Randomized Trial Comparing Cyclophosphamide, Methotrexate, and 5–Fluorouracil (CMF) with Rotational CMF, Epirubicin and Vincristine as Primary Chemotherapy in Operable Breast Carcinoma

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BACKGROUND. According to the overview of Early Breast Cancer Trialists’ Collaborative Group, anthracycline containing regimens are superior to cyclophosphamide, methotrexate, and 5–fluorouracil (CMF) as adjuvant chemotherapy for breast carcinoma, but no comparative information is available in terms of primary chemotherapy. In the current randomized controlled trial, the authors compared CMF with a chemotherapy regimen including CMF, epirubicin, and vincristine (CMFEV).

METHODS. Two hundred eleven patients with Stages I and II palpable breast carcinoma and tumor diameter > 2.5 cm or ≤ 2.5 cm with cytologically proven axillary lymph node involvement were randomized to receive CMF (arm A) or CMFEV regimen (arm B) for four cycles before surgery. After surgery, patients in both arms received adjuvant CMF for three cycles; the postmenopausal patients also received tamoxifen for two years.

RESULTS. There were no significant differences in the complete response (CR) and in the CR plus partial response (PR) rates between the two arms. In the subset analysis, among premenopausal patients, significantly higher rates of CR (26% vs 4%, P = 0.004) and of CR + PR rates (80% vs 54%, P = 0.007) were observed in the CMFEV, as compared to the CMF arm. Multivariate analysis confirmed the presence of a significant interaction between menopausal status and type of treatment on the probability of achieving CR (P = 0.02) or CR + PR (P = 0.01). There were no major differences in the side effects of the two treatments, with the exception of more frequent alopecia in the experimental arm.

CONCLUSIONS. The results of the current study are in line with those of previous published randomized clinical trials comparing regimens without and with anthracycline as adjuvant treatment, indicating an agreement between the short term response to primary chemotherapy and the long term results observed in the adjuvant setting. Cancer 2002;95:228–35. © 2002 American Cancer Society.

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KEYWORDS: breast carcinoma; primary chemotherapy; neoadjuvant chemotherapy; cyclophosphamide, methotrexate, and 5–fluorouracil; anthracycline-containing regimen; randomized controlled trial.

Primary chemotherapy was first developed as a means of tumor reduction in locally advanced breast carcinoma and later widely adopted as a conventional treatment for this disease stage, despite the fact that no randomized studies documented its efficacy in improving survival. More recently it has been used in the treatment of operable breast carcinoma with the aim of favoring conservative surgical pro-
cedures. Response to primary chemotherapy can be used to predict the individual chemosensitivity of breast carcinomas in vivo, and it has recently been recognized as providing prognostic information concerning the achievement of a complete response (CR).1,2

Primary chemotherapy can also be used as a model for comparing the activity of different drugs or combinations. It has been reported that anthracycline containing regimens are superior to cyclophosphamide, methotrexate, 5-fluorouracil (CMF) in advanced disease in terms of response rates and, less consistently, survival,3–7 furthermore, when given in the adjuvant setting, those regimens have also been shown to reduce the risk of recurrence and death.8 However, to the best of our knowledge, there is no information in the literature concerning the relative merits of CMF versus anthracycline containing combinations as primary treatments.

The aim of the current prospective randomized controlled trial was to compare CMF with a regimen including the same agents plus epirubicin and vincristine as primary chemotherapy in operable breast carcinoma. The primary endpoints were complete response (CR) and CR / partial response (PR) rates. Results in term of outcome (relapse free survival, overall survival) will be reported separately.

PATIENTS AND METHODS

Eligibility Criteria, Diagnosis and Staging

The main eligibility criteria were: a palpable tumor mass > 2.5 cm or a palpable tumor mass ≤ 2.5 cm with a cytologically-proven positive axillary node involvement; clinical Stage I or II according to the American Joint Committee on Cancer/International Union Against Cancer;9,10 age < 70 years; the absence of distant metastases following a complete staging process that included a physical examination, chest X-ray, bone scan, liver echography, or computed tomography; the absence of additional primary tumors; and adequate bone marrow, kidney, liver, and heart function. Clinical Stage III tumors, i.e. T3 N1, or T4 any N, or any T N2, were not eligible.

Conservative surgery was not included among the endpoints of the current study because even patients for whom there was no initial indication for a conservative surgical approach (i.e. multicentric, multilocal tumors) were eligible.

The diagnosis of breast carcinoma was made on the basis of the results of a fine needle aspiration (FNA) biopsy. Tru-cut or small incisional opening biopsy techniques were initially allowed, but they were discouraged within a few months on the ground that they might interfere with the clinical and pathologic assessment of response. We considered negligible the probability of occurrence of an in situ histology, not assessable with FNA, in palpable tumors. In order to include as many patients as possible with proven axillary lymph node involvement, ultrasound-guided FNA cytology was performed. At the time of the initial FNA biopsy, some biologic tumor parameters (estrogen and progesterone receptor, Ki 67) were determined using immunocytochemic techniques and tested for their ability to predict response to chemotherapy in a parallel study. All patients underwent complete hemogram, blood chemistry, and electrocardiographic examinations.

The study protocol was approved by the Ethics Committees of the participating institutions, and all of the patients gave informed consent.

Study Design and Treatment

This was a multi-institutional study carried out by the Medical Oncology Units of Parma, Reggio Emilia, Terni, Perugia, Piacenza, and Fermo of the Italian Oncology Group for Clinical Research (GOIRC); Parma and Reggio Emilia contributed the vast majority (93%) of the enrolled patients. The study design is shown in Figure 1.

The patients were centrally randomized via a telephone call to the operations office of the GOIRC in Parma. Allocation was made within strata defined by institution, menopausal status (premenopausal vs postmenopausal), clinical tumor diameter (≤ 3.5 vs > 3.5 cm), and clinical axillary nodal status (negative vs positive). Patients were assigned to receive four cycles of CMF (arm A) or four cycles of CMFEV (arm B), and then, after evaluation of clinical response, patients underwent surgery (quadrantectomy and axillary dissection, or mastectomy and axillary dissection), thus allowing the assessment of pathologic complete response (pCR) in all patients. The patients in both arms received the same adjuvant chemotherapy (CMF for three cycles); those who were postmenopausal also received oral tamoxifen 20 mg per day for two years.

The CMF combination (Table 1) consisted of
TABLE 1
Doses and Schedules

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<thead>
<tr>
<th></th>
<th>CMF Combination</th>
<th>CMFEV (Rotational)</th>
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<tbody>
<tr>
<td>Cyclophosphamide</td>
<td>600 mg/m², iv short infusion Days 1 and 8</td>
<td>Cyclophosphamide 600 mg/m², iv short infusion Days 1 and 8</td>
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<tr>
<td>Methotrexate</td>
<td>40 mg/m², iv bolus Days 1 and 8</td>
<td>Methotrexate 40 mg/m², iv bolus Days 1 and 8</td>
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<tr>
<td>Fluorouracil</td>
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<td>Fluorouracil 600 mg/m², iv bolus Days 1 and 8</td>
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<tr>
<td>Vincristine</td>
<td>1.4 mg/m², iv bolus Day 1</td>
<td>Vincristine 1.4 mg/m², iv bolus Day 1</td>
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<td>(every 4 weeks)</td>
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<td>CMFV Combination</td>
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<td>CMEV combination</td>
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<tr>
<td>Cyclophosphamide</td>
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<td>Methotrexate</td>
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<td>CMEV Combination</td>
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<td>Cyclophosphamide</td>
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<td>MFEV Combination</td>
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<td>Methotrexate</td>
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<td>Fluorouracil</td>
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iv: intravenously.

monthly cycles of cyclophosphamide 600 mg/m² intravenously (iv), Days 1 and 8; methotrexate 40 mg/m² iv, Days 1 and 8; and 5-fluorouracil 600 mg/m² iv, Days 1 and 8. In the CMFVE group, epirubicin (E) and vincristine (V) were added in such a way that each of the four cycles were administered as four drug combinations by means of the sequential omission of E, F, M and C (thus effectively becoming CMFV, CMEV, CF EV, and MFEV). The CMF doses and times of administration were the same as those used in arm A; the doses of epirubicin and vincristine were 40 mg/m² iv (Days 1 and 8) and 1.4 mg/m² iv (Day 1), respectively.

Blood chemistry and liver function tests were repeated on Day 1 of each cycle; complete blood cell counts were obtained on Days 1 and 8. On Day 1, treatment was delayed by one week if the white blood cell (WBC) count was lower than 4,000/m² and/or the platelet count was lower than 120,000/m². On Day 1 after the one week delay, and on Day 8, the dosages of C, M, F, and E were reduced by 30% when the WBC count ranged from 3,900 to 3,600 and/or the platelet count ranged from 119,000 to 100,000, and by 50% when the WBC count ranged from 3,500 to 2,500 and/or the platelet count ranged from 99,000 to 70,000. No drugs were administered when the WBC count was less than 2,500 and/or the platelet count was less than 70,000. No dose reductions were planned for vincristine. Cardiotoxicity was monitored on the basis of the baseline radionuclide ejection fraction and electrocardiograms before each cycle given to the patients assigned to arm B.

Response and Toxicity Criteria
Objective responses were assessed just prior to surgery using the World Health Organization criteria (WHO). All patients underwent both a clinical examination and mammography, and, in the case of disagreement, the previously described criteria for attributing the final response were used. The pathologic response was assessed at time of surgery. A pathologic complete response meant the absence of any residual infiltrating or noninfiltrating tumor in the breast and axilla. The toxicity grades were evaluated according to the WHO criteria and the patients classified by the worst degree of treatment complication.

Statistical Methods
With the number of patients actually enrolled, the study had a power of 75% to detect an increase in the rate of CR of about 15% and a power of approximately 90% to detect an increase in the rate of CR + PR of about 20% in the experimental arm over estimated values in the CMF arm of 15% and 60%, respectively, at a 5% two-sided level of significance.

The chi-square test and Fisher exact test were used to compare the distribution of patients’ characteristics, the rates of response, and toxicities in the two treatment groups. To assess the presence of a significant influence of menopausal status on the activity of the experimental treatment, the multivariate logistic regression model was used, including in the model the appropriate interaction terms between treatment and menopausal status.

Patients not eligible and mistakenly randomized were excluded from the analyses. Due to the explanatory objectives of this trial at assessing response rates, patients eligible but nonevaluable for response were also excluded from the evaluation of response. All randomized patients will be included in the evaluation of long term outcome according to the intention to treat principle. We used SPSS (Chicago, IL) software in all analyses.

RESULTS
Between November 1990 and April 1995, 211 patients were randomized to CMF (107) or to CMFEV (104). Six
patients were not eligible: four in the CMF (all with Stage III disease) and two in the CMFEV group (one aged 70 years, and one with distant metastases).

Table 2 shows the characteristics of the 205 eligible patients by treatment arm. There were only negligible differences between the two groups. About half of the patients were premenopausal. Given the eligibility criteria, only a small proportion of the patients in each arm had a tumor diameter ≤ 2.5 cm (i.e., those having cytologically proven axillary involvement as the only eligibility criterion; 10% and 13%, respectively) or more than 5 cm (i.e., those having T3 N0, or Stage II B tumors; 7% and 7%, respectively); axillary status was considered clinically positive in 38% of the patients and proven to be positive by FNA cytology in 57 of the 205 eligible patients (28%). About 35% of the patients had estrogen receptor negative tumors. Eight patients were not evaluable for response to primary chemotherapy: four on CMF (two were lost to followup and two refused further chemotherapy after the first cycle), and four on CMFEV (one was lost after randomization, and three refused further chemotherapy after the first cycle). A total of 197 patients (99 on CMF, 98 on CMFEV) were therefore evaluable for response.

Table 3 shows the response rates by chemotherapy regimen. Among patients treated with CMFEV, higher rates of CR (21% vs 12%, \( P = 0.08 \)), of CR + PR (73% vs 66%, \( P = 0.23 \)), and of pCR (6% vs 2%, \( P = 0.16 \)) were seen, but none of these differences achieved statistical significance.

Table 4 shows the pathologic axillary response in the 57 patients with cytologically proven axillary node involvement prior to the start of chemotherapy. Pathologic downstaging from Stage II to Stage I disease was much more frequent in the 31 patients treated with CMFEV than in the 26 patients treated with CMF (23% vs 4%, \( P = 0.059 \)).

Table 5 shows the subgroup exploratory analysis by menopausal status. In premenopausal patients, the percentages of CRs and the percentages of CRs + PRs were significantly greater in the patients treated with CMFEV than with CMF (26% vs 4%, \( P = 0.004 \), and 80% vs 54%, \( P = 0.007 \), respectively). A higher proportion of pCRs was also observed (8% vs 0%, respectively). Conversely, among postmenopausal patients, no significant differences were observed in the percentages of CRs (19% vs 17%, \( P = 0.77 \)) and of CRs + PRs (75% vs 67%, \( P = 0.32 \)).
There were no significant differences between CMF and CMFEV in terms of objective response rates (CR + PR) in relation to tumor diameter (≤ 3.5 cm and > 3.5 cm: 76% vs 65% and 72% vs 65%) or clinically negative or positive axillary node status (73% vs 59% and 74% vs 66%).

To formally assess the modifying effect of menopausal status on the relative activity of either chemotherapy regimen, two multivariate logistic models were fitted to the data, with probability of complete response and of overall objective response as dependent variables, and menopausal status, type of chemotherapy and the interaction term as covariates. The results of these analyses are shown in Tables 6 and 7 and indicate the presence of a significant interaction between menopausal status and type of chemotherapy in determining the probability of achieving complete response (odds ratio [OR] = 0.11, 95% confidence interval [CI] 0.02 to 0.71, P = 0.02) and overall objective response (OR = 7.7; 95% CI 1.6 to 36.3) and of overall objective response (OR = 3.4; 95% CI 1.4 to 8.3) was not seen in postmenopausal women. Surprisingly, among women treated with CMF, response rates were higher among postmenopausal than among premenopausal women (OR = 5.1, 95% CI 1.1–24.6 and OR = 2.6, 95% CI 1.1–6.1 for complete responses and overall responses, respectively).

At the time of surgery, 52% of the CMF and 58% of the CMFEV patients had axillary node involvement. Overall, a large proportion of the patients had 4 or more positive nodes (27%), and a small but not negligible proportion had 10 or more (9%). There was no significant difference between the two treatment groups in terms of any of the node status categories. The axillary node status was unknown in two cases.

Although the protocol did not include any specific guidelines concerning the type of surgery, the general approach was to perform conservative surgery whenever the surgical tumor diameter was ≤ 2.5 cm. A total of 58% of the patients underwent conservative surgery (55% of those treated with CMF and 62% of those treated with CMFEV).

Both treatments were reasonably well tolerated (Table 8). Hematologic toxicity was similar in the two groups, but alopecia was more frequent and severe in the patients receiving CMFEV (P < 0.0005); mild mucositis (P = 0.023) and mild neurologic side effects (P < 0.0005) were slightly more frequent in the same group. There was no difference in the incidence of chemically induced amenorrhea between the two arms (79% versus 76%).
The feasibility of the two chemotherapy programs was confirmed by the high proportion of the planned cycles that were actually administered: 98.7% of the 396 planned CMF cycles and 98.5% of the 392 planned CMFEV cycles.

**DISCUSSION**

The CMFEV regimen used in the current trial is an innovative schedule aimed at administering five partially or totally noncross-resistant cytotoxic agents, first designed and tested by our group as a means of late intensification after CMF in metastatic disease, and whose rotational strategy is different from that of alternating or sequential schemes. All five agents are administered at full dose, but, to avoid excessive toxicity and consequent dose reductions, each cycle involves the administration of only four drugs, always including vincristine, and is organized in such a way that only three among the four potentially myelotoxic drugs (C, M, F, E) are rotatively included (CMFV, CMEV, CFEV, MFEV); the planned dosages of C, M, F, and E in each cycle were therefore either 100% or 0%.

Vincristine was empirically included to continue our previously tested policy in advanced disease. It is active as a single agent in metastatic breast carcinoma and was included in the historically important CMFVP regimen designed by Cooper to treat metastatic disease and also repeatedly administered in an adjuvant setting. A few studies have reported a statistically significant superiority of the CMFVP regimen over CMF in the adjuvant setting. However, there is no proof that vincristine offers any additional activity when included in multi-drug combinations for the treatment of advanced disease. On the basis of the available results, we very much doubt that its presence had any real impact on the current results.

In the current study, although the CMFEV regimen did not offer an overall significant advantage over CMF in terms of objective response, it was associated with an increase in the rate of CRs and with a more frequent downstaging from pathologically positive to pathologically negative axilla (23% vs 4%, \( P = 0.059 \)). Moreover, a statistically significant interaction between type of treatment and menopausal status was seen, with the anthracycline-containing regimen greatly favoring the achievement of CR and CR + PR only in premenopausal patients.

To the best of our knowledge, only a few previous randomized trials involving women with operable breast carcinoma have compared different types of primary chemotherapy in terms of clinical and pathologic response, none of which compared CMF with an anthracycline-containing regimen. A comparative approach in the primary chemotherapy setting should be encouraged because it allows differences in short term effects to be directly assessed in a much shorter period of time than that needed to assess long term effects in the adjuvant setting. In fact, both adjuvant and primary chemotherapy provide a systemic approach to the control of micrometastatic disease, and CR and particularly pCR rates correlate with a better outcome. On this basis, if a given chemotherapy is shown to be more active than another one in these terms, it is possibly associated with a better outcome.

The current study has two particular characteristics: the first is that the strategy used in the experimental treatment, retained all three agents of the CMF combination, in addition to anthracycline; the second is that the results showed a selective advantage in premenopausal patients. To discuss the potential meaning of these two particular characteristics, we cannot rely on similar studies in the primary chemotherapy setting (because they are not available), but we can consider by analogy previous randomized studies in the adjuvant setting.

With respect to the first aspect, an analysis of the available randomized studies of adjuvant chemotherapy comparing regimens with and without anthracycline suggests that it is important to retain in the experimental arm all or most of the agents making up the reference treatment; if this is not the case, the potentially positive effect of the addition of anthracycline may not be revealed. In fact, all of the so-called positive studies (i.e., those showing differences in outcome in favor of the anthracycline-containing regimen, globally or in one or other of the sub-groups)
have this characteristic, whereas the negative studies (those not showing differences) include those in which the experimental arm did not retain any or retained only one of the drugs administered in the reference arm. In the case of the studies in which CMF was the reference treatment, the only positive studies used FEC or FAC (i.e., at least two of the drugs in the CMF combination) in the experimental arm, whereas the negative studies used only anthracycline (i.e., none of the CMF drugs) or the combinations AC or EC (i.e., only one of the CMF drugs). Moreover, in a systematic review concerning the medical treatment of advanced disease, a significant survival advantage by the addition of anthracycline was found only in randomized trials in which the experimental arm included anthracycline plus all, or a minimum of two, agents included in the comparison arm. On the basis of these considerations, our experimental combination CMFEV (or possibly the combination CMFE), which retains all three drugs of the CMF combination, should also be optimal for testing in an adjuvant setting. In this light, a randomized study comparing CMF with this rotational CMFEV in the adjuvant setting was carried out by the GOIRC Group; the results will become available soon.

With regard to the second noteworthy finding of the current trial, analysis of some of the available randomized studies of adjuvant chemotherapy suggests that anthracycline-containing regimens may be more efficacious only in premenopausal or relatively younger women. First, the Oncofrance study, which compared CMF with the FACV combination, reported significantly positive overall results, but subgroup analysis showed that only premenopausal patients contributed to these results. Second, a Scandinavian study comparing CMF with the FEC combination reported significantly positive results in premenopausal but not in postmenopausal patients. Finally, the apparently contradictory results reported by the NSABP Group in their B-11 (positive) and B-12 (negative) studies, which both used 1-PAM plus fluorouracil without and with doxorubicin (PF vs PAF), could be explained by the fact that the first study enrolled a majority of premenopausal patients, or at any rate younger patients than those enrolled in the second study.

This discussion is intended to highlight the potential relevance of our strategy in designing and testing the CMF combination as a primary chemotherapy and to support the hypothesis that the current positive results (selectively observed in premenopausal patients) may have been due to an underlying biologic difference rather than chance. However, the current study was not planned to assess the results selectively by menopausal status, and the increased risk of detecting false positive associations by making subgroup analyses is well known; consequently, the current results can only be considered a suggestion for future research.

To the best of our knowledge, this is the first time a study has compared CMF with an anthracycline-containing regimen as primary chemotherapy in operable breast carcinoma, and therefore the first time that favorable results have been reported in relation to the latter. These data therefore allow us to bring together (albeit indirectly) the short term objective response results obtainable in a neoadjuvant setting with those demonstrable in the long term (i.e., DFS and OS) in an adjuvant setting.

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