Circulating Tumor Cells: Will They Be Clinically Useful?

By Nancy J. Nelson

Since the mid-19th century, scientists have described microscopic tumor cells in the blood of cancer patients. Known as circulating tumor cells (CTCs), they are thought to play a role in metastasis by breaking loose from a solid tumor, entering the bloodstream, and eventually migrating to distant organs to develop secondary tumors.

The relative number of CTCs is low—perhaps one cell among 100 million blood cells—and until recently, they have been hard to find. But in the past 10 years, new technologies have made it easier to detect and count CTCs, leading to visions of these small cancer cells as wide-ranging biomarkers of disease progression and drug response.

“With CTCs you can see tumor cells in the act of spreading,” said Harvard’s Daniel Haber, M.D., Ph.D., director of the Massachusetts General Hospital Cancer Center in Boston and co-inventor of an assay for detecting CTCs. “With a noninvasive blood test you can question them—see what genes are abnormal and whether they are likely to respond to this drug versus that drug. And you can do that repeatedly through the course of a patient’s treatment. You can follow treatments during real time.”

A blood-based assay that could detect high-risk patients, help guide treatment options, analyze gene expression profiles, identify new drug targets, and illuminate metastasis are some of the hopes pinned on CTCs. Researchers still have a long way to go to realize those aims. But a number of trials are now testing CTCs as prognostic and predictive markers (see sidebar), and some investigators are convinced that they will be clinically useful one day.

Prognostic and Predictive

One early indication of CTCs’ prognostic potential was a 2004 report in the New England Journal of Medicine by Massimo Cristofanilli, M.D., now at Fox Chase Cancer Center in Philadelphia. In a multicenter study of 177 metastatic breast cancer patients who were about to start a new treatment, CTC levels were measured before and after treatment. Women with fewer than five CTCs per 7.5 mL of blood before therapy had a statistically significantly longer overall survival (18 months versus 10.1 months) and progression-free survival (7.0 versus 2.7 months) than those with five or more CTCs per 7.5 mL of blood. Three to 4 weeks later, after therapy, the difference between the groups remained statistically significant.

And after therapy, the number of women in the lower-level CTC group increased, presumably because of a positive response.

In a follow-up study with the same patients, published in Clinical Cancer Research in 2006, G. Thomas Budd, M.D., professor of medicine at the Cleveland Clinic in Ohio, reported that the number of CTCs in this population was a better indicator of disease progression than traditional imaging techniques such as computed tomography and magnetic resonance imaging; CTCs were more reproducible, were better predictors of survival, and estimated disease progression earlier. A 2009 Annals of Oncology article reported that CTC levels were a good predictor of overall survival in metastatic breast cancer patients.

Colon cancer patients have shown similar results. Neal Meropol, M.D., from University Hospitals Case Medical Center in Cleveland, was part of a team that tested whether CTCs were associated with prognosis in patients with metastatic colorectal cancer. Among the 430 patients who took part in this study, there was a statistically significant difference associated with CTC levels.
CTCs in Clinical Trials

Clinical trials are testing whether CTCs can serve as biomarkers of risk and/or response to therapy, using various detection techniques. For example, at least three breast cancer treatment trials in the U.S.—these led by the Cancer and Leukemia Group B—and a large German trial, SUCCESS, are using the CellSearch technique to see whether levels of CTCs can predict the risk of recurrence. And the Southwest Oncology Group in the U.S. is conducting a study with CellSearch in 500 metastatic breast cancer patients to test whether CTC levels can predict their response to therapy near the beginning of the treatment cycle.

Using a modified CellSearch technique, Massimo Cristofanilli, M.D., at Fox Chase Cancer Center in Philadelphia, has begun a breast cancer trial using CTCs as biomarkers to detect changes in HER2 expression as the disease progresses. If HER2 levels increase, patients may become eligible for Herceptin treatment months after their diagnosis.

Two prostate cancer trials are underway using the next generation of detection techniques. With the CTC chip, Daniel Haber, M.D., Ph.D., and Mehmet Toner, Ph.D., Harvard University Medical School, Boston, are testing whether levels of CTCs or specific molecular markers can predict relapse among men with early-stage disease. And using the recently patented MagSweeper, a group at Stanford University in Palo Alto, Calif., led by Sandy Srinivas, M.D., and Stefanie Jeffrey, M.D., is hoping to find specific gene expression patterns or genetic mutations in CTC DNA that predict response to therapy in prostate cancer patients.

Studies in an adjuvant setting are more difficult, both because of the number of CTCs detected and because the proportion of patients with detectable CTCs are lower than in metastatic disease. But in 2008 in Clinical Cancer Research, Jean-Yves Pierga, M.D., from the Curie Institute in Paris, reported detecting low levels of CTCs in 23% of 118 patients receiving neoadjuvant chemotherapy; these levels turned out to be associated with early relapse. Along with these positive results come caveats. Although the level of CTCs seems to be associated with disease progression, CTCs do not themselves predict metastases. Budd said that they have found CTCs in the blood of patients whose cancers have not recurred, at least in the time frame of their studies. Howard Scher, M.D., chief of genitourinary oncology at Memorial Sloan–Kettering Cancer Center in New York, and author of the prostate cancer study, put it this way: “If you took 1,000 patients with detectable CTCs versus 1,000 with no detectable cells, the group with cells would not do as well, but there would be a lot of patients with CTCs who would be cured.”

Detection Methods . . .

A critical issue for CTC researchers, and the eventual clinical use of CTCs, is the assay used to detect them. All the groups mentioned above used the CellSearch System, developed by Immunicon Corporation and Veridex, LLC, the only method so far with approval from the U.S. Food and Drug Administration (for metastatic breast, colorectal, and prostate cancer).

Because CTCs are so few in number relative to circulating blood cells, they must be initially enriched or separated from other blood cells. In CellSearch, this task is carried out by having them bind to an antibody for epithelial cell adhesion molecule, EpCAM, which is often overexpressed in carcinomas of the breast, prostate, colon, head, and neck but is absent from blood cells. The EpCAM antibodies are attached to microscopic iron particles, called ferrofluid, and once the CTCs bind to these antibodies, powerful magnets pull them out of the blood. To make the final cut, cells must be positive for cytokeratin, an epithelial cell marker and nucleic acid, and negative for CD45, a leukocyte cell surface marker. They also must have the signatures of malignant cells—large size, large nuclei, and visible nucleoli.

A group from Massachusetts General Hospital, including Haber, has recently developed an alternative method, known as the CTC chip. The initial separation from other blood cells in this system also involves binding to the EpCAM antibody. The blood sample is continuously pumped through an array of 78,000 microscopic columns coated with EpCAM antibodies that are sitting on a silicon chip the size of a microscope slide. The captured cells undergo a screening process similar to that in CellSearch.

In a 2008 New England Journal of Medicine article, the group reported that DNA extracted from CTCs by using the CTC chip could be analyzed for specific mutations in the epidermal growth factor receptor gene. Eleven of 12 patients who

Meropol said one of the results he found provocative was that patients who continued to have high levels of CTCs a few weeks after starting therapy had a particularly poor prognosis. This finding made him wonder whether CTCs could change the course of treatment early in therapy, sparing patients from additional therapy that would probably not be effective. Also, because CTCs were the strongest predictors of outcome, he speculated that they might be useful in identifying patients who could afford prolonged treatment breaks or a reduction in treatment intensity.

Metastatic prostate cancer studies also have shown CTCs to be associated with survival. A study in 2008 by Johann S. de Bono, M.D., from the Royal Marsden Hospital in London, involving 231 men, found statistically significantly longer overall survival times among men with lower CTC levels. The association between low CTC counts and increased survival was stronger than that between low CTC counts and the prostate specific antigen test at all time points. And patients whose CTC levels changed from the high group to the low during treatment had longer survival times than those whose counts didn’t change.

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In a 2008 New England Journal of Medicine article, the group reported that DNA extracted from CTCs by using the CTC chip could be analyzed for specific mutations in the epidermal growth factor receptor gene. Eleven of 12 patients who
had these mutations in their non–small-cell lung tumors also had them in CTC DNA.

“We have shown that the CTC chip reproducibly isolated circulating tumor cells in sufficient quantity and with sufficient purity to allow molecular analyses,” said Harvard’s Mehmet Toner, Ph.D., one author of the report and a co-inventor or the CTC chip, which was licensed to Cellpoint Diagnostics. “The next step will be to optimize and automate the CTC chip for high-throughput processing for large-scale clinical trials,” Toner said.

About a half-dozen additional CTC enrichment techniques are in development. These take advantage of either tumor markers such as MUC1 and HER2 or the larger size of CTCs compared to other blood cells. However, they have not been standardized, tested in large trials, or compared to each other in the same population, according to Bianca Mostert, M.D., at Erasmus Medical Center in Rotterdam, The Netherlands, who is first author of a recent report on CTCs in Cancer Treatment Reviews.

...and Issues
Once the various techniques have been standardized and tested in large, multi-institutional trials, many questions can be addressed. But problems with the current methods need to be worked out. For instance, Mostert said, research groups should focus on improving the sensitivity of current techniques because CTCs can be detected in only 60% of patients with advanced disease.

Another issue is whether the various technologies are detecting the same cells. “The number of cells detectable and the proportion of patients in whom cells can be detected vary widely between studies,” Budd said. “There is concern that different detection platforms may be detecting different subsets of circulating cells.” That concern seems justified by a recent report that an EpCAM-dependent system did not detect a subtype known as normal-like breast cancer cells (J. Natl. Cancer Inst. 2009;101:61–6).

Budd describes CTC research as a growing, still immature field with many unanswered questions. But he said that the answer to the most important question—whether CTCs are clinically relevant—seems to be yes.

Disclosures; Drs. Toner and Haber are included as coinventors on pending patents for technological and diagnostic applications of the CTC technology. Dr. Budd has received past grant support and honoraria from Immunicon Corporation and Veridex, LLC, developers of CellSearch.