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ORIGINAL PAPER

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

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Abstract
Purpose Treatment efficiency of adjuvant therapy in breast cancer is only revealed after several years by statistical evaluation and gives no answer for the individual patient. We here present a method to analyze the response to adjuvant chemotherapy online in individual patients.
Methods/results In 25 consecutive non-metastatic primary breast cancer patients adjuvant fluorouracil/epirubicin/cyclophosphamid (FEC) or EC followed by taxane (EC-T) or cyclophosphamid/methotrexate/fluorouracil (CMF) therapy were given. Circulating epithelial tumor cells (CETC) were quantified before and after each second cycle of the therapy regimen, between the anthracycline and the taxane block of the regimen and in some cases repeatedly during CMF treatment. Independent of the initial cell number CETC numbers showed a decline, no change or a minor increase in 15 patients of which 14 remained in complete remission and 1 suffered local relapse. Ten patients showed an increase at the end of therapy of which 4 have relapsed during the observation time of between 2 months and up to 54 months. This patient group was compared to a previously published group of 25 patients who have all reached a follow-up of 4.5 years or until relapse.
Conclusion As in the previous report, Kaplan–Meier analysis revealed a high correlation between the response of CETC to therapy and relapse ($p < 0.0001$) and curves of both patient groups were super imposable. Multivariate analysis revealed the response of CETC to therapy to be an independent predictive marker for relapse.

Keywords Increase in number of circulating tumor cells · Relapse

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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 has improved the results (Jones 2005), relapse is not infrequent, with fatal outcome after diagnosis of metastatic disease. It has long been known that 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 CETC has been reported by different groups in patients with primary (Solomayer et al. 2001; Mansi et al. 1999; Janni et al. 2005) or metastatic (Cristofanilli et al. 2004) breast cancer.

In patients with metastatic disease with higher cell numbers in bone marrow (Janni et al. 2005) or blood (Cristofanilli et al. 2004) a shorter survival was observed, but there is no

clear evidence that more aggressive treatment will lead to improved survival (Carrick et al. 2005). In the adjuvant situation, in contrast, therapy is applied to eliminate minimal residual systemic disease and cure is still feasible. Although it is well established that adjuvant therapy leads to statistically significantly improved survival (Bonadonna et al. 2005), to date, no tool is available to monitor the effect of adjuvant treatment apart from statistical analyses of the frequency of relapses (Bonadonna et al. 2005) and with the methods used so far it was not possible for the individual patient to predict whether she will benefit from the treatment or not (Muller et al. 2005).

We have recently shown that the application of the MAINTRAC® method using laser scanning microfluorimetry (Pachmann and Pachmann 2000) for repeated real-time analysis of CETC in 25 non-metastatic breast cancer patients allows monitoring the response to adjuvant therapy (Lobodasch et al. 2007). We could follow the changes in numbers of CETC during therapy and show that an increase in the number of these cells at the end of treatment was highly predictive for relapse during the subsequent 4.5 years. Here, we report on an additional cohort of 25 patients who have not yet reached a comparably long observation time. Kaplan–Meier analysis confirms the predictive value also for these patients.

Materials and methods

A cohort of 25 sequential newly diagnosed breast cancer patients without metastases (11 patients stage T1 (4 N0, 7 N1) 10 stage T2 (1 N0, 7 N1, 1 N2 and 1 NX) 3 patients T3 (1 N0 and 2 N1) and 1 patient T4 (N1) was evaluated for decrease or increase in cell numbers before and after each second cycle of the therapy regimen, between the anthracycline and the taxane block of the regimen and in some cases repeatedly during CMF treatment and time to last visit or relapse and compared to 25 previously analyzed patients (11 patients stage T1 (7 N0 and 4 N1), 9 stage T2 (6 N0 and 3 N1), two patients T3 (1 N0 and 1 N1) and three patients T4 (3 N1)). Total white cells from 10 ml of anti-coagulated peripheral blood, drawn with informed consent according to the ethics committee approval, were subject to the MAINTRAC® analysis which includes incubation of the whole white cells from 10 ml of blood with human epithelial antigen (HEA)-magnetic beads and fluorescein isothiocyanate (FITC)-conjugated mouse anti-HEA (HEA, human epithelial antigen, Miltenyi Bergisch Gladbach, Germany) as extensively described (Pachmann et al 2001), enrichment of epithelial cells using a magnet and analysis with a Laser Scanning Cytometer® (Compucyte Corporation, Cambridge, MA, USA)) enabling relocation of cells for visual examination of vital epithelial cells. Numbers of cells varied between 1000 and 5 million cells/5l of blood in the circulation corresponding to between 2 and 10000 cells/10 ml which is well in the range of 5–20,000 cells/7.5 ml blood sample as reported by Cristofanilli et al. (2004) in metastatic breast cancer and, using the appropriate improved methods (Lara et al. 2004), now is also reported from bone marrow aspirates. Combination staining of anti-HEA with anti-CD45-PE and restaining with anti-cytokeratin had shown that CD45+ blood cells could easily be discriminated from epithelial-antigen positive cells, and that all cells staining with anti-HEA also stained with cytokeratin. Nonspecific staining with anti-HEA did not occur when using live cells, which were defined as cells exhibiting exclusive surface staining. No live epithelial antigen positive cells were detected in 97% of healthy donors aged between 17 and 75 years; and in none of the 25 patients with hematological malignancies, whether full blown, in complete remission with regenerating haematopoiesis, or in relapse, could we detect cells staining with the HEA antibody (Pachmann et al. 2005b). Repeated analyses of blood drawn from patients at 1–3 h intervals showed less than 10% variation and, in some patients without therapy, less than two-fold changes over more than half a year were observed (Pachmann 2005a). The design of the method and fluoromicrographs of such cells with green fluorescence, exclusively surface-located, are shown in Fig. 1. Statistical analysis of

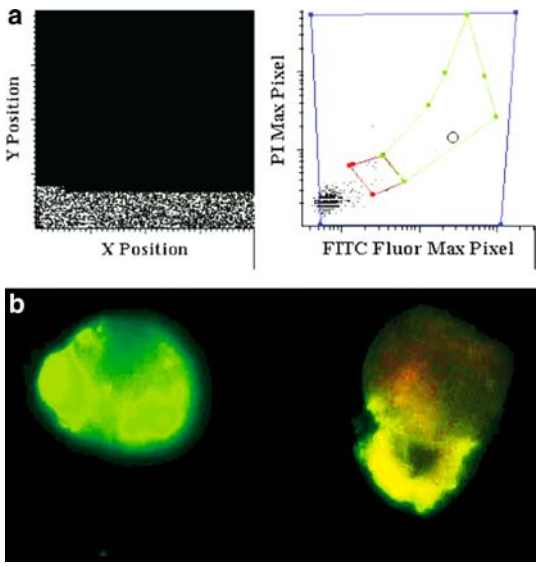

Fig. 1 Design of the approach for detection of circulating tumor cells and pictures of such cells. The microscope scans over a defined area (upper left scheme) and recognizes all white cells by light scatter and measures the fluorescence over each cell. A typical histogram is displayed in the right upper scheme with a circle around a positive cell (green gate). Two typical such cells are displayed below: an epithelial antigen positive cell (left cell, green cap) and another such cell counterstained for the estrogen receptor (right cell, epithelial antigen green cap and orange staining over the whole cell for the estrogen receptor)

relapse free survival was performed in a multivariate analysis and a Kaplan–Meier analysis using the SPSS program.

Results

From July 2000 to March 2002 32 consecutive non-metastatic patients were recruited randomly in the order in which they presented at the institution for diagnosis and therapy of breast cancer. Twenty-five patients were evaluable follow-up of until September 2005 and this group was compared to a previously analyzed group of 25 patients from another institution (Lobodasch et al. 2007). The distribution of disease stages comprised a patient population with 12 patients with good prognostic markers whereas 13 patients were grouped as having poor prognostic markers in comparison to the previous patient population with 13 patients with good prognostic markers. 1 male patient, although with an ER + tumor and no involved lymph nodes was grouped among the poor prognostic patients due to his male gender (Table 1).

For better comparison of changes in CETC numbers due to therapy, values before therapy were set 100% and an increase or decrease calculated accordingly. CMF was given more frequently (12 pts) than in previous group (3 pts) and (F)EC to 13 patients (20 pts, respectively), followed by taxane in three patients (8 pts, respectively). 15 patients showed a decrease during therapy, 6 × during EC therapy in 1 patient followed by taxane and 5 × during CMF therapy (typical examples are shown in Fig. 2a). Three patients showed little changes in cell numbers (Fig. 2b). All were relapse free at last follow-up visit. Ten patients showed either a continuous increase or an initial good reduction in CETC between 15- and 100-fold, however, followed by an increase up to 1000-fold. Of these 6 are still in remission (typical examples in Fig. 2c) whereas 4 have already relapsed (typical examples in Fig. 2d).

In summary, until now five patients have suffered relapse, the male patient with more than ten-fold increasing CETC numbers during CMF therapy, three patients with the typical initial decrease and subsequently increasing CETC numbers have experienced distant relapses and 1 patient in spite of stable circulating CETC number suffered local relapse (Tables 2 and 3) as compared to six patients from the previous cohort.

Both patients groups, the previous and the present patient population, were analyzed together in a multivariate analysis. Those in complete remission differed significantly from relapsed patients with respect to age, estrogen receptor expression and lymph node positivity (Table 4). As in the previous patient population there was again no significant difference between relapse free and relapsed patients in the number of CETCs before or at the end of therapy. In contrast, the dynamics of the cells (increase or decrease) differed highly significantly between patients who remained in complete remission and patients experiencing relapse and in the multivariate analysis this showed up as single independent highly-significant predictor of relapse ($p < 0.0001$).

In the Kaplan–Meier plot patients with a more than ten-fold increase in CETC numbers and patients with less than ten-fold increase or decreasing CETC numbers were compared separately and together. Among the patients with a more than ten-fold increase of the first group of 25 patients there were no censored cases, since all patients with these characteristics have suffered relapse during the observation time of 4.5 years. In the second group with shorter observation time there are censored patients also in the group with ten-fold increase in CETC (Fig. 3a). But both curves are super imposable indicating that the time to relapse is comparable. The combined analysis of all 50 patients showed a highly significant difference in relapse

Table 1 Characteristics of the patients under investigation

	25 Patients	Percent
Gender		
Male		4
Female		96
Race		
Caucasian		100
Age (years)		
Minimum	31	
Maximum	72	
Mean	56	
Tumor size/node		
T1		44
T2		40
T3		12
T4		4
N0		24
>N1		76
Receptor stage		
ER^-/PR^-		20
ER^+/PR^+		80
Metastases		20
Time to relapse (days)		
Minimum	273	
Maximum	1,691	
Mean	708	
Presurgery		
Ultrasound		100
X-ray thorax		100
Szintigraphy		100

Er, Estrogen receptor; *Pr*, progesteron receptor; *T*, tumor; *N*, nodes; *M*, metastases

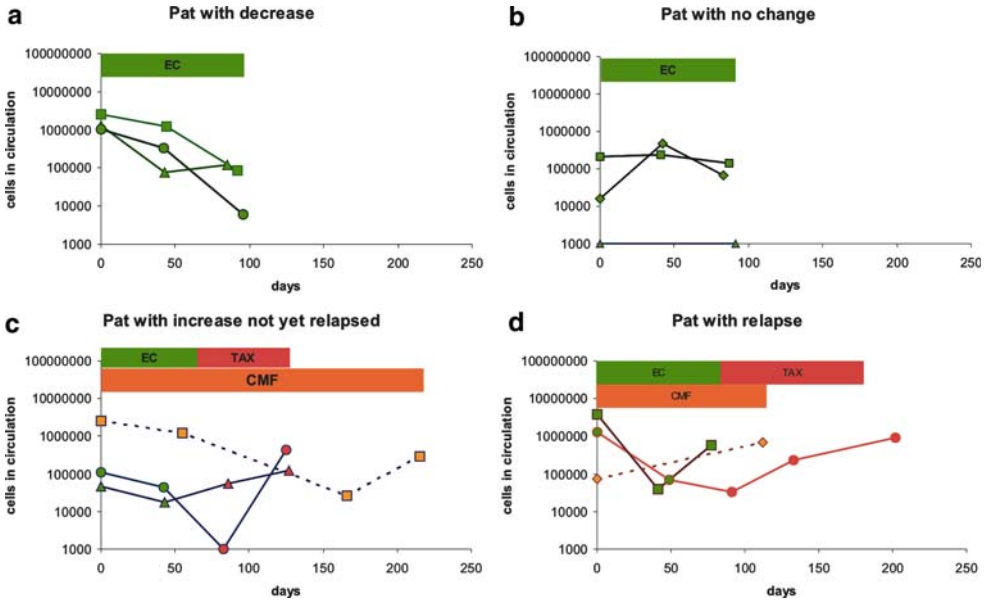

Fig. 2 Changes in CETC numbers during adjuvant therapy **a** in three typical patients with a decrease in cell numbers during EC therapy; **b** 3 patients with no or little changes during therapy, **c** 4 patients who show an increase during therapy but have not yet relapsed; and **d** 4 patients who have relapsed

Table 2 Relationship between remission stage and prognostic markers

	Total	Patients in complete remission	Relapsed patients	*p*-value
No. of patients	50	39	11	
Mean age		52	42	0.01
ER pos (%)	68	62 of total	6 of total	0.004
ER neg (%)	32	17of total	15 of total	
Node neg (%)	46	44 of total	2 of total	0.009
Node pos (%)	54	40 of total	14 of total	
Cell number before therapy in 5 l blood		9,19,015	1,46,069	n. s.
Cell number end of therapy in 5 l blood		5,46,032	1,70,485	n. s.
Increase >ten-fold (%)	32	12 of total	20 of total	<0.0001
Decrease or no change (%)	68	66 of total	2 of total	

Table 3 Correlation of cell dynamics and relapses to risk stages of breast cancer in group 1

Group 1	Good prognosis N < 3 ER^+ $Her2^-$		Poor prognosis N1 > 3 $ER^{+/-}$	
Cell number	CR	Rel	CR	Rel
Reduction at end of therapy	12	0	5	0
Increase at end of therapy	1 (slight)	0	1 (slight)	6

Table 4 Correlation of cell dynamics and relapses to risk stages of breast cancer in group 2

Group 2	Good prognosis N < 3 ER^+ $Her2^-$		poor prognosis N1 > 3 $ER^{+/-}$	
Cell number	CR	Rel	CR	Rel
Reduction at end of therapy	8	0	6	1
Increase at end of therapy	4	0	2	4

free survival between patients with decreasing or marginally changing CETC numbers during therapy and patients who showed a more than ten-fold increase in CETC numbers at the end of therapy as compared to the previous analysis (Fig. 3b). This difference was highly significant ($p < 0.0001$) in all the three comparisons (log rank, Breslow and Taxon Ware).

Discussion

Assuming that tumor cells in the circulation are the source of metastases, in recent years much work has been entered into the detection of CETC. Due to different methods used and to different tumor entities investigated, results are still controversial (Ditsch et al. 2002; Fehm et al. 2004). The

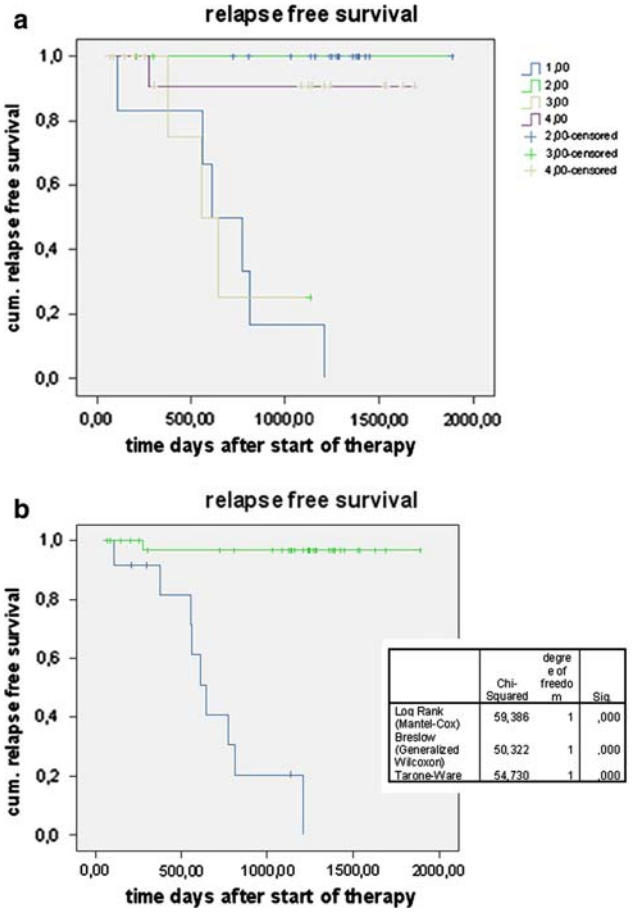

Fig. 3 Kaplan–Meier analysis of relapse free survival. **a** Overlay of the to patients' groups showing identical kinetics of relapse free survival for both groups. **b** Combining analysis of groups 1 and 2 (50 patients) for their response of circulating tumor cells to therapy in relation to relapse free survival

longest experience is available from breast cancer patients where mainly bone marrow was analyzed because of the observation that this tumor frequently metastasizes into bone (Braun et al. 2005). Although comparable numbers and frequencies as those detected by us in blood have now been reported even in bone marrow (Tong et al. 2006), using improved enrichment procedure, bone marrow aspirates can not be performed frequently, thus follow-up aspirations are difficult to obtain. One such study indicates that breast cancer patients with epithelial cells persistent in bone marrow have a poorer prognosis than patients without such cells. There are, however, patients with cells in bone marrow who do not relapse and patients with no such detectable cells who do suffer relapse, thus no clear answer can be given for the individual patient (Janni et al. 2005).

Peripheral blood, in contrast, is easy to obtain, is a well defined material and can be drawn repeatedly according to the requirements for repeated analysis of epithelial cells. We have shown that repeated analyses of a sample from the same patient exhibit less than 10% variation. In the neoadjuvant setting CETC in breast cancer patients respond to therapy in an identical fashion as the tumor (Pachmann et al. 2005b), and repeated analyses from lung cancer patients after therapeutic manipulations revealed only patients with increasing numbers of CETC over time to be at risk for relapse (Rolle et al. 2005). Our first preliminary studies in adjuvant chemotherapy have shown that close therapy control is feasible. Even after an initial response, numbers of tumor cells may rise again containing cells that are capable to settle in distant organs and to grow into metastases which are detectable only after months or years. Here, we show that comparable results could be obtained also in subsequent analyses in patients with a somewhat different risk profile. Patients were treated according to their risk profile; therefore different therapy schedules were not compared. Rather our intention was to study the response of individual patients to therapy, therefore, cell changes in CETC before and at the end and, if different therapy blocks were combined at the end of each therapy block, were correlated to individual outcome for the next 25 patients. In these patients, there occurred four distant and one local relapses (20%). Due to a shorter observation interval this was in a lower range than in the previous patient sample. According to their risk profile (Feldman et al. 2002) patients with good prognostic parameters N(0–3) were treated with EC only whereas patients with inferior prognostic parameters N1(>3) received additional taxane cycles (Campone et al. 2005) or CMF.

Fifteen patients showed a decrease, no change or less than ten-fold increase during EC or CMF therapy. Fourteen have not relapsed during the observation time. one local relapse occurred in a patient with a decrease in CETC numbers at the end of therapy.

As in the previous report, the characteristic feature of the patients experiencing distant relapse again was an increase in the number of CETCs of more than ten-fold at the end of therapy or the last therapy block as compared to the previous analysis. Distant relapses again occurred preferentially in the patients with less favorable prognostic markers. However due to a partly short follow-up, in the present patient population there are patients with increasing cell numbers without relapse.

Therefore, the results, obtained from the initial sample with an observation time of more than 4.5 years were compared to the present patients in a Kaplan–Meier analysis. Relapse free survival differed highly significantly between patients with marginal or good reduction in CETC numbers in response to therapy and those patients who showed increasing CETC numbers in spite of therapy, and this was true for the both patients' groups analyzed concurrently or separately. In the multivariate analysis of all 50 patients comparing the presence or absence of infiltrated lymph

nodes, the expression of the estrogen receptor, the initial number of CETCs before therapy, and the ability of the CETCs to increase in spite of continuous treatment the latter was the most significant independent predictor of early relapse. Our results also explain why testing the mere presence or absence of CETC at the end of adjuvant treatment as a prognostic factor so far did not yield consistent results (Muller et al. 2005; Xenidis et al. 2003; Pierga et al. 2004). Comparably to the well differentiated ER positive tumors also their CETC may respond only marginally to inhibitors of DNA replication whereas in the less differentiated (ER negative) tumors a large population of the initially present cells may have a high proliferative activity reflected by their initially good response to anthracycline-based therapy. It is a well known phenomenon in the treatment of leukemia that patients with excellent initial responses can show a rapid re-increase in leukemic cells with disease relapse. This may be comparable to the re-increase in CETC occurring in spite of continued treatment. A small fraction of cells resistant to the previous treatment may settle in remote loci and restart proliferating in spite of therapy (Naumov et al. 2003). The increase in cell number during taxane therapy might also be due to micro-metastases shedding cells into the circulation. Taxanes have been reported to preferentially damage endothelial cells leading to reduced intracellular fluid pressure (Griffon-Etienne et al. 1999) and may act on remnant occult tumor (metastases) to release cells into the circulation resulting in the observed increase in CETC. In this case the increase in cell numbers may reflect the aggressiveness of tumor cells shed from micrometastases, which are able to settle again in distant organs and grow into new metastases.

It is also obvious from the present results and other observations (Pachmann 2005a; Meng et al. 2004) that complete elimination of epithelial cells is rarely achieved and in part of the patients there may be no need for complete elimination of all suspect cells in order to achieve long lasting remissions. Such cells may be dormant for long times but it will be helpful to regularly repeat monitoring in order to early detect a renewed increase in CETC numbers as an indicator of imminent relapse.

We here confirm our previous evidence, that quantitative monitoring of CETC is feasible even under adjuvant conditions in breast cancer patients. We have strong indications that these present results can be further confirmed in a larger patient cohort from other treatment centers awaiting final evaluation. Not a single analysis, but the dynamics of the cells with a final increase in CETC numbers at the end of therapy as detected by repeated monitoring is predictive for relapse. It precedes clinical overt relapse by 5+ months if no further therapy is applied. Thus, there would remain sufficient time to apply additional therapies under the control of the MAINTRAC® analysis to achieve a response of these cells.

Monitoring of CETC may become a valuable tool for therapy surveillance in adjuvant therapy. It may complement other approaches for analysis of therapy resistance (Sawyers 2005), lead to new treatment considerations and a more exact tailoring of therapy.

References

Bonadonna G, Moliterni A, Zambetti M, Daidone MG, Pilotti S, Gianni L, Valagussa P (2005) 30 years' follow up of randomised studies of adjuvant CMF in operable breast cancer: cohort study. BMJ 330:217–220

Braun S, Vogl FD, Naume B, Janni W, Osborne MP, Coombes RC, Schlimok G, Diel IJ, Gerber B, Gebauer G, Pierga JY, Marth C, Oruzio D, Wiedswang G, Solomayer EF, Kundt G, Strobl B, Fehm T, Wong GY, Bliss J, Vincent-Salomon A, Pantel K (2005) A pooled analysis of bone marrow micrometastasis in breast cancer. N Engl J Med 353:793–802

Campone M, Fumoleau P, Bourbouloux E, Kerbrat P, Roche H (2005) Taxanes in adjuvant breast cancer setting: which standard in Europe? Crit Rev Oncol Hematol 55:167–175

Carrick S, Parker S, Wilcken N, Ghersi D, Marzo M, Simes J (2005) Single agent versus combination chemotherapy for metastatic breast cancer. Cochrane Database Syst Rev. Apr 18:CD003372

Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, Reuben JM, Doyle GV, Allard WJ, Terstappen LW, Hayes DF (2004) Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 351:781–791

Ditsch N, Funke I, Mayer B, Untch M (2002) Detection of disseminated tumor cells in bone marrow-currently of no practical therapeutic value. MMW Fortschr Med 144:37–39

Fehm T, Becker S, Pergola-Becker G, Kramer B, Gruber I, Sotlar K, Kurek R, Wallwiener D, Solomayer E (2004) Influence of tumor biological factors on tumor cell dissemination in primary breast cancer. Anticancer Res 24:4211–4216

Feldman M, Stanford R, Catcheside A, Stotter A (2002) The use of a prognostic table to aid decision making on adjuvant therapy for women with early breast cancer. Eur J Surg Oncol 28:615–619

Griffon-Etienne G, Boucher Y, Brekken C, Suit HD, Jain RK (1999) Taxane-induced apoptosis decompresses blood vessels and lowers interstitial fluid pressure in solid tumors: clinical implications. Cancer Res 59:3776–3782

Janni W, Rack B, Schindlbeck C, Strobl B, Rjosk D, Braun S, Sommer H, Pantel K, Gerber B, Friese K (2005) The persistence of isolated tumor cells in bone marrow from patients with breast carcinoma predicts an increased risk for recurrence. Cancer 103:884–891

Jones AL (2005) Reduction in mortality from breast cancer. BMJ 330:205–206

Lara O, Tong X, Zborowski M, Chalmers JJ (2004) Enrichment of rare cancer cells through depletion of normal cells using density and flow-through, immunomagnetic cell separation. Exp Hematol 32:891–904

Lobodasch K, Dengler R, Fröhlich F, Rengsberger M, Schubert R, Pachmann U, Pachmann K (2007) 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. Breast 16:211–218

Mansi JL, Gogas H, Bliss JM, Gazet JC, Berger U, Coombes RC (1999) Outcome of primary-breast-cancer patients with micrometastases: a long-term follow-up study. Lancet 354:197–202

Meng S, Tripathy D, Frenkel EP, Shete S, Naftalis EZ, Huth JF, Beitsch PD, Leitch M, Hoover S, Euhus D, Haley B, Morrison L, Fleming TP, Herlyn D, Terstappen LW, Fehm T, Tucker TF, Lane N,

Wang J, Uhr JW (2004) Circulating tumor cells in patients with breast cancer dormancy. Clin Cancer Res 10:8152–8162

Muller V, Stahmann N, Riethdorf S, Rau T, Zabel T, Goetz A, Janicke F, Pantel K (2005) 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

Naumov GN, Townson JL, MacDonald IC, Wilson SM, Bramwell VH, Groom AC, Chambers AF (2003) Ineffectiveness of doxorubicin treatment on solitary dormant mammary carcinoma cells or late-developing metastases. Breast Cancer Res Treat 82:199–206

Pachmann K (2005a) Longtime recirculating tumor cells in breast cancer patients. Clin Cancer Res 11:5657; author reply 5657–5658

Pachmann U, Pachmann K (2000) Minimale residuale Tumorerkrankung bei soliden epithelialen Tumoren: Neues automatisiertes Analyseverfahren. Dtsch Ärztebl 97(51–52):A-3511

Pachmann K, Heiß P, Demel U, Tilz G (2001) Detection and enumeration of minimal numbers of circulating tumour cells in peripheral blood using Laser Scanning Cytometry (LSC®). Clin Chem Lab Med 39:811–817

Pachmann K, Camara O, Kavallaris A, Schneider U, Schünemann S, Höffken K (2005b) Quantification of the response of circulating epithelial cells (CETC) to neodadjuvant treatment of breast cancer: a new tool for therapy monitoring. Breast Cancer Res 7:R975–R979

Pierga JY, Bonneton C, Vincent-Salomon A, de Cremoux P, Nos C, Blin N, Pouillart P, Thiery JP, Magdelenat H (2004) 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

Rolle A, Günzel R, Pachmann U, Willen B, Höffken K, Pachmann K (2005) Increase in number of circulating disseminated epithelial cells after surgery for non-small cell lung cancer monitored by MAINTRAC® is a predictor for relapse: a preliminary report. World J Surg Oncol 3:18

Sawyers CL (2005) Calculated resistance in cancer. Nat Med 11:824–825

Solomayer EF, Diel IJ, Salanti G, Hahn M, Gollan C, Schutz F, Bastert G (2001) 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

Tong X, Melnik K, Braun S, Zborowski M, Chalmers JJ (2006) Detection of disseminated tumor cells in bone marrow of breast cancer patients. 6th International Conference on Scientific and Clinical Applications of Magnetic Carriers May 17th–20th. Krems, Austria

Xenidis N, Vlachonikolis I, Mavroudis D, Perraki M, Stathopoulou A, Malamos N, Kouroussis C, Kakolyris S, Apostolaki S, Vardakis N, Lianidou E, Georgoulias V (2003) Peripheral blood circulating cytokeratin-19 mRNA-positive cells after the completion of adjuvant chemotherapy in patients with operable breast cancer. Ann Oncol 14:849–855