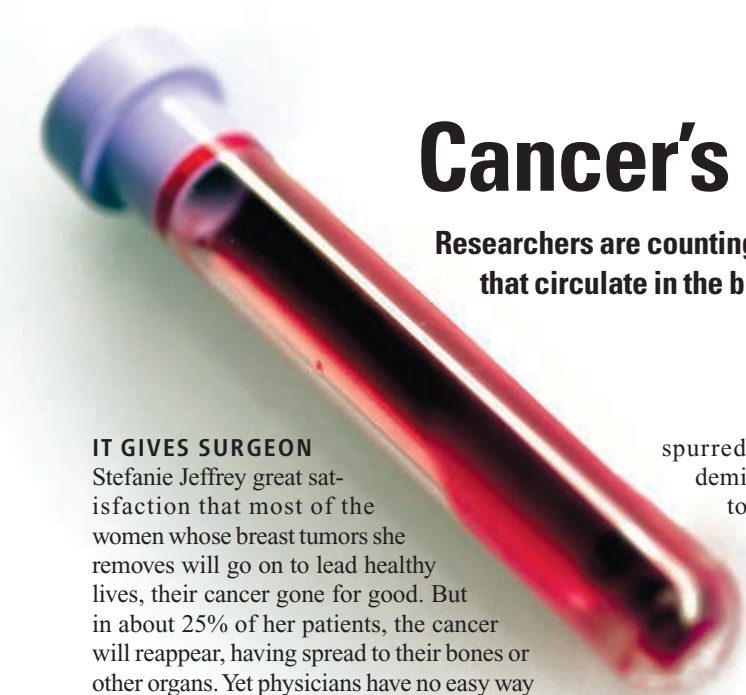

Cancer's Circulation Problem

Researchers are counting and examining the rare cells shed by a primary tumor that circulate in the blood, but will these studies help patients?

IT GIVES SURGEON Stefanie Jeffrey great satisfaction that most of the women whose breast tumors she removes will go on to lead healthy lives, their cancer gone for good. But in about 25% of her patients, the cancer will reappear, having spread to their bones or other organs. Yet physicians have no easy way to know whether a woman's breast tumor has metastasized or whether treatments are keeping a cancer in check. Often they and their patient find out only when a new tumor is large enough to cause symptoms or show up with imaging technologies. By then, "it's often too late" for a cure, says Jeffrey. "It's very frustrating not knowing which patients will develop metastases or how best to treat those who do."

A solution to Jeffrey's frustration may come at her lab bench at Stanford University in Palo Alto, California, rather than in her operating room. She and other researchers are exploring a new window for tracking the spread of cancer: the vanishingly few tumor cells that circulate in a patient's blood.

It's been recognized for decades that cancers metastasize because primary tumors shed cells into the blood, which carries them to other organs where they seed new tumors. It's these metastases, not the primary tumors, that cause 90% of cancer deaths. Only in the past 10 years, however, have researchers figured out how to efficiently capture circulating tumor cells (CTCs) from a blood sample. Clinical researchers are now counting CTCs every few weeks in patients with several types of metastatic cancer, a crude but potentially useful measure for gauging whether a treatment is working. Researchers have also begun to analyze CTCs for certain gene variants or proteins that indicate a patient's tumor is susceptible to a particular drug. This kind of "liquid biopsy," which may allow physicians to follow cancer changes over time and tailor treatment, has spurred at least two dozen academic groups and companies to come up with new CTC detection devices.

The growing ability to detect and analyze CTCs could also galvanize the development of drugs designed to block metastasis, says Joan Massagué, a metastasis researcher at Memorial Sloan-Kettering Cancer Center in New York City. Compared to waiting for secondary tumors to appear, monitoring CTC counts may give companies a shortcut for measuring whether an antimetastasis drug works.

At the same time, CTC research faces hurdles. Any new CTC detection technology is considered a disease monitoring device by U.S. regulators and must be validated in clinical trials—a slow, costly process. And because CTCs are so rare and hard to capture—there may be as few as one cancer cell in a billion blood cells—most separation strategies are thought to miss some of the cancer cells. Moreover, researchers don't yet have a good handle on whether the cells they're collecting from people's blood are the ones that can seed new tumors.

Online
sciencemag.org
Podcast interview with author Jocelyn Kaiser.

Further analysis of CTCs could answer that question and confirm that the picture of metastasis developed over the past decade in animal studies is the same in people. "In some way, the big missing piece has been access to these cells," says Daniel Haber, director of the Massachusetts General Hospital (MGH) Cancer Center in Boston.

Rare, but important

The first report of blood-borne cells shed by a solid tumor came in 1869 from an Australian physician named Thomas Ashworth. Using a microscope, he examined blood from a patient who died of metastatic cancer, spotting cells that looked identical to the cells in the patient's tumors. Such cells "may tend to throw some light upon the mode of origin of multiple tumours existing in the same person," Ashworth presciently wrote.

But only in the 1990s did clinicians fully realize the potential value of CTCs. The inspiration came in part from work on rare primary tumor cells found lodged in a cancer patient's bone marrow long before metastasis was evident. Studies in Europe suggested that patients with these so-called disseminated tumor cells in their bone marrow had a poorer prognosis. Frequent bone marrow biopsies aren't practical, however, so researchers began to look to CTCs in blood samples, says Klaus Pantel, a medical oncologist at the University Medical Center Hamburg-Eppendorf in Germany.

Also motivating the interest in CTCs has been the recent development of molecularly targeted cancer therapies that work best on patients whose tumors have a particular mutation. This spurred a push to develop devices to efficiently capture and analyze CTCs, which could potentially serve as a surrogate for the tumor itself, Pantel says.

One of the first widespread CTC detectors, called CellSearch, works for cancers that arise in the epithelial tissue lining organs such as the breast and colon. The device traps CTCs using magnetic beads coated with an antibody that sticks to a protein called epithelial cell adhesion molecule (EpCAM) that's found on the tumor cells but not on blood cells. But before technicians can use a microscope to count the CTCs, they must stain the trapped cells with other antibodies to distinguish the tumor-derived ones from white blood cells that linger as contamination.

CellSearch demonstrated its potential in 2004, when a study of 177 breast cancer

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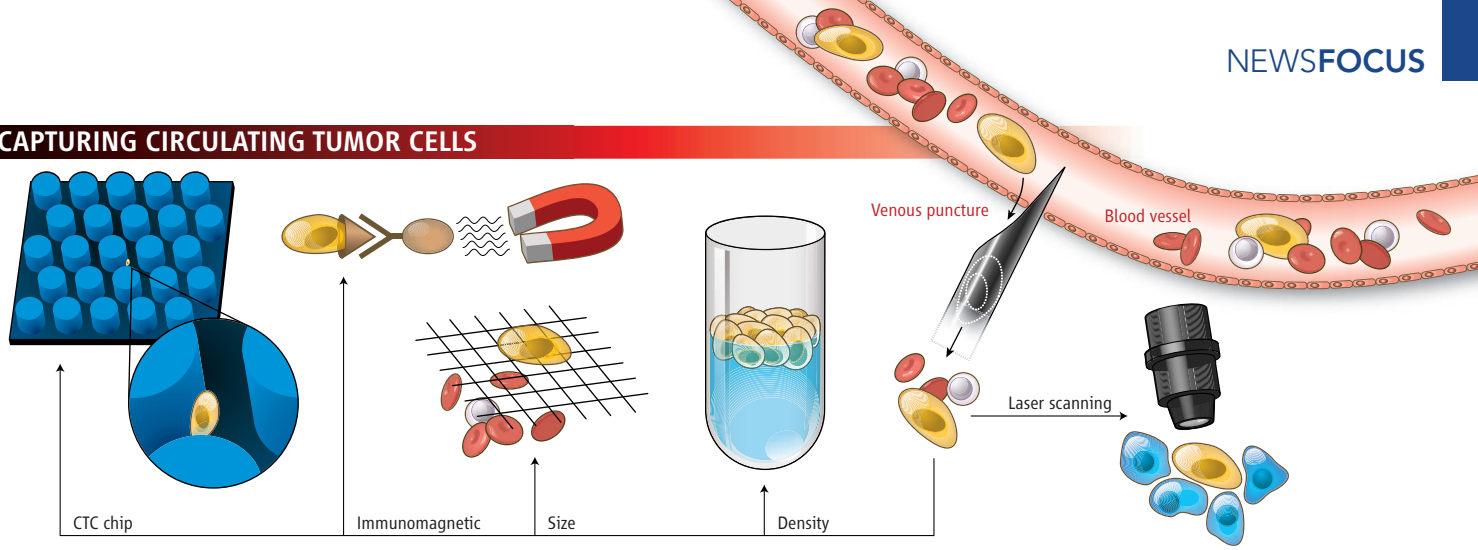

Needle in a haystack. Devices for separating the rare tumor cells (yellow) in a blood sample include a silicon chip studded with microscopic posts, magnetic beads coated with antibodies, filters, density-based centrifuges, and laser detection. Antibodies or genetic analyses are then used to identify and characterize the cells.

patients showed that women with at least five CTCs per 7.5 milliliters of blood had a poorer prognosis than those with fewer or no CTCs. Based on these results, that year the U.S. Food and Drug Administration approved CellSearch as a device for managing the progression of metastatic breast cancer. Manufacturer Veridex later won approval for using CellSearch to monitor metastatic colon and prostate cancers.

One CellSearch test on a blood sample costs about $600, far less than $1500 or more for a PET or CT scan that may only spot significantly sized tumors, notes Massimo Cristofanilli of the Fox Chase Cancer Center in Philadelphia, Pennsylvania, who led the breast cancer trial. CellSearch "will be extremely useful for oncologists," he says.

Just how useful remains uncertain. When the American Society of Clinical Oncology issued its most recent guidelines for treating breast cancer in 2007, it cautioned against making treatment decisions based on CTCs just yet. To confirm the utility of CTC monitoring, experts want a prospective study to show that switching treatments when a patient's CTC levels rise, rather than waiting until an imaging scan shows progression, extends lives. A randomized breast cancer trial now enrolling 500 women in the United States with metastatic breast cancer aims to provide this evidence within the next few years.

More than a number

Counting cells barely scratches the surface of how oncologists want to use CTCs. "My problem with counting is, say the cell count is up and the drug isn't working. What drug do you switch to?" says Jeffrey. She and others want to analyze CTCs for molecular changes in a person's cancer that would point toward particular treatments. For example, only women with breast cancer whose tumor cells express the receptor HER2 respond to the drug Herceptin. Researchers reported 6 years ago that some women whose primary tumors were HER2-negative later had CTCs that were positive for HER2, suggesting that their cancer had mutated. Monitoring CTCs might therefore identify women who were initially ineligible for Herceptin but who would later qualify for the drug.

To detect such molecular changes routinely, researchers say they need to improve on CellSearch, which often finds only a few, if any, CTCs in a cancer patient and yields impure cells that can't be analyzed in much depth. One newer device is a silicon chip developed by Haber and MGH biomedical engineer Mehmet Toner. Called the CTC Chip, it has 78,000 microscopic posts coated with EpCAM antibodies that let blood cells pass by but trap live tumor cells; as with CellSearch, these cells are then dyed with markers and detected with a microscope. In a 2007 *Nature* paper on the CTC Chip, the MGH team reported that 67 of 68 mostly metastatic cancer patients had CTCs, while controls had none.

The MGH group later used the chip to capture CTCs from lung cancer patients for genetic analysis. In a 2008 *New England Journal of Medicine* article, the MGH group reported extracting DNA from these CTCs and detecting key mutations in the gene for a cell surface protein called EGFR—including a genetic change indicating that some patients' cancer had become resistant to the potent drug they were receiving.

Haber's team has a $15 million, 3-year grant from Stand Up To Cancer, a U.S. telethon to raise money for cancer research, to further improve the test—it's slow, requiring about 6 hours per sample—and incorporate it into clinical trials at several other cancer centers.

Devices like the CTC chip and CellSearch that use EpCAM to fish out cancer cells in blood have a potentially significant drawback: They miss CTCs that lack EpCAM, possibly because the cells have gone through the so-called epithelial-mesenchymal transition (EMT), a process in which some tumor cells become less sticky as they're breaking free and entering the blood. Nor can EpCAM methods detect non-epithelial cancers, such as sarcomas.

To combat these problems, some labs are using cocktails of antibodies to try to pick up more CTCs. Others are experimenting with so-called negative filtration, which uses antibodies to remove blood cells from a sample and leave behind tumor cells. Still others are working on CTC trapping methods that don't rely on antibodies. A size-based membrane filter—CTCs tend to be larger and less dense than blood cells—developed by a team led by pathologist Richard Cote of the University of Miami in Florida wins the record for speed: It's essentially a big syringe, and in just 90 seconds it squeezes a blood sample through a filter, leaving behind tumor cells. Yet another approach is to smear whole blood on a slide, stain the cells with various fluorescent markers, then use a laser to rapidly count the cells.

A particular device "may be better depending on what you're trying to do. It's up to you to prove it," says oncologist Howard Scher of Sloan-Kettering. CTC detectors may also soon face a challenge from growing efforts to analyze blood samples for free-floating DNA shed by tumor cells (see sidebar, p. 1074).

One reason researchers are hotly pursuing better CTC detectors is the prospect of a test that is sensitive enough to detect a person's initial tumor early in its development.

SOURCE: ADAPTED FROM K. PANTEL *ET AL.*, *NATURE REVIEWS CANCER* 8 (MAY 2008)

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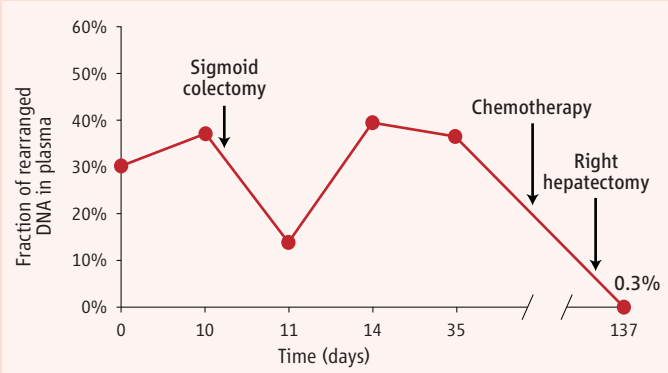
Keeping Tabs on Tumor DNA

While many researchers are working on using tumor cells in blood to track cancer (see main text), Bert Vogelstein's group at Johns Hopkins University has been doggedly pursuing a related idea: monitoring free-floating tumor DNA in blood. A new study suggests that advances in DNA sequencing may make this approach a strong alternative.

Detecting naked DNA shed by a tumor isn't quite as daunting as capturing circulating cancer cells, which requires physically separating multiple kinds of cells. But it isn't easy, either. For several years, Vogelstein's team has tried to detect tumor DNA in a cancer patient's blood by searching for DNA with subtle mutations in known cancer genes. But not all patients' tumors carry such mutations. And the polymerase chain reaction (PCR) technique used to amplify and detect these single-DNA-base changes can generate false positives.

While cataloging mutations in cancers, the Johns Hopkins group hit upon a potentially better way to fish out tumor DNA. They noticed that all solid tumors had large chromosomal rearrangements that were unique to that patient's tumor. Rapid advances in DNA-sequencing technology have now made it practical to systematically look for such large-scale changes. In *Science Translational Medicine* this week, the Johns Hopkins team reports taking biopsies of breast or colon tumors from six people and sequencing the entire genome in each tumor's cells. In each case, the researchers identified rejiggered chromosomes specific to the tumor but not seen in a person's normal DNA.

The researchers then showed they could use PCR to pick out scant amounts of this distinctive tumor DNA from normal DNA, even if it constituted as little as 0.001% of the overall DNA sample. They next analyzed the blood of two people who had colon tumors that had been biopsied and genome-sequenced but not yet been removed. The blood of both tested positive for their specific cancer biomarker, whereas blood from healthy people tested negative. Finally, the researchers used the genetic fingerprint of one colon tumor to track that patient's response to various treatments (see graph). The amount of tumor DNA in the person's plasma declined in the hours after surgery, rose during the next few weeks, and then dropped again after chemotherapy and surgery for a secondary tumor in the liver.



Cancer clue. Levels of free-floating tumor DNA in a colon cancer patient's blood plasma fluctuated during treatment.

Compared with single-base mutations, the chromosomal biomarkers are "extraordinarily specific. The chance of getting a false positive is essentially zero," says Johns Hopkins's Victor Velculescu, who led the pilot study. They could also be used to detect tumor DNA in other fluids and tissues.

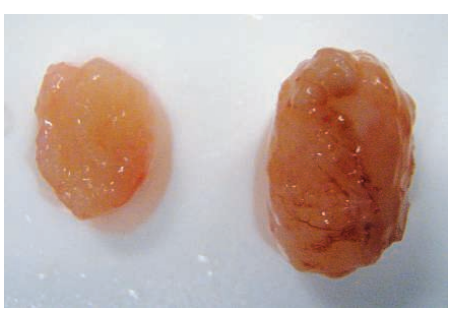
Klaus Pantel of the University Medical Center Hamburg-Eppendorf in Germany, who studies both circulating whole tumor cells and free tumor DNA, enviously admits that his group has thought about the same genome-sequencing strategy. "It is a great approach," he says. But as Pantel and the study authors themselves note, the costs—$5000 per patient just to find the unique chromosome changes—must still come down significantly before the strategy could be available for routine use. And Howard Scher of the Memorial Sloan-Kettering Cancer Center in New York City cautions that tests for whole tumor cells and free-floating tumor DNA needed to be compared head to head in clinical trials to know which is more useful for a specific purpose.

–J.K.

"We're not ready for early detection, but the principle is there," says Haber. Bioengineers are also working on ways to physically filter CTCs out of the blood to prevent metastasis. Some oncologists seem skeptical that you could get rid of every last cancer cell without causing harmful side effects, but the National Cancer Institute is funding research on the topic.

Dangerous seeds?

Whatever the technique, CTC researchers are still struggling with the question of whether the cells they're capturing are the seeds for new tumors. Teams are chipping away at the problem by probing CTCs for the expression of genes thought to be involved in the EMT process or for stem cell markers. The latter is a more recent development as more cancer researchers have begun to believe that the cells initiating tumors have stem cell properties (*Science*, 24 August 2007, p. 1029). Several teams, including Cristofanilli's and Jeffrey's, have been finding CTCs that express EMT or stem cell genes.



Growth spurt. A mouse tumor generated from cultured CTCs (*right*) was twice as large as a regular tumor after 3 weeks.

A few labs are going a step further, trying to directly show that human CTCs can cause new tumors. "If anybody can prove that the few CTCs in human samples grow in mice, it would be almost a proof of their stem cell nature," says Pantel.

This, however, requires isolating sufficient numbers of living CTCs, which only a few labs have managed to do. Jeffrey's group has implanted cells cultured from a human cancer cell line in mouse mammary tissue, waited for tumors to grow, then isolated live CTCs from these mice using a new device that her team invented. When these CTCs were cultured and implanted in another set of mice, the resulting tumors grew larger and spread faster than tumors from the original cell line, her team reported online in January in the *British Journal of Cancer*. "These cells definitely were involved in metastasis," Jeffrey says. She eventually wants to culture CTCs from patients and implant them in mice to see if tumors form.

Cancer biologists are paying more and more attention to such experiments. "I'm thinking of CTCs all the time," says Massagué. If the details of these cells can reveal how human cancers spread, they may offer new ways of stopping it in its tracks.

–JOCELYN KAISER

CREDITS (TOP TO BOTTOM): LEARY *ET AL.*, *SCIENCE TRANSLATIONAL MEDICINE*/AAAS; K. AMERI *ET AL.*, *BRITISH JOURNAL OF CANCER* 102 (2010)

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