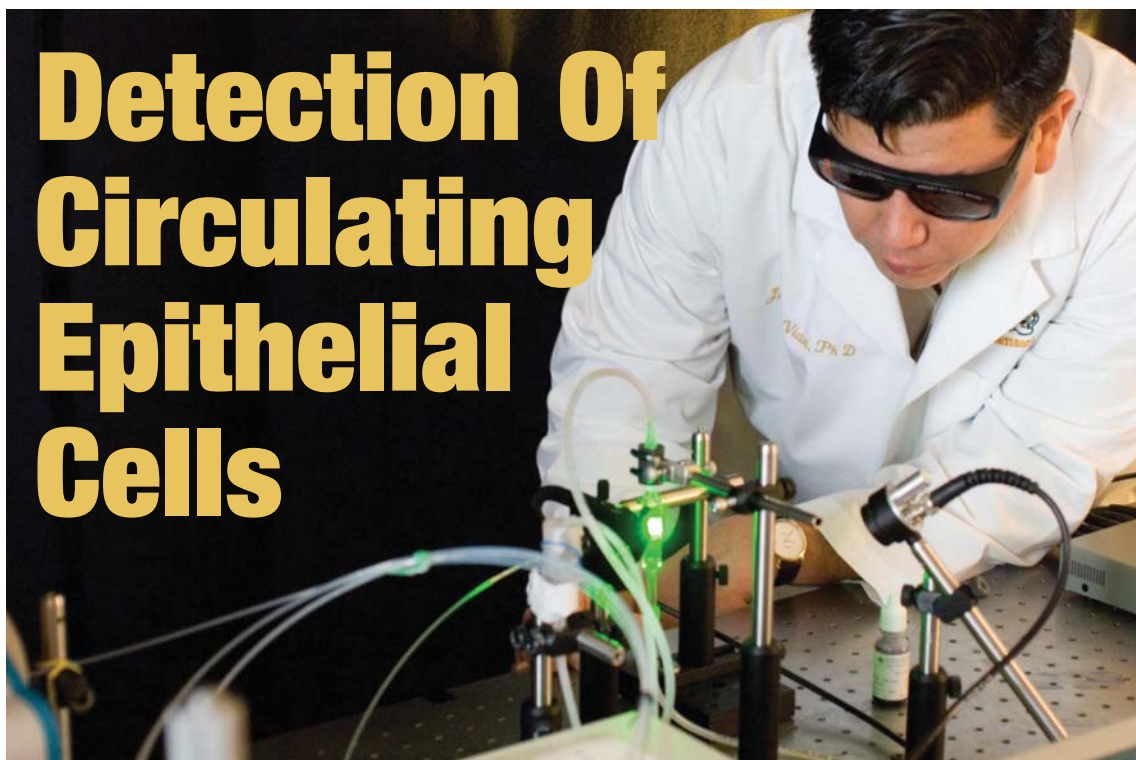


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## Detection Of Circulating Epithelial Cells



by Mike May, PhD

**T**umor cells escape. In fact, scientists learned long ago — as early as 1869 — that cancer cells can end up in the blood. By the mid-1950s, some researchers thought that these so-called circulating tumor cells (CTCs) might help

Figure 1. John Viator aims lasers at samples of blood in a flow cell, and then “listens” with a piezoelectric sensor for photo-acoustic waves that could indicate the presence of cancer cells in the sample. (Image courtesy of John A. Viator).

clinicians catch cancer earlier or measure the efficacy of a treatment. To be specific, researchers mostly look for circulating epithelial cells — not necessarily circulating tumor cells per se

— but the term CTCs remains widely used. Terminology aside, CTCs prove very difficult to find — making up only one cell out of millions even in the blood of someone who has cancer. “It’s like looking for the smallest needle in a moving haystack,” says Mehmet Toner, PhD, of Massachusetts General Hospital.

Even if a researcher can pull a needle from this flowing haystack, it’s not easy to tell one needle from another. When it comes to sensitive ways to tell cells apart, though, scientists often turn to the reverse-trans-

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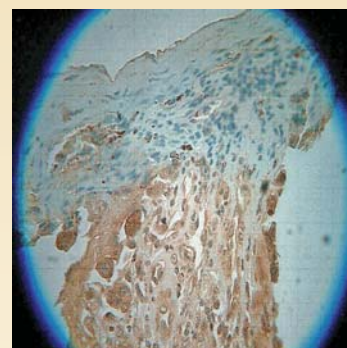
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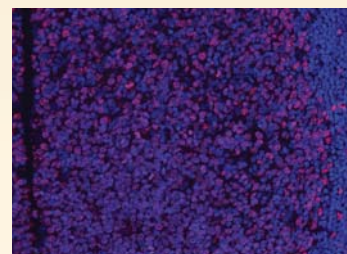
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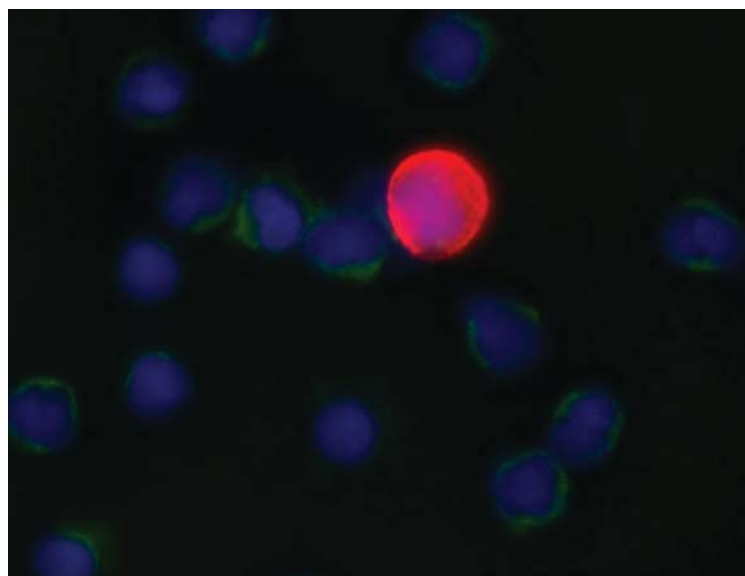
scription polymerase chain reaction (RT-PCR), which can be used to distinguish cells genetically. “The real problem with using this technique,” says Tony Godfrey, PhD, of the Mount Sinai School of Medicine, “is that we don’t have a marker for RT-PCR that is cancer specific. So we use markers that are tissue-type specific.” For example, researchers could look in the blood for messenger RNA from epithelial cells of the lung. Finding this marker in the blood could mean that a patient has a lung-cancer tumor, and that it is producing CTCs.

But just because that marker finds its way to the blood doesn’t prove that a person has cancer. “All genes are expressed a little almost all the time,” says Godfrey, “because it’s almost impossible to turn off anything 100 percent. So maybe a blood cell has this ‘leaky expression’ — producing mRNA that looks like cancer — and even though it’s a tiny level, RT-PCR is sensitive enough to find it.”

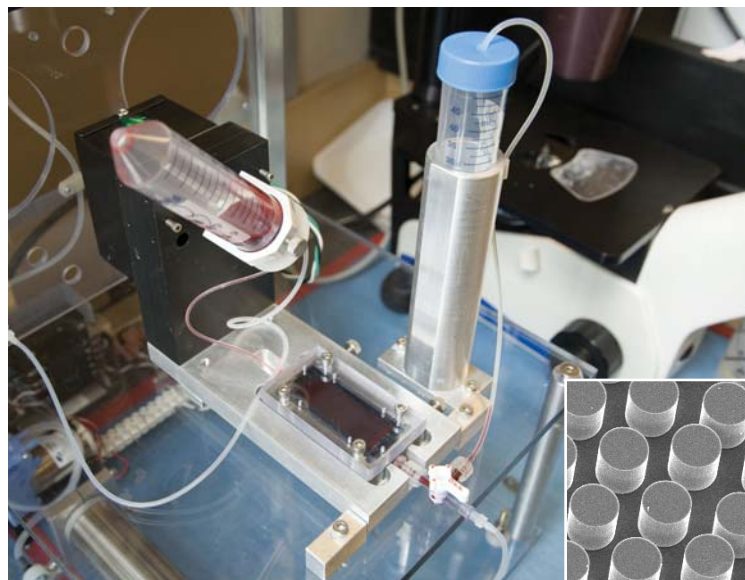
To use RT-PCR to distinguish between leaky expression and CTCs, scientists need some way to enrich the samples. “You actually need to enrich the CTCs by 1,000-fold,” says Godfrey. “Right now, there’s no simple way to do this.”

Not everyone agrees, however, about the difficulty in enriching CTCs. For example, Frank Feist, MBA, executive director of Advalytix, which was founded in Munich, Germany, and is now a product group within Olympus, says, “Standard flow cytometers or immunomagnetic techniques can do this very reliably.”

Various clinical researchers and companies believe in the value of RT-PCR for analyzing CTCs. Once CTCs get isolated and enriched, scientists can look for differences in the CTCs with Advalytix’s AmpliGrid,



Using fiber-optic array screening technology, this circulating epithelial cell—and 254 others—were identified in a tube of blood from a patient with progressive metastatic colon cancer. Moreover, this cell was positively characterized as a circulating tumor cell by a pathologist’s review. (Image courtesy of Peter Kuhn.)



Mehmet Toner and his colleagues developed a chip (above) that captures circulating tumor cells with antibody-encrusted microposts, which are shown in a scanning electron micrograph (right). (Images courtesy of Mehmet Toner.)

which runs RT-PCR on individual cells. “Once you find a CTC,” says Feist, “you can take its genetic fingerprint to study the clinical relevance of subpopulations delineated by differences in gene expression.”

#### An antibody edge

Most cancers arise from mutations in epithelial cells. “More than 85 percent of all cancers are of epithelial origin,” says Toner. So it’s possible to make antibodies that grab

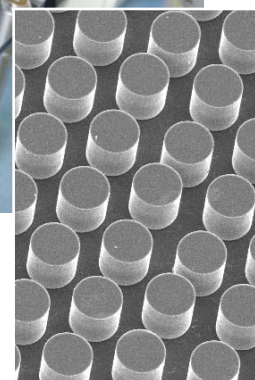
surface markers on CTCs, such as the epithelial cell–adhesion molecule. The non-epithelial cancers will just need different antibodies, according to Toner. The question is: Once an antibody binds to CTCs, how do you find and measure them?

Toner and his colleagues made a device about the size of a business card, which is covered with 80,000 microscopic posts, each covered with the antibody being used to grab CTCs. A 4–5 milliliter sample of blood gets pumped into this microfluidic chip, and the forest of posts makes sure that a CTC hits one of them before passing through the chip. Toner designed this chip so that posts are offset by a fraction of their diameter from one to the next. That way, if a blood cell blocks a CTC from hitting one post, the CTC is likely to eventually hit another post.

The captured cells can be stained and then analyzed in various ways, from simply looking for the stained cells with an ordinary microscope or counting them with an automated imaging platform. The captured cells can also be gathered from the chip and used for molecular studies. For example, captured CTCs could be lysed to release their DNA, which could then be sequenced.

So far, Toner’s chip costs about \$150, depending on how many he makes. The current silicon chip, though, could eventually be converted to plastic. “Then these would cost pennies, maybe dollars,” says Toner. “It would be very cheap.”

Veridex, a Johnson & Johnson company in Raritan, NJ, also markets a device that uses antibodies to capture CTCs. The CellSearch System identifies and counts CTCs that come from epithelial cells and is



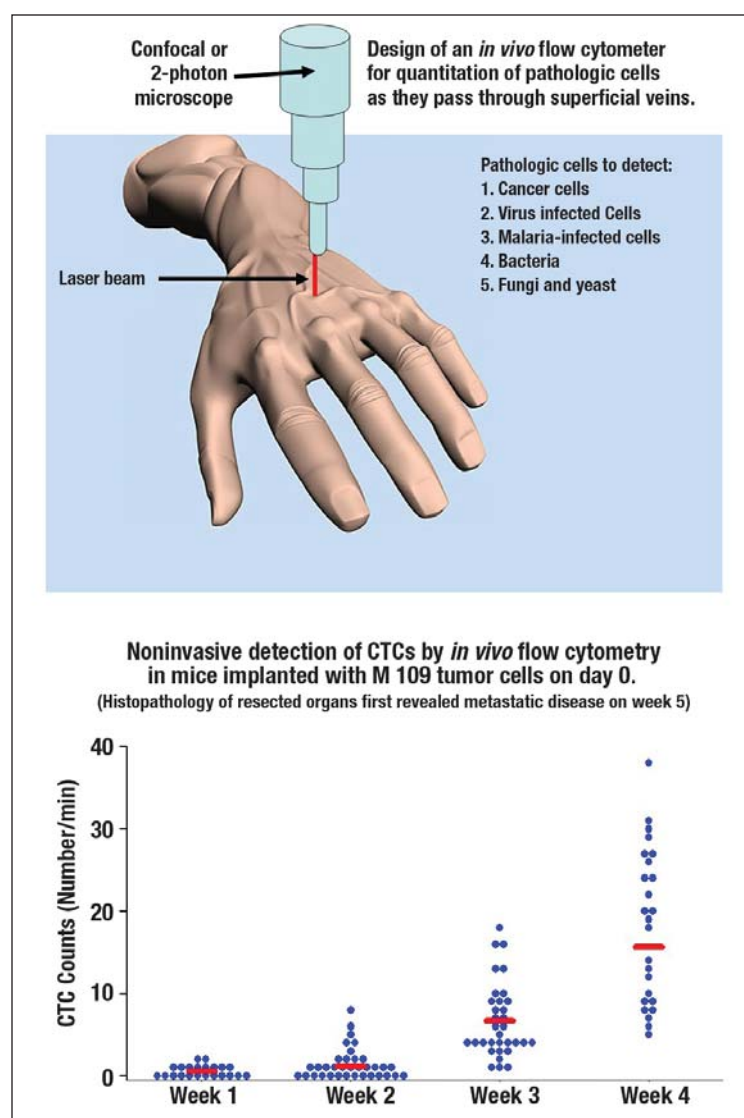
designed to work with metastatic breast, colorectal, or prostate cancer.

### Laser vision

What if a physician could just look to see if there were any CTCs floating in a patient's blood? That's just what Philip S. Low, PhD, of Purdue University, has in mind. He can already place the objective of a multiphoton microscope above a blood vessel that is near the surface, and then use a laser to light up any labeled CTCs in the flowing blood. "We're basically using the blood stream as a flow cytometer," he says. The trick, of course, is finding the right labels. "We've identified several high-affinity ligands that are selective in binding to malignant cells," says Low. Then, Low and his colleagues attach fluorescent dyes to the ligands.

Low's team already tested this technique in animals that had their blood "spiked" with CTCs. "Down to very low numbers, we could detect 99 percent of the cells that we introduced," Low says. The cells for spiking, though, were cultured cells, and real CTCs in humans could be — probably are — more heterogeneous. "So, we could miss some of those," Low adds.

So far, though, Low and his team are aiming at an *ex vivo* approach for humans. Using 2 milliliters of drawn blood, Low can add fluorescent dye-labeled markers and then hit the sample with a laser to look for CTCs. This approach proved very sensitive in finding CTCs in blood samples from patients with prostate or ovarian cancer. Lasers and labeled cells can also be used in other ways to detect CTCs. Peter Kuhn, PhD, of the Scripps Research Institute, looks for CTCs with a laser-based technique called fiber-optic array screening technology (FAST), which was developed at the Palo



A laser beam can be used to cause labeled circulating tumor cells to fluoresce, which can be imaged with a confocal or multiphoton microscope (top). When Philip Low implanted tumors in mice, an increasing number of circulating tumor cells appeared in the blood over time, as measured with Low's non-invasive technique. Low could definitely detect tumor cells in circulation by week 2, but a board-certified pathologist found no evidence of metastatic disease until week 5. (Image courtesy of Philip S. Low.)

Alto Research Center. As a simple explanation of FAST: nucleated cells from about 10 milliliters of blood get spread into a monolayer; epithelial cells get labeled with a fluorescent marker; and, finally, a laser scans the monolayer. Cells that fluoresce get detected with a fiber-optic sensor. "This approach enables very high quality imagery by coupling it to a fluorescent microscope," says Kuhn, "and

this allows for easy and repeat staining of various markers for each cell." So far, this technique works well in determining the source of CTCs, such as a primary breast-cancer tumor. "In the future," says Kuhn, "this technique could also determine the destination of these circulating cells as well as their metastasis potential."

There are even more ways to search for CTCs with lasers.

John A. Viator, PhD, of the University of Missouri, got interested in such an approach after Paul Dale, MD, a surgical oncologist at the University of Missouri, asked if a laser might be able to find CTCs. Lasers would certainly work with CTCs from melanoma, Viator thought, because those cells contain melanin. Viator had already worked with melanin in cells for dermal applications. "Melanin is black," explains Viator, "so it absorbs all colors of light." Because of that, a laser can be used to heat up the melanin, and that makes the cells expand, which creates a mechanical vibration — so-called photo-acoustic waves. Viator can detect these waves with a custom piezoelectric sensor.

So Viator takes a sample of blood, uses a centrifuge to spin down the cells, and then subjects them to a red and a green laser. The white blood cells absorb no color. The red blood cells absorb the green light but not the red. So if a photoacoustic response arises from both the red and green lasers, then the sample includes CTCs with melanin in them.

This technique might also work for cancers beyond melanomas. That would take attaching some sort of label to the CTCs. Viator has already attached a colored label to an antibody for the HER-2 receptor on breast-cancer cells, and he could detect them. Other antibodies could work for other forms of cancer.

Some of these CTC-searching techniques could eventually end up in the clinic. Then, CTCs might help physicians catch cancer much earlier. Likewise, accurate and sensitive measurements of CTCs could be used to track the efficacy of a treatment. Then, the discovery of CTCs — nearly a century and a half old — could move from intriguing to therapeutic. ■