Annals of Oncology 18: 1484–1492, 2007
doi:10.1093/annonc/mdm206

The relevance of circulating epithelial tumor cells (CETC) for therapy monitoring during neoadjuvant (primary systemic) chemotherapy in breast cancer

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Received 27 March 2007; accepted 23 April 2007

Background: Having demonstrated in a previous report that the response of circulating epithelial tumor cells (CETC) during the first cycles of primary (neoadjuvant) chemotherapy perfectly reflects the response of the tumor, in the present study the changes in cell numbers during subsequent cycles and their possible impact on the therapy's outcome were examined.

Patients and methods: In 58 breast cancer patients CETC were quantified during therapy with either EC (epirubicin/ cyclophosphamid) or dose intensified E (epirubicin) followed by taxane, with or without trastuzumab, and subsequent CMF (cyclophosphamid/methorexate/ fluorouracil).

Results: CETC numbers declined more than 10-fold (good response) in 65% (her2/neu-negative) and 55% (her2/neu-positive) of patients during EC, and in 60% during dose intensified E, respectively, followed by an increase of CETC in all patients. CETC remained increased, decreasing only when adding CMF. A good initial response correlated with estrogen-receptor negativity, a poor response with early distant relapse ($P < 0{,}0001$, hazard ratio = 11.91).

Conclusion: Response of CETC already during the first cycles of neoadjuvant treatment predicts the final response of the tumor. Hitherto unknown effects of the release of tumor cells during therapy further our understanding of tumor-blood interaction and may improve access of agents like antibodies to cells. The impact on the further course of disease remains to be evaluated.

Key words: circulating tumor cells, neoadjuvant therapy monitoring.

introduction

Neoadjuvant or primary chemotherapy, initially used only in non-resectable breast cancer to reduce the tumor's size [1], is now also an option in resectable tumors [2–3]. In addition to tumor-size reduction aiming to achieve higher rates of breast conservation [4], this therapy may also provide information about the responsiveness of the individual tumor to chemotherapy for later application in case of relapse [5]. Different chemotherapeutic agents have shown reactivity the metastatic situation and an improvement in relapse-free survival in the adjuvant situation. Various clinical studies currently examine these agents in different combinations with respect to their effectiveness in neoadjuvant treatment against the primary tumor [6]. Neoadjuvant treatment allows controlling the therapeutic success by imaging and verification through pathologic analysis of the remaining tumor tissue [7]. Lastly, it can be correlated to the final outcome [8–9], although the hypothesis that neoadjuvant treatment improves the survival rate could not be corroborated [10].

One of the main reasons for inadequate response of primary solid tumors to chemotherapy is the poor penetration and distribution of such agents in the tumor tissue due to interstitial hypertension, which is a universal characteristic of solid tumors [11]. It has been demonstrated that taxanes reduce this pressure [12]. The addition of taxanes to neoadjuvant regimen has increased the frequency of complete pathological responses (pCR) in neoadjuvant therapy in breast cancer [13–14]. This has led to improved outcome, according to some studies [15–16] but was not conclusively confirmed by others [17–18]. Patients with ER (estrogen receptor) positive tumors had a lower pCR rate than patients with ER-negative tumors [19]. On the other hand, preoperative trastuzumab in her2/neu-positive patients, regarded as high-risk patients, has resulted in a dramatically increased pCR rate [20].

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In preceding studies we have shown that quantification of circulating tumor cells is possible. In breast cancer patients the reduction of tumor size—starting with the initial magnetic resonance tomography (MRT) analysis up to the final pathological response of the tumor at the time of surgery—was compared with the reduction of circulating epithelial tumor cells (CETC). The comparison revealed that CETC responded during the first 3–4 cycles of neoadjuvant therapy in an identical manner as the tumor. Therefore, the response of the CETC to neoadjuvant therapy may be used for monitoring the efficacy of chemotherapy, and it enables to determine at an early stage the patient's response to the therapy [21].

We observed, however, that tumor cells or small cell clusters can also be shed from the tumors during therapy. In the present work we have more closely investigated the cells in peripheral blood of patients with breast cancer during the subsequent cycles of neoadjuvant chemotherapy.

patients and methods

Included in the study were 58 patients who were diagnosed between 2003 and 2005 with breast cancer stages T2 to T4 or inflammatory tumor. Her2/neu-negative patients were assigned randomly to two different chemotherapy arms: 28 patients were treated with four cycles of epirubicin–cyclophosphamid (EC) (E/C 90/600 mg/m^2 q 21d) followed by four cycles of taxane (Paclitaxel 175 mg/m^2 q 21d); 16 patients were treated with three cycles of dose-intensified epirubicin (Epirubicin 150 mg/m^2 q 14d), three cycles of taxane (Paclitaxel 225 mg/m^2), and three additional cycles of cyclophosphamid/methotrexat/fluorouracil (CMF) (500/40/600 mg/m^2 q 14d); 14 patients who were her2/neu-positive in immunohistochemistry or positive in the FISH test were treated with four cycles of EC (E/C 90/600 mg/m^2 q 22 d) and subsequently with four cycles of taxane (Paclitaxel 175 mg/m^2 q 21 d) with the addition of trastuzumab 8mg/kg cycle 5 and 6mg/kg during cycles 6–8.

Before each cycle of chemotherapy, samples of 1 ml of anti-coagulated peripheral blood were obtained with the patient's consent, according to the ethics committee approval, and analyzed using the previously described microfluorimetric method [22, 23]. In short, samples were subject to red blood cells lysis using 10 ml of erythrocyte lysis solution (Qiagen, Hilden, Germany) for 10 min in the cold. The white cell pellet was then spun down at 700g and rediluted in 1 ml of PBS. 10 µl of fluoresceinisothiocyanate (FITC)-conjugated mouse anti-human epithelial antibody (HEA) (Milteny, Bergisch Gladbach Germany) and 1 µl of phycoerythrin (PE) labeled anti CD45 were added to 100 µl of cell suspension, incubated for 15 min in the dark, readjusted to 1 ml and 20 µl of this suspension and used for measuring epithelial-antigen positive cells .

In order to measure the cells they were applied to a defined area on adhesion slides (Menzel Gläser, Braunschweig, Germany). After adding the cell suspension to the slides, vital cells became adherent to the slide surface after 10–15 s. Measurements were started when the cells had settled and took about 20–30 min, depending on cell density. For optimal measurements it was imperative to have a single cell suspension with about 2–3 cell diameters space between the cells. The adherent cells were measured using a Laser Scanning Cytometer (LSC® Compucyte Corporation, Cambridge, MA, USA). The cells could easily and unequivocally be contoured using forward scatter as a thresholding parameter at 20x magnification. Background fluorescence was determined dynamically to calculate both peak and integral fluorescence on a per-cell basis. This unique method corrects for variation in background fluorescence so that the fluorescent calculation is the same for all cells. The FITC-HEA positive cell fluorescence was collected using a 530/30 nm bandpass filter and amplified using a photomultiplier (PMT). Values are displayed in scattergrams and histograms and percentages and mean values of positive and negative cells are calculated from the region comprised of single cells only. The LSC®enables the user to locate cells contained within the positive population for visual examination through the microscope. In addition, a CCD camera attached to the microscope allowed to record simultaneously photo- and fluoromicrographs [22–23]. Figure 1a depicts an example of the procedure used for analysis of epithelial cells suspect to be of tumor origin. The microscope scans a defined area on the slide to which a certain volume of cell suspension has been applied. This allows calculation of the absolute number of epithelial cells per ml of blood. Viability of the cells detected by laser scanning cytometry was visually verified by looking for exclusive surface staining. Typical pictures of such cells detected by their green fluorescing cap are shown in Figure 1b.

results

Table 1 sums up the applied regimen including tumor characteristics (ER-positivity, PR-positivity, Her2/neu score), tumor size before therapy and at surgery, the number of circulating tumor cells per ml before therapy onset, the response of CETC to therapy and the outcome at the last appointment and time to relapse with the 58 patients who had been assigned to the three different neoadjuvant chemotherapy regimen. Vital circulating epithelial cells were detected in almost all peripheral blood samples before the first cycle of chemotherapy. Initial pre-therapy numbers of CETCs varied considerably between patients from the highest value of 273 150 to the lowest below the threshold of detection, with a mean of 26 079 cells/ml. The numbers are higher than those reported for bone marrow (1–25 in 2x 10^6 mononuclear cells) [24] or blood (5–25 000/7.5 ml) [25] due to differences in methodological approaches. Thus we applied only one washing and no enrichment step. Before the first cycle of therapy there was an exponential and significant correlation (correlation coefficient 0.621; $P = 0.001$) between the tumor volume, as measured by magnetic resonance tomography, and the number of CETC (Figure 2). Because of the high variation in pre-therapy CETC numbers, CETC values were normalized to make the curves between different patients comparable. For this purpose the CETC number immediately before surgery was set 100% and the other values calculated accordingly.

Numbers of circulating tumor cells were determined before each chemotherapy cycle if possible and before surgery.

Pre-surgery values were available for 20/28 patients from the 4xEC/4xTax arm and typical analyses are shown in Figure 3a, b. Altogether, 65% of patients (13/20) responded to the EC therapy with a more than 10-fold decrease in cell numbers, whereas 35% of patients (7/20) responded marginally to EC therapy, with only a 10-fold decrease, or less. Surprisingly, CETC numbers reached a nadir in all patients, followed by a subsequent increase, usually during the taxane treatment. CETC numbers in some cases re-increased 10 000-fold. A reduction in tumor size was recorded during this increase in cell number suggesting that the increase in CETC numbers in peripheral blood was mainly due to release of cells during the decay of the tumor. In patients responding with a less than 10-fold decrease to EC therapy, the increase in CETC during

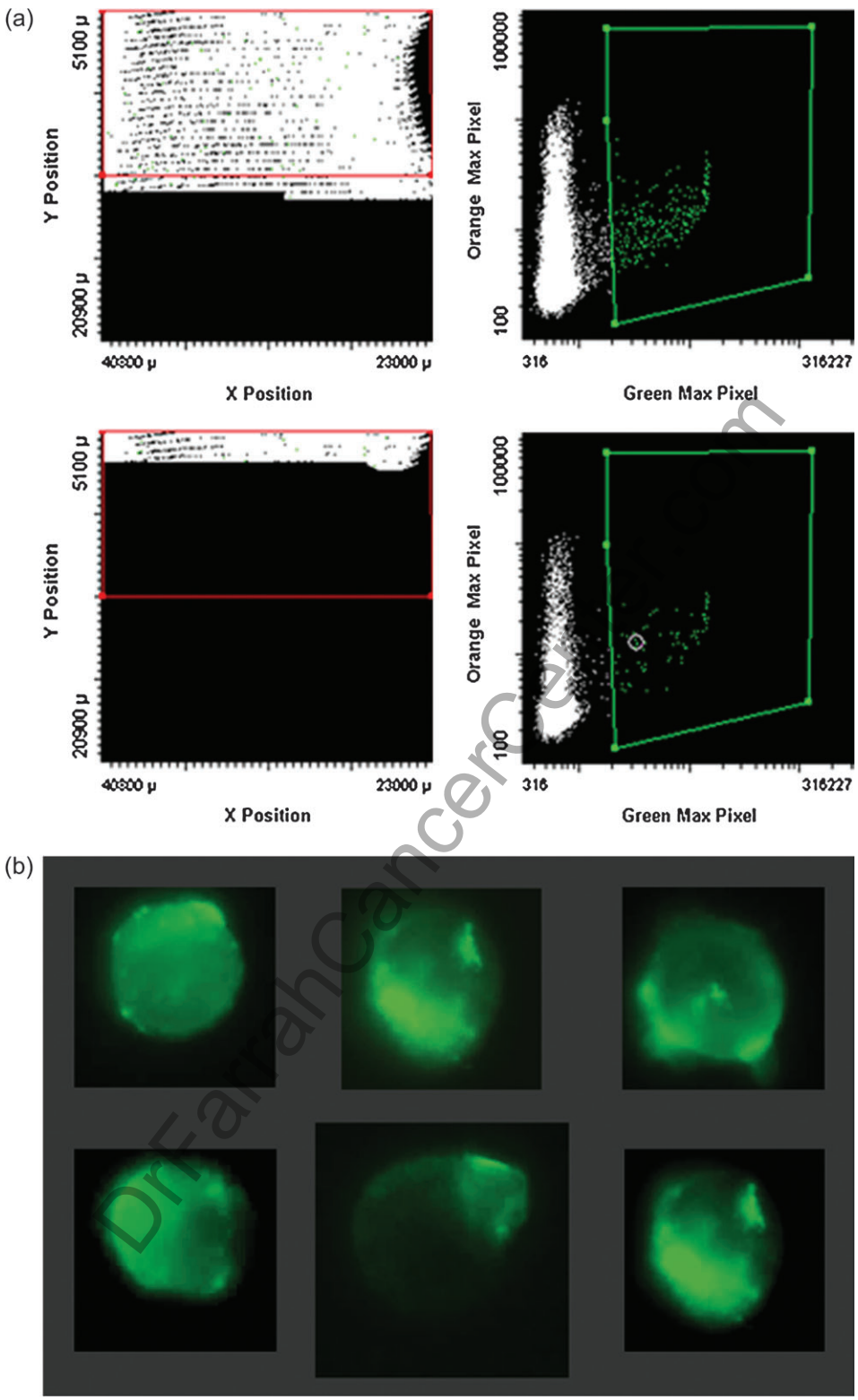

Figure 1. (a) Example of the procedure used for analysis of epithelial cells. The microscope scans a defined area on a slide to which a defined volume of cell suspension is applied. The majority of cells are normal blood cells, showing only background fluorescence. Positively stained green fluorescing cells are gated in the green window Cells in this window can be localized again (event in the circle), viewed, photographed and re-analyzed; (b) typical pictures of such cells detectable by their green fluorescing cap.

taxane treatment was less marked (Figure 3b). In most patients cell numbers remained at almost the same high level until surgery and even thereafter (not shown), indicating that at least some of these cells were viable cells. One patient in this treatment arm experienced a pCR (no tumor detection); 21% of the patients (6/28) have relapsed, and 7% (2/28) died.

Table 1. Characteristics of 58 patients treated with neoadjuvant chemotherapy CETC numbers

Pat. No.	Treatment	ER	PR	Her2/neu	Tumour size before treatment	Tumor size at surgery	CETC/ml before treatment	CETC good/ poor response	Status relapse or CR	Time months until relapse
1	EC-T	+	+	++	25x21x17	marginal reduction	n.a.	n.a.	CR	
2	EC-T	–	–	+	20x15x20	pCR	43000	good response	CR	
3	EC-T	+	–	–	22x15x28	13x10x8	7200	poor response	CR	
4	EC-T	n.a.	n.a.	n.a.	24x16x20	5x3x4	3600	good response	CR	
5	EC-T	+	+	+	50x30x30	25x35x17	7131	poor response	relapse	1
6	EC-T	+	+	++	20x16x16	15x15x15	400	n.a.	CR	
7	EC-T	+	+	++	20x20x20	12x12x12	33480	good response	CR	
8	EC-T	+	+	++	15x11x13 + 8x9x8	15x17x9	n.a.	n.a.	CR	
9	EC-T	+	+	++	36x30x36	20x20x20	4135	poor response	CR	
10	EC-T	–	–	–	31x25x25	20x17x15	200	good response	CR	
11	EC-T	–	–	+++	20x20x20	12x12x12	3000	good response	relapse	11
12	EC-T	+	+	+	25x27x27 + 9x10	n.a.	200	poor response	relapse/died	3
13	EC-T	+	–	+++	25x25x25	7x6x5	6900	poor response	CR	
14	EC-T	–	–	–	50x50x50	15x10x12	n.a.	n.a.	relapse	28
15	EC-T	–	–	–	40x50x50	10x10x10	2520	good response	CR	
16	EC-T	–	–	+	20x20x20	7x6x6	n.a.	n.a.	CR	
17	EC-T	+	+	FISH -	20x20x20	17x17x17	n.a.	n.a.	n.a.	
18	EC-T	n.a.	n.a.	n.a.	42x30x30	27x27x27	2500	poor response	relapse	13
19	EC-T	+	–	++	17x19x14 + 10x10x10	12x12x12	6122	good response	CR	
20	EC-T	–	+	+++	16x10x9 + 6x4x4	n.a.	3264	good response	CR	
21	EC-T	–	–	–	25x25x25+DCIS	7x7x7	1800	good response	CR	
22	EC-T	–	+	+	20x20x20	12x12x12	3212	good response	CR	
23	EC-T	n.a.	n.a.	n.a.	20x32x20	5x5x5	n.a.	n.a.	CR	
24	EC-T	–	+	+	20x20x20+DCIS	17x17x17	5400	good response	CR	
25	EC-T	–	+	+++	35x35x35	25x25x25	5600	poor response	relapse/died	8
26	EC-T	+	+	++	38x20x20	35x19x19	n.a.	n.a.	CR	
27	EC-T	–	–	–	15x17x17	15x15x15	7400	good response	CR	
28	EC-T	+	+	+	19x14x15	10x12x12	560	good response	CR	
29	EC-TH	–	+	+++	23x15x25	9x9x8	46980	good response	CR	
30	EC-TH	–	–	+++	80x70x70	75x80x55	15744	good response	CR	
31	EC-TH	–	+	+++	20x20x20	12x12x12	2600	good response	CR	
32	EC-TH	+	+	++	50x30x40	pCR	200	poor response	CR	
33	EC-TH	+	+	+++	30x40x20 + 5x5x5	30x10x22	n.a.	n.a.	relapse	12
34	EC-TH	+	+	+++	22x17x17	3x4x4	7743	good response	CR	
35	EC-TH	+	+	FISH +	56x25x25	16x10x10	4200	poor response	CR	
36	EC-TH	+	–	+++	17x16x16	pCR	n.a.	n.a.	CR	
37	EC-TH	+	+	+++	28x21x21	27x28x27	2600	poor response	relapse	27
38	EC-TH	+	+	n.a.	20x24x20	pCR	600	poor response	relapse	27
39	EC-TH	+	+	++	38x26x27 + 27x16x18	17x13x13	27200	good response	CR	
40	EC-TH	n.a.	n.a.	n.a.	30x20x30	18x19x18	n.a.	n.a.	n.a.	
41	EC-TH	–	–	FISH +	45x45x45	17x18x17	10800	poor response	relapse	5
42	EC-TH	n.a.	n.a.	n.a.	32x18x20	1x0,3x1	273150	good response	died w.o. relapse	
43	E-T-CMF	+	+	+++	85x85x85	38x26x22	6600	good response	CR	
44	E-T-CMF	–	+	+	50x60x60	9x6x7	192372	good response	CR	
45	E-T-CMF	+	+	+++	10x10x10	17x17x17	10	poor response	CR	
46	E-T-CMF	+	+	–	25x25x25	12x12x12	7400	poor response	relapse	11
47	E-T-CMF	–	–	n.a.	40x40x40	n.a.	6052	good response	n.a.	

Table 1. (*Continued*)

Pat. No.	Treatment	ER	PR	Her2/neu	Tumour size before treatment	Tumor size at surgery	CETC/ml before treatment	CETC good/ poor response	Status relapse or CR	Time months until relapse
48	E-T-CMF	n.a.	n.a.	n.a.	20x50x25	70x50x20	8900	poor response	relapse	28
49	E-T-CMF	−	−	+	30x40x40 + 40x50x50	1x1x1	22460	good response	CR	
50	E-T-CMF	+	+	+++	39x39x39	12x12x12	900	poor response	relapse	19
51	E-T-CMF	+	+	+	20x20x20	10x10x10	12408	n.a.	CR	
52	E-T-CMF	+	−	+	25x20x25	9x12x8 s	4400	good response	CR	
53	E-T-CMF	+	+	++	22x13x20	10x10x10	3800	poor response	relapse	23
54	E-T-CMF	−	−	++	60x60x60	52x53x55	28800	poor response	relapse	25
55	E-T-CMF	−	−	n.a.	20x50x50	pCR	53200	good response	CR	
56	E-T-CMF	+	+	++	43x32x42	1x1x1	32000	good response	CR	
57	E-T-CMF	+	+	++	27x21x35+DCIS	17x17x17	28960	good response	CR	
58	E-T-CMF	+	+	+	16x9x9	1x1x1	8600	good response	CR	

E, epirubicin; C, cyclophosphamid; T, = taxane; H, herceptin; M, methorexate; F, fluorourcil; n.a., not available; CR, complete remission; pCR, pathological complete response.

Good response of CETC corresponds to a more than 10-fold reduction in CETC numbers during anthracycline containing therapy and poor response to a 10-fold or less reduction

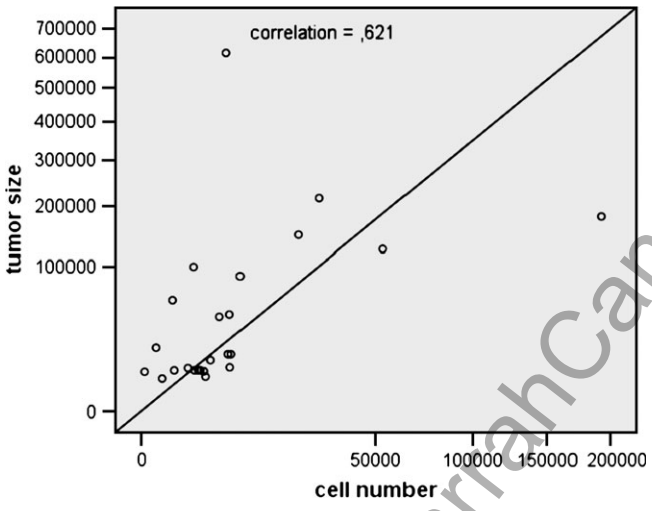

Figure 2. Correlation between initial tumor sizes as determined by magnetic resonance tomography and pre-therapy numbers of CETC.

Of patients with her2/neu-positive tumors 14 received 4 cycles of EC followed by 4 x taxane with the addition of trastuzumab; 55% (6/11) of the evaluable patients responded to the EC cycles with a more than 10-fold decrease in cell numbers and 45% (5/11) with a marginal decrease (less than 10-fold) or even an increase. It was remarkable that no comparably marked increase in CETC, as in the taxane-only group, was observed during the treatment with trastuzumab together with taxane. Typical curves are shown in Figure 4a, b. A pCR was observed in 21% of the patients (3/14) during this treatment, independent of the initial response to EC. Thus the combination of taxane and trastuzumab was obviously capable of effectively reducing the circulating tumor cells, as well as the tumor. Of the patients in the trastuzumab treatment arm, 28% (4/14)have relapsed and one patient died without signs of relapse.

An initial decrease in cell numbers was also observed in the 16 patients from the treatment arm with dose-intensified epirubicin. 60% of the evaluable patients (9/15) responded with a strong (more than 10-fold) decrease (Figure 5a). Among the 40% of patients (6/15) with only 10-fold or less decrease, there were 2 patients with an increase in cell numbers during therapy (Figure 5b). After the initial response there was an increase in cell numbers in all patients of the dose-dense treatment arm during taxane treatment, which was more pronounced in those patients who initially responded more strongly. This increase in circulating epithelial cell numbers, again, was accompanied by a reduction in tumor size and was measurable by ultrasound.

The patients in this treatment arm then received additionally 3 CMF cycles before surgery. In contrast to the patients with EC/Tax treatment, whose CETC numbers remained at the same high level until surgery, CETC numbers in this group were reduced in part of the patients by the CMF cycles until surgery. This was more effective in patients with good response to the initial anthracycline treatment (Figure 5c) than in patients with marginal or no (less than 10-fold) initial response (for typical examples see Figure 5d). One out of sixteen patients (6%) from the good response group experienced a pCR. All 5 patients with early relapses in this treatment arm (33%) belong to the poor response group.

As previously published, the initial reduction of CETC closely correlates with the final tumor-size reduction reached by neoadjuvant therapy [22], and this was corroborated by the present results. Moreover, we were now able to correlate numbers under neoadjuvant treatment with prognostic factors. Circulating cells from estrogen-receptor positive tumors responded significantly inferior to the anthracycline treatment than cells from estrogen-receptor negative tumors (Figure 6a). Correlating the response of CETC to clinical outcome revealed that only the correlation to anthracycline response was significant. Patients with a 10-fold or less

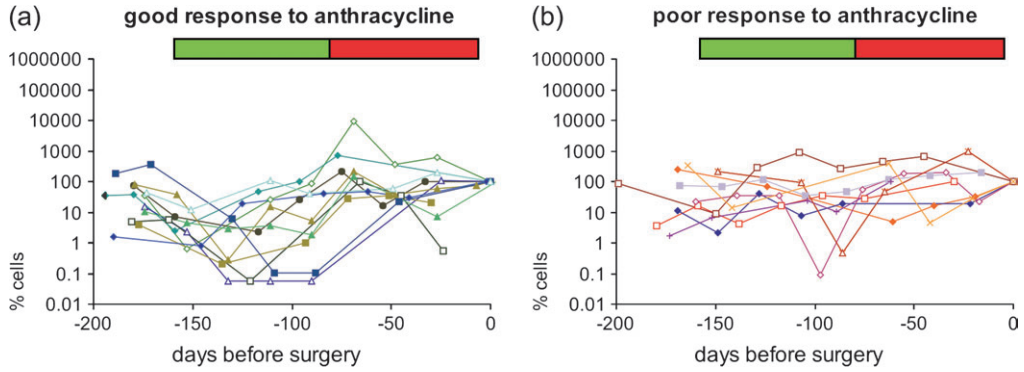

Figure 3. Longitudinal analysis of the pattern of CETC from patients treated in the EC-T arm. Values at surgery were set 100% and the other values calculated accordingly: (a) patients from the good response group; (b) patients from the poor response group.

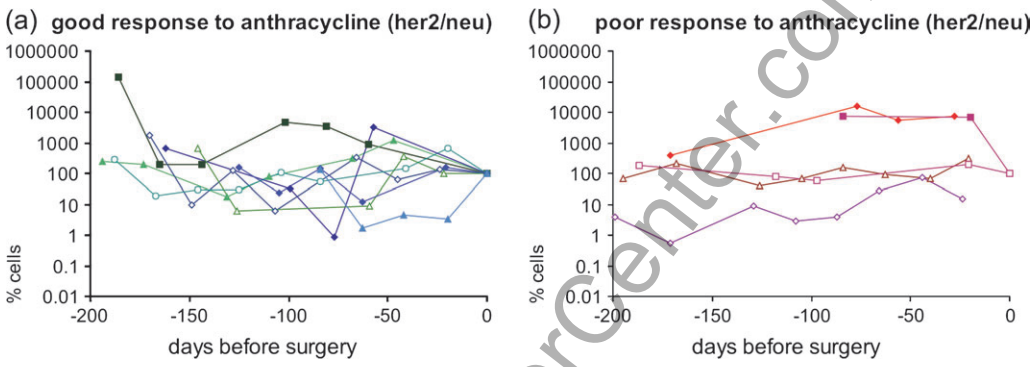

Figure 4. Longitudinal analysis of the pattern of CETC from patients treated in the EC-TH arm. Values at surgery were set 100% and the other values calculated accordingly: (a) patients from the good response group; (b) patients from the poor response group.

reduction in CETC in response to the anthracycline regimen had a significantly higher probability to develop early distant metastases ($P < 0.0001$; hazard ratio = 11.91) during the postoperative observation interval of 1 to 28 months (mean = 16 months) than patients with a good response (more than 10-fold reduction) of CETC (Figure 6b), irrespective of subsequent treatment (Tamoxifen, aromatase inhibitors or trastuzumab).

discussion

Circulating epithelial cells can be detected in many patients with solid tumors but rarely in healthy subjects [22]. Since most solid tumors are of epithelial origin or have an epithelial component, it is assumed that these circulating epithelial cells are shed from the tumor. We have developed a method for easy, rapid, reliable and reproducible quantification of epithelial cells in peripheral blood [22–23]. We were able to detect circulating epithelial cells in 47/48 breast cancer patients scheduled for treatment before onset of neoadjuvant therapy for which blood samples were available before therapy. Although the number of CETC detected is several logs higher than reported by other groups [24–25], we could demonstrate for the first time that this number correlates with tumor size at a level of significance of $P = 0.001$. Neoadjuvant chemotherapy of patients with breast cancer is applied in well-defined studies [26–27]. The patients monitored in the present report were treated within the compass of the PREPARE- and TECHNO-study [26, 28]. Changes in numbers of CETC were quantified at defined intervals during the chemotherapy regimen and compared to tumor response.

The high correlation between initial reduction in CETC with final tumor-size reduction documented at surgery reported in a previous study during the first 3–4 courses of EC or dose-intensified E therapy, respectively [21], was corroborated by the present results.

Investigation of CETC was now extended to the subsequent 3–4 cycles of taxane and, in the dose-dense arm, to the additional 3 cycles of CMF. The most remarkable result of the taxane treatment was a rapid increase in CETC numbers, although a reduction in tumor size was observed by the patients and verified by ultrasound. Without further treatment cell numbers remained at heightened levels until surgery.

Reduction in tumor size can be due to different effects: (1) cell death; (2) draining of intra-tumoral fluid and, indeed, it has been demonstrated [12] that taxane leads to a reduction in intra-tumor pressure and a widening of tumor vessels; (3) moreover, we assume that there occurs a release of cells from the tumor tissue. CETC numbers increased concomitantly with taxane-induced tumor size-reduction. A reduction in cellularity in the residual tumor tissue in neoadjuvantly treated tumors has been observed by

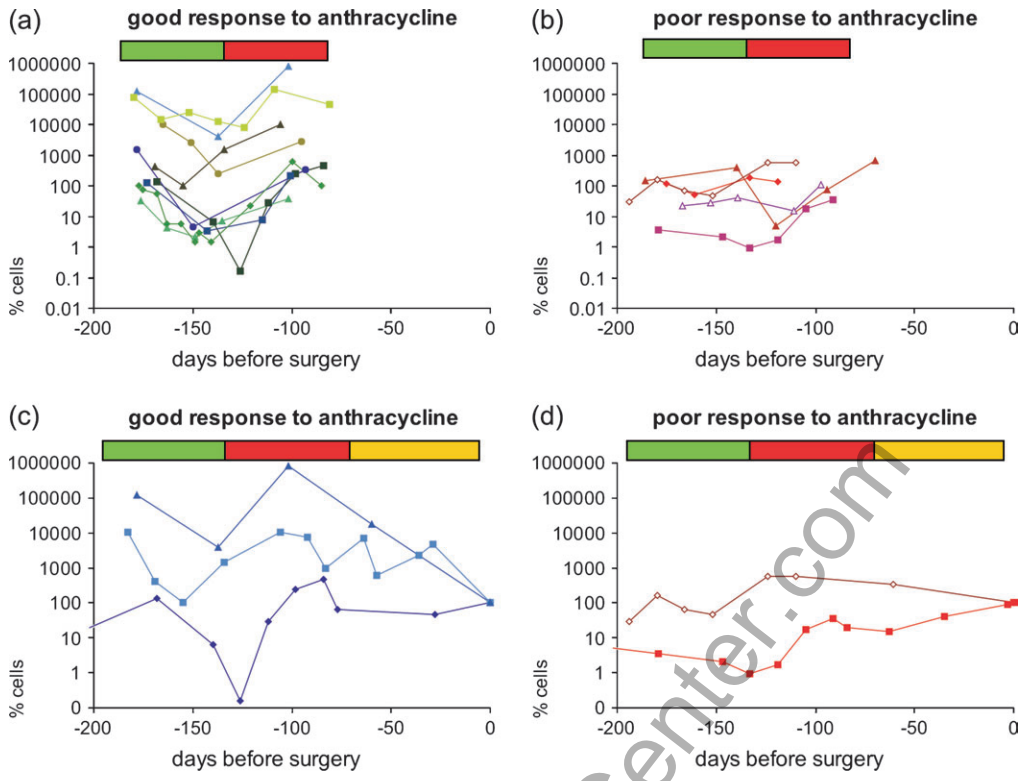

Figure 5. Longitudinal analysis of the pattern of circulating cells from patients treated in the E-T-CMF arm. Values at surgery were set 100% and the other values calculated accordingly: Initial response during the dose-dense part of Epirubicine and taxane in (a) patients from the good-response group; (b) patients from the poor-response group. Examples of pattern of CETC during the complete therapy schedule including CMF in (c) patients from the good-response group and (d) patients from the poor-response group.

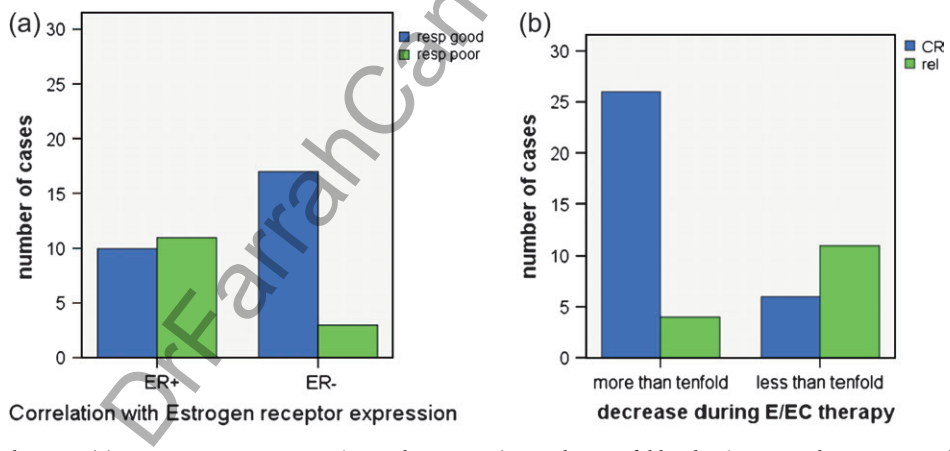

Figure 6. Correlation between (a) estrogen-receptor expression and response (more than 10-fold reduction = good response; 10-fold or less = poor response) of CETC numbers (from pre-therapy number to nadir) during the cycles of anthracycline treatment and (b) clinical outcome (CR or distant relapse) and response of CETC to anthracycline treatment.

Rajan et al. [29] but pCR may sometimes be difficult to evaluate [30] after neoadjuvant chemotherapy. Draining of intra-tumoral fluid may wash out cells of the tumor at the same time. The reduction in intra-tumoral pressure possibly leads to a better access of the therapeutic agents to the tumoral cells [31] but may, simultaneously, lead to tumor cell dissemination.

The role of the released cells for the patient's course of disease is not clear. pCR is doubled by addition of preoperative taxane to anthracycline/cyclophosphamid [32], but not if added to anthracycline alone [17–18]. Patients with pCR are assumed to have a better relapse-free and overall survival [33–34]. Still, the addition of taxane does not affect overall survival significantly, and improves disease-free survival only slightly [15, 35]. In addition to making it more difficult to set appropriate resection margins [36], tumor-cell dissemination may contribute to make the prognostic value of pCR during taxane treatment equivocal [37]. There was no

decrease in the number of circulating cells during or after taxane treatment. Thus, most of the cells detected in the circulation may be taxane surviving cells.

Long-time monitoring results after the neoadjuvant treatment confirm this assumption. Stable numbers of CETC have now been observed in some of these patients for more than two years after surgery accompanied by a slow decline during sustained treatment with herceptin or tamoxifen (not shown). These cells seeded into the circulation are obviously able to survive for long periods [38–39]. However, most of these cells may not be able to metastasize or be dormant cells, and only a very small fraction of the circulating tumor cells may be able to resettle and re-grow and form metastases [40–41]. In some of the patients who obtained CMF cycles following taxol treatment, a subsequent reduction in circulating cell numbers was observed, but this did not translate into better relapse-free survival in this small group of patients.

A comparably pronounced increase in CETC as in the her2/neu-negative tumors was not noticed in her2/neu-positive patients if trastuzumab was added to the taxane. One could speculate that cells released during taxane treatment may have become better accessible to the antibody, which otherwise has difficulties penetrating into the tumor tissue [42]. Indeed, in trials in which trastuzumab was added to a chemotherapy containing taxane, a significant increase in pCR [20, 43] has been observed in patients with her2/neu-positive disease. This was also confirmed in the small sample of patients in this trial, with 3/14 patients achieving complete pathological response. Some agents may not be efficient in tumor reduction, possibly because they are not able to penetrate into the tumor tissue. Other agents leading to efficient tumor-size reduction may only partly act by cell destruction and rather reduce tumor pressure and release tumor cells. This, in turn, may allow more efficient cell killing by additional agents. This observation may open new aspects in the treatment of breast cancer with taxanes: by reduction of intra-tumoral pressure they might provide a window for improved penetration of chemotherapeutic agents into the tumor [31]. In addition, taxanes may also allow more efficient action of such agents on the released cells, because in blood these cells may be better accessible and exposed to higher concentrations. The number of patients was, however, too small for evidence of clinical improvement.

Such mechanisms become detectable only by quantitative analysis of CETC. Thus far, such effects have rarely been understood and taken advantage of.

A predictive factor that defines the response of the primary tumor to chemotherapy is the hormone-receptor status [19]. This was confirmed for the circulating tumor cells as well. Thus, CETC from ER- tumors showed significantly more frequently a more than 10-fold decrease upon anthracycline containing therapy than CETC from ER+ tumors.

One goal of primary systemic therapy is to identify non-responders early in the course of therapy and to allow for a change in therapy with potentially better response and less toxicity. Our results indicate that patients from all three treatment arms with a good (more than 10-fold) response already to the first cycles of anthracycline have a significantly better disease-free survival (hazard ratio = 11.91). This corresponds to data from meta-analyses [16], the NSABP B27 trial, and the Scottish neoadjuvant trial, which all indicate that patients not benefiting from primary anthracyclines are unlikely to benefit from subsequent taxanes. This was also true in the trastuzumab treatment group, and it concurs with a report that the response of her2/neu-positive tumors depends on anthracycline treatment [44].

Monitoring the systemic part of the tumor, the circulating tumor cells may contribute to attain the goal of identifying good responders more reliably than measurement of tumor-size reduction and to better define the response to therapy. Early identification of good responders could permit shortening neoadjuvant therapy thus minimizing toxicity.

Monitoring of the response of the tumor cells in peripheral blood during therapy allows, for the very first time, the monitoring of therapy success online, instead of waiting years for statistical evaluation of empirically designed trials. To sum up, then, tracing and quantification of circulating tumor cells can become an essential tool for therapy monitoring in solid tumors.

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