Is There a Role for Circulating Tumor Cells in the Management of Breast Cancer?

Daniel F. Hayes and Jeffrey Smerage

Abstract Circulating tumor cells (CTC) can be identified and characterized in blood of patients with many solid tumors, particularly breast cancer. Between 10% and 30% of patients with stage I to III breast cancer and 50% to 70% of women with metastatic breast cancer have detectable CTCs. In both cases, presence and elevation of CTCs are associated with worse prognosis. In the metastatic setting, persistent CTC after 3 to 5 weeks of a new therapy seem to indicate lack of activity of that regimen, and an ongoing prospective randomized clinical trial is addressing the relative worth of changing to an alternative treatment rather than waiting for classic clinical and radiologic evidence of progression. Recent technical advances offer the promise of further geno-typing and phenotyping for important tumor-associated genes and proteins.

Tumor cells were first identified in the bloodstream of patients in 1869 (1). Recently, several technical advances have led to important studies that suggest that tests for circulating tumor cells (CTC) may gain a role in clinical care of patients with breast and other malignancies. This review will focus on the potential utility of CTC in breast cancer, although exciting advances have recently been reported in detecting CTC in other solid malignancies.

CTCs are a rare component among the billions of cells within the human bloodstream. To identify CTC, investigators have exploited differences between epithelial cells and normal hematopoeitic cells or between malignant and normal epithelial cells. These differences include physical characteristics, such as size and weight, or biological characteristics, such as expression of molecules specific to epithelium or epithelial malignancies. Two major strategies have been used to exploit these differences. In one, whole blood is subjected to density gradient centrifugation and/or erythrocyte lysis to separate nucleated cells from erythrocytes, and then the nucleated cell fraction is examined by a variety of means to identify the CTCs. For example, reverse transcription PCR (RT-PCR) has been used to detect transcription of genes, such as cytokeratin, which should normally not be present in blood (2). In addition to cytokeratin transcripts, investigators have reported detection of CTC using RT-PCR against *MUC*-1, *carcinoembryonic antigen,* *epithelial cell adhesion molecule, mammoglobin, HER*-2, and other genes. A second strategy to separate epithelial cells from whole blood exploits immunomagnetic or other immunosepa-ration techniques. These cells are then further characterized with labeled probes, such as antibodies against other epithelial or putative tumor-specific antigens. Recently, a fully automated immunomagnetic and immunofluorescent system for detection of CTCs has been developed (CellSearch; Immunicon, Inc.). This system uses microscopic ferrofluids that have been coated with an antibody against epithelial cell adhesion molecule to magnetically separate epithelial cells from whole blood (3). The isolated cells are then characterized as CTC based on cellular size and morphology, staining with 4′,6-diamidino-2-phenyl-indole, staining with a fluorescently labeled cocktail of pan anticytokeratin antibodies, and absence of staining for the leukocyte-specific antigen, CD45. Other systems using immu-nomagnetic and immunocharacterization of CTC have been reported, but their development seems less mature than that for CellSearch with regard to specific clinical utility.

RT-PCR and immunomagnetic/fluorescent approaches have relative pros and cons. Broadly, although not without argu-ment, the RT-PCR method seems to be the more sensitive and the immunomagnetic/fluorescent approach seems to be more specific. The latter may also provide the opportunity for further characterization of the cells. The two strategies, however, have not been rigorously compared head-to-head in clinical trials. Indeed, they are not mutually exclusive, as some investigators have used an initial enrichment immunoseparation step followed by RT-PCR (4).

Recently, other innovative methods have been described to isolate CTC based on physical and biological differences between epithelial malignant and normal cells. For example, microposts coated with anti–epithelial cell adhesion molecule antibody have been used to capture epithelial cells, which are then characterized for cytokeratin expression using fluorescent microscopy (5), in a manner similar to the commercially available CellSearch. Other investigators have shown that CTC can be identified based on secretion of epithelial-associated or even tumor-associated soluble proteins using the so-called Epi-spot technology (6), and a recently published technique

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has exploited microfiltering coupled with electrolysis and RT-PCR (7). Preliminary reports of these assays suggest remarkable sensitivity and potential for further cellular characterization, but the specificity and clinical utility of these assays is undetermined.

Setting and Possible Utility of CTC

As with all tumor markers, CTC might be useful in one of several clinical situations in breast cancer, including risk determination, screening, differential diagnosis, determination of prognosis in either the adjuvant or metastatic settings, prediction of specific benefit from particular therapies, or monitoring patients who are either free of disease for occult recurrence or those who have established metastases to determine disease course (8). At this time, there is no indication that any assay for CTC has sufficient sensitivity or specificity to be useful as a risk or screening tool, or to distinguish malignant from benign lesions or one type of malignancy from another. There is a growing body of literature to suggest, however, that CTC might provide useful clinical information either in newly diagnosed patients or in those being monitored for recurrence or during treatment of metastatic disease.

CTC in patients with operable, early-stage breast cancer. Adjuvant systemic therapy, including antiestrogen and anti-HER treatments and chemotherapy, have clearly resulted in remarkable decreases in breast cancer mortality (9, 10). These treatments, however, are not without substantial toxicity, inconvenience, and cost. Therefore, it would be preferable to avoid treating those patients unlikely to benefit, either because their prognosis is so good that they simply do not need it, or their tumor is so unlikely to respond to a specific therapy that it will not do them any good (8). For example, anatomic tumor stage (T, N, M) is prognostic, whereas estrogen receptor or HER2 are powerful predictive factors for antiestrogen and anti-HER2 therapies, respectively. CTCs are currently being investigated for both their prognostic and predictive utility.

Studies have suggested that CTC can be found in up to 40% of patients with newly diagnosed breast cancer when assayed by RT-PCR and in ~ 10% using CellSearch (11). It seems that 20% to 30% of patients with stage I or II breast cancer have positive RT-PCR results for cytokeratin 19 transcripts and these patients have a poorer prognosis than those without CTC (11–21). The presence of CTCs seems to be associated with a poorer prognosis at diagnosis regardless of nodal status or whether patients receive adjuvant systemic therapy. Recent reports have suggested that increasing CTC results during or residual CTCs after adjuvant may predict that the ongoing or prior therapy is ineffective (22, 23). These provocative results are consistent with findings by other investigators regarding bone marrow micrometastases (24).

Presently, it is not clear how CTC levels might be used to make clinical decisions in the adjuvant setting. Each of these studies has been conducted within patient cohorts in which therapy was not prospectively dictated, and no study has used CTC results to direct therapy compared with a group of patients treated using standard prognostic and predictive criteria. Given the overall survival benefit of adjuvant chemotherapy, use of a tumor marker, such as CTC, to make clinical decisions must be considered very carefully. For example, the American Society of Clinical Oncology Tumor Marker Guidelines Panel has made its

recommendations on high levels of evidence, based on a scale of I (best) to V (worst; ref. 25). Current CTC results in the early breast cancer setting must be considered levels of evidence III at best, and insufficient to apply in standard practice.

CTC in patients with metastatic breast cancer. Although an occasional patient with metastatic breast cancer seems to be cured, most are destined to ultimately die of their disease (26). Nonetheless, a number of new therapies has been introduced for patients in this setting, resulting in modest prolongation of survival and substantial improvements in palliation. Thus, the goal of therapy for most patients with metastatic breast cancer is to choose the therapy with the highest likelihood of response
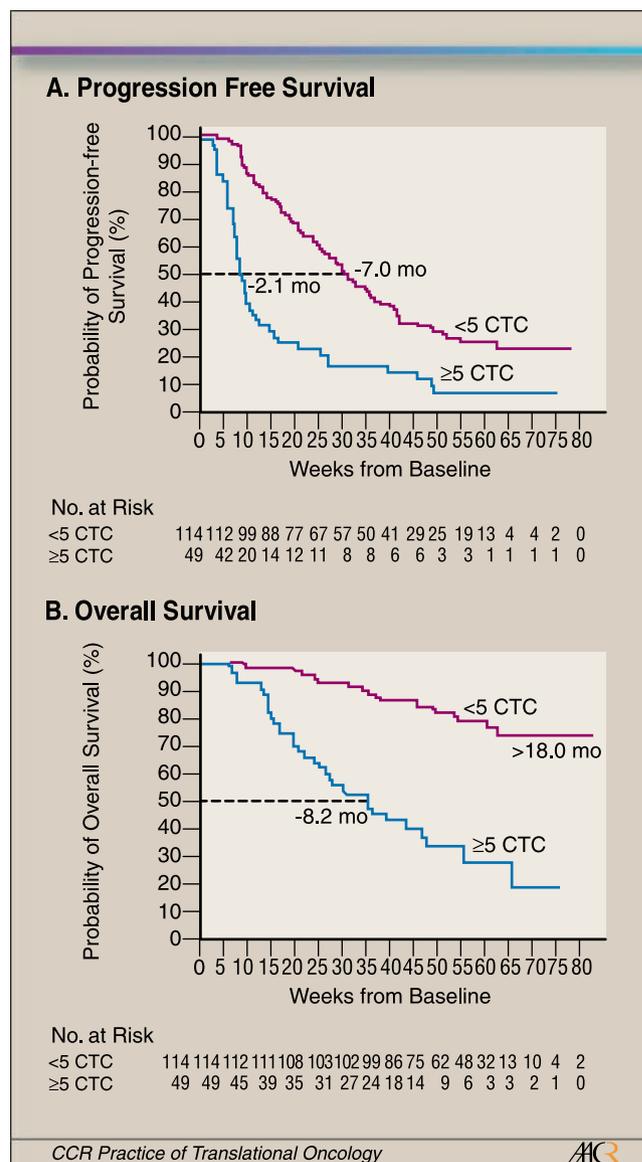

A. Progression Free Survival

No. at Risk

<5 CTC	114	112	99	88	77	67	57	50	41	29	25	19	13	4	4	2	0
≥5 CTC	49	42	20	14	12	11	8	8	6	6	3	3	1	1	1	1	0

B. Overall Survival

No. at Risk

<5 CTC	114	114	112	111	108	103	102	99	86	75	62	48	32	13	10	4	2
≥5 CTC	49	49	45	39	35	31	27	24	18	14	9	6	3	3	2	1	0

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Fig. 1. Kaplan-Meier estimates of probabilities of progression-free survival and overall survival in patients with metastatic breast cancer for those with <5 CTCs per 7.5 mL of whole blood and those in the group with ≥5 CTCs in 7.5 mL of whole blood at the first follow-up visit after initiation of a new line of therapy. All patients in the trial (*n* = 177) are included in these figures. *A,* progression-free survival from baseline. *B,* overall survival from baseline. Adapted from M. Cristofanilli, G.T. Budd, M.J. Ellis, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 2004;351:781–97 with permission. Copyright © 2004 Massachusetts Medical Society. All rights reserved (27).
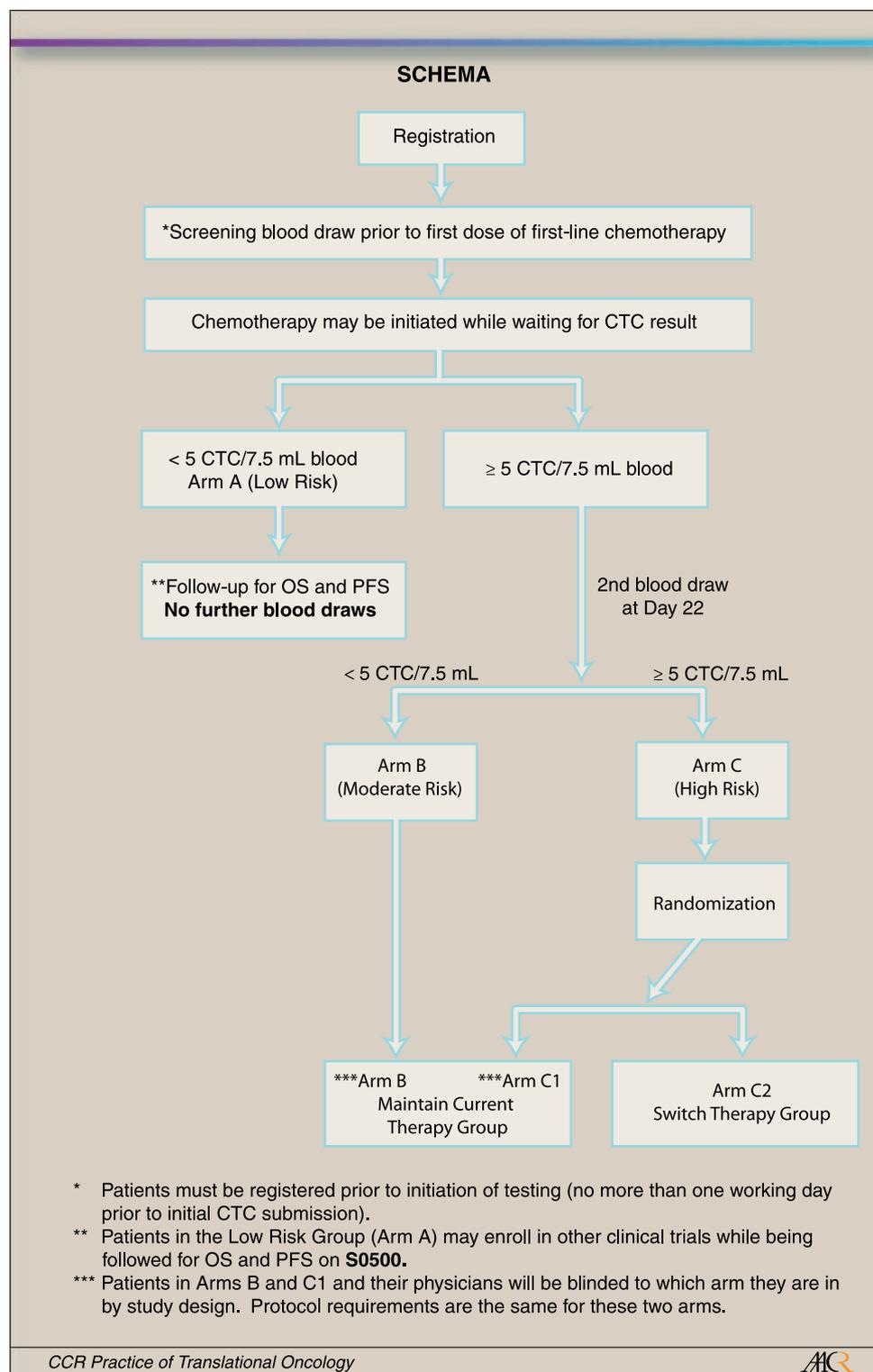
SCHEMA

Registration

↓

*Screening blood draw prior to first dose of first-line chemotherapy

↓

Chemotherapy may be initiated while waiting for CTC result

↓

< 5 CTC/7.5 mL blood
Arm A (Low Risk)

≥ 5 CTC/7.5 mL blood

↓

**Follow-up for OS and PFS
No further blood draws

2nd blood draw
at Day 22

< 5 CTC/7.5 mL   ≥ 5 CTC/7.5 mL

Arm B
(Moderate Risk)

Arm C
(High Risk)

↓

Randomization

↓

***Arm B   ***Arm C1
Maintain Current
Therapy Group

Arm C2
Switch Therapy Group

* Patients must be registered prior to initiation of testing (no more than one working day prior to initial CTC submission).
** Patients in the Low Risk Group (Arm A) may enroll in other clinical trials while being followed for OS and PFS on **S0500.**
*** Patients in Arms B and C1 and their physicians will be blinded to which arm they are in by study design. Protocol requirements are the same for these two arms.

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Fig. 2. Schema of Southwest Oncology Group S0500.

and the lowest possibility of toxicity, thus balancing symptoms of the cancer with side effects of treatment. Indeed, a wide array of strategies and agents are now available to treat these patients, including antiestrogen therapies, chemotherapies, and other biological therapies. Once a palliative treatment regimen is selected for a patient with metastatic breast cancer, it is generally continued until either undue toxicity or evidence of progression. Current methods of determining progression include history, physical examination, serologic testing, and radiographic evaluation. History and physical examination are notoriously unreliable, due to subjectivity of assessment and because most patients do not have palpable lesions during the examination. Nonspecific serologic examinations, such as enzymes derived from bone (e.g., alkaline phosphatase) and

liver (e.g., alkaline phosphatase and serum glutamate oxalate transferase) lack both sufficient sensitivity and specificity to be very helpful in this setting (26). The American Society of Clinical Oncology Tumor Marker Guideline Panel has recommended that assays for MUC-1 proteins (CA15-3 and CA27.29) and for carcinoembryonic antigen may be helpful in monitoring selected patients with metastatic breast cancer (25).

In this regard, elevated CTCs are found in 50% to 75% of patients with metastatic breast cancer by using either RT-PCR or immunomagnetic/fluorescence approaches. In a prospective, multi-institutional clinical trial involving women with measurable, progressive metastatic disease who were about to start a new therapy, elevated CTC at any time point, using CellSearch, were associated with a high likelihood of a very short time to progression (27–30). In this trial, 50% of these patients had elevated CTC before starting a new treatment, and their prognosis was worse than those without elevated CTC. Importantly, at the first follow-up visit after starting a new therapy (usually 3-5 weeks), only 30% had elevated CTC levels. Patients with persistently elevated CTCs had a worse prognosis than patients who did not, and the latter patients had a favorable prognosis, similar to those women without CTC at baseline, suggesting a therapeutic response (Fig. 1; refs. 27, 28). Likewise, elevated CTCs at later time points were also associated with rapid subsequent progression (29). Although the recently released American Society of Clinical Oncology Tumor Marker Guidelines did not recommend use of CTCs in metastatic disease (25), we believe it is reasonable to use CTCs to make clinical decisions after several months of therapy in patients with metastatic disease, especially those with nonmeasurable disease in whom classic clinical, serologic, and radiographic findings are nondiagnostic.

Very early determination of treatment response or failure with classic serologic markers, such as CA15-3, CA27.29, or carcinoembryonic antigen, is confounded by the phenomenon of the so-called tumor marker spike (31). Tumor marker spikes are defined as an increase in marker level for a few weeks to months before they return to or below baseline, and may occur in as many as 25% of patients who are ultimately felt to have responded to therapy. Thus, serologic circulating tumor markers may often provide a false-positive signal for progression during the first 1 to 2 months of a newly started therapy. In contrast, residual CTC at first follow-up is an indication of a very high likelihood of rapid progression (Fig. 1; ref. 27). It is possible that changing therapy at this time point might be more beneficial than maintaining an apparently futile treatment regimen. Such a recommendation would establish a new and unfamiliar paradigm in the treatment of metastatic breast cancer. Therefore, a prospective randomized clinical trial addressing the value of immediate change in therapy versus waiting until classic clinical and radiographic findings of progression is now being conducted within the North American Breast Cancer Intergroup, led by the Southwest Oncology Group (S0500; Fig. 2).

Future Directions

Simple enumeration of CTCs is just the tip of the iceberg with regard to the enormous biological and clinical contributions these assays may provide. As noted, RT-PCR–based assays have already been reported for *HER2*. Several investigators have reported secondary phenotyping and genotyping of immunomagnetically detected cells for a variety of tumor-associated markers, including HER2, IGFR1, BCL-2, measures of apoptosis, telomerase, Notch1, UUPAR and, in prostate cancer, even gene expression profiling (32–36). No trials have been reported describing how these assays might be used to direct clinical care, but their potential is provocative and exciting.

In summary, technology to detect and characterize CTCs is advancing rapidly. We believe that early studies already suggest a role in selected patients with metastatic disease. We anticipate that, coupled with an increasing understanding of the need for well-designed and well-conducted trials, better understanding of the biology of CTC will result in their becoming a routine part of the clinical evaluation of at least patients with metastatic breast and other cancers, and perhaps even in early stage disease.

Disclosure of Potential Conflicts of Interest

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