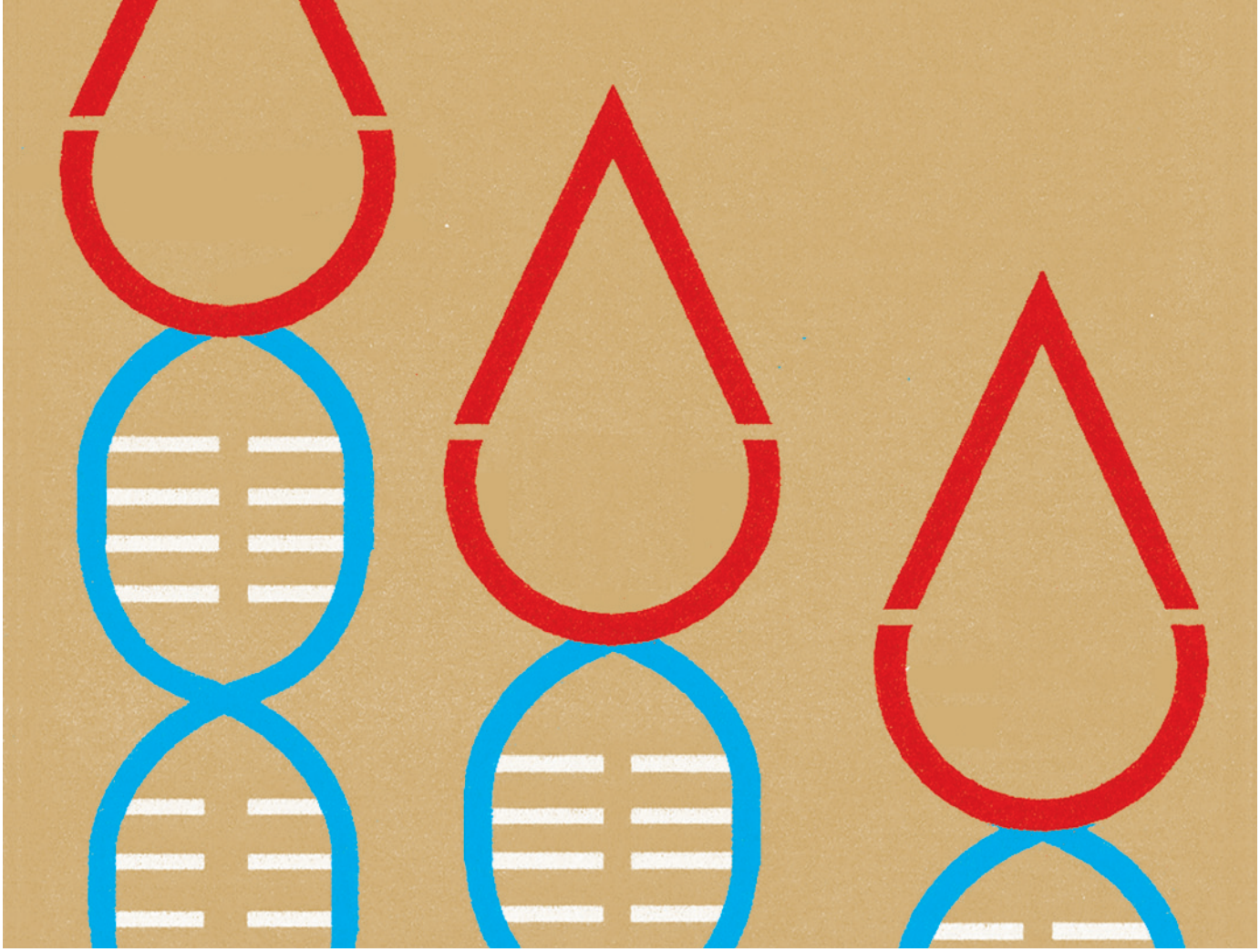
If researchers can clear those hurdles, liquid biopsies could help clinicians to make better choices for treatment and to adjust those decisions as conditions change, says Victor Velculescu, a genetic oncologist at Johns Hopkins. Moreover, the work might provide new therapeutic targets. "It will help bring personalized medicine to reality," says Velculescu. "It's a game-changer."

DELAYED ACTION

Scientists first reported finding DNA circulating in human blood in 1948 (ref. 2), and specifically in the blood of people with cancer in 1977 (ref. 3). It took another 17 years to show that this DNA bore mutations that are hallmarks of cancer — proof that it originated from the tumours[4,5].

The first practical use of circulating DNA came in another field. Dennis Lo, a chemical pathologist now at the Chinese University of Hong Kong, reasoned that if tumours could flood the blood with DNA, surely fetuses could, too. In 1997, he successfully showed that pregnant women carrying male babies had fetal Y chromosomes in their blood[6]. That discovery allowed doctors to check a baby's sex early in gestation without disturbing the fetus, and ultimately to screen for developmental disorders such as Down's syndrome without resorting to invasive testing. It has revolutionized the field of prenatal diagnostics (see *Nature* **507,** 19; 2014).

"Cancer has been slower to catch on," says



ILLUSTRATIONS BY OLIVER MUNDAY

Nitzan Rosenfeld, a genomicist at the Cancer Research UK Cambridge Institute. This is partly because tumour DNA is much harder to detect than fetal DNA. There is typically less of it in the blood, and the amounts are extremely variable. In people with very advanced cancers, tumours might be the source of most of the circulating DNA in the blood, but more commonly, ctDNA makes up barely 1% of the total and possibly as little as 0.01%. Early sequencing technologies were not up to the task of detecting it — at least, not consistently or reliably enough to use ctDNA as a biomarker.

But the past decade has brought sensitive techniques that can detect and quantify minute amounts of DNA. For example, an amplification method known as BEAMing — which fastens circulating DNA to magnetic beads that can then be isolated and counted — can detect ctDNA even if it is outnumbered by healthy cell DNA by a factor of 10,000 to 1.

Genetic oncologists Bert Vogelstein and Kenneth Kinzler at Johns Hopkins developed the technique, and in 2007 they described[7] using it to track ctDNA in 18 people who were being treated for bowel cancer. After surgery, the patients' ctDNA levels fell by 99%, but in many cases the signal did not disappear completely. In all but one of the people with detectable ctDNA at the first follow-up appointment, the tumours eventually returned. None of the people with undetectable levels after surgery experienced a recurrence.

These results suggested that ctDNA can reveal how well a patient has responded to surgery and whether they need chemotherapy to finish off any lingering cancer cells. Researchers soon found similar results for other types of cancer. Rosenfeld and his Cancer Research UK colleagues James Brenton and Carlos Caldas showed that ctDNA provides a precise portrait of advanced ovarian and breast cancers[8]. And in the largest study yet, Diaz and other members of the Johns Hopkins group detected ctDNA in at least 75% of patients with advanced tumours, in organs as diverse as the pancreas, bladder, skin, stomach, oesophagus, liver and head and neck[9]. (Brain cancers were a notable exception, because the blood–brain barrier stops tumour DNA from reaching the bloodstream.)

BETTER BIOMARKERS

Circulating DNA might perform better than the protein biomarkers that researchers have been seeking and refining for decades. Proteins are used in the clinic to diagnose illnesses and monitor people undergoing treatment. For example, prostate-specific antigen is a biomarker for prostate cancer, but it can give false positives because there are other reasons that the antigen can be elevated in the blood. False positives should be rarer with ctDNA because it is defined by mutations and other genomic changes that are hallmarks of cancer cells. And although most protein biomarkers stay in the blood for weeks, ctDNA has a half-life of less than two hours, so it gives a clearer view of a tumour's present, rather than its past. The Cambridge and Johns Hopkins teams have found that ctDNA is more sensitive than protein biomarkers when it comes to detecting breast[10] and bowel[9] cancers, respectively, and it is more accurate at tracking tumour disappearance, spread and recurrence.

Both teams also showed that ctDNA was more sensitive than circulating tumour cells — intact cancer cells that also travel around the bloodstream and have been an intense area of research. In a sub-study of 16 people, Diaz's team found that where both were present, ctDNA fragments outnumbered circulating tumour cells by 50 to 1 (ref. 9). And although ctDNA was always there if the circulating cells were, 13 people with detectable tumour DNA had no trace of such cells.

But most exciting to scientists, says Diaz, is the ability to watch tumours evolve and adapt over time: "It'll help us answer questions in oncology that have never been answered before."

For example, why do so many targeted therapies eventually fail? Gefitinib and panitumumab are among several drugs that block the epidermal growth factor receptor (EGFR), a protein involved in cell growth and division that is overactive in a number of cancers. People taking these drugs do very well — briefly. But after a few months, their cancers almost always develop resistance, often through

changes to other genes, such as *KRAS*, which is mutated in many cancers.

To monitor patients and decide on the next course of action, clinicians would normally need to take multiple biopsies. But people with advanced cancer often have several tumours to test, and different parts of any single tumour could be resistant in different ways. Biopsies are invasive and risky, and difficult for inaccessible and fragile organs such as the lungs. "You can't just go to the patient and get five more biopsies after the treatment fails," says Velculescu. Taking blood is simple in comparison.

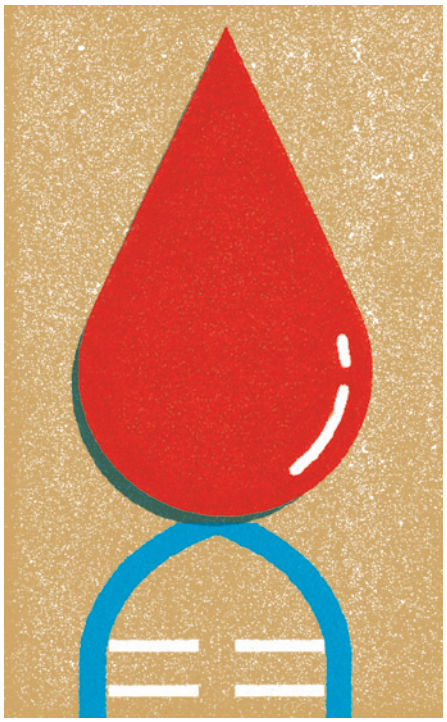
In 2012, Diaz's team reported[11] using ctDNA to study patients who were being treated with EGFR inhibitors. The researchers found 42 different *KRAS* mutations that confer resistance; on average, these turned up 5 months before imaging techniques showed that the tumours were progressing. The team was specifically looking for *KRAS* mutations, but Rosenfeld's group has used ctDNA to identify resistance mutations from a blind start. Last year, the researchers described how they had sequenced the complete exomes — the 1% of the genome that encodes protein — in blood samples from six people being treated for advanced breast, lung or ovarian cancers. In five cases, the unguided search revealed routes to resistance, such as mutations that prevent drugs from binding to their target proteins[12].

Spotting resistance early would let clinicians take patients off toxic and expensive drugs that are unlikely to keep working. And by identifying the mutations that underlie the resistance, they could find effective alternatives or drug combinations. "The hope is that we can turn cancer from a deadly disease into a chronic one," says Velculescu. "You treat someone with one therapy and when it stops working, you switch, or alternate back and forth."

CLINICAL CAVEATS

Despite its promise, ctDNA is not yet ready for a starring role in the clinic. For one thing, the most sensitive techniques for detecting it, such as BEAMing, rely on some knowledge of which mutations to look for. This knowledge can be provided by taking a biopsy, sequencing its mutations, designing patient-specific molecular probes that target them, and using those probes to analyse later blood samples — a laborious approach that must be repeated for each patient. The alternative is to use exome sequencing, as Rosenfeld's team did. This requires no previous knowledge about the cancer, but it is prohibitively expensive to sequence and analyse every sample at the depth required to detect rare mutant fragments.

Maximilian Diehn, a radiation oncologist at Stanford University in California, has tried to combine the best of both worlds. His team identified a small proportion of the genome — just 0.004% — that is repeatedly mutated in lung cancers[13]. Whenever the researchers get a new blood sample, they sequence this fraction



"IT'LL HELP US ANSWER QUESTIONS IN ONCOLOGY *THAT HAVE NEVER BEEN ANSWERED BEFORE.*"

10,000 times over. This picks up even rare mutant fragments, and the focused approach keeps costs down. Because almost everyone with lung cancer has at least one mutation in these regions, the method should work in almost every patient, says Diehn. The team is now working to develop similar mutation panels for other types of cancer, and to validate the technique in clinical trials — work that could take several years.

Like practically all ctDNA biopsy techniques, Diehn's approach does not do well at picking up early forms of cancer. In a small study[13], it detected every lung cancer of stage II or higher, but only half of stage I tumours. This is understandable — advanced cancers simply discharge more DNA — but it limits ctDNA's potential as a cancer-screening tool.

Diehn says that more-sensitive techniques could overcome this problem, but Diaz disagrees. "The limiting factor is biology," he says. "There just aren't a lot of fragments in circulation." And if ctDNA hints at the presence of an undetected cancer, what then? "If you detect a mutation in the circulation, you don't know where it's coming from," says Diaz.

There are other unknowns, too. Does ctDNA paint a truly representative portrait of a cancer? Do tumours that have spread to other organs release as much DNA as the original tumours? Do all the cells in a tumour release as much ctDNA as each other? Diaz says that the only way to answer these questions is to do 'warm autopsies' — to take samples and characterize all of a person's tumours very soon after death, and compare them with ctDNA extracted in life. "This is the heavy lifting that'll need to be done in the field," he says.

And the biggest question remains: does an accurate picture of tumour burden, or a real-time look at emerging mutations, actually save patients or improve their quality of life? Even if doctors discover that someone's tumour has developed a resistance mutation, that insight is useless if there are no drugs that target the mutation. "The limitation is the reality of targeted therapies," says Velculescu. "You get all this information — but so what? Our approaches to understanding cancer are outstripping our clinical options."

Even if ctDNA does not yet affect outcomes, scientists say that it is an invaluable research tool, and clinicians are starting to collect it routinely. Swanton, for example, is leading a £14-million (US$24-million) lung-cancer study called TRACERx (Tracking Cancer Evolution Through Therapy), which will use both conventional biopsies and ctDNA collected once every three months. The circulating DNA may or may not provide clues that help the study participants, but at the very least, it will give Swanton a much better understanding of how lung cancer evolves, and how to control that evolution.

As Rosenfeld argues, it is better to have this information than not to. Currently, he says, "we're groping in the dark. Why would you do that if you have a tool that allows you to see what's happening?" ■

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