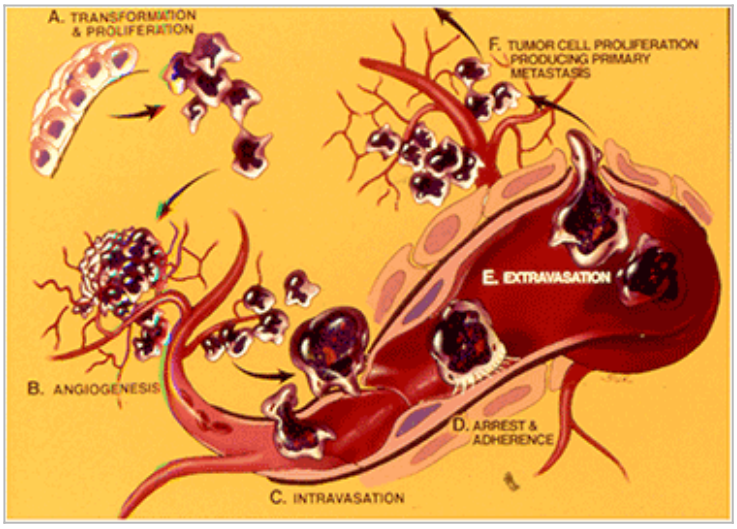
Unraveling metastasis with circulating tumor cells

Anne Paxton

May 2014—Some diseases have clear origins and unfold in predictable ways, but cancer isn't one of them. Despite legions of studies over the decades, cancer tumorigenesis and its deadly sequel, metastasis, essentially remain a riddle, wrapped in a mystery, inside an enigma, as Winston Churchill once described Russia.

But scientists do know that circulating tumor cells (CTCs) are one of the keys to metastasis. Like migrants, invaders, or colonizers from nation-states seeking to expand, CTCs leave the primary tumor, travel through the body's vasculature, and somehow serve as seeds for cancer's spread.

It's been 10 years since seminal research led by Massimo Cristofanilli, MD, reported in the New England Journal of Medicine (2004;351:781–791), showed that the presence of CTCs revealed by a diagnostic assay of the blood is predictive of overall survival in patients with metastatic breast cancer. That study helped lead the Food and Drug Administration to approve CellSearch, by Veridex (now Janssen Diagnostics LLC), the first test to count CTCs in a blood sample to help clinicians with prognosis in breast cancer. FDA approval of CellSearch use in prostate and colorectal cancer followed soon after.



Circulating tumor cells are "seeds" of fatal metastasis, a process consisting of sequential and rate-limiting steps where rare metastasis-competent CTCs shed from primary tumors are able to survive in the circulation and colonize organs distant from the primary lesion. CTC-induced brain metastasis results from a CTC subpopulation with defined characteristics. Illustration courtesy of Dr. Marchetti.

With the mass of research and new test development completed since then, does it appear that CTCs can shed light on tumorigenesis and metastasis—and more importantly, that their use can help stage and treat patients with metastatic cancer? CAP TODAY asked pathologists and clinicians in the field that question and found that circulating tumor cells are yielding up the secrets of their role in cancer progression reluctantly, but that clinical use of CTCs continues to grow.

As with any biomarker, not all the hoped-for applications have panned out, and widespread clinical use remains several years away. Still, tantalizing research findings, innovative test technologies, and promising clinical applications are re-stoking optimism about CTCs' future.

“To me and to many of my colleagues, CTC is a very important test right now to help us understand whether or not therapy is working, and if it isn’t, to really make some judgments about what to do next in those patients,” says Richard J. Cote, MD, chair of the Department of Pathology at the University of Miami School of Medicine, chief of pathology at Jackson Memorial Hospital, and director of the Dr. John T. Macdonald Foundation Biomedical Nanotechnology Institute. He predicts an even more central role for CTCs in the future. “In the broader perspective, CTCs will perhaps be the single most important general test that we can perform in cancer, particularly in patients with metastasis.”

That broader perspective may be needed, because recently the field of CTC research was unsettled by disappointing results of a major clinical trial. Hopes that CTCs could be used to guide breast cancer therapy were dealt a setback in December at the San Antonio Breast Cancer Symposium, when investigators reported on results of a phase three clinical trial funded by the National Cancer Institute. The study, known as SWOG S0500, found that when metastatic breast cancer patients were switched from one form of chemotherapy to another based on a continuing elevated CTC count, using the CellSearch test, it made no difference in the patients’ overall survival or time to progression.

Those findings, of course, could relate more to shortcomings of the therapies than to the value of CTCs as a biomarker. The study did validate that patients with elevated CTC counts (five or more cells per 7.5 mL sample), at both baseline and 21 days after starting their first chemotherapy, have a worse prognosis, and SWOG study coordinator Jeffrey B. Smerage, MD, PhD, said this result could indicate that this patient population needs more effective treatment options beyond traditional chemotherapy.

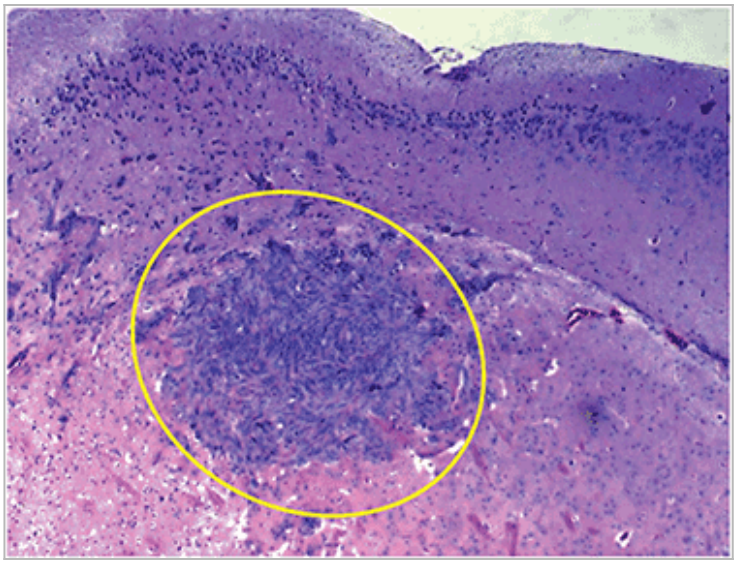
Dr. Marchetti

But the report was not particularly encouraging news, says Dario Marchetti, PhD, professor of pathology and immunology and director of the CTC Core Facility at Baylor College of Medicine in Houston. Unfortunately, most insurance companies do not consider tests for CTCs to be reimbursable based on the clinical results so far, and this new study will not help the field of CTC research. He sees the findings as affirming the need for strong characterizing studies, looking at CTC properties by marker presence, functionality, and other parameters.

“We need to further our knowledge regarding CTCs and cancer, and we also need to potentially make additional CTC platforms available for clinical testing,” Dr. Marchetti says. He stresses that CellSearch detects only a subset of CTCs, and it’s important not to leap to conclusions about the clinical usefulness of CTCs based on this one study.

In fact, he believes CTCs are fundamental in combating metastases, in particular those targeting the brain, which have been his main interest since his father died of brain cancer many years ago. Several studies have shown that CellSearch does not detect the CTCs that invade and colonize the brain. His center was the first to bring this to the forefront, Dr. Marchetti says.

Of several hundred patients he has studied who were clinically diagnosed with brain cancer metastasis, CTCs in the vast majority—65 percent to 70 percent—were not detectable by the CellSearch platform, which uses the epithelial cell adhesion molecule, or EpCAM. Last year in Science Translational Medicine, his center reported that four different proteins, HER2+, EGFR, heparanase, and Notch1, spell out the signature of CTCs that colonize the brain (Zhang L, et al. 2013;5 [180]:180ra48).



Representative image of breast cancer brain metastasis induced by human CTCs following injection into mice. The work published by Dr. Marchetti's laboratory in Science Translational Medicine (2013) provides the first-time evidence of CTC isolation from patients' blood, and the characterization of CTCs possessing metastatic competence in experimental animals. Photo courtesy of Dr. Marchetti.

Dr. Marchetti emphasizes that he has great respect for the CellSearch platform and for the investigators who developed what he calls a "marvelous piece of precision engineering." But, he adds, the observation about the CTCs that CellSearch did not detect "stimulated our desire to look into alternative methodologies, like flow cytometry, microfiltration, and dielectrophoretic-based CTC platforms. The whole field is a puzzle, a work in progress."

Since CTCs are the seeds of further metastases, the CTC Core Facility at Baylor is focusing on predicting metastases of the brain as well as other organs, and how to adjust therapy according to biomarkers and progress over time. "By understanding more about the biology of these cells, we have the ultimate goal to develop an assay that can be a clinically useful tool to interrogate and monitor the progression of cancer within the patient in real time," Dr. Marchetti says. "Taking a blood aliquot is certainly less painful than a biopsy." And through a real-time "liquid biopsy," "we can monitor the course of the disease longitudinally in relation to the properties of the CTCs characteristic of these metastases."

His objective would be one day to put into the clinic the first diagnostic test to predict breast cancer brain metastases. Right now, though, that's a faraway goal. To develop clinically useful tools for oncologists, Dr. Marchetti stresses the need to learn more about the properties of the CTCs and CTC subsets, especially which cells are able to invade or colonize an organ, versus those that remain dormant or quiescent, or die. "We need to know far more about the molecular characteristics at the base of CTC heterogeneity. This investigation will help towards our goal to monitor therapy effectiveness in patients and adjust it according to the presence or absence of defined biomarkers."

CTCs are not the only culprit in cancer progression, he cautions, pointing to circulating DNA and exosomes as other possible factors. "But within the complex microenvironment of the blood, CTCs can be one important aspect. Certainly improving our understanding of these cells will be very important, if not essential, in better understanding the biology of cancer metastases. We are convinced, and we are not the only ones who are convinced, that CTCs will be a fundamental piece of the entire puzzle of metastasis."

Aside from CellSearch, at least five other methods of capturing CTCs are in development or undergoing

analytical validation: flow cytometry, which sorts cells by size and surface antigen expression; microchips to capture CTCs as blood flows past EpCAM-coated microposts; filters with pore size that retains CTCs but permits smaller cells to pass; imaging techniques relying on Fiber-Optic Array Scanning Technology (FAST) to use fluorescent labels to identify CTCs; and negative enrichment that eliminates all cells from blood samples except CTCs.

Dr. Linden

But for the time being, CellSearch is the only FDA-approved test, and the CellSearch instrument is finding a home in more and more laboratories. When Michael A. Linden, MD, PhD, was a hematopathology fellow at the University of Washington a few years ago, the laboratory there was one of the early ones to bring the CellSearch instrument online. The University of Minnesota, where Dr. Linden is now assistant professor and hematopathologist in the Department of Laboratory Medicine and Pathology, has acquired CellSearch instrumentation, is conducting clinical validation, and plans to offer the test in-house early this summer.

Interestingly, purchase of the instrument was enabled in part because a patient heard about the test and, considering it to be important, donated a significant amount of money to the institution for its purchase. (The purchase ultimately was made possible by contributions from the private donor, the university, and Fairview Health Services.) “Our initial plan is to offer the test for clinical use, but we’ll also have it as an opportunity for researchers who want to study CTCs as biomarkers in clinical trials, as well as use the platform to investigate new diagnostic assays,” he says.

He considers the CellSearch test remarkable, given that the standard diagnostic cutoff in a 7.5 mL sample of blood is only three to five CTCs amid the billions of red and white blood cells in the sample. “The technology is very powerful,” Dr. Linden says. “But as the test is designed to enumerate very rare events, there is some degree of imprecision at low concentrations of CTCs. This is an important consideration, especially when the measured concentration of CTCs is near the diagnostic cutoff.” In addition, the stains used to detect CTCs in patients with metastatic carcinoma can also detect non-neoplastic epithelial cells, he says. “We don’t normally have detectable epithelial cells circulating in our blood. However, if a single CTC is detected in a patient with a low pretest probability of metastatic carcinoma, the data should not be overinterpreted.”

The kits most commonly used with CellSearch are validated to detect CTCs or carcinoma cells only in breast, prostate, and colorectal cancer, but Dr. Linden is confident that the technology can be adapted to find other cell types. “You’d just have to modify the reagents used to include antibodies that recognize antigens on the surface of your CTC of interest.” Some institutions are already doing studies of that possibility, he says. “I think the technology will be really informative in learning about the biology of metastatic neoplasia.”

While his institution plans to focus on enumeration for now, characterization is becoming a priority for many in the field. “Researchers are interested in elucidating the immunophenotypic and genotypic differences between a primary neoplasm and the CTCs that are shed. Do they express different surface antigens or have different genetic properties that make them capable of circulating rather than remaining localized to the main neoplasm? There are still unanswered questions about the biology of metastatic neoplasia, and characterizing the CTCs that seed metastases may lead to greater understanding of the processes, with implications for new diagnostic and therapeutic strategies.”

Dr. Linden would like to encourage pathologists to take ownership of and be leaders in recommending clinical tests to their clinician partners. “At a certain point, clinicians treating cancer patients are going to order a CTC test and

may look to their pathologist for help. Pathologists play a vital role, even if they do not perform the test in-house. We as pathologists can help clinicians understand the data supporting test clinical utility, interpretation of test results, and the analytical limitations of the test."

Minetta Liu, MD, of the Department of Medical Oncology and the Department of Laboratory Medicine and Pathology at Mayo Clinic Rochester, has already incorporated the use of CTC enumeration in her clinical practice. "The prognostic value of CTC enumeration is repeatedly demonstrated with each reported clinical trial in metastatic breast or prostate cancer," she says. "Those patients with elevated CTCs are much more likely to have inferior outcomes compared to patients with undetectable levels or low numbers. The questions are: How can we take advantage of this information, convert those patients with unfavorable to favorable CTC counts, and improve overall survival?"

In patients with metastatic breast cancer, she explains, clinicians typically obtain restaging scans at nine- to 12-week intervals to assess for responsive, stable, or progressive disease. However, "inter- and intrareader concordance in determining progression versus no progression by imaging studies alone is not 100 percent," she says. "We need to improve our ability to identify the proper point in a patient's disease course to change therapy. I don't want to waste time and expose patients to toxicity if the drug is no longer helping, but I also don't want to abandon an intervention if it is still providing benefit."

CTCs are reflective of underlying tumor biology, she says. "In my clinical practice, I use serial CTC enumeration by the FDA-cleared technology as an adjunct to routine bloodwork, clinical evaluations, and imaging studies. In my laboratory research efforts, we are focused on the molecular characterization of CTCs by various platforms, with the goal of establishing CTC analyses as reliable predictors of treatment benefit to specific agents," Dr. Liu says.

Dr. Liu

Outside of major academic research institutions, there has not yet been widespread uptake of CTCs in clinical practice. "In this era of personalized medicine, physicians are really looking beyond enumeration and prognosis toward using CTCs as a means of guiding drug selection and improving survival." That is the focus now in the development of related technologies, Dr. Liu says.

Most research on CTCs has come from epithelial tumors such as those of the breast, colon, lung, and prostate, says Terence Friedlander, MD, assistant clinical professor of hematology/oncology at the University of California, San Francisco. His research in medical oncology has focused on development of novel therapeutics by looking at tumor cells in advanced cancer of the prostate.

Dr. Friedlander

“The CellSearch test allows us to count or enumerate CTCs in a very reliable way,” he says, noting the well-established link between the number of CTCs a patient has at baseline and prognosis for survival, and that patients whose CTC numbers drop in response to chemotherapy for prostate cancer have better overall survival.
But an estimate of patient prognosis has limited usefulness, he points out. He is much more interested in CTCs as a predictive marker that can be used to tailor the most clinically effective therapy. The liquid biopsy concept, though not yet validated clinically, would have special value in metastatic prostate cancer, Dr. Friedlander believes.

“We know no two cancers are alike between two people, or within the same person, because the cancer cells have different mutations. Prostate cancer is a great example of a disease that spreads mostly to the bone, and that is pretty inaccessible. It’s a costly and expensive procedure to biopsy bone just to get a piece of prostate tissue, and because there’s generally not enough tissue to get useful information, a lot of different labs are studying the genetics of CTCs to see how well they correlate with tumor biopsies and how they change over time in response to therapy.”

Metastatic prostate cancer patients can live up to four or five years, Dr. Friedlander points out. “Chemotherapy is a one-size-fits-all blanket approach that we take, hoping that it kills off a lot of the cancer cells. But it doesn’t address molecular changes that have happened over time in the cells. The hope is that by learning what’s driving the cancers molecularly, clinicians can select more appropriate therapy for individual patients. And CTCs are just one way of doing that.”

Over the last five or 10 years, he adds, the field has been moving from simply counting CTCs to characterizing them. With a treatment called enzalutamide that is used for metastatic castration-resistant prostate cancer, for example, “there is some evidence now that if you see a certain pattern of the androgen receptor staining in the cytoplasm of the prostate cancer cell, this may predict whether the patient is likely to respond to enzalutamide, and help us avoid wasting two months of therapy only to find that it didn’t work.”

Many mysteries about CTCs’ role in cancer progression remain, however. “We know patients have primary tumors and that metastases presumably develop from these cells getting into the circulation and establishing themselves at distant sites. But what is somewhat unclear when we capture CTCs is what they really represent. Are they cancer cells that have detached from the primary tumor or from the metastatic one, and more importantly, are they destined to become metastases in another spot? There’s not very good hard evidence about this.”

His bias is that they probably represent the advanced cancer, the most active part of the cancer that’s growing fast, and the cells are leaving tumors from different parts of the body and are being detected in the bloodstream. “But a lot of this is speculation. It’s what makes the field more challenging than the solid tumor field, where you can just biopsy the primary or metastatic tumor.”

Dr. Friedlander is excited by one recent study of hormonal therapy for advanced prostate cancer. “What the investigators showed was that change in CTC counts, along with change in lactate dehydrogenase—which is a simple biochemical marker—taken together, actually predicts survival for the trial. That means that if a patient had a decline in those two numbers, they were statistically likely to live longer.”

The striking thing was that the investigators found that CTCs met strict criteria for surrogacy, meaning that a biomarker completely captures and replicates an endpoint being studied. This is an important development, Dr. Friedlander says. “The reason is that generally when we do a clinical trial, we have to show that patients actually live longer in order to get the drug approved. You can imagine if we could say that a CTC was a complete surrogate for survival, then a clinical trial could just be run and the endpoint would just be the change in CTC count” (Goodman OB Jr., et al. Cancer Epidemiol Biomarkers Prev. 2009;18[6]: 1904–1913).

Such a surrogacy would mean that clinical trials could be years shorter, probably with fewer patients and lower cost, and would speed up delivery of new drugs into clinical use, Dr. Friedlander says. “Theoretically, a clinical trial that now takes four to five years to complete could be done in a year or two, and the implications would go far beyond the cancers being dealt with right now. There are a lot of challenges to confirming CTCs as a surrogate endpoint, but I think this looks very promising.”

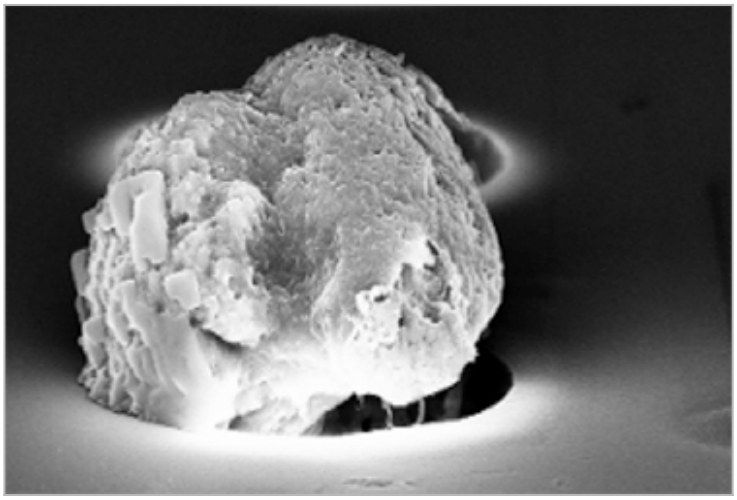
For pathologists, he believes there is huge potential for CTCs to inform clinicians about what molecular changes are occurring in the patient in real time. “In the future, we may not be getting tissue biopsies; this may represent a whole different source of metastatic tissue for analysis, and you can imagine the amount of discoveries we could make using CTCs as a platform for learning about mechanisms of disease progression.”

UCSF has been collaborating with the State University of New York Stony Brook on a test platform that will yield live CTCs that can be grown in the lab to study the biology of the cells, and he notes that Janssen is developing a next-generation platform for CellSearch that will involve much more investigation of genomics. “Several other companies are really pushing to get CTC analysis into the hands of clinicians, so in the next 10 years I expect CTCs will become much more widely used by them.”

Dr. Cote

When Dr. Cote took up study of CTCs, publishing his first paper in 1988 and working closely with the Ludwig Cancer Research Institute, which pioneered much of the early key research, the field was called occult metastasis or micrometastasis. “We were interested in identifying these very rare populations of cancer cells in a massive population of normal cells; that is the CTC problem, and the CellSearch technology was a way to enrich the epithelial cells to target CTCs.”

A frustration in the early days was that researchers were targeting bone marrow aspirates for analysis. “The problem is that aspirate is a difficult and painful procedure, and that as rare as tumor cells were in bone marrow, they were about a log order more rare—about 10 times less common—in blood on a volume basis than they were in bone marrow. CellSearch answered the question of how to use blood to identify system tumor cells, but even with this technology, it was very difficult to identify cancer cells in patients with early-stage disease.”
That remains true to this day, Dr. Cote says. It was for that reason that the field shifted focus from patients with early-stage cancer to patients with established metastasis, using the CTCs as a tool of prognostication at first, and more recently, as a tool to predict whether a course of therapy is working.



An electron microscopy photo of a circulating tumor cell being captured by the filter-based microdevice developed by a

group of scientists and physicians at the Miller School of Medicine, University of Miami. Reprinted from Cancer Biomarkers, 9(1–6); "Micrometastases: detection methods and clinical importance," pp. 397–419, Balic M, Williams A, Dandachi N, Cote RJ. ©2010, with permission from IOS Press.

"The evidence is very clear that CellSearch technology can predict patients who are going to have a worse outcome, but it can also indicate which patients with breast, prostate, and colorectal cancer are responding or not responding to the specific therapeutic intervention with which they are being treated," Dr. Cote says. Patients are generally not going to be cured, but they could have prolonged survival—in the case of prostate cancer, substantially prolonged. "So these were meaningful observations and a step up from just saying 'your prognosis is poor.'" Providing proof that changing therapy would benefit the patient will be the next step, he believes, despite the findings of the SWOG study.

Although he considers CellSearch to be the clinically validated gold standard for detecting CTCs, Dr. Cote and his laboratory have developed a novel filter-based microdevice as an alternative technology to CellSearch because they are seeking a broader array of targets than the epithelial cancers. "All solid tumors that we've studied are candidates for the filter technology, because cancer cells arising from solid tumors have one consistent characteristic: They tend to be larger than normal blood cells. Our filter works by enriching the tumor cell population away from the vast majority of the normal blood population."

From a cost point of view, the filter technology is not significantly different from CellSearch, he believes. "But one advantage of filter technology is that it is very rapid, so processing for that initial enrichment procedure in the blood takes about five minutes. We can also handle much larger volumes of blood than can be handled by a single CellSearch test. This then allows you to use blood in earlier stages of the disease, in order to look at more blood and have a better chance of capturing a cancer cell."

Dr. Cote does not expect that the filter will be useful as a cancer screening tool, but for early detection of metastases, he sees it as extremely promising. It's a key reason why he thinks CTCs will become ever more important as a biomarker. "Testing for CTCs can potentially direct our therapeutic management, indicate prognosis, and if we can get it sensitive enough in patients with early-stage disease, it can really better direct systemic therapy in those patients. If the things we are seeing can be further validated, this would be the one general test that virtually every cancer patient would undergo."

Dr. Boffa

In the future, he hopes, the liquid biopsy will become standard practice for patients with metastatic disease. "In other words, you would simply do a blood test to assess the status of that tumor, then monitor the response and whether or not new targets are emerging, again with a simple blood test."

The potential ability of CTCs to home in on appropriate targets for therapy is likewise the feature of most interest to Daniel J. Boffa, MD, associate professor of surgery at Yale School of Medicine. A thoracic surgeon, he operates mostly on lung cancer and esophageal cancer patients, but for select patients, he also removes deposits of metastatic cancer that originate from other parts of the body that end up in the lungs in hopes of curing them.
"Frustratingly few patients with metastatic cancer are eligible for a curative-intent approach. Even in that highly selected population with only a few metastatic lesions, two-thirds will succumb to their disease despite complete

removal of all radiographically identifiable areas of cancer. We have been studying CTCs not only to prognosticate among patients with limited spread of cancer but, perhaps more interestingly, to understand how some patients appear to contain the traditionally lethal process of metastatic progression."

The prognostic ability of CTCs, as measured by the CellSearch test, has not shaped up as clearly as hoped in the oligometastatic cancer population, Dr. Boffa says. "What has been painfully clear is the first generation of CTC assays turned up as many questions as they answered, with clear inconsistencies between measured cell numbers and clinical outcome. All of us who evaluate CTCs in cancer patients have identified some early-stage patients who were apparently cured yet had persistent CTC populations, and other patients experience rapid progression, despite the absence of CTCs."

It's become more and more evident, he says, that the fluid base of cancer progression is not as simple as previously thought. The circulating population of cells originally thought to be tumor cells includes a mixture of epithelial-derived cells that are not all cancer cells but likely play a role in the process and may have independent prognostic potential. CTC enumeration has offered many new perspectives on the process of metastatic progression. The simplistic notion that a single cell leaves a tumor, enters the bloodstream, and then exits at the next available opportunity and forms a metastasis does not appear to be the case at all, Dr. Boffa says. "There's much more to the circulation than just a simple downstream flow pattern, and CTC study has exposed flaws in this model and has real potential to clarify the true pathway."

When he meets a patient, he tries to consider what is the "driver of their demise." "Once you get a sense for whether it is the established sites of disease that are going to get the patient into trouble or yet-to-be manifested progression, you can plan treatment. For patients whose outcomes are dominated by established areas of tumor, we have a wide range of progressively more effective and less invasive surgical and ablative techniques to eradicate these areas of cancer. Some patients have tumors whose natural history is to grow where they start with little potential for hematogenous spread. These 'local growers' would benefit from surgery, even if it was high risk. On the other hand, 'early spreaders' are destined for systemic progression from a seemingly unimpressive primary tumor. Even low-risk surgery is less likely to help these patients. In this way, we need a 'metastameter' to estimate potential for dissemination to optimize treatment. CTC analysis has real potential for this type of information."

One of the most exciting aspects of CTC research is the potential to target the circulating phase of hematogenous tumor progression. "The important tumor cell attributes for successful dissemination likely vary at different phases of progression. Just as a triathlete uses different skills as she swims, runs, and bikes to complete the race, there are likely tumor cell attributes that are uniquely important for the successful circulating phase of progression. It may be possible to target these attributes and contain disseminated cancers," Dr. Boffa says. "Converting patients from disseminated cancers back to local growers is one of the ultimate frontiers for CTCs research."

He hopes that CTCs could bring about a change in the surgeon's role in cancer treatment. "As we gain an understanding of how a disease is going to behave, it could mean we don't operate on some traditional patients and we do operate on some higher-risk patients we traditionally haven't operated on. For the stage four cancer patient who has five areas deposited in a single lung, you would go as far as removing the entire lung, if necessary, to completely eradicate those lesions if you knew those five areas were the only areas that would ever give that patient problems. So that is my hope—that using CTCs as a biomarker to identify those patients, we might be able to reset our understanding of a patient's tumor cell biology and adapt our approach to them."

The field has evolved from counting cells that were atypical in the circulation of healthy patients, Dr. Boffa says, to sophisticated profiling and characterization of the cells as a reflection of the patient's global disease burden. "We now have a better understanding of what role cells play in cancer progression and a better ability to estimate prognosis. I hope the evolution will continue as CTCs provide a window to the patient's cancer, exposing treatable, targetable aspects of the tumor, and that ultimately CTCs will serve not only as a window of what is happening with the patient's established tumor burden, but also as the Achilles' heel for metastatic progression of cancer."□□

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