

recommend the routine use of serum biomarkers to monitor response for early disease but only for advanced breast cancer.⁶ Furthermore, persistence of micrometastatic disease in the bone marrow of patients with primary breast cancer that have completed adjuvant therapy identifies patients with high-risk of recurrence.⁷ However, this is not routinely used in clinical practice. Nevertheless, Pachmann et al¹ appear to introduce a new concept of complete response in a clinical scenario in which measurable or detectable disease by standard imaging techniques is not present. Therefore, it must be assumed, even though not clearly stated, that patients in whom detectable mononuclear cells disappear with treatment have achieved a complete response. Moreover, other response categories are based on criteria that lack supportive validation data, such as a) marginal changes (< 10 -fold decrease or > 10 -fold increase) b) increase in mononuclear cells. Other problems with the manuscript include the relatively small size of the patient group (91 patients), the variety of different therapies that were employed, and the inability to prognosticate from the presence of the mononuclear cells since 90% of the patients had them. Finally, the lack of decrease of CTCs at completion of therapy was claimed to identify different prognostic groups even though their relationship to standard prognostic factors is not clearly stated.

In summary, those results raise many issues and concerns among clinicians and researchers in this field. How should we interpret and use this information? Are CTCs readily detectable in every patient with breast cancer that has completed adjuvant therapy or is the interpretation of those cells as CTCs incorrect? We suggest that classification of mononuclear cells as CTCs demands rigorous scrutiny through the conduct of well-designed protocols with defined cytomorphology, expression of epithelial markers without hematopoietic cell markers, and genetic analysis whenever possible.⁸⁻¹⁰ The major effort should then be to define the molecular pathways in these cells and their potential biologic/clinical impact.

Massimo Cristofanilli

Department of Breast Medical Oncology, The University of Texas, M.D. Anderson Cancer Center, Houston, TX

James Reuben

Department of Hematopathology, University of Texas, M.D. Anderson Cancer Center, Houston, TX

Jonathan Uhr

Cancer Immunobiology Center, University of Texas Southwestern Medical Center, Dallas, TX

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

REFERENCES

1. Pachmann K, Camara O, Kavallaris A, et al: Monitoring the response of circulating epithelial tumor cells to adjuvant chemotherapy in breast cancer allows detection of patients at risk of early relapse. *J Clin Oncol* 26:1208-1215, 2008
2. Al-Hajj M, Wicha MS, Benito-Hernandez A, et al: Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci U S A* 100:3983-3988, 2003
3. Pierga JY, Bonneton C, Vincent-Salomon A, et al: Clinical significance of immunocytochemical detection of tumor cells using digital microscopy in peripheral blood and bone marrow of breast cancer patients. *Clin Cancer Res* 10:1392-1400, 2004
4. Cristofanilli M, Budd GT, Ellis M, Stopeck A, Matera J, Miller MC, et al: Circulating tumor cells predict progression free survival and overall survival in metastatic breast cancer. *N Engl J Med* 351:781-791, 2004
5. Kuerer HM, Newman LA, Smith TL, et al: Clinical course of breast cancer patients with complete pathological primary tumor and axillary lymph node response to doxorubicin-based neoadjuvant chemotherapy. *J Clin Oncol* 17:460-469, 1999
6. Harris L, Fritsche H, Mennel R, et al: American Society of Clinical Oncology 2007 update of recommendations for their use of tumor markers in breast cancer. *J Clin Oncol* 25:5287-5312, 2007
7. Janni W, Rack B, Schindlbeck C, et al: Persistence of isolated tumor cells in the bone marrow of breast cancer patients predicts increased risk for relapse. *Cancer* 103:884-891, 2005
8. Riethdorf S, Fritsche H, Mueller V, et al: Detection of circulating tumor cells in peripheral blood of patients with metastatic breast cancer: A validation study of the CellSearch system. *Clin Cancer Res* 13:920-928, 2007
9. Fehm T, Morrison L, Saboorian H, et al: Patterns of aneusomy for three chromosomes in individual cells from breast cancer tumors. *Breast Cancer Res Treat* 75:227-239, 2002
10. Meng S, Tripathy D, Shete S, et al: HER-2 gene amplification can be acquired as breast cancer progresses. *Proc Natl Acad Sci U S A* 101:9393-9398, 2004

DOI: 10.1200/JCO.2008.18.0356

IN REPLY: Thank you for transmitting to us the Letter to the Editor from Cristofanilli, Reuben, and Uhr in response to our publication in *Journal of Clinical Oncology*. Regarding their issues we would like to answer as follows:

First, Cristofanilli et al claim that "even with the use of enrichment technologies the detection of CTCs can vary between 10% and 15% in primary breast cancer." However, in earlier publications from the same authors,²⁻⁴ they report on higher percentages of CTC: 13 of 14 patients with breast cancer without detectable spread⁴; 13 of 36 dormancy candidates, 7 to 22 years after mastectomy and without evidence of clinical disease²; 12 of 37 patients with no evidence of disease³; respectively. These discrepancies may, in part, depend on methodological modifications:

- The use of preservatives may change epitope accessibility.⁵
- Epitope expression may be too low on some CTCs to allow magnetic enrichment.⁶
- Enrichment technologies, while generating a higher number of the requested events in a defined volume, do not imply that all events present in the original sample are recovered. We

have analyzed this issue thoroughly in a previous publication⁷ and shown that the enrichment procedures lead to a considerable loss of epithelial tumor cells from blood samples. This may explain why circulating epithelial tumor cells (CETCs) can often not be detected in primary tumors just because of the enrichment procedures used.

- In two previously published reports,^{8,9} still using magnetic bead enrichment, we were able to detect circulating tumor cells in almost all patients with early breast cancer in a comparable range as published by Cristofanilli et al¹⁰ for patients who were metastatic, and we were able to follow these patients during adjuvant chemotherapy.
- Due to loss of epithelial cells during the enrichment process we have omitted this step and obtained even more reliable results.
- A recently published approach¹¹ was for the first time able to detect CTCs in the same range as published by us and CTCs were isolated in seven of seven patients with early-stage prostate cancer.

Second, Cristofanilli et al also criticize that patients with metastatic disease were not included as an additional control group. The

aim of the present research was the analysis of the behavior of CETCs during adjuvant chemotherapy in breast cancer; therefore metastatic patients were not included. We have, however, previously compared the numbers in patients with metastatic versus primary breast cancer,⁸ and we detect a significantly higher number in patients with metastatic than in primary breast cancer, although with a broad overlap.

Third, the question raised by the authors whether tumor volume has any effect on the number of CETCs has already been addressed in a previous publication.¹² In patients with untreated breast cancer, CETC numbers correlate with tumor volume.

Fourth, in the same publication,¹² monitoring of treatment response was investigated in the neoadjuvant setting revealing an excellent correlation of the reduction in CETC to the reduction in tumor volume ($r^2 > 0.9$). Tumor volume reduction was preferred to pathological complete response as a measure of treatment response because in smaller tumors, even if a complete response can be obtained, the reduction may be less than in large tumors with still remnant tumor mass.

Fifth, all patients included in the present investigation were by clinical definition in complete remission. Adjuvant treatment is only given to patients in whom measurable or detectable disease by standard imaging techniques is not present. The term complete remission was used as clinically defined and we did not aim at introducing a new concept of complete remission.

Sixth, we would like to emphasize that our analyses were restricted to monitoring therapy response.

Seventh, the mere presence or absence of CETC with a single analysis is not sufficient to serve for prognostication and complete elimination of CETC (not mononuclear cells) seems not to be necessary for long-lasting complete remissions. It was not the aim of this study to use the presence of CETC to establish an additional prognostic factor. But obviously the response of CETC to any therapy tells us something about their aggressiveness and their ability to metastasize. Therefore this analysis is independent of the therapy schedule applied.

Eighth, we admit that the patient group is small. On the other hand, even with this restricted patient sample size, statistical analyses showed highly significant results with a higher hazard ratio than, for example, persistence of micrometastatic disease in bone marrow. Contrary to Cristofanilli et al's allegation the response of the CETCs to therapy was well correlated to standard prognostic factors especially lymph node positivity as shown in Figure 4 of the publication.¹ Together with the two additional patient groups analyzed previously^{8,9} with even longer observation intervals, it now adds up to 141 patients, and more patients will be included into the next update.

In summary, in our hands neither the mere presence nor absence of surface epithelial cell adhesion molecule–CETC before or after adjuvant therapy was of prognostic relevance. Such cells can be present obviously in a dormant state for long years even after completion of adjuvant therapy. It is the behavior of these cells in response to therapy that predicts the further course of disease.

The behavior of tumor cells in the circulation may be more difficult than previously thought. Certainly it may not be easy for the clinician as well as for the patient to understand that a single analysis of CETC can hardly allow a statement with respect to prognosis. Tumor cells in the circulation that do not have the capability to adhere and

grow in distant sites may be irrelevant for prognosis, although they may recirculate and survive in a dormant state for long times as also indicated by the studies of Chambers et al.¹³

On the other hand, increasing numbers of CETC may signal the release from initial occult metastatic loci and subsequent presence of a population of aggressive tumor cells. There exist numerable homologies in biology. The right timing of therapies, aiming at the CETC discharged on the first round of chemotherapy, especially taxanes, to destroy them in a second round of therapy might help to improve the success of systemic chemotherapies.

A multicenter study testing this hypothesis should be the next step comparing the outcome of patients which have been identified as high risk due to their increasing CETC numbers receiving additional cycles of chemotherapy under the monitoring of their CETC with patients treated in the conventional way without further chemotherapy. This might help to clarify the impact of CETC monitoring.

Katharina Pachmann

Department of Experimental Hematology and Oncology, Clinic for Internal Medicine II, Friedrich Schiller University Jena, Jena, Germany

AUTHOR'S DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

REFERENCES

1. Pachmann K, Camara O, Kavallaris A, et al: Monitoring the response of circulating epithelial tumor cells to adjuvant chemotherapy in breast cancer allows detection of patients at risk of early relapse. *J Clin Oncol* 26:1208-1215, 2008
2. Meng S, Tripathy D, Frenkel EP, et al: Circulating tumor cells in patients with breast cancer dormancy. *Clin Cancer Res* 10:8152-8162, 2004
3. Terstappen LW, Rao C, Gross S, et al: Peripheral blood tumor cell load reflects the clinical activity of the disease in patients with carcinoma of the breast. *Int J Oncol* 17:573-578, 2000
4. Racila E, Euhus D, Weiss AJ, et al: Detection and characterization of carcinoma cells in the blood. *Proc Natl Acad Sci U S A* 95:4589-4594, 1998
5. GoenAM, Yaziji H, Battifora H: Correspondence Re: Varma et al: Effect of Formalin Fixation and Epitope Retrieval Techniques on antibody 34BE12 Immunostaining of Prostatic Tissues. *Mod Pathol* 13:834-835, 2000
6. Rao CG, Chianese D, Doyle GV, et al: Expression of epithelial cell adhesion molecule in carcinoma cells present in blood and primary and metastatic tumors. *Int J Oncol* 27:49-57, 2005
7. Pachmann K, Clement JH, Schneider CP, et al: Standardized quantification of circulating peripheral tumor cells from lung and breast cancer. *Clin Chem Lab Med* 43:617-627, 2005
8. Lobodasch K, Dengler R, Fröhlich F, et al: Quantification of circulating tumour cells for monitoring of adjuvant therapy in breast cancer: An increase in cell number at completion of therapy is a predictor of early relapse. *The Breast* 16:211-218, 2007
9. Pachmann K, Dengler R, Lobodasch K, et al: Quantification of circulating tumor cells (CETC) for monitoring of adjuvant therapy in breast cancer. An increase in cell number at completion of therapy may develop as an indicator of early relapse. *J Cancer Res Clin Oncol* 134:59-65, 2008
10. Cristofanilli M, Budd GT, Ellis MJ, et al: Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N Engl J Med* 351:781-791, 2004
11. Nagrath S, Sequist LV, Maheswaran S, et al: Isolation of rare circulating tumour cells in cancer patients by microchip technology. *Nature* 450:1168-1169, 2007
12. Camara O, Rengsberger M, Egbe A, et al: The relevance of circulating epithelial tumour cells (CETC) for therapy monitoring during neoadjuvant (primary systemic) chemotherapy in breast cancer. *Ann Oncol* 18:1484-1492, 2007
13. Brackstone M, Townson JL, Chambers AF: Tumour dormancy in breast cancer: An update. *Breast Cancer Res* 9:208, 2007

DOI: 10.1200/JCO.2008.18.0927