Trial Results Boost Circulating Tumor Cell Field

Tumor cells that invade the bloodstream and reach distant organs are the seeds of death. “Patients with cancer … don’t die from the primary tumor, they always die from metastases,” said Leon Terstappen, M.D., Ph.D., chief scientific officer of Immunicon, a Pennsylvania biotechnology company. Because tumor cells make their way to the site of distant metastases via the bloodstream, “there must be cells there,” Terstappen pointed out.

In theory, finding such circulating tumor cells, or CTCs, could identify metastatic disease at its very earliest stage. But the technical hurdles for creating such a clinical test are enormous. “We’re looking for really one cell, a weird cell, in a background of maybe 20 million cells,” said David Krag, M.D., professor of surgery at the University of Vermont. “Boy, that can be really tough.”

But research into circulating tumor cells took a big leap forward in June. In a talk at the American Society of Clinical Oncology (ASCO) annual meeting, University of Michigan medical oncologist Dan Hayes, M.D., reported prospective clinical trial results using a test developed by Immunicon to detect CTCs in patients with metastatic breast cancer. Of 177 cancer patients, nearly 50% had at least five CTCs in a 7.5-milliliter blood sample before chemotherapy treatment, and 30% of these patients still exceeded this five-CTC threshold at a follow-up exam 3 to 4 weeks later. (No patients with benign conditions had more than two CTCs in a blood sample at any time.)

Patients with a high CTC count had a much worse prognosis, but Hayes’ most striking data showed that CTC count was associated with treatment response: for patients with five or more CTCs at first followup, progression-free survival was 2.1 months, compared with 7.0 months for patients with fewer than five CTCs. “These data suggest that, for a patient who has an elevated CTC level
at ... 3 to 4 weeks after starting a new therapy, it is very unlikely she will benefit from that therapy,” Hayes said in his presentation. Doctors typically do not know for months whether a given course of treatment is working.

Finding CTCs in the blood has been technically feasible (if not very reliable) for several decades, but the trial Hayes led provides the strongest evidence to date that such cells make a difference in patients. “It was, up to now, pretty much unclear what circulating tumor cells really mean for the clinical outcome of patients with breast cancer,” said Klaus Pantel, M.D., Ph.D., a tumor biologist at the University Hospital Hamburg-Eppendorf in Hamburg, Germany. “It’s really a very important proof of principle that it’s useful to measure these cells in the circulation.”

Many methods for isolating CTCs have been tried. Immunicon uses “immunomagnetic selection”: magnetized iron particles, coated in albumin and suspended as crystal balls in so-called “ferrofluids,” are added to the blood sample. The particles are linked to antibodies that target epithelial cell markers. (Epithelial cells, because they are not normally found in blood, are assumed to be cancer cells.) Magnets pull the cells from the blood. Then a second epithelial cell antibody, with a fluorescent marker, is added, along with a cell-specific marker and a marker designed to exclude any falsely stained white blood cells.

Following separation, the fluorescently labeled cells are viewed in a microscope and automatically counted by computer. “Technically, this is a highly reproducible assay,” Hayes said. “It’s hard to screw up.” Johnson & Johnson subsidiary Veridex incorporated Immunicon’s technology into a product called the CellSearch Epithelial Cell Kit that in January received marketing clearance from the U.S. Food and Drug Administration for the management of metastatic breast cancer. Veridex plans to introduce the system commercially later this year.

A Word of Caution

Should medical oncologists use the system to, for example, monitor patient response to breast cancer treatment? Experts say the data are persuasive but still incomplete. “On a first pass, to see something like that, that’s pretty cool,” said Krag. “That’s going to open the door [but it] certainly needs to have validation.”

John Park, M.D., a medical oncologist at the University of California at San Francisco, agreed. Park is waiting for the peer-reviewed version before making any recommendations. (A paper is in press.) The results presented at the ASCO meeting “are very encouraging,” Park said, “but we’re still not sure how to best use this for patients.”

In fact, even if the trial results stand up to scrutiny, their usefulness to doctors treating metastatic breast cancer is far from obvious. “The big caveat is, ‘so what?’” Hayes himself commented. “What are you going to do with the information? Does it mean you should change therapy, or does it mean you should just stop all therapy?”

Hayes is planning a clinical trial to test whether switching treatments based on CTC count will make a difference in survival in patients with metastatic breast cancer. For early-stage cancer applications, CTCs have even farther to go. For screening purposes, they may never measure up. “There are so few [cancer] cells in that setting that … many cancers will still go undetected,” Park said. “However, it may be useful in combination with other screening strategies.”

Nor has anyone shown yet that counting CTCs in patients with primary (nonmetastatic) breast cancer can predict response to treatment. (Right now, oncologists do not know which node-negative breast cancer patients are cured by surgery and which ones need adjuvant chemotherapy, so such a test is badly needed.) The Immunicon test is not yet sensitive enough to do this, although Terstappen says the company is working on improvements.

The Prognosis for Prognosis

The presence of micrometastases in the bone marrow, on the other hand, has shown promise as a prognostic indicator for early-stage breast cancer. Pantel and colleague Stephan Braun, M.D., reported 4 years ago in the New England Journal of Medicine that the presence of bone marrow epithelial cells is a strong predictor of survival for such patients. At last December’s San Antonio Breast Cancer Symposium, Braun reported similar results in a pooled analysis involving more than 3,000 patients.

Several hospitals in Europe now routinely look for bone marrow epithelial cells in cancer patients before deciding whether to give chemotherapy, although the practice remains experimental and is not used in the United States. Adoption here “may require at least one other confirmatory study or a prospective study,” said Park. The American College of Surgeons Z10 study, which has finished accruing patients, could provide the answer.

In the meantime, Pantel is actively testing different CTC detection methods to see if he can find CTCs in the blood as reliably as in the bone marrow. (Pantel is using the Immunicon system, among others.) “That would be very, very exciting to know whether the bone marrow analysis could be replaced by blood analysis in patients with primary breast cancer,” Pantel said. “This information could then be used to tailor adjuvant treatment and monitor adjuvant treatment.”

The CTC field, compared with genomics and proteomics-based prognostic testing, remains low-profile. But several other companies besides Immunicon now have devices for detecting, counting, and characterizing CTCs. For example, ChromaVision Medical Systems in San Juan Capistrano, Calif., has an immunomagnetic capture system. Becton-Dickinson’s immunomagnetic bead capture system uses a flow cytometer instead of a microscope to count the cells. Other companies are
working on filtration (taking advantage of cancer cell size), density gradient methods (cancer cells are heavier and so can be captured in a sucrose gradient), and RT-PCR (to detect cancer cell marker RNA in the blood).

In general, immunomagnetic separation is highly specific—positive results are reliable—but lags in sensitivity, whereas reverse transcription–polymerase chain reaction (RT-PCR) is highly sensitive but lacks specificity. Filtration, density gradient, and immunohistochemical staining of blood all remain unproven. “The technology involved in rare cell event detection and interpretation is still pretty immature,” Krag said.

**Biopsy in Blood?**

Investigators are now moving beyond counting circulating tumor cells to actually characterizing them. They are hoping, in the long run, that CTCs can provide a molecular snapshot, or “real-time biopsy,” of tumor response to therapy. Multiple tumor biopsies must now be done to check molecular markers on tumor cells for response to targeted therapy. Such biopsies are invasive and often not possible, so CTCs offer a noninvasive alternative. “The question is, can you use [CTCs] to substitute for response data in terms of phase II studies, that would get you where you want to go—red light, green light—for a new drug, faster?” Hayes said. Immunicon is planning several clinical trials to test this application. Meanwhile, ChromaVision, together with researchers at the University of California at Los Angeles, has launched a clinical trial to see if monitoring Her-2 and vascular endothelial growth factor (VEGF) on CTCs from patients receiving bevacizumab (Avastin) and trastuzumab (Herceptin) combination therapy predicts treatment response.

So the next few years may see a flood of new data on both CTCs and bone marrow micrometastases, with the potential for real impact on patient treatment. The Hayes trial gives the measurement of CTCs a role in predicting metastatic breast cancer outcome, even if the trial falls short of telling doctors what to do with the information. And the potential of CTCs to enable “real-time biopsies” will soon be tested. “This is exactly why I think this technology is so exciting,” Park said. “But … it’s still definitely a work in progress.”

—Ken Garber