A randomized phase II trial comparing preoperative plus perioperative chemotherapy with preoperative chemotherapy in patients with locally advanced breast cancer

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The aim of this study was to investigate in a randomized trial the activity of perioperative chemotherapy in patients treated with preoperative chemotherapy for locally advanced breast cancer and to compare it with the preoperative chemotherapy alone. Patients with cT2–3 N0–2 M0 histologically proven breast cancer, with estrogen receptors and progesterone receptors in less than 20% of cells, or with absence of progesterone receptors, received epirubicin 25 mg/m^2 days 1 and 2, cisplatin 60 mg/m^2 day 1, and fluorouracil 200 mg/m^2 daily as continuous infusion. Responding patients were randomized to continue fluorouracil until 2 weeks after surgery (perioperative chemotherapy) or to stop fluorouracil 1 week before surgery. Fifty-eight patients completed six courses of epirubicin, cisplatin and fluorouracil, and were randomized to perioperative chemotherapy (29 patients) or to control (29 patients). The median Ki-67 index remained stable (32–27.5%) in the perioperative chemotherapy arm ($P=0.3$) and decreased from 55 to 22.5% in the control arm ($P=0.01$). The rate of pathological complete remission was 41% in both arms ($P=1.0$). No significant difference in terms of disease-free survival and overall survival was observed between the two arms. Perioperative chemotherapy failed to show an increase in the pathological complete remission rate. A biological effect on Ki-67 expression was demonstrated. *Anti-Cancer Drugs* **17:1201–1209 © 2006 Lippincott Williams & Wilkins.**

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Introduction

Primary systemic treatment has become a common therapy in operable breast cancer, particularly in cases with unfavorable breast-to-tumor size ratio, in order to allow breast conservative surgery [1,2]. No survival advantage has emerged from most randomized clinical trials comparing primary and adjuvant administration of chemotherapy. Nonetheless, the optimal timing of systemic treatment remains a crucial research question. Perioperative chemotherapy, which is defined as a short course of chemotherapy that starts within a few days after surgery, aims to control the tumor growth burst elicited by the surgical procedure and related wound repair processes. Several randomized clinical trials evaluating the efficacy of perioperative treatments, alone or associated with a prolonged adjuvant therapy, have shown a significant advantage for the perioperative approach in terms of disease-free survival, although no clear impact on overall survival was demonstrated [3–5]. This is confirmed by a meta-analysis, showing that early initiation of chemotherapy reduced the risk of relapse by 11% [6].

We previously established the feasibility of perioperative chemotherapy with continuous infusion of fluorouracil, administered as primary therapy until 30 min before surgery, restarted immediately after surgery and then continued for 2 weeks, thus covering the surgical phase and acute wound-healing period [7].

We subsequently designed a randomized phase II study to test the activity of perioperative chemotherapy with fluorouracil in continuous infusion, administered after primary systemic therapy with epirubicin, cisplatin and fluorouracil (ECF) [8]. Enrolled patients had locally advanced breast cancer, with both estrogen receptors (ERs) and progesterone receptors (PgRs) expressed in less than 20% of tumor cells, or with the absence of PgRs and any expression of ERs. This patient selection was

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based primarily on previous results from our group, indicating a higher response rate to primary chemotherapy for tumors with low expression of PgRs [9]. Moreover, an analysis of predictive features from a study that randomized patients to a perioperative cycle of cyclophosphamide, epirubicin and fluorouracil versus no perioperative therapy showed a significant survival advantage for the perioperative arm only in the subgroup of patients with ER-negative tumors [5].

Methods

Patients

Women with histologically proven primary breast cancer in clinical stage T2–3 N0–2 M0 according to the fifth edition of the tumor–node–metastasis staging system [10], with both ERs and PgRs expressed in less than 20% of tumor cells, or with absence of PgR and any expression of ER, were eligible for the study. Other inclusion criteria were: no previous chemotherapy/hormonotherapy, performance status 0–2 (Eastern Cooperative Oncology Group scale), measurable or evaluable lesions, age between 18 and 70 years, no relevant concomitant illnesses, adequate bone marrow reserve (white blood cell count $\geq 4.0 \times 10^9$/l, platelet count $\geq 100 \times 10^9$/l), renal function [creatinine ≤ 1.25 upper normal limit (UNL)] and hepatic function (aspartate aminotransferase, alanine aminotransferase and γ-glutamyl transpeptidase ≤ 2.5 UNL, total bilirubin ≤ 1.25 UNL).

The study has been approved by the Ethical Committee of the European Institute of Oncology and was conducted in compliance with the Helsinki Declaration.

All patients had histological diagnosis of invasive breast carcinoma performed through tru-cut biopsy and hormone receptor expression assessed by immunohistochemistry. Physical examination, bilateral mammography and breast ultrasound, chest radiograph, abdominal ultrasound, bone scan, blood withdrawal for complete blood count, blood chemistry, CA15/3, and carcinoembryonic antigen were performed within 2 weeks from the start of treatment.

Treatment and study design

After insertion of a central venous catheter (port-a-cath), patients were treated with epirubicin 25 mg/m^2 intravenous (i.v.) bolus on days 1 and 2, cisplatin 60 mg/m^2 i.v. on day 1 over 1 h with appropriate saline Hydration, and fluorouracil 200 mg/m^2 i.v. daily as continuous infusion over 21 days through a portable elastomeric infusion system. Antiemetic prophylaxis included a serotonin receptor antagonist i.v. and dexamethasone 8 mg i.v. before cisplatin and epirubicin. Courses were repeated every 3 weeks. Patients achieving a partial or complete remission after three cycles received three further courses of ECF and were then randomized to continue the infusion of fluorouracil until 2 weeks after surgery (perioperative treatment arm) or to stop fluorouracil infusion on day 21 of the sixth cycle, 1 week before surgery (control arm). A computer-generated randomization list, which was concealed from the investigators, was used to allocate patients to the two treatment arms. The surgical treatment was planned on day 29 of the last course of chemotherapy in both arms. Patients with stable or progressive disease after three cycles of ECF underwent immediate surgery and were not enrolled into the study. In patients randomized to perioperative chemotherapy, infusion of fluorouracil continued until 30 min before surgery and was restarted immediately after surgery, lasting for 15 days afterward. Patients randomized to the control arm stopped the infusion of fluorouracil on day 21 of the last cycle and underwent surgery 1 week later.

The surgical procedure consisted of quadrantectomy with axillary dissection according to Veronesi *et al.* [11] or modified radical mastectomy, depending on the size and site of the residual tumor in relation to breast size.

Adjuvant treatment was prescribed according to the size and biological features of residual disease at surgery. Twenty-eight patients (14 in each arm) received no further therapy; 20 patients (10 in each arm) received chemotherapy with i.v. continuous infusion of fluorouracil for 2 months (five patients), cyclophosphamide, methotrexate and fluorouracil (CMF) for three cycles (one patient), taxane-based regimens (five patients), anthracycline-based regimens (four patients) or high-dose chemotherapy (five patients); 10 patients (five in each arm) received endocrine therapy with tamoxifen alone or in association with gonadotropin releasing hormone analogues on the basis of the menopausal status and one patient received three cycles of CMF followed by tamoxifen.

Women undergoing conservative surgery received irradiation of the breast, extended to the axilla in case of involvement of four or more lymph nodes; those undergoing radical mastectomy were irradiated both on the chest wall and on regional lymph nodes if four or more axillary nodes were involved.

Toxicity evaluation and dose modifications

Patients were assessed for toxicity at day 21 of each cycle, with clinical evaluation and laboratory examinations, and complete blood count was repeated weekly for the entire treatment period. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria version 2 [12]. The treatment was delayed for 1 week if neutrophil granulocytes were less than 1.0×10^9/l and/or platelets were less than 100×10^9/l. If the blood cell count had not recovered on day 8, therapy was delayed for another week after which, in case of nonrecovery, the patient went off the study for marrow toxicity. In case of

elevated serum creatinine up to 1.5 times the UNL, cisplatin was omitted and the dose of epirubicin was reduced by 25%. In case of elevated total bilirubin up to 1.5 times the UNL, the doses of fluorouracil and epirubicin were reduced by 25%. If serum creatinine and/or total bilirubin were higher than 1.5 times the UNL, treatment was stopped until recovery. In the case of grade 1 plantar-palmar erythema, fluorouracil was continued and pyridoxine was added at 300 mg daily. For grade 2–3 plantar-palmar erythema, fluorouracil was interrupted until recovery and then restarted at 75% of the dose, with associated pyridoxine. Other grade 1–2 nonhematological side-effects were managed with the common supportive measures, whereas for grade 3–4 toxic effects treatment was temporarily withheld and then restarted with a 25% dose reduction after resolution of symptoms.

Response assessment

Assessment of response was performed before each cycle by physical examination, measuring the size of the primary tumor and of ipsilateral axillary nodes when palpable. Mammography and breast ultrasonography were repeated after three and six courses of chemotherapy. Tumor response was defined according to World Health Organization criteria [13]. The disappearance of any sign of disease both at physical examination and with instrumental evaluation was considered a clinical complete remission. A reduction greater than, or equal to, 50% in the sum of the products of the largest perpendicular diameters of all measurable lesions (or an estimated decrease in tumor size of 50% or more for nonmeasurable lesions) was defined as a partial response. A 25% or greater increase in the size of one or more lesions or the appearance of new disease manifestations were defined as tumor progression. A less than 50% decrease or less than 25% increase in total tumor size was classified as no change. Whenever the results of the different modalities of tumor evaluation diverged, a conservative approach was held, considering the least favorable response. Pathological response was classified according to the scale described by Sataloff *et al.* [14]. Pathological complete remission is defined as a total or near total disappearance of the tumor, including the presence of isolated foci of invasive tumor.

Pathology and immunohistochemistry

Surgical specimens were extensively sampled for the evaluation of residual tumor after primary chemotherapy, as described previously [7]. Immunostaining for the localization of ERs, PgRs, Her2/*neu* protein and Ki-67 antigen was performed on consecutive tissue sections of the core biopsies obtained before primary treatment and from the residual tumor after surgery [7]. The following primary antibodies were used: the monoclonal antibody (mAb) to ER (Dako, Glostrup, Denmark; at 1/100 dilution), the mAb to PgR (Dako; at 1/800 dilution), the Molecular Immunology Borstel-1 (MIB-1) mAb to the Ki-67 antigen (Immunotech, Marseille, France; at 1/1200 dilution) and the polyclonal antiserum (Dako; at 1/3200 dilution) to the Her2/*neu* protein.

The immunostained slides were evaluated independently by two pathologists. Only nuclear reactivity was taken into account for ERs, PgRs and Ki-67 antigen, whereas only an intense and complete membrane staining of the tumor cells was taken as evidence of Her-2/*neu* overexpression. The results were recorded as the percentage of immunoreactive cells over at least 2000 neoplastic cells.

Statistical considerations

In a pooled analysis of our previous trials, primary chemotherapy yielded a mean decrease in Ki-67 of 41%, with a reasonably normal distribution and a standard deviation of 30%. This study was designed to detect a difference in the mean percentage decrease in Ki-67 of 15% between the two arms: 40% decrease in the standard therapy arm compared with 55% decrease in the perioperative arm. With a 90% power and a 5% significance level, 86 patients were required per arm in the study using a two-sided test.

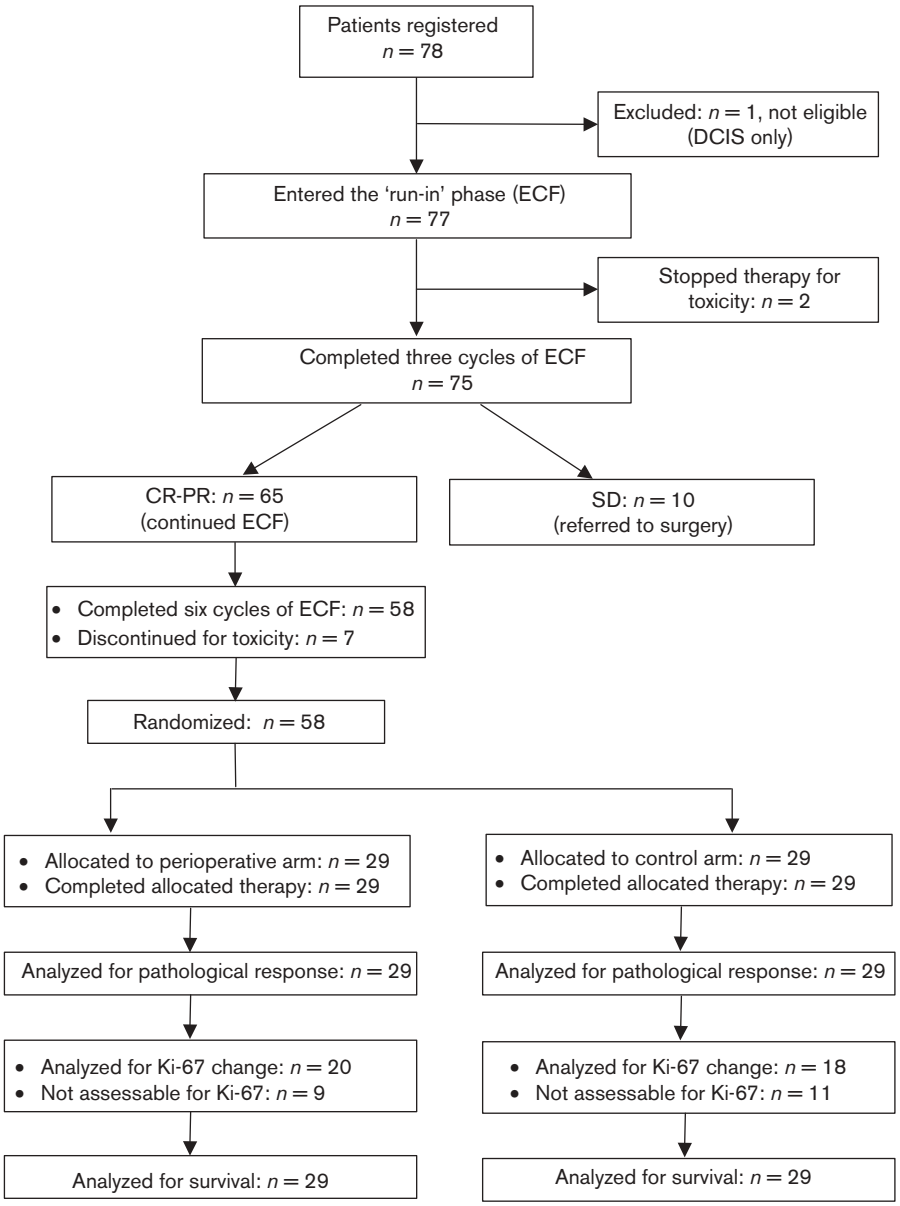
The limited patients' acceptance made the accrual slower than expected. Therefore, as we defined better which biological features predicted response to chemotherapy (ERs and PgRs absent tumors) [15], we decided to close the study after the enrollment of 58 patients. It thus has 80% power at the 10% significance level to detect a difference in the mean percentage decrease in Ki-67 of 20% between the two groups (40% in the standard arm compared with 60% in the perioperative arm), using a two-sided test.

The distribution of Ki-67 values was not normal according to the Shapiro–Wilk test. Therefore, Wilcoxon's rank-sum test was used to test the main hypothesis and Wilcoxon's signed-rank test to compare baseline versus surgical values of Ki-67 within each arm. The differences in proportions were assessed by Fisher's exact test. Time-to-event end-points were estimated by the product-limit method and were compared between groups with the log-rank test. Disease-free survival and overall survival were measured from the first day of chemotherapy to the date of disease recurrence and to the date of death, respectively.

Results

Between February 2000 and December 2004, 78 consecutive women referred to the Division of Medical Oncology of the European Institute of Oncology were registered for this study. One turned out to be ineligible because of lack of tumor invasiveness at baseline biopsy

Fig. 1



Study flow diagram. ECF, epirubicin, cisplatin and fluorouracil; DCIS, ductal carcinoma *in situ*; SD, standard deviation; CR, complete remission; PR, partial remission.

and 77 were entered onto the 'run-in' phase, starting primary chemotherapy with ECF. Of these, 65 had an objective response after three cycles and continued primary chemotherapy. Ten patients had stable disease and two patients interrupted therapy for toxicity (one epirubicin extravasation and one neurological), and were referred to the surgeon. No patient had disease progression. Overall, the clinical objective response rate to primary chemotherapy for 77 eligible patients was 84.4% [95% confidence interval (CI): 74.4–91.7%] with 30 (39%; 95% CI: 28.0–50.8%) clinical complete remissions. Twenty-five patients (32.5%; 95% CI: 22.2–44.1%) had pathological complete remission of the primary tumor (complete disappearance of primary or residual carcinoma *in situ* or microscopic foci of invasive tumor) at pathological examination of the surgical specimen and 20 patients (26%; 95% CI: 16.6–37.2%) had no evidence of residual tumor in lymph nodes too.

Of 65 responding patients, 58 completed six cycles of treatment and were randomized to stop fluorouracil infusion 1 week before surgery (control arm, 29 patients) or to continue fluorouracil infusion until 2 weeks after surgery (perioperative arm, 29 patients). Seven patients

Table 1 Patients' and tumors' characteristics at baseline (on 58 enrolled patients)

	No. of patients		
	Perioperative arm ($n=29$)	Control arm ($n=29$)	*P* value[a]
Age at randomization (years)			
≤ 39	11	14	0.25
40–49	11	5	
≥ 50	7	10	
CT			
2	20	18	0.78
3	9	11	
CN			
0	8	6	0.44
1	21	21	
2	0	2	
ER (%)			
0	22	25	0.43
1–9	2	0	
≥ 10	5	4	
PgR (%)			
0	28	28	1
1–9	1	1	
≥ 10	0	0	
Ki-67 (%)			
<20	1	4	0.35
≥ 20	28	25	
Her-2/*neu*			
0/+/++	18	24	0.14
+++	11	5	

cT, clinical tumor stage; cN, clinical nodal stage; ER, estrogen receptor; PgR, progesterone receptor.
[a]Fisher's exact test

Table 2 Treatment results

	No. of patients		
	Perioperative arm ($n=29$)	Control arm ($n=29$)	*P* value[a]
Ki-67 on surgical samples[b] (%)			
<20	6	8	0.10
≥ 20	14	10	
Ki-67 change from baseline[b]			
decreased	10	14	0.10
stable or increased	10	4	
Her-2/*neu*[b]			
0/+/++	13	14	0.49
+++	7	4	
ypT			
0	6	9	0.37
is	3	2	
x	3	1	
1	14	9	
2	3	7	
3	0	1	
No. of axillary nodes involved			
0	20	18	0.13
1–3	4	3	
4–9	5	3	
≥ 10	0	5	
Peritumoral vascular invasion[b]			
absent	12	10	1.00
present	6	5	
not available	2	3	
Tumor response			
pCR (ypT0 + ypTis + ypTx)	12[c]	12[d]	0.58
cCR	2	0	
cPR	15	17	
Pathological response of T			
pCR (ypT0 + ypTis)	9	11	0.78
no pCR	20	18	
Pathological response of T and N			
PCR (ypT0 + ypTis + ypN0)	9	10	1
no pCR	20	19	
Type of surgery			
quadrantectomy	21	24	0.53
radical mastectomy	8	5	

ypT, pathological tumor stage; pCR, pathological complete remission; cCR, clinical complete remission; cPR, partial remission.
[a]Fisher's exact test.
[b]On 20 assessable patients in the experimental arm and 18 assessable patients in the control arm.
[c]Three patients with 'focal invasive tumor residuals'.
[d]One patient with 'focal invasive tumor residuals'

discontinued therapy before completing the sixth cycle due to toxicity (two cases for deep venous thrombosis, two for peripheral neurological toxicity, one for hematological toxicity, one for nausea and vomiting, and one for pain in the site of fluorouracil infusion). All 58 randomized patients completed treatment according to the protocol were assessable for pathological response and toxicity; 38 patients were assessable for Ki-67 response on invasive carcinoma, whereas 20 patients were not assessable due to the complete disappearance of primary tumor (ypT0: 15 patients; six in the perioperative arm and nine in the control arm) or to the presence of residual carcinoma *in situ* only (ypTis: five patients; three in the perioperative arm and two in the control arm). A flow diagram of the study is shown in Fig. 1 and patient's characteristics are listed in Table 1. The following results refer to the randomized 58 patients.

All patients had good performance status and most were premenopausal, with a median age of 41 years (range 22–64 years). Approximately, 65% had a T2 primary tumor and 35% a T3 tumor. About 75% presented with clinically positive axillary nodes. Eighty-one percent had ER-negative tumors (defined by the immunohistochemical detection of receptors in 0% of neoplastic cells) and 97% had progesterone receptor-negative tumors. Twenty-eight percent had Her2/*neu* overexpression, defined as an intense and complete membrane staining in at least 10% of the tumor cells. The median baseline value of Ki-67 in the whole cohort of 58 patients was 35% (range 3–85%). Baseline biological features were well balanced between the two treatment arms.

Treatment results are summarized in Table 2. The degree of change in the Ki-67 labeling index before and after chemotherapy was assessable on 20 patients in the experimental arm and on 18 patients in the control arm. Among these assessable patients, median Ki-67 index changed from 32% at baseline to 27.5% at surgery in the perioperative arm ($P = 0.30$, Wilcoxon's signed-rank test) and from 55 to 22.5% in the control arm ($P = 0.01$). Ten patients had a decrease in Ki-67 in the experimental arm and 14 in the control arm ($P = 0.1$, Fisher's exact test). The median ratio between Ki-67 at surgery and Ki-67 at baseline was 0.99 in the experimental arm and 0.63 in the control arm, which did not differ significantly ($P = 0.14$,

Table 3 Side-effects of preoperative treatment (on 58 enrolled patients) [*N* (%)]

Side-effect	Grade 1		Grade 2		Grade 3–4	
	Perioperative arm	Control arm	Perioperative arm	Control arm	Perioperative arm	Control arm
Anemia	6 (21%)	6 (21%)	7 (24%)	6 (21%)	–	–
Leukopenia	10 (34%)	9 (31%)	13 (45%)	13 (45%)	1 (3%)	1 (3%)
Neutropenia	3 (10%)	1 (3%)	4 (14%)	9 (31%)	15 (52%)	14 (48%)
Thrombocytopenia	–	–	–	–	–	1 (3%)
Nausea	16 (55%)	10 (34%)	9 (31%)	15 (52%)	1 (3%)	1 (3%)
Vomiting	11 (38%)	10 (34%)	7 (24%)	8 (28%)	–	–
Diarrhea	5 (17%)	6 (21%)	2 (7%)	–	–	–
Stipsis	10 (34%)	9 (31%)	5 (17%)	9 (31%)	–	–
Mucositis	11(38%)	9 (31%)	6 (21%)	5 (17%)	–	1 (3%)
Alopecia	–	2 (7%)	11 (38%)	10 (34%)	19 (66%)	17 (59%)
Transaminases	–	–	–	1 (3%)	–	–
Fever	2 (7%)	1 (3%)	–	–	1 (3%)	–
Skin toxicity	–	–	1 (3%)	–	1 (3%)	–
Hand foot	9 (31%)	7 (24%)	4 (14%)	4 (14%)	–	1 (3%)
Neurological	5 (17%)	4 (14%)	1 (3%)	2 (7%)	–	–
Infections	–	1 (3%)	–	–	–	–
Febrile neutropenia	–	–	–	–	1 (3%)	–
Asthenia	10 (34%)	11 (38%)	7 (24%)	3 (10%)	–	–
Conjunctivitis	3 (10%)	3 (10%)	–	–	–	–
Deep venous thrombosis	–	–	–	–	–	–

Wilcoxon's rank-sum test). Four patients in the experimental arm and eight in the control arm had a percentage reduction greater than 50% ($P = 0.16$, Fisher's exact test). Ki-67 index measured on surgical samples was lower than 20% in six patients in the perioperative arm and in eight patients in the control arm ($P = 0.50$, Fisher's exact test). The distribution of pathological tumor and nodal stage at surgery is reported in Table 2 separately for each treatment arm. No significant difference was present between the two groups. The rate of pathological complete remission on primary tumor, according to the definition of Sataloff *et al.* [14], was 41% in both arms ($P = 1$, Fisher's exact test). When considering only cases with no evidence of residual invasive tumor, neither in the breast nor in the lymph nodes, the rate of pathological complete remission was 31.0% in the perioperative arm and 34.5% in the control arm ($P = 1$, Fisher's exact test). The rate of peritumoral vascular invasion did not differ between the two groups. Among patients allocated to perioperative chemotherapy, 21 had conservative surgery (quadrantectomy) and eight had a radical mastectomy, whereas 24 patients had a quadrantectomy and five had a radical mastectomy in the control group ($P = 0.53$, Fisher's exact test). So far, 20 patients have relapsed, 12 in the perioperative arm and eight in the control arm, after a median follow-up of 43 months. Three-year disease-free survival is 65.8% in the perioperative arm and 68.4% in the control arm ($P = 0.52$, log-rank test). Three-year overall survival is 86.4% in the perioperative arm and 84.0% in the control arm ($P = 0.44$). In the endocrine unresponsive subgroup, 3-year disease-free survival is 54.1% in the perioperative arm and 57.8% in the control arm ($P = 0.78$), whereas 3-year overall survival is 81.5% in the perioperative arm and 77.7% in the control arm ($P = 0.45$).

All patients who were randomized received six cycles of ECF. Side-effects are summarized in Table 3. All patients who were allocated to perioperative chemotherapy completed 2 weeks of postoperative fluorouracil starting in the operating room. No significant toxicity was registered during perioperative treatment; in particular, no infection was observed and no detrimental effect to wound healing was reported. No delay or dose reduction was necessary.

Discussion

The administration of chemotherapy during the surgical phase has been demonstrated to be relevant. Preclinical studies indicated that the time interval between primary tumor removal and chemotherapy is critical. The most effective control of metastases was achieved with the administration of chemotherapy at the time of or before primary tumor removal, providing a biological rationale for the use of perioperative adjuvant chemotherapy [16]. As tumor cell repopulation may be expected to occur when the effect of chemotherapy ends, prolonging primary treatment up to the moment of surgery could increase the chance of a pathological complete remission. Surgical trauma and processes of wound healing induce the production of growth factors for epithelial and endothelial cells, which might stimulate the growth of micrometastases [17–24], and have been shown to produce an increase in proliferation index particularly in tumors overexpressing Her2/*neu* [25]. The primary tumor may shed angiogenesis inhibitors in the blood and its removal may shift the angiogenic balance toward neovascularization, inducing the development of micrometastases [26]. In the immediate postsurgical period, the tumor burden is lowest, the tumor should be in the steeper portion of

its gompertzian growth curve and its growth fraction should be high. This implies a steeper dose–response curve for most anticancer drugs and a greater chance of response to cell-cycle-specific agents, particularly with protracted exposure through continuous i.v. infusion.

We hypothesized that a perioperative treatment with continuous infusion of fluorouracil could improve the results of systemic therapy in early breast cancer. The treatment that we propose covers the immediate presurgical and postsurgical period, continuing until 30 min before surgery and restarting immediately after surgery, then lasting for 15 days afterwards.

Despite the theoretical background, in the present study we did not find significant differences in the rate of pathological complete remissions between the two treatment arms.

We, however, registered a significant decline of Ki-67 in the control group and no significant changes in the perioperative group.

We chose a surrogate marker as primary end-point for this study. Ki-67 has been shown to have a prognostic value in early breast cancer and some studies also indicate its potential as a predictive marker [27]. Anyway, Ki-67 labeling index could be a surrogate marker more apt for the study of cytostatic drugs, like hormonal agents, where slowing the growth rate is expected, than for cytotoxic agents, which could in theory indirectly induce an increase in growth fraction by reducing the tumor burden, without directly influencing the pace of the cell cycle. Furthermore, preclinical models have shown that fluorouracil induces cell cycle arrest in the G_1/S phases, in which proliferating antigens such as Ki-67 are highly expressed, particularly with protracted exposure to the drug [28–30]. This fact could have contributed to produce the trend toward a higher median value of Ki-67 at surgery in the perioperative group compared with the control group.

The selection of patients enrolled in the study might at least partially explain the results reported herein. We enrolled patients whose tumors were PgR absent (with any expression of ERs) or with both low estrogen and progesterone receptors (expressed in less then 20% of tumor cells). This was based on the results of a previous retrospective analysis of our trials of primary chemotherapy, in which the absence of PgRs emerged as the most relevant factor predicting response [9]. Subsequent analysis on a larger series of patients revealed that the absence of both hormone receptors is the most important independent predictor of response at multivariate analysis [15]. Moreover, studies assessing the genetic profile of breast tumors have shown the existence of different tumor subtypes, confirming the previous clinical hypothesis that hormone receptor-negative tumors are a separate disease entity in comparison with tumors expressing steroid hormone receptors [31–33]. An analysis of predictive features from trial V of the International Breast Cancer Study Group, which randomized patients with node-negative breast cancer to receive a course of perioperative CMF chemotherapy or no adjuvant treatment, showed a significantly reduced risk of relapse in the treatment arm for postmenopausal patients with tumors not expressing hormone receptors, whereas no difference was found for patients with tumors expressing steroid hormone receptors and for the group of premenopausal patients [34]. A retrospective evaluation of the results of adjuvant chemotherapy according to the timing of treatment start showed a better outcome, in patients with tumors not expressing hormone receptors, when treatment began within 3 weeks from surgery compared with later starts, further underlying the relevance of treatment timing [35].

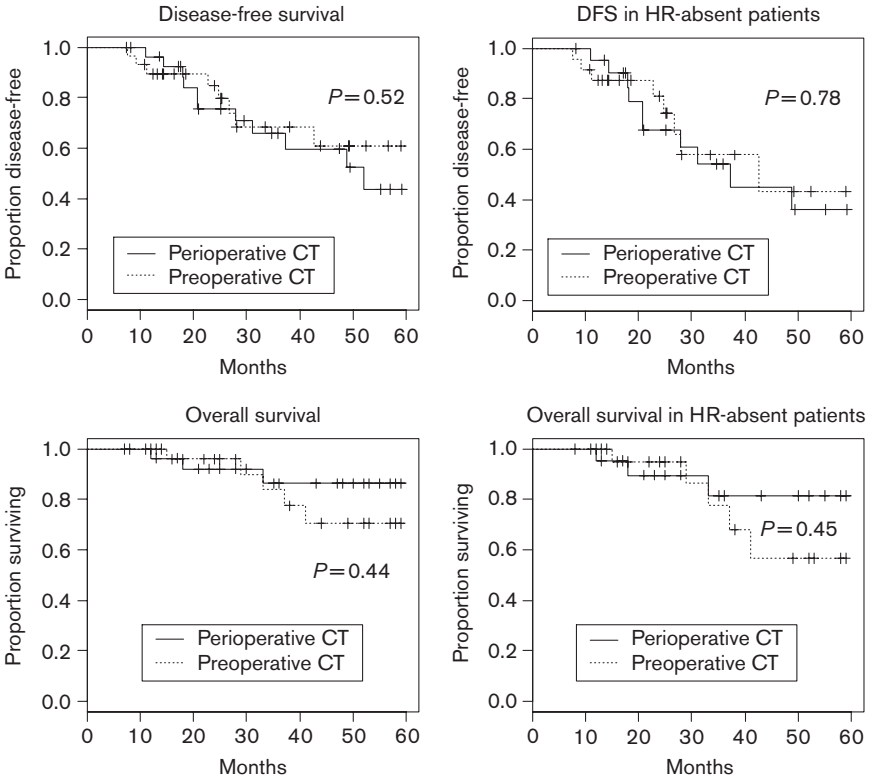
Therefore, patients with absence of both ERs and PgRs might benefit form early start of chemotherapy. In our study, the results for the subgroup of patients with endocrine unresponsive tumors (defined as tumors with no expression of both ERs and PgRs) did not differ from those of the whole cohort of patients (data not shown). Anyway, as shown in Fig. 2, the overall survival curves tend to diverge with long-term follow-up, favoring the experimental arm, particularly when the analysis is restricted to patients with hormone receptor-negative tumors, supporting further studies in this subgroup of patients.

Tumors with overexpression of growth factor receptors, such as Her1 and/or Her2, are probably at higher risk of stimulation by growth factors released at the time of surgery, and could also benefit from a perioperative treatment [25]. These tumors are also frequently hormone receptor-negative, belonging to the Her2/*neu* subtype, overexpressing Her2, or to the basal-like subtype, which frequently overexpresses Her1 [32,33]. The latter frequently expresses genes involved in matrix remodeling and angiogenesis, and could thus particularly benefit from therapies inhibiting the angiogenic process [36]. Among our patients, only 16 had tumors showing Her2/*neu* overexpression, therefore not supporting an analysis in this subgroup of patients.

The release of growth factors and the consequent tumor growth burst associated with surgery might be related to the extent of surgery [24]. This could therefore be a further criterion to consider when assessing the effectiveness of perioperative treatment.

Overall, this patient cohort, with nonendocrine responsive or doubtful endocrine responsive tumors, obtained a

Fig. 2



Disease-free survival (DFS) and overall survival. DFS (top plots) and overall survival (bottom plots) for all patients (left) and for the subgroup with hormone receptor (HR) absent tumors (right). CT, chemotherapy.

high rate of pathological complete remissions in both treatment arms, but this did not translate into good prognosis. This confirms our previous results showing that patients with nonendocrine responsive tumors achieve a significantly higher incidence of pathological compete remissions after primary chemotherapy than those with endocrine responsive disease, but have worse disease-free survival [15]. In our study, patients with hormone receptor-positive tumors received adjuvant endocrine therapy, but most had a low expression of receptors and this may have limited the impact of endocrine therapy. Sixteen patients had tumors showing Her2/*neu* overexpression and could have benefited from treatment with trastuzumab, as recently established [37,38]. For other categories of tumors, particularly for 'triple-negative' (hormone receptor and Her2/*neu* negative) tumors, the impact of a short course of perioperative chemotherapy might be insufficient and more prolonged treatments may be necessary to improve survival. In these patients, metronomic chemotherapy with low-dose oral agents is particularly appealing, being well tolerated and apt for long-term treatment. We are therefore investigating its role both as adjuvant and as perioperative therapy.

Perioperative chemotherapy with continuous infusion of fluorouracil, covering the period from the last cyclic bolus administration of primary chemotherapy up to 2 weeks after surgery, does not reduce Ki-67 labeling index on surgical tumor samples. Anyway, it is not possible to draw definite conclusions on perioperative chemotherapy from our study, due to the small number of patients and the heterogeneity of tumor subtypes. Therefore, considering its preclinical and rational bases, and preliminary results on endocrine unresponsive tumors, further studies including perioperative chemotherapy with a different patient selection and with different treatment schedules and outcome measures are worthwhile.

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