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ORIGINAL REPORT

Monitoring the Response of Circulating Epithelial Tumor Cells to Adjuvant Chemotherapy in Breast Cancer Allows Detection of Patients at Risk of Early Relapse

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ABSTRACT

Purpose
To demonstrate that it is possible to monitor the response to adjuvant therapy by repeated analysis of circulating epithelial tumor cells (CETCs) and to detect patients early who are at risk of relapse.

Patients and Methods
In 91 nonmetastatic primary breast cancer patients, CETCs were quantified using laser scanning cytometry of anti–epithelial cell adhesion molecule–stained epithelial cells from whole unseparated blood before and during adjuvant chemotherapy.

Results
Numbers of CETCs were analyzed before therapy, before each new cycle, and at the end of chemotherapy. The following three typical patterns of response were observed: (1) decrease in cell numbers (> 10-fold); (2) marginal changes in cell numbers (< 10-fold); and (3) an (sometimes saw-toothed) increase or an initial decrease with subsequent reincrease (> 10-fold) in numbers of CETCs. Twenty relapses (22%) were observed within the accrual time of 40 months, including one of 28 patients from response group 1, five of 30 patients from response group 2, and 14 of 33 patients from response group 3. The difference in relapse-free survival was highly significant for CETC (hazard ratio = 4.407; 95% CI, 1.739 to 9.418; $P < .001$) between patients with decreasing cell numbers and those with marginal changes and between patients with marginal changes and those with an increase of more than 10-fold (linear Cox regression model).

Conclusion
These results show that peripherally circulating tumor cells are influenced by systemic chemotherapy and that an increase (even after initial response to therapy) of 10-fold or more at the end of therapy is a strong predictor of relapse and a surrogate marker for the aggressiveness of the tumor cells.

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INTRODUCTION

Solid malignant tumors of the breast are the most frequent cause of death in women in the developed world. Although early detection, precise surgery with wide margins, and adjuvant therapy have improved results,[1] relapse is not infrequent. In premenopausal women, a first narrow peak occurs approximately 8 to 10 months after mastectomy, and a second peak occurs at 28 to 30 months. Postmenopausal patients display a peak at approximately 18 to 20 months.[2] After diagnosis of metastatic disease, the outcome is fatal. To date, there is no tool to monitor the effect of adjuvant treatment apart from statistical analyses[3]; however, prediction for the individual patient is restricted.

Solid tumors can seed tumor cells into the peripheral blood, which may, even after complete resection of the tumor, eventually grow into metastases. Detection of such circulating tumor cells has been reported in patients with primary[4-6] and metastatic[7] breast cancer, with a shorter survival in patients with cells in bone marrow[8] or in patients with metastatic disease with higher cell numbers in blood.[7] In metastatic disease, the clinical consequence of this result is questionable because there is no indication that treatment will lead to improved survival in patients with poor prognosis.[9] In patients with primary tumor, only 40% of patients carrying isolated tumor cells in bone marrow experience recurrence,[6] indicating that a portion of the circulating epithelial tumor cells (CETCs) may be biologically irrelevant and tumor cells may differ in

their proliferative and metastatic potential.[10,11] Thus, so far, it has not been possible for the individual patient to predict whether she will benefit from treatment or not.[12]

We have previously demonstrated that therapeutic manipulations such as surgery can contribute to the seeding of epithelial cells[13] and that such cells can persist in the circulation for a long time.[14] The present article examines the application of the MAINTRAC (SIMFO GmbH, Bayreuth, Germany) method[15,16] for repeated quantitative analysis of epithelial, presumably tumor cells during adjuvant therapy in primary breast cancer patients. CETCs were analyzed directly without magnetic enrichment,[17] omitting all enrichment procedures and yielding higher numbers of cells. During neoadjuvant therapy,[18] this has been shown to mirror the response of the primary tumor. Previous results[19,20] showed that an increase in the number of these cells towards the end of treatment is predictive of early relapse; these results were confirmed in the present study in a larger population.

PATIENTS AND METHODS

Beginning in January 2002, of 111 patients with newly diagnosed breast cancer scheduled for surgery at our institution, 91 (82%) consented to blood drawing before surgery and during adjuvant chemotherapy according to the ethics committee approval. Applied adjuvant chemotherapies were epirubicin/cyclophosphamide (EC), epirubicin/taxane, fluorouracil/epirubicin/cyclophosphamide (FEC) with and without taxane, cyclophosphamide/methotrexate/fluorouracil (CMF), or capecitabine for older patients with and without trastuzumab according to HER-2/neu status.

Of 7.5 mL of blood anticoagulated with EDTA drawn before surgery and at each visit before administration of chemotherapy or during follow-up, 1 mL was lysed with ammonium chloride (Qiagen, Hilden, Germany). For detection of CETCs, white cells from the sediment were subject to the MAINTRAC analysis, diluting the pellet in 500 μL of phosphate-buffered saline (pH 7.4) and adding 12.5 μL of fluorescein isothiocyanate–conjugated mouse antihuman epithelial antibody (Miltenyi Biotec, Bergisch Gladbach, Germany) and 5 μL of phycoerythrin-labeled CD45 (Miltenyi Biotec) simultaneously for 15 minutes in the dark. Analysis of red and green fluorescence of the cells was performed using a laser scanning cytometer (Compucyte Corporation, Cambridge, MA), enabling relocation of cells for visual examination of vital epithelial cells as extensively described in a previous study.[17]

Fluoromicrographs of epithelial cells with green fluorescence, exclusively surface located, and CD45 red fluorescing normal blood cells are shown in Figures 1A and 1B. Note the red fluorescing normal leukocytes in the neighborhood of the green fluorescing CETCs. A defined volume of the cell suspension was applied to a defined area on an adhesion slide (Menzel Gläser, Braunschweig, Germany), and laser scanning was performed on this area. Cells were detected by their forward scatter, and red and green fluorescence was recorded. Dot plots of the cells are shown in Figure 1C. Each dot on the left-hand plot (Fig 1C) represents a cell. The epithelial cells selected by their green fluorescence (green gate in Fig 1C) were relocated and analyzed for vitality. Only vital cells were counted.

Numbers of CETCs were calculated per milliliter and varied between 0 and 100,000. These numbers are 10-fold higher than the numbers reported in a previous publication[19] and as detected by Cristofanilli et al[7] using the CellSearch system (Veridex, Warren, NJ) in metastatic breast cancer. This difference may be a result of omission of magnetic separation, which leads to a loss of specific cells together with the enrichment process, as discussed extensively in a previous study.[17] Normal blood cells could easily be distinguished from epithelial antigen-positive cells. No live epithelial cells were detected in 97% of healthy donors and in 38 of 40 patients with hematologic malignancies. The two positive hematologic patients were patients with Hodgkin's disease. We used Kaplan-Maier plots for all survival analyses. The influence of several prognostic factors on the event-free survival time was tested in univariate analysis using the log-rank test under the null hypothesis that the factors would exhibit no influence at all. Furthermore, we used the Cox regression model to

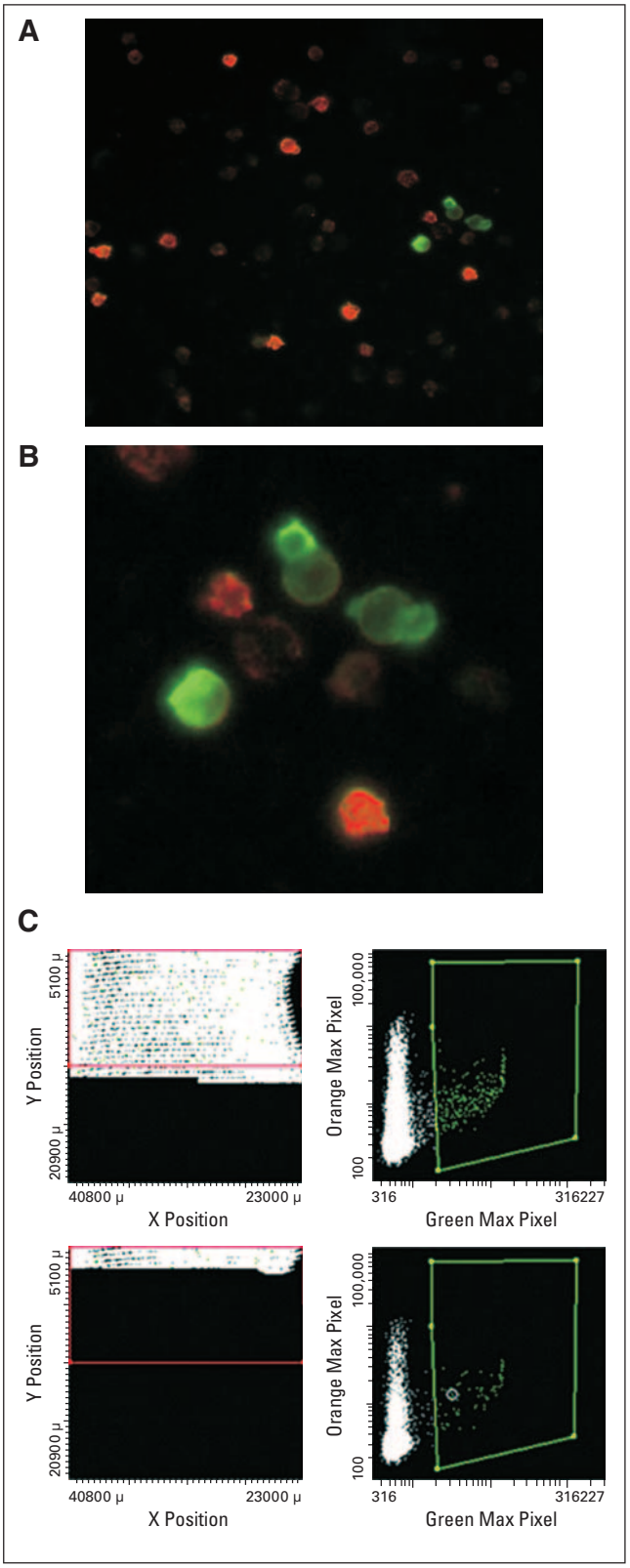


Fig 1. (A) Typical epithelial antigen-positive cells with green caps among blood leukocytes stained orange for CD45 (×250 magnification). (B) Higher magnification (×500) of the group of epithelial antigen-positive cells. (C) Design of the approach for detection of circulating epithelial tumor cells. The microscope scans over a defined area and recognizes all white cells, WBCs, and tumor cells by light scatter and measures the fluorescence over each cell. Typical histograms are displayed with positive cells in the green gate.

model the influence of CETCs and determine the corresponding hazard ratios (HRs). For the linear Cox regression model, the quotients between the highest value (decrease) or between the nadir and the value at the end of therapy (increase) were calculated. We also used the Cox regression with a backward selection procedure with likelihood ratio test to investigate the joint effects of CETCs, tumor size, nodal status, and estrogen receptor (ER) status. All analyses were performed using the SPSS statistical program (SPSS Inc, Chicago, IL).

RESULTS

Ninety-one consecutive nonmetastatic patients were observed prospectively for changes in CETC numbers during adjuvant chemotherapy and were assessable for all data and follow-up (Fig 2). The following therapies were applied (Table 1): 32 patients received FEC, nine patients received FEC/taxane, one patient received FEC/taxane/gemcitabine, six patients received EC, 17 patients received EC/taxane, four patients received epirubicin/taxane, two patients received E/C/T, two patients received EC/CMF, eight patients received CMF, four patients received capecitabine, one patient received epirubicin/fluorouracil, one patient received taxane weekly, three patients were under observation, and therapy was unknown for one patient. All ER-positive patients were administered hormone therapy after the adjuvant therapy. CETC numbers were analyzed before the start of treatment, before each new cycle of chemotherapy, and at the end of chemotherapy (but not during subsequent hormone therapy) and were influenced by therapy as follows: 28 patients showed a decrease in cell numbers of 10-fold or more (Fig 3A) calculated from the highest value until the end of therapy; 30 patients showed only marginal changes in cell numbers (< 10-fold) during the course of therapy despite applied chemotherapy (Fig 3B); and 33 patients showed an increase in cell numbers of more than 10-fold from the nadir (lowest value) towards the end of therapy. Surprisingly, in these last 33 patients, frequently an initial decrease and then a subsequent reincrease in cell numbers during therapy (Fig 3C) were observed.

Response patterns were not restricted to different therapy schedules. However, it should be mentioned that decreases in CETCs were more frequent (17 decreases, 10 marginal changes, and 16 increases) in patients receiving anthracycline-based therapy including fluorouracil than in patients receiving anthracycline therapy schedules without fluorouracil, in whom marginal changes (< 10-fold) or increases (> 10-fold from nadir) were more frequent (six decreases, 13 marginal changes, and 12 increases; Table 1). An increase after previous reduction occurred in 9% of patients treated with FEC compared with 22% of patients treated with FEC/taxane and in 17% of patients treated with EC compared with 53% of patients treated with EC/taxane.

Twenty-seven percent (n = 25) of all patients had good prognostic markers (N0, ER positive, HER-2/*neu* negative). In these patients, a reduction in CETC numbers (> 10-fold) was observed in nine patients (36%), and a marginal change in CETC numbers was observed in seven patients (28%); none of these patients experienced relapse. Nine patients (36%) had an increase in cell numbers (> 10-fold), and three of these patients experienced relapse. Sixty-six patients (73%) had adverse prognostic markers (N1, ER positive or negative, HER-2/*neu* positive or negative). Of these patients, 19 (29%) had a reduction of CETCs at the end of therapy, and one experienced relapse. Twenty-three patients (35%) had marginal changes, and five of these patients experienced relapse. Twenty-four patients (36%) had an increase in CETCs at the end of therapy with or without previous reduction. Of these patients, 11 have experienced relapse during the observation time of up to 40 months (Table 2).

Patients in complete remission and patients with relapse did not differ significantly regarding mean age, tumor size, or ER expression; however, there was a significant difference in lymph node positivity between patients remaining in complete remission and patients experiencing relapse during the observation interval (Table 3). Most importantly, the behavior of the CETC (increase > 10-fold from nadir to end of therapy) correlated significantly with relapse (Table 3). The earliest relapses occurred 2 months after the end of therapy, and the latest occurred approximately 28 months after the end of therapy, with a mean time to relapse of 397 days (range, 71 to 833 days) after the

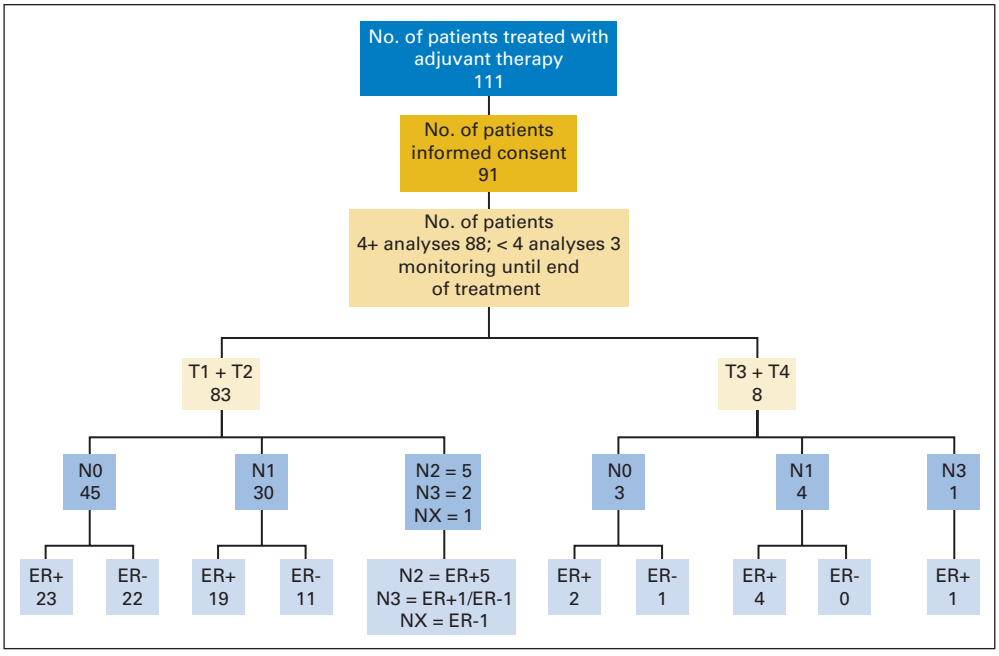


Fig 2. Distribution disease stages of patients accrued during the years 2002 to 2006 for repeated analysis of circulating epithelial tumor cells. ER, estrogen receptor.

Table 1. Relationship Between Applied Therapy Schedules, Response Pattern, and Relapse

Therapy and Tumor Stage	No. of Pts	No. of Patients With Increase in CETCs	No. of Patients With No Change in CETCs	No. of Patients With Decrease in CETCs	No. of Patients With CR	No. of Patients Experiencing Relapse
FEC	32					
N0	22	8	7	7	21	1
N1	10	3	2	5	8	2
FEC/taxane	9					
N0	5	2	1	2	4	1
N1	4	2	0	2	3	1
FEC/taxane/gemcitabine	1					
N0	1	0	0	1	1	0
EC	6					
N0	2	0	1	1	2	0
N1	3	2	0	1	3	0
N3	1	0	1	0	0	1
EC/taxane	17					
N0	3	1	1	1	1	2
N1	14	7	6	1	7	7
ET	4					
N0	2	1	0	1	2	0
N1	2	0	1	1	0	2
E/C/T	2					
N1	2	1	1	0	2	0
EC/CMF	2					
N1	2	0	2	0	2	0
CMF	8					
N0	7	1	4	2	7	0
N1	1	1	0	0	0	1
Capecitabine	4					
N1	3	1	0	2	3	0
N0	1	0	1	0	1	0
EF	1					
N1	1	1	0	0	1	0
Docetaxel	1					
N0	1	1	0	0	0	1
Observation	3					
N0	3	1	1	1	2	1
Unknown	1					
N0	1	0	1	0	1	0

Abbreviations: CETCs, circulating epithelial tumor cells; CR, complete remission; FEC, fluorouracil/epirubicin/cyclophosphamide; EC, epirubicin/cyclophosphamide; ET, epirubicin/taxane; E/C/T, epirubicin/cyclophosphamide/taxane; CMF, cyclophosphamide/methotrexate/fluorouracil; EF, epirubicin/fluorouracil.

observed increase until the relapse (metastases) became detectable with conventional diagnostic tools. Figures 4A to 4D show the cumulative relapse-free survival in Kaplan-Meier plot for patients with small tumors compared with patients with large tumors, for patients with and without positive lymph nodes, for patients with and without ER expression, and for patients from group 1 (reduction in cell numbers $>$ 10-fold), group 2 (marginal change in cell numbers), and group 3 (increase in cell numbers $>$ 10-fold or initial decrease and subsequent reincrease $>$ 10-fold from nadir). The univariate Cox regression analysis showed significant results for the nodal status ($P = .03$), with an HR of 2.927 (95% CI, 1.110 to 7.716) between node-negative and node-positive patients, as well as highly significant results for the CETC numbers ($P < .001$), with an HR of 4.407 (95% CI, 1.739 to 9.418) between patients with decreasing cell numbers and those with marginal changes and between patients with marginal changes and those with an increase more than 10-fold (linear Cox regression model). The joint regression analysis of CETC, nodal status, tumor size, and ER status resulted in a regression model in which only CETC significantly influenced the relapse-free survival. Because follow-up is still shorter than in previous reports,[19,20] more patients with a more than 10-fold increase in cell numbers are expected to experience relapse. ER expression and nodal status of patients who are still in complete remission during the observation time were not significantly different from those of patients who have already experienced relapse, but patients in complete remission have marginally more T1 tumors than larger size tumors compared with patients experiencing relapse (12 *v* seven tumors, respectively).

DISCUSSION

The longest experience with respect to disseminated tumor cells is available from breast cancer. Even if cumulated, long-term follow-up studies of more than 4,000 patients indicate that breast cancer patients with epithelial cells have a poorer prognosis than patients without such cells,[8] there is still a considerable portion of patients with positive bone marrow findings who never experience relapse.[12] In addition, positive bone marrow findings do not correlate with increased relapse

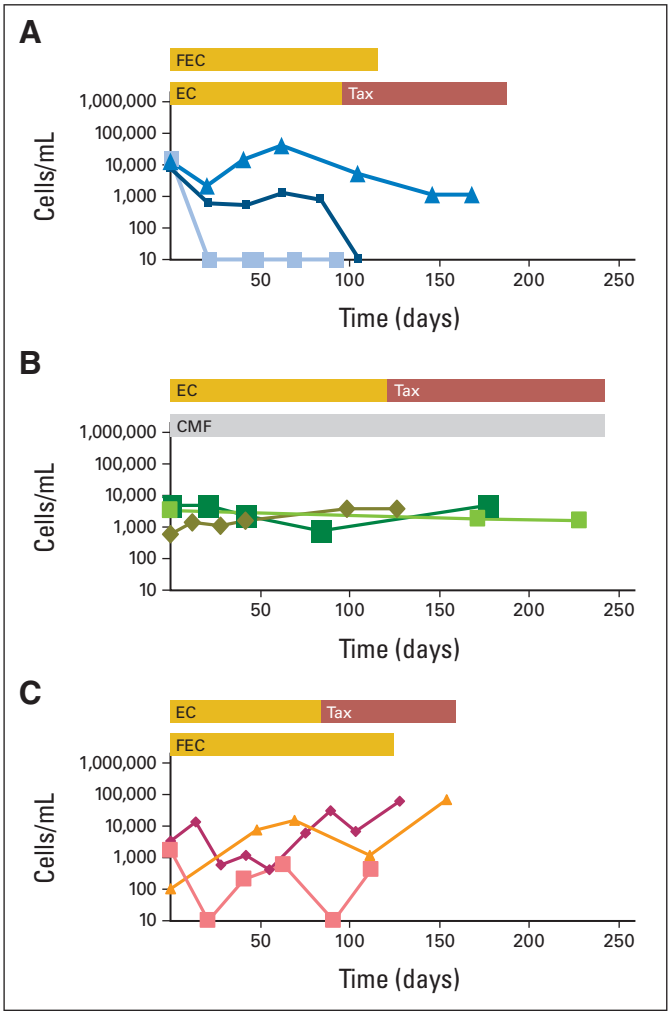


Fig 3. (A) Three typical response curves of individual patients responding to adjuvant chemotherapy with a more than 10-fold decrease in circulating epithelial tumor cell (CETC) numbers (no relapse). (B) Three typical response curves of patients reacting to adjuvant chemotherapy with marginal changes in CETC numbers (no relapse). (C) Three typical response curves of individual patients either with increase of CETCs from the beginning or with an initial decrease and a subsequent reincrease of more than 10-fold during adjuvant chemotherapy (two relapses). FEC, fluorouracil/epirubicin/cyclophosphamide; EC, epirubicin/cyclophosphamide; Tax, taxane; CMF, cyclophosphamide/methotrexate/fluorouracil.

to the bone. Thus, no clear answer can be given for the individual patient, and testing the mere presence or absence of CETCs as a prognostic factor so far has not yielded consistent results.[21,22]

Peripheral blood for analysis of epithelial cells is easy to obtain; it is a well-defined material and can be drawn repeatedly according to the requirements. In contrast to the approach used for bone marrow analyses, we noticed that sucrose density centrifugation depletes epithelial cells from the cells in the interface in blood samples; therefore, this step was omitted. Much higher numbers of epithelial cells were detected using magnetic bead enrichment,[16,19] and this was confirmed by others.[7] However, we noticed that blood epithelial cells, too, were lost during magnetic bead enrichment, which may be explained by their partly low expression of epithelial cell adhesion molecules.[23] Although there was a correlation between cell numbers detected with and without magnetic bead enrichment, this step was omitted as well, and instead, the direct simplified detection was optimized.[17] Setting no threshold, detection of CETCs was possible in 90% of all tumor patients with automated quantification using laser scanning cytometry and subsequent visual control. During neoadjuvant therapy, CETCs in breast cancer patients responded to neoadjuvant therapy in an identical manner as the primary tumor.[18]

Patients with good initial response to neoadjuvant treatment with anthracycline-based therapy have improved relapse-free survival.[24] Stable numbers of CETCs can be detected without disease recurrence even after several years.[14]

Subsequently, the effect of adjuvant therapy on CETCs in individual patients was investigated. Retrospective analysis demonstrated that patients at risk of relapse could be distinguished from patients remaining relapse free by the response of their CETCs to therapy.[19,20] The present prospective approach was designed to extend these previous results and to observe more closely the response of individual patients to therapy during therapy cycles. Patients were treated according to their risk profile and to different study designs in which they were included. The changes in cell numbers were easy to analyze during therapy in 91 patients, and three response groups were distinguished. The first group includes patients with good response to therapy (> 10-fold decrease), although CETCs were not completely eliminated in all patients. One of these patients experienced relapse. The second group includes patients with marginal response to therapy, sometimes even with a slight increase in cell numbers during therapy. In this group, five patients experienced relapse. The patients in this second group may not benefit from therapy because of the inherently good prognosis and also, regarding patients experiencing relapse, because of unresponsiveness to therapy. The third group includes patients with cells either already increasing from the beginning during therapy or with an initial response but with subsequent reincrease in cell numbers more than 10-fold compared with the nadir. In this group, the majority of relapses occurred.

Table 2. Relationship Between Response Pattern and Relapse in Established Prognostic Tumor Stages

	Good Prognosis: N0/ER Positive/HER-2 Negative (No. of patients)			Poor Prognosis: N1 or Greater/ER Positive or Negative (No. of patients)		
Cell No.	Total	CR	Relapse	total	CR	Relapse
Reduction at end of therapy	9	9	0	19	18	1
Marginal changes at end of therapy	7	7	0	23	18	5
Increase at end of therapy	9	6	3	24	13	11

Abbreviations: ER, estrogen receptor; HER-2, human epidermal growth factor receptor 2; CR, complete remission.

Table 3. Relationships Between Established Prognostic Factors and Survival and Response Pattern of CETC and Survival

Factor	Total No. of Patients (N = 91)	Patients in Complete Remission (n = 71)		Patients Experiencing Relapse (n = 20)		Log-Rank *P*
		No.	%	No.	%	
Time to relapse, days						
Mean				397		
Range				71-833		
Age, years						
Mean	55	55		55		
Range	31-78	36-71		31-78		
Tumor stage						.123
T1	52	43		9		
T2-4	39	28		11		
Nodal status						.023
Positive	43	29	41	14	70	
Negative	48	42	59	6	30	
ER status						.751
Positive	55	43	61	12	60	
Negative	36	28	39	8	40	
CETC number						< .001
Decrease > 10-fold	28	27	38	1	5	
Minor changes < 10-fold	30	25	35	5	25	
Increase > 10-fold	33	19	27	14	70	

Abbreviations: CETC, circulating epithelial tumor cell; ER, estrogen receptor.

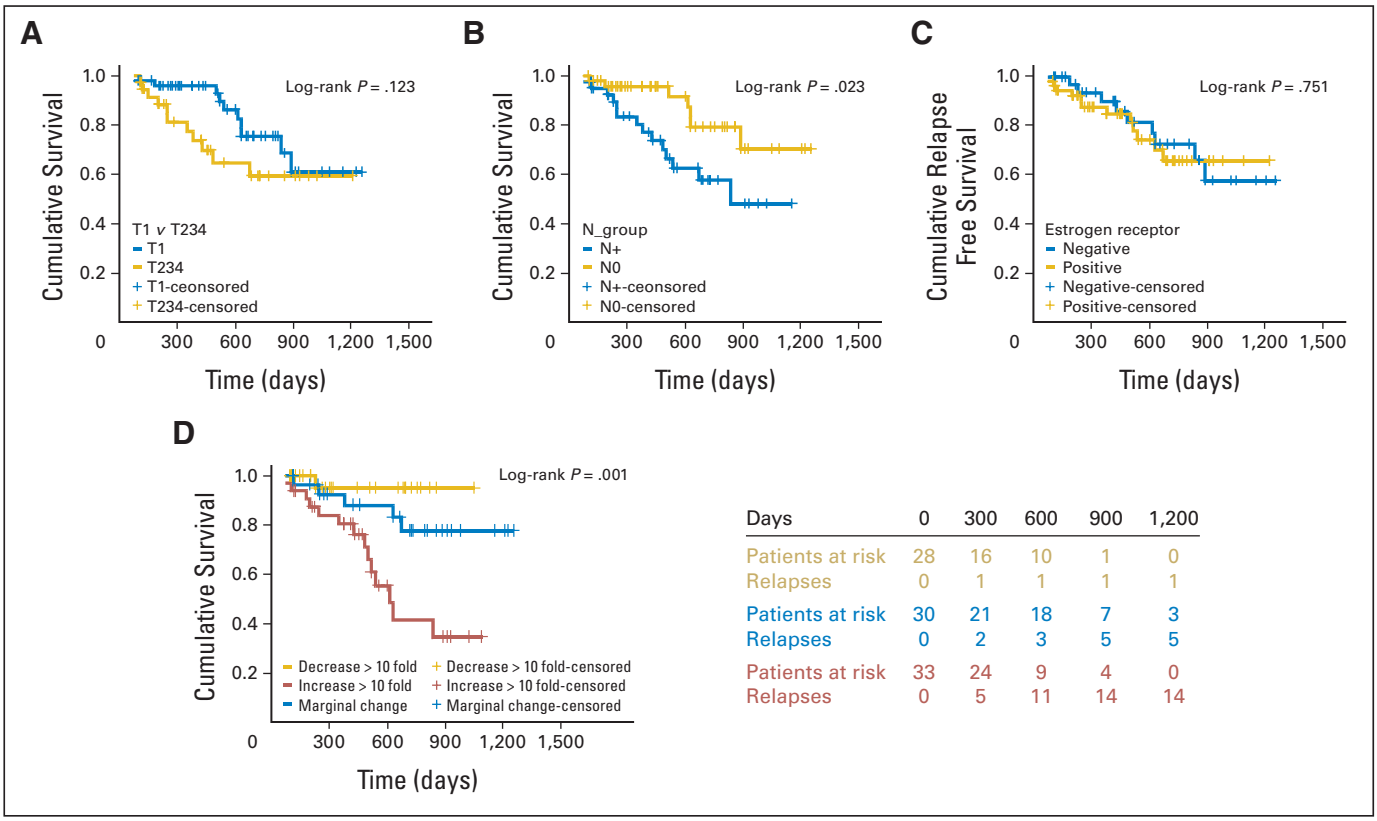
In sum, the 20 distant relapses (22%) were within the same range as published by the Early Breast Cancer Trialists' Collaborative Group.[25] Follow-up time is still at 40 months or less. As in previous reports, relapses occurred almost exclusively in patients with less favorable prognostic markers, with only three relapses in patients with N0, ER-positive disease, all three of whom showed increasing cell numbers during therapy.

Effectiveness of therapy may vary between circulating cells and manifest metastases. One patient with adverse prognostic parameters responded with a more than 10-fold decrease to therapy but experienced relapse after a short increase at the end of therapy. She had high overall CETC numbers and may have had cells disseminated into distant organs on which chemotherapy only had a temporary effect, with detection of metastases grown to detectable size at the end of chemotherapy. In some patients without relapse despite increasing cell numbers during chemotherapy, subsequent trastuzumab or hormone treatment may prevent settling of mobilized cells in distant loci or at least inhibit growth. Analyses of longtime treatment of patients with tamoxifen or aromatase inhibitors have shown a gradual decrease in cell numbers over years (unpublished data). Whether this will also be true for these patients remains to be seen.

A characteristic behavior of CETCs in part of the patients was an initial good response to inhibitors of DNA replication. A large population of these cells initially may have a high proliferative activity.[26] Also seen in some of our patients were high apoptotic rates, even before initiation of treatment.[27] Subsequent reincrease in CETCs frequently occurred despite taxane treatment.[28] Komarova and Wodarz[29] predict that drug-resistant subclones almost certainly exist before the start of therapy. Our results would be compatible with the hypothesis that rare, drug-refractory subclones generated during tumor evolution or, possibly, tumor stem cells constitute the re-emerging dominant tumor population and may restart proliferating under the selective pressure of drug exposure. The high probability of subsequent relapse indicates that these resistant cells have increased fitness. Therefore, genomic profiling[30] should not only be performed from the primary tumor, but also from the reincreasing population to explore sensitivity to targeted therapy. However, the (re)increase of CETCs might also derive from already growing metastases shedding cells into the circulation.[31] Such metastases might be pre-existing in a state of dormancy or suppressed angiogenesis while at the same time being insensitive to the applied chemotherapy.[32] Taxanes have been reported to preferentially damage endothelial cells, leading to reduced intracellular fluid pressure.[33] Release of CETCs from the primary tumor during taxane-based therapy has been consistently observed by us during primary systemic therapy.[24] A comparable effect might also occur from pre-existing metastases in the adjuvant setting. Some of these cells may then be able to settle in distant organs and grow into new metastases consistent with known biologic properties of tumor cells. Thus, longitudinal monitoring of CETC behavior is superior to a single analysis, and a more than 10-fold increase in CETC numbers towards the end of therapy is highly predictive for relapse.

It is also obvious from these results and other observations[14,34] that, depending on the tumor cells' growth potential, it is neither possible nor necessary to completely eliminate all suspect cells to achieve long-lasting remissions. However, it might be crucial to regularly repeat monitoring to detect early a renewed increase in cell numbers as an indicator of imminent relapse.

This study confirmed monitoring of CETCs as a valuable tool for therapy surveillance because the dynamics of the circulating cells cannot be captured by one single snapshot. Such therapy monitoring using CETCs in breast cancer should now be included in larger adjuvant trials and might lead to new treatment considerations and personal tailoring of therapy.



Days	0	300	600	900	1,200
Patients at risk	28	16	10	1	0
Relapses	0	1	1	1	1
Patients at risk	30	21	18	7	3
Relapses	0	2	3	5	5
Patients at risk	33	24	9	4	0
Relapses	0	5	11	14	14

Fig 4. (A) Relapse-free survival of patients with T1 tumors versus patients with T2-4 tumors showing no significant difference ($P = .2$). (B) Relapse-free survival of patients with or without positive lymph nodes showing a significant difference ($P = .028$). (C) Relapse-free survival of patients with positive or negative estrogen receptor expression showing no significant difference ($P = .8$). (D) Relapse-free survival of patients responding with a more than 10-fold decrease in circulating epithelial tumor cells during adjuvant chemotherapy (green line), with marginal change (blue line), or with a more than 10-fold increase (red line), showing a highly significant difference ($P < .001$) between response groups 1 and 3 in relapse-free survival.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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

AUTHOR CONTRIBUTIONS

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Manuscript writing: Carola Rabenstein
Final approval of manuscript: Katharina Pachmann, Ingo Runnebaum, Klaus Höffken

REFERENCES

1. Jones LA: Reduction in mortality from breast cancer. BMJ 330:205-206, 2005

2. Demicheli R, Bonadonna G, Hrushesky WJM, et al: Menopausal status dependence of the timing of breast cancer recurrence after surgical removal of the primary tumor. Breast Cancer Res 6:R689–R696, 2004

3. Bonadonna G, Moliterni A, Zambett M, et al: 30 years' follow up of randomised studies of adjuvant CMF in operable breast cancer: Cohort study. BMJ 330:217-220, 2005

4. Solomayer EF, Diel IJ, Salanti G, et al: The independence of the prognostic impact of tumor cell detection in the bone marrow of primary breast cancer patients. Clin Cancer Res 7:4102-4108, 2001

5. Mansi JL, Gogas H, Bliss JM, et al: Outcome of primary-breast-cancer patients with micrometastases: A long-term follow-up study. Lancet 354:197-202, 1999

6. Janni W, Rack B, Schindlbeck C, et al: The persistence of isolated tumor cells in bone marrow from patients with breast carcinoma predicts an increased risk for recurrence. Cancer 103:884-891, 2005

7. 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

8. Braun S, Vogl FD, Naume B, et al: A pooled analysis of bone marrow micrometastasis in breast cancer. N Engl J Med 353:793-802, 2005

9. Carrick S, Parker S, Wilcken N, et al: Single agent versus combination chemotherapy for metastatic breast cancer. Cochrane Database Syst Rev 18:CD003372, 2005

10. Smerage JB, Hayes DF: The measurement and therapeutic implications of circulating tumor cells in breast cancer. Br J Cancer 94:8-12, 2006

11. Muller V, Stahmann N, Riethdorf S, et al: Circulating tumor cells in breast cancer: Correlation to bone marrow micrometastases, heterogeneous response to systemic therapy and low proliferative activity. Clin Cancer Res 11:3678-3685, 2005

12. Janni W, Rack B, Lindemann K, et al: Detection of micrometastatic disease in bone marrow: Is it ready for prime time? Oncologist 10:480-492, 2005

13. Camara O, Kavallaris A, Nöschel H, et al: Seeding of epithelial cells into circulation during surgery for breast cancer: The fate of malignant and benign mobilized cells. World J Surg Oncol 4:67, 2006

14. Pachmann K: Longtime recirculating tumor cells in breast cancer patients. Clin Cancer Res 11:5657-5658, 2005

15. Pachmann U, Pachmann K: Minimale residuale tumorerkrankung bei soliden epithelialen tumoren: Neues automatisiertes analyseverfahren. Deutsches Ärzteblatt 97:pA-3511, 2000

16. Pachmann K, Heiss P, Demel U, et al: Detection and quantification of small numbers of circulating tumour cells in peripheral blood using laser scanning cytometer (LSC). Clin Chem Lab Med 39:811-817, 2001

17. 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

18. Pachmann K, Camara O, Kavallaris A, et al: Quantification of the response of circulating epithelial cells to neoadjuvant treatment of breast cancer: A new tool for therapy monitoring. Breast Cancer Res 7:R975–R979, 2005

19. Lobodasch K, Fröhlich F, Rengsberger M, et al: Quantification of circulating tumor cells for monitoring of adjuvant therapy in breast cancer: An increase in cell number at completion of therapy is a predictor of early relapse. Breast 16:211-218, 2007

20. Pachmann K, Dengler R, Lobodasch K, et al: An increase in cell number at completion of therapy may develop as an indicator of early relapse: Quantification of circulating epithelial tumor cells (CETC) for monitoring of adjuvant therapy in breast cancer. J Cancer Res Clin Oncol134:59-65, 2008

21. Ditsch N, Funke I, Mayer B, et al: Detection of disseminated tumor cells in bone marrow: Currently of no practical therapeutic value. MMW Fortschr Med 144:37-39, 2002

22. Fehm T, Becker S, Pergola-Becker G, et al: Influence of tumor biological factors on tumor cell dissemination in primary breast cancer. Anticancer Res 24:4211-4216, 2004

23. 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

24. Camara O, Rengsberger M, Egbe A, et al: The relevance of circulating epithelial tumor cells (CETC) for therapy monitoring during neoadjuvant (primary systemic) chemotherapy in breast cancer. Ann Oncol 18:1484-1492, 2007

25. Early Breast Cancer Trialists' Collaborative Group: Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: An overview of the randomised trials. Lancet 365:1687-1717, 2005

26. Sawyers CL: Calculated resistance in cancer. Nat Med 11:824-825, 2005

27. Bernhard EJ, Muschel RJ: Apoptosis: An early event in metastatic inefficiency. Cancer Res 61:333-338, 2001

28. Campone M, Fumoleau P, Bourbouloux E, et al: Taxanes in adjuvant breast cancer setting: Which standard in Europe? Crit Rev Oncol Hematol 55:167-175, 2005

29. Komarova NL, Wodarz D: Drug resistance in cancer: Principles of emergence and prevention. Proc Natl Acad Sci U S A 102:9714-9719, 2005

30. Zanetti-Dällenbach R, Vuaroqueaux V, Wight E, et al: Comparison of gene expression profiles in core biopsies and corresponding surgical breast cancer samples. Breast Cancer Res 8:R51, 2006

31. Butler TP, Gullino PM: Quantitation of cell shedding into efferent blood of mammary adenocarcinoma. Cancer Res 35:512-516, 1975

32. Naumov GN: Ineffectiveness of doxorubicin treatment on solitary dormant mammary carcinoma cells or late-developing metastases. Breast Cancer Res Treat 82:199-206, 2003

33. Griffon-Etienne G, Boucher Y, Brekken C, et al: Taxane-induced apoptosis decompresses blood vessels and lowers interstitial fluid pressure in solid tumors: Clinical implications. Cancer Res 59:3776-3782, 1999

34. Meng S, Tripathy D, Frenkel EP, et al: Circulating tumor cells in patients with breast cancer dormancy. Clin Cancer Res 10:8152-8162, 2004