Infusional fluorouracil, epirubicin, and cisplatin followed by weekly paclitaxel plus bevacizumab in locally advanced breast cancer with unfavorable prognostic features

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The objective of this study was to evaluate the clinical and biological activities of bevacizumab in combination with preoperative anthracyclines and taxane-based chemotherapy in locally advanced breast cancer selected for unfavorable prognostic features. Patients with cT2–4c, cN0–2, estrogen and progesterone receptors less than 10% of the cells or cT4d and any estrogen/progesterone receptors expression received four courses of ECF-chemotherapy (epirubicin, cisplatin, fluorouracil as continuous infusion) followed by three courses of weekly paclitaxel in combination with bevacizumab. Thirty patients were included in the study. An objective response, either complete or partial, was observed in 26 patients (87%; 95% confidence interval: 69–96%), stable disease was observed in two patients (7%), and two patients (7%) progressed. A pathological complete response was obtained in 10 patients (33%; 95% confidence interval: 17–53%). Side effects related to bevacizumab with grade ≥ 2 included headache and hypertension. A nonstatistical significant decrease in the median value of circulating endothelial cells was observed at surgery (3.0/μl vs. 5.7/μl, $P=0.19$). In conclusion, high rates of both clinical and pathological responses with anthracycline-containing chemotherapy followed by weekly paclitaxel plus bevacizumab were observed in locally advanced breast cancer with unfavorable prognostic features. A non-negligible rate of progressive disease was observed, suggesting careful monitoring of the patients. Further studies evaluating the potential benefit of bevacizumab in neoadjuvant treatment need to be tested. ***Anti-Cancer Drugs*** **20:197–203 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Preoperative treatment is indicated for patients with operable breast cancer for whom a reduction of primary tumor size may allow breast conservation, whereas it is mandatory in patients with locally advanced breast cancer [1]. Different clinical scenarios are commonly considered in this category and include patients with breast carcinomas with direct extension to the skin or chest wall and inflammatory breast cancer (IBC). IBC is commonly considered as a distinct clinico-pathological entity [2]. In fact, several retrospective studies that compared patients with primary IBC and those with non-IBC led to the notion that IBC is clinically and biologically more aggressive [3,4].

Hormone receptor-negative breast cancer is more likely to benefit from chemotherapy in terms of pathological complete response (pCR) as compared with hormone receptor-positive tumors, but the long-term outcome of those patients remains worse, even in those experiencing a complete response after primary treatment [5]. Although many cytotoxic agents have been shown to be effective, hormone receptor-negative disease and inflammatory disease continue to have dire prognoses and new treatment strategies are needed. The combination of anthracyclines and taxanes shown to improve the pCR rate [6] and the administration of at least six courses of anthracycline-containing and taxane-containing preoperative regimens is considered an adequate approach [7]. Angiogenesis inhibitors showed activity when administered with chemotherapy in breast cancer [8–11]. Bevacizumab is a monoclonal antibody (MAb) that inhibits several activities of vascular endothelial growth factor (VEGF), including endothelial cell growth, vascular permeability, and angiogenesis [12,13].

Recently, data reported in metastatic breast cancer patients showed a statistically significant improvement

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in objective response rate and progression-free survival for the combination of weekly paclitaxel and bevacizumab versus paclitaxel alone [10]. Similar results were reported with the combination of bevacizumab and docetaxel in a double-blind randomized phase III study as first-line therapy for patients with locally recurrent or metastatic breast cancer [11].

However, there is a strong rationale for the exploration of bevacizumab in earlier stages of disease. In fact, the role of this drug might be more relevant at the beginning of the course of the neoplasia where other growth factors might have a less important role in the growth of the tumor when compared with later stages of disease [14].

On the basis of these considerations we investigated the clinical efficacy, in terms of clinical outcome (pCR and clinical response), and biological activity of the ECF regimen (epirubicin, cisplatin, fluorouracil as continuous infusion) followed by paclitaxel plus bevacizumab in endocrine nonresponsive locally advanced tumors and in patients with inflammatory (T4d) breast cancer. The regimen was selected considering both the reported synergism by the combination of paclitaxel and bevacizumab and the significant activity observed in terms of objective remission and pCR rate, in a series of locally advanced triple negative breast cancer treated with preoperative of ECF followed by weekly paclitaxel [15].

Patients and methods

Patients with cT2–4c, cN0–2, M0, estrogen and progesterone receptors (ER and PgR) less than 10%, and patients with cT4d breast cancer and any ER/PgR expression, were enrolled into this study. In agreement with T4d classification of the American Joint Committee on Cancer system, clinical diagnosis of IBC required the presence of erythema, heat, ridging or peau d'orange on the breast. A trucut was performed at baseline, for diagnosis and assessment of biological characteristics of the tumor. Investigations (chest radiograph, abdomen ultrasound, and bone scan, or abdomen scan and total body positron emission tomography scan) were carried out to exclude distant metastasis, and blood tests were carried out to assess bone marrow, renal, and hepatic function (white blood cells $\geq 3000/mm^3$, platelets $\geq 100\,000/mm^3$, creatinine $\leq$ upper limit of normal, aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase $\leq 2.5 \times$ upper normal limit and total bilirubin ≤ 1.5 mg/dl). Blood pressure assessment plus urine analysis (or dipstick) were performed at baseline and before each bevacizumab administration.

The protocol was notified to the ethics committee and written informed consent was obtained from all patients. This was a single institution study. All included patients had pathological evaluation performed at the European Institute of Oncology.

Pathology evaluation

ER and PgR status, assessment of the proliferative activity (% of Ki-67-stained cells) and overexpression of HER2 were determined on core biopsies obtained for diagnosis, as previously described [5]. Tumors were defined ER and PgR negative if less than 10% of neoplastic nuclei stained positively. HER2 status was defined at immunohistochemistry as negative (faint and partial staining in >10% of cells = 1+), equivocal (faint and complete staining in >10% of cells = 2+), and positive (intensive and complete staining in >10% of cells = 3+). In the latter cases, fluorescence in-situ hybridization (FISH) was performed to assess the amplification of the HER2 gene. The following primary antibodies were used: the MAb to ER (at 1/100 dilution; Dako, Glostrup, Denmark), the MAb to PgR (1/800 dilution; Dako), the MIB-1 MAb to the Ki-67 antigen (1/1200 dilution; Immunotech, Marseille, France), the polyclonal antiserum (1/3200 dilution; Dako) to the HER2 protein.

Biological evaluation

Peripheral blood samples were collected for measurements of circulating cells. Circulating endothelial cells (CECs) and their progenitor subpopulation (CEPs) were measured by six-color flow cytometry as previously described [16]. Cell suspensions were evaluated by FACSCanto (Becton Dickinson, San Jose, California, USA). The antibodies used were: CD31 and CD146 (EC marker), CD45 (pan-hematopoietic marker), CD133 (AC133, progenitor/stem cell marker), CD34 (progenitor/stem cells, EC), VEGFR-1, VEGFR-2, and VEGFR-3. Fluorescently labeled isotype-matched IgG1 antibodies were used as control for analysis. Appropriate analysis gates were used to enumerate viable and apoptotic CECs and CEPs. After acquisition of at least 1×10^6 cells per blood sample, analyses were considered as informative when adequate numbers of events (i.e. >100) were collected in the CEC enumeration gates. CECs were defined as, negative for the hematopoietic marker CD45, positive for the endothelial markers CD31 and CD146, and negative for the progenitor marker CD133. CEPs were depicted by the expression of CD133. 7AAD was used to gain insight into CEC/CEP viability according to Philpott *et al.* [17]. Blood samples were collected at baseline, immediately before surgery and 1 week after surgery, to estimate any change in CECs and CEPs.

Study treatment

All patients were treated with the ECF regimen [epirubicin 25 mg/m^2 intravenously (i.v.) on days 1 and 2; cisplatin 60 mg/m^2 i.v. on day 1; and fluorouracil 200 mg/m^2 as a continuous infusion from day 1 to 21] for

four courses. Cycles were repeated every 21 days. Paclitaxel was administered at the dose of 90 mg/m^2 i.v. on days 1, 8, and 15 every 28 days for three courses. Bevacizumab was administered at the dose of 10 mg/kg i.v. every 14 days for six courses during paclitaxel treatment, no premedication was required.

Patients with inflammatory endocrine responsive breast cancer received chemotherapy as previous indicated plus endocrine therapy with letrozole (2.5 mg o.s. daily) +/− GnRH analogue (11.25 mg intramuscularly every 3 months), according to menopausal status. Patients were assessed at each cycle for clinical response, by physical examination with a caliper; for instrumental response with breast ultrasound and mammography, at baseline, at the end of the fourth course of ECF and after the end of chemotherapy with paclitaxel plus bevacizumab.

Clinical evaluation

Clinical responses were evaluated according to both radiological (breast ultrasound and/or mammography) and clinical evaluations, by measuring the largest diameters of the tumors and were graded according to standard Response Evaluation Criteria In Solid [18]. pCR was evaluated according to Kuerer *et al.* [19]. A pCR was defined as a total disappearance of invasive tumor both in the breast and in the axilla. The presence of intraductal carcinoma qualified for pCR.

Toxicity was reported and classified according to the National Cancer Institute of Canada–Clinical Trials Group criteria. The treatment was postponed by 1 week if the blood count on day 21 showed a neutrophil count less than 1000/mm^3 and/or platelet count less than 100 000/mm^3. In the case of febrile neutropenia, or anemia, mucositis, hand and foot syndrome, gastrointestinal, biochemical and neurological toxicity $\geq$ grade 2, dose reduction by 25% of the related drug was performed.

Bevacizumab infusion was stopped in case of uncontrolled hypertension (sustained systolic blood pressure greater than 160 mmHg or diastolic blood pressure greater than 100 mmHg), and in case of allergic-type reaction. The therapy was reinitiated within 1 h at the discretion of the investigator. In the presence of the first occurrence of proteinuria the bevacizumab: (i) was administered as scheduled (if proteinuria: 1+ by dipstick), (ii) was administered as scheduled and collected 24-h urine to determine the total protein within 3 days before the next administration (if proteinuria: $\geq$ 2+ by dipstick) was requested, (iii) was postponed and collected 24-h urine to determine the total protein within 3 days before the next administration (if proteinuria: 4+ by dipstick) was requested.

Surgery was performed 28 days after the last paclitaxel plus bevacizumab administration to allow recovery from toxicity. Surgical specimens were extensively sampled for the evaluation of residual tumor as previously reported [5]. All patients were considered to be assessable for response and toxicity by intent-to-treat analysis if they had received at least one preoperative chemotherapy cycle.

Statistical considerations

The primary endpoint of this study was the pCR proportion among all registered patients (intent-to-treat analysis). According to historical data collected at our institute on approximately 200 cT2–T3, N0–3, ER and PgR less than 10% of the cells, or cT4d breast cancer patients receiving primary therapy (chemotherapy and/or endocrine therapy), about 15% of patients had a pCR. A sample size of 30 patients was planned, yielding an 80% power to detect an increase in the proportion of pCR equal or greater than 20%, when compared with historical controls (i.e. 35 vs. 15%). The type I error rate was set to 5%.

Secondary endpoints included clinical responses (complete and partial), the evaluation of decrease of Ki-67 levels in breast cancer tissue between the values taken at biopsy and at surgery, the determination of toxicity of the regimen and the evaluation of changes in the levels of circulating markers of angiogenic activity. The Fisher's exact test and the Wilcoxon's rank-sum test were used to evaluate differences in the distribution of categorical and continuous variables, respectively. The Wilcoxon's signed-rank test was used to evaluate differences within the same patients of continuous variables measured at two different time points. All *P* values were two sided. The statistical analyses were run using SAS version 8.2 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Between April 2006 and July 2007, 30 patients were enrolled into this study and were valuable for response and side effects. Three patients did not receive bevacizumab (patient's preference, one patient; disease progression, one patient; central nervous system benign disease, one patient). Baseline characteristics of patients and tumors are reported in Table 1. Twelve patients (40%) had IBC, six patients of them had endocrine responsive disease (ER and/or PgR $\geq$ 10%). HER2 was overexpressed in 11 patients (37%), six patients of them with inflammatory disease.

Twenty-five patients obtained a partial response (83%), a complete response was observed in one patient (3%), for an overall response rate of 87% [95% confidence interval (CI): 69–96%] (Table 2). Two patients had stable disease (7%), and two patients, both with T4 tumors, had a progression, one of whom after one course of ECF, and the other one during paclitaxel plus bevacizumab. Responses according to chemotherapeutic regimen are

Table 1 Patient characteristics at baseline

Characteristic	No. of patients	%
Age (years)		
Median	43	
Range	25–63	
Menopausal status		
Premenopausal	22	73
Postmenopausal	8	27
Clinical tumor size		
T2	8	27
T3	8	27
T4b	2	6
T4d	12	40
Clinical nodal status		
N0	2	7
N1	25	83
N2	3	10
HER2/neu		
Not overexpressed	19	63
Overexpressed	11	37
Ki-67		
$<20\%$	0	0
$\geq 20\%$	30	100
Nuclear grade		
2	12	40
3	18	60

Table 2 Responses after treatment

	No. of patients	%
Pathological complete response		
Yes	10	33
No	20	67
Clinical response		
Complete	1	3
Partial	25	83
Stable disease	2	7
Progressive disease	2	7
Pathological tumor size		
T0	10	37
Tis	3	10
T1	4	13
T2	2	7
T3	6	20
T4	1	3
TX	3	10
Nodal status at surgery		
N0	16	53
N1	7	23
N2	7	23

Table 3 Clinical responses according to chemotherapeutic regimen

Response after ECF *N* (%)	Response after paclitaxel + bevacizumab *N* (%)
PR 24 (80)	CR 1 (3)
	PR 20 (67)
	SD 2 (7)
	PD 1 (3)
SD 5 (17)	PR 5 (17)
PD 1 (3)	–

CR, complete response; ECF, epirubicin, cisplatin, fluorouracil; PD, progression disease; PR, partial response; SD, stable disease.

reported in Table 3. All patients underwent radical surgery, breast conservative treatment was feasible in 11 patients (37%; 95% CI: 20–56%). Biopsy of sentinel node with no further axilla clearance was performed in five cases (17%), whereas 25 patients (83%) underwent to axillary dissection. Radiotherapy was performed in all patients submitted to breast conserving surgery or patients who had an IBC at the diagnosis.

A pCR was observed in 10 patients (33%; 95% CI: 17–53%), all treated with bevacizumab. Moreover, two cases presented at surgery with only infiltrating ductal carcinoma foci and isolated tumor cells, respectively.

A higher percentage of pCR was observed in receptor-negative disease (nine out of 10 patients, 90%). Interestingly, five (45%) patients with HER2-positive disease obtained a pCR. Among 12 patients with inflammatory disease at the diagnosis, nine (75%) achieved a clinical response and two (16.7%), one of whom had hormone receptor positive breast cancer, obtained a pCR.

The Ki-67 expression was evaluated at baseline in all patients and at surgery in 17 patients with residual tumor at surgery. The median relative decrease was 38%. No difference at baseline was showed in Ki-67 distribution within pCR cases and non-responders ($P = 0.83$).

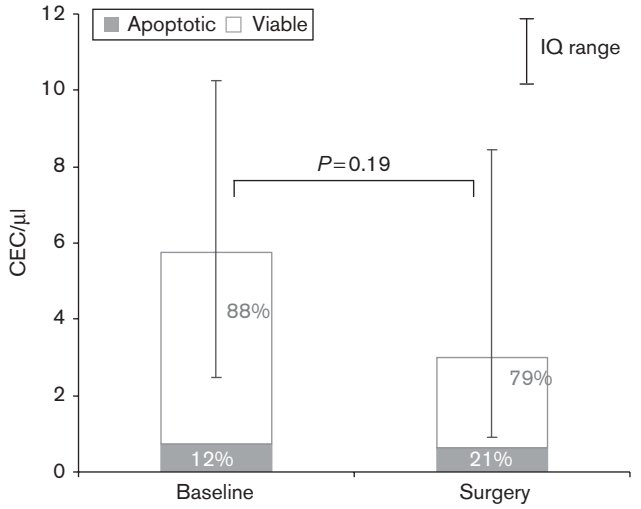
The main toxicities are summarized in Table 4. ECF was administered for four courses in 29 patients, and discontinued after the first cycle because of progression in one patient. Paclitaxel was administered at reduced dosage in 10 (33%) patients because of grade ≥ 2 transaminitis, hematological and neurological toxicity. Paclitaxel and bevacizumab were discontinued early in three patients, because of a deep thrombosis of the vein where the port-a-cath was inserted (one patient), because of disease progression after two courses of this regimen (one patient), or for persistent hypertension of grade 3 (one patient). No patients experienced proteinuria of grade ≥ 2 during bevacizumab treatment.

Table 4 Main adverse events

	Grade 2		Grade 3		Grade 4	
	No. of patients	%	No. of patients	%	No. of patients	%
Leukopenia	11	37	5	17	1	3
Neutropenia	8	27	11[a]	37	4	13
Anemia	6	20	–	–	–	–
Nausea	19	63	5	17	–	–
Vomiting	8	27	3	10	–	–
Diarrhea	7	23	–	–	–	–
Stipsis	10	33	–	–	–	–
Mucositis	14	47	2	7	–	–
Transaminitis	3	10	–	–	–	–
Neurological	12	40	1	3	–	–
Asthenia	10	33	–	–	–	–
Epigastralgya	5	17	–	–	–	–
Hand–foot syndrome	9	30	–	–	–	–
Deep venous thrombosis	0	0	1	3	–	–
Hypertension	1	3	1	3	–	–
Headache	4	13	–	–	–	–

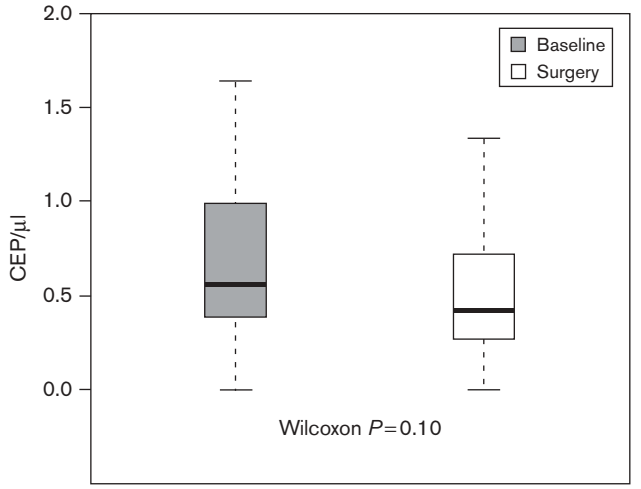
[a]Two patients (7%) with febrile neutropenia.

Fig. 1



Circulating endothelial cells (CECs) at baseline and after surgery. The fraction of viable and apoptotic CECs contributing to the absolute CEC count is shown. IQ range, interquartile range.

Fig. 2



Circulating endothelial progenitor cells (CEPs) at baseline and after surgery.

CECs and CEPs were evaluated at baseline in 29 of 30 enrolled patients. Eighteen patients also had CECs and CEPs measured at surgery; the median value of CECs at baseline was 5.7/µl, and 3.0/µl at surgery ($P = 0.19$) (Fig. 1). The viable CECs were the main contributors to the CEC count decrease, without reporting a significant variation of the apoptotic CECs.

Moreover, we observed a nonsignificant decrease of CEPs between baseline and surgery ($P = 0.10$) (Fig. 2). No correlation was found between CECs/CEPs number and the clinical outcome (data not shown). Finally, the number and kinetics of CEC subpopulations expressing VEGFR1, VEGFR2, or VEGFR3 did not significantly correlate with the clinical outcome.

Discussion

Relatively little progress has been made on the treatment of patients with locally advanced breast cancer with unfavorable features (e.g. IBC and/or nonendocrine responsive breast cancer). On account of the lack of data, no specific systemic regimen is currently recommended for the treatment of this disease. There is, therefore, current interest in the multimodality therapeutic strategies to increase the outcome (pCR, disease-free survival, and overall survival) of these patients. The systemic neoadjuvant treatment of breast cancer is constantly evolving, as more active chemotherapeutic agents become available and biological factors have been incorporated into decisions on treatment.

Recent literature data on bevacizumab administered with chemotherapy as primary systemic therapy in locally advanced or IBC indicated a significant clinical activity [20–22]. A recent study exploring primary systemic therapy containing bevacizumab, docetaxel, and cyclophosphamide, followed by doxorubicin in locally advanced breast cancer showed a pCR of 29%. All 36 patients enrolled into this study had a relatively manageable toxicity [22]. In a second trial, Greil *et al.* [23] treated 18 patients with locally advanced HER2-negative breast cancer with bevacizumab, capecitabine, and docetaxel as primary therapy. Clinical response rate of 83% and pCR of 22% were observed.

In this study, we treated a heterogeneous breast cancer population with dire prognosis. In fact, we selected both locally advanced hormone receptor-negative disease and inflammatory (T4d) breast cancer. The latter population, characterized by a low probability of pCR, represented 40% of this study group. We observed an overall pCR rate of 33%, which doubled that reported in historical controls.

Among 12 patients with inflammatory disease at the diagnosis, nine (75%) of them achieved a clinical response and two (16.7%) of them obtained a pCR. Although, the number of included IBC patients is small, our results compare favorably with previous experiences. Wedam *et al.* [20] treated 21 patients with inflammatory and locally advanced breast cancer with bevacizumab-based therapy in association with doxorubicin and docetaxel. Fourteen patients had a clinical partial response and an overall response rate of 67% (95% CI: 43–85.4%) was observed. Only one patient (stage IIIB) had a pCR. Moreover, 11 (37%) cases had HER2-

overexpressed breast cancer, another unfavorable prognostic factor, and five of them achieved a pCR. At the time when this study was designed, trastuzumab was not included as a part of the standard preoperative treatment of HER2-positive locally advanced breast cancer. In contrast, preliminary data showed that upregulation of VEGF expression occurs in HER2-overexpressing breast cancer cells [24,25]. The high rate of pCR (45%) observed in this subgroup indicates the activity of an antiangiogenic agent in HER2-positive tumors and further supports the combination of bevacizumab and trastuzumab.

Interestingly, either the five nonresponding patients and 21 out of 24 responding patients after ECF obtained a further partial or complete response to the second regimen, suggesting that a significant proportion of clinical and pathological responses may be attributable to the combination of bevacizumab plus paclitaxel.

Besides the confirmation of different patterns of response for selected subgroups of patients who are candidates for preoperative therapy, the results of this study offer an opportunity to identify other important items to be considered in the preoperative setting. In this study, two patients progressed, both with T4 tumors, a rate lower than that previously observed in another study focusing on patients with T4 breast cancer treated with a dose-dense regimen including anthracyclines and docetaxel, which closed prematurely because of a high rate of progressive disease (27%) [26]. These results, together with other reports in the literature, indicate that patients with locally advanced breast cancer selected for unfavorable prognostic features (e.g. IBC) might present a relevant risk of disease progression during treatment requiring a careful monitoring. Conversely, no progression was observed in T2–T3 tumors, different from that reported in our previous study [15] with the same chemotherapy regimen without bevacizumab.

CECs and bone marrow-derived endothelial precursor cells play an important role in neovascularization and tumor growth [27–29]. Several new pieces of evidence indicates that tumor angiogenesis is supported by the mobilization and functional incorporation of other cells, including CEPs [30–32].

In this study, we observed a nonsignificant decrease of CECs between baseline and surgery. Moreover, no statistical correlation within CECs/CEPs number and the clinical outcome has been found, although a trend to a reduction in viable CECs number after bevacizumab treatment was registered. Further studies on a larger number of patients are therefore required to evaluate the role of CECs and CEPs measurement as a potential marker for antiangiogenic treatment.

In conclusion, in this study a high clinical and pathological response rates with anthracycline-containing chemotherapy followed by weekly paclitaxel plus bevacizumab were observed in locally advanced breast cancer with unfavorable prognostic features. Further studies evaluating the potential benefit of bevacizumab and its prolonged administration in neoadjuvant treatment need to be tested. Moreover, the identification of markers predictive of response to antiangiogenic treatments to be used for the selection of patients to be candidated to bevacizumab remains a priority.

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