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CHEMOTHERAPY WITH CYCLOPHOSPHAMIDE, ADRIAMYCIN, AND 5-FLUOROURACIL COMPARED TO CHEMOTHERAPY PLUS HORMONAL THERAPY WITH TAMOXIFEN IN THE TREATMENT OF ADVANCED BREAST CANCER: AN INTERIM ANALYSIS

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Summary—Between February 1980 and August 1982, the Cancer and Leukemia Group B (CALGB) performed a randomized study aimed to compare chemotherapy with CAF (Cyclophosphamide, Adriamycin, 5-Fluorouracil) versus the same chemotherapeutic regimen plus tamoxifen (T-CAF) in stage IV breast cancer patients. Patients were stratified on the basis of menopausal status, estrogen receptors (ER) status, dominant site of metastasis and prior adjuvant treatment. Overall 474 patients were entered into the study of whom 433 were assessable for response. 314 patients were postmenopausal, 85 premenopausal and 34 patients were unknown as far menopausal status was concerned. No difference was evident among postmenopausal patients in overall response rate and duration of responses between T-CAF and CAF (52% vs 50% respectively). Similarly no difference was shown among premenopausal patients, response rates being 63% with T-CAF and 60% with CAF. Lack of benefit from adding T to chemotherapy was seen also according to the different strata, including patients with ER positive tumors. The failure for this combination to be synergistic might reflect an effect of T on tumor kinetics interfering with the activity of chemotherapy.

INTRODUCTION

Metastatic breast carcinoma may be responsive to cytotoxic chemotherapeutic drugs, to hormonal manipulation, or to both therapeutic modalities. The use of currently available cytotoxic drugs in a variety of combination chemotherapy programs has led to objective response rates of 50–80% with approx 10–15% complete responses. The median response durations are from 9 to 16 months. Similarly, responses to hormonal manipulation are observed in 25% of unselected breast cancers and in 55% of breast cancers which contain estrogen receptor protein. Although both cytotoxic chemotherapy and hormonal therapy can produce acceptable response rates, including complete remissions, these responses are temporary and few, if any, patients are cured. Since neither form of therapy is a curative by itself new approaches are needed. Cytotoxic chemotherapy and hormonal therapy probably have different mechanisms of action and they each may have their major effects on a different cell population within a breast cancer [1, 2]. Cancer and Leukemia Group B (CALGB) decided to combine these two modalities of therapy to determine if an increased response rate and improved response duration would result.

A previous CALGB study demonstrated that the combination of cyclophosphamide, adriamycin, 5-fluorouracil, vincristine, and prednisone (CAFVP) was superior to the combinations of cyclophosphamide, methotrexate, 5-fluorouracil, vincristine, and prednisone, (CMFVP) [3]. In that study, the combination of cyclophosphamide, adriamycin, and 5-fluorouracil (CAF) yielded results similar to that of CAFVP and was less toxic. Thus, CALGB chose CAF as the chemotherapy combination for use in the present trial. Tamoxifen was chosen as the hormonal agent because of its demonstrated efficacy and relative lack of toxicities [4].

This paper represents the third interim analysis of this CALGB study [5, 6].

EXPERIMENTAL

Patients and methods

From February 1980 to August 1982 the Cancer and Leukemia Group B conducted a prospective randomized trial comparing chemohormonal therapy with T-CAF (tamoxifen plus cyclophosphamide, adriamycin, 5-fluorouracil) to chemotherapy alone (CAF) in women with metastatic, locally recurrent,

or locally advanced breast cancer. The study was initially designed for postmenopausal women but an addendum in April 1980 opened the study to premenopausal women as well. Women with histologically documented carcinoma of the breast were eligible if they had measurable metastatic, locally recurrent, or surgically incurable (stage IV) disease. Only patients with their first recurrence were eligible. Patients were not eligible if they had performance status of greater than 3, or a second primary malignant neoplasm or a malignant neoplasm of the breast other than carcinoma. A history of recent myocardial infarction, congestive heart failure, or documented angina also rendered the patient ineligible. Patients who had completed adjuvant chemotherapy greater than 6 months prior to entry were eligible provided it was their first documented recurrence. Prior therapy with tamoxifen rendered the patient ineligible. Informed consent was obtained from all patients.

Stratifications

Premenopausal patients were randomized from within a single stratum. Postmenopausal patients were stratified on the basis of estrogen receptor (ER) status, dominant site of metastatic disease prior to randomization, and by no prior therapy versus prior adjuvant chemotherapy (Table 1). The estrogen receptor assays were quality controlled by internal monitoring utilizing reference powders provided by James Witliff, Ph.D. of the University of Louisville. Detailed results of the quality control program will be the subject of a subsequent publication. There was good agreement for all laboratories analyzing reference powders which were negative, and only one of 14 laboratories tested reported a negative result on an ER-positive reference powder. Patients were stratified into 3 groups on the basis of ER:

Table 1. Stratifications for postmenopausal patients

1.	Estrogen receptor (ER) status	
	A. ER-negative	<7 fmol/mg protein
	B. ER-positive	≥ 7 fmol/mg protein
	C. ER-unknown	—test not performed
2.	Dominant site of metastatic disease	
	A. Visceral	
	B. Other (osseous and soft tissue)	
3.	Prior therapy	
	A. No prior therapy	
	B. Prior adjuvant chemotherapy	

ER-positive ≥ 7 fmol/mg protein;

ER-negative < 7 fmol/mg protein;

ER-unknown (test not performed).

The cut-off value of 7 fmol/mg was selected based upon the data of Hilf *et al.* [7]. Stratification by site of metastatic disease was into 2 groups: visceral dominant or other (osseous and/or soft tissue).

Randomization

Based upon the appropriate stratifications, patients were randomized to receive CAF chemotherapy alone or T-CAF chemohormonal therapy (tamoxifen + CAF). The schema for CALGB study 8081 is illustrated in Fig. 1.

Treatment schedule

Patients randomized to receive T-CAF received the tamoxifen continuously in a dose of 10 mg twice daily. The chemotherapy was the same in each treatment arm and was given in intermittent cycles over

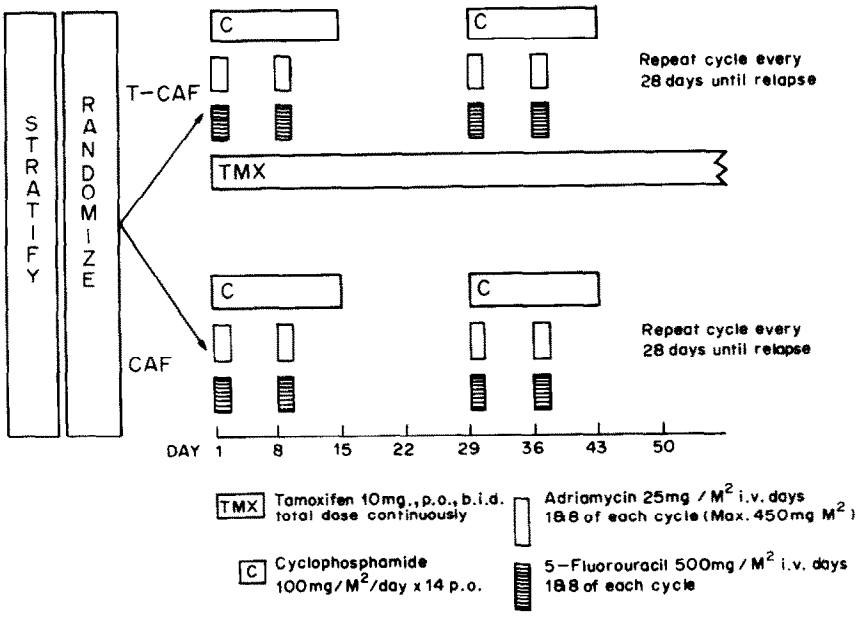

Fig. 1. Schema of cancer and leukemia group B study 8081.

a 28-day period with 14 days of cytotoxic drug administration followed by a 14-day rest as follows (see Fig. 1): cyclophosphamide 100 mg/m^2/day p.o. days 1–14; adriamycin 25 mg/m^2/i.v. days 1 and 8; and 5-fluorouracil 500 mg/m^2 i.v. days 1 and 8. Treatment cycles were repeated on day 29, 57, 85, etc. After a total cumulative dose of adriamycin of 450 mg/m^2 had been administered, methotrexate was substituted. The methotrexate dose was 40 mg/m^2 i.v. on days 1 and 8 unless the patient was over age 60 in which case it was reduced to 30 mg/m^2. Dose calculations were based on the patient's ideal body weight or actual weight, whichever was lower.

Dose modifications for cyclophosphamide, adriamycin, and 5-fluorouracil were based on the total white blood cell count and platelet count on the day of treatment. Reductions were also made for elevations of serum bilirubin, and SGOT and for the occurrence of stomatitis, diarrhea, or cystitis.

Response criteria

A complete response (CR) was defined as complete disappearance of all signs and symptoms attributable to the tumor including the disappearance of all measurable lesions for at least 1 month and the appearance of no new lesions. For osseous disease a CR required recalcification of all osteolytic lesions. A partial response (PR) was defined as greater than 50% reduction in the sum of the products of the two largest perpendicular diameters of all measured lesions with no deterioration in performance status, and without the appearance of any new lesions.

For patients achieving an objective response, the date that the tumor met the criteria for response was the onset of response. Response duration was calculated from the onset of CR or PR until the documentation of progression. Patients were evaluated after 2 courses (8 weeks) of therapy. If there was evidence of progressive disease, the patient was considered a treatment failure and taken off protocol. Responding patients or those with stable disease continued on therapy until there was evidence of tumor progression or prohibitive drug toxicity.

There was no provision in the study for ER-positive or ER-unknown patients randomized to CAF alone who did not respond or who responded and then failed to automatically receive tamoxifen alone as secondary treatment.

Ancillary therapy

Once a patient was started on protocol, palliative radiation was not administered, except for cranial radiation for documented intracranial metastases. Chemotherapy was not witheld when patients required such radiation.

Statistical methods

Treatment assignment was done by Latin square design, balancing within and across institutions for each stratum. In performing the analyses, differences in pretreatment characteristics and response frequencies were evaluated using the chi square technique for contingency tables. Differences in remission duration were evaluated by the generalized Wilcoxan test. Multivariate regression analyses were performed using Cox's multiple linear logistic model.

RESULTS

A total of 474 patients were enrolled in the study. Of these 433 cases were evaluable for response. Thirteen cases were ineligible for the study and 5 died prior to receiving any therapy. An additional 23 cases were disqualified because of a major protocol violation, inadequate records, or improper randomization. There were 314 post-menopausal patients, 85 premenopausal patients, and 34 patients whose menopausal status was unknown at the time of this interim analysis.

Postmenopausal patients

Postmenopausal patients were evenly divided between the two treatment groups. The comparability of the two treatment groups is seen in Table 2.

The two treatment groups were similar with regard to type of menopause, dominant site of metastatic disease at entry, performance status, prior adjuvant chemotherapy, and estrogen receptor status. The one difference between the 2 groups was that the patients randomized to CAF were slightly older than the patients randomized to T-CAF (median age of 60 years vs median age of 58 years, $P = 0.085$).

The premenopausal patients were evenly divided between the 2 treatment groups. The comparability of the treatment groups is seen in Table 3. The two treatment groups were similar with regard to age at diagnosis and performance status. More estrogen receptor-positive patients were randomized to T-CAF than to CAF (38% vs 26%, $P = 0.25$). Also more premenopausal patients on T-CAF had received prior adjuvant chemotherapy (12% vs 2%, $P = 0.11$).

Table 2. Comparability of treatment groups—postmenopausal patients

Number evaluable	*T-CAF* 155	*CAF* 159
Dominant site of metastases		
Visceral	57%	51%
Non-visceral	43%	49%
Estrogen receptor status		
ER-negative	34%	33%
ER-positive	29%	30%
ER-unknown	37%	37%
Prior therapy		
Adjuvant chemotherapy	10%	14%
No prior chemotherapy	90%	86%
Performance status		
0–1	75%	77%
2–3	25%	23%
Type of menopause		
Natural	61%	69%
Surgical	28%	22%
Median age (years)	58	60

Table 3. Comparability of treatment groups—premenopausal patients

Number evaluable	*T-CAF* 42	*CAF* 43
Estrogen receptor status		
ER-negative	40%	44%
ER-positive	38%	26%
ER-unknown	21%	30%
Prior therapy		
Adjuvant chemotherapy	12%	2%
No prior chemotherapy	88%	98%
Performance status		
0–1	77%	83%
2–3	23%	17%
Median age (years)	42	42

Table 4. Frequency of response to therapy—postmenopausal patients

No. evaluable	*T-CAF* 153	*CAF* 155	*P*
No. CR (%)	25 (16%)	21 (14%)	0.53
No. PR (%)	55 (36%)	57 (37%)	
No. CR + PR (%)	80 (52%)	78 (50%)	0.73

The overall response frequency for all postmenopausal patients is outlined in Table 4. There is no difference in overall response rate (CR + PR) between T-CAF (52%) and CAF (50%) nor is there a difference in CR rates between T-CAF (16%) and CAF (14%). Figure 2 illustrates the duration of remission for the 158 postmenopausal patients who responded to therapy. At the time of this analysis 62 out of the 158 patients had relapsed. There is no difference in the duration of remission between T-CAF and CAF.

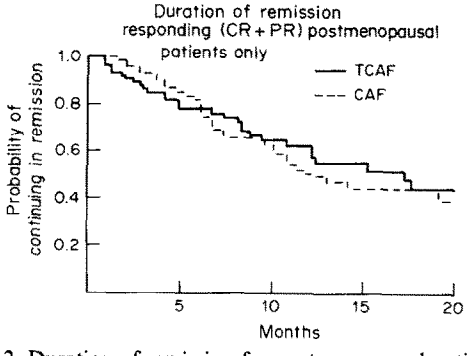

Fig. 2. Duration of remission for postmenopausal patients who responded to therapy.

The response rates for postmenopausal patients were analyzed by ER status (Table 5). Among ER-negative patients the response rate to CAF alone (68%) was somewhat greater than the response rate to T-CAF (55%), but the difference was not statistically significant ($P = 0.22$). Response rates among ER-positive patients were the same for T-CAF and for CAF (both 53%). The ER-unknown patients responsed more frequently to T-CAF (49%) than to CAF alone (33%) but the difference did not achieve statistical significance ($P = 0.09$). Among patients treated with CAF, the ER-negative patients had a higher response rate (68%) than the ER-positive patients (53%). However, this difference is not statistically significant ($P = 0.15$).

When the response rates for postmenopausal patients were analyzed by dominant site of metastatic disease (Table 6) there was a suggestion that visceral dominant patients responded more frequently to T-CAF (58 vs 45% response rate to CAF) and that non-visceral dominant patients responded more frequently to CAF (57 vs 48% response rate to T-CAF), but these differences were not statistically significant.

Table 7 compares the response rates of the postmenopausal patients who had received previous adjuvant chemotherapy to the response rates of those who had not had prior chemotherapy. Among patients who had not received any prior chemotherapy the response rates to T-CAF and CAF are the same (51 and 52%). For patients who had received prior adjuvant chemotherapy, response rates were 63% in the T-CAF group and 43% in the CAF group ($P = 0.32$). Among patients treated with CAF on this study, there were no statistical significances in response frequencies between those who had not received prior adjuvant chemotherapy (52%) and those who had received prior chemotherapy (43%).

Premenopausal patients

The overall response frequencies for all premenopausal patients is outlined in Table 8. There was

Table 5. Response by estrogen receptor status—postmenopausal patients

	T-CAF CR + PR/Total (%)	*CAF* CR + PR/Total (%)	*P*
ER-negative	10 + 18/51 (55%)	3 + 31/50 (68%)*	0.22
ER-positive	4 + 20/45 (53%)	11 + 14/47 (53%)*	>0.9
ER-unknown	11 + 17/57 (49%)	7 + 12/58 (33%)	0.09
			*0.15

Table 6. Response by dominant site of metastatic disease—postmenopausal patients

	T-CAF CR + PR/Total (%)	*CAF* CR + PR/Total (%)	*P*
Visceral	19 + 28/81 (58%)	10 + 24/75 (45%)	0.15
Nonvisceral	6 + 24/62 (48%)	10 + 28/67 (57%)	0.38
Site unknown	0 + 3/10 (30%)	1 + 5/13 (46%)	0.67

Table 7. Response by prior adjuvant chemotherapy (CT) vs no prior adjuvant CT—postmenopausal patients

	T-CAF	*CAF*	
	CR + PR/Total (%)	CR + PR/Total (%)	*P*
No prior CT	24 + 45/135 (51%)	19 + 48/128 (52%)*	0.90
Prior CT	1 + 9/16 (63%)	2 + 7/21 (43%)*	0.32
			*0.48

Table 8. Frequency of response—premenopausal patients

	T-CAF	*CAF*	
No. evaluable	41	43	*P*
No. with complete response (%)	13 (32%)	8 (19%)	0.21
No. with partial response (%)	13 (32%)	18 (42%)	
No. CR + PR(%)	26 (63%)	26 (60%)	0.82

Table 9. Response by menopausal status

	% *CR + PR*		
	Postmenopausal	Premenopausal	*P*
T-CAF	52%	63%	0.22
CAF	50%	60%	0.30

no statistically significant difference in overall response rate (CR + PR) between patients treated with T-CAF (63%) and those treated with CAF (60%) nor was there a significant difference ($P = 0.21$) in complete response rate between patients treated with T-CAF (32%) and those treated with CAF (19%).

Table 9 shows the response frequencies according to menopausal status. There were no statistically significant differences in response frequency associated with menopausal status.

Toxicity

The side-effects of therapy consisted of mild-to-moderate myelosuppression, nausea and vomiting, alopecia, and stomatitis. Toxicity was similar in the two treatment groups and no additional side-effects appeared to be caused by tamoxifen.

DISCUSSION

Despite its theoretical superiority, no advantage was observed in this study for the combined chemotherapy and hormonal therapy approach (T-CAF) over chemotherapy alone (CAF) even in the subset of patients known to have estrogen receptor-positive tumors. Osborne has suggested three reasons for the failure of combined hormonal therapy and chemotherapy to exert a major impact on tumor cell reduction [8]. First, a large fraction of tumor cells may be resistant to both treatments. Second, a large fraction of the cells present may be sensitive to either of the modalities of therapy, thus minimizing the effect of a combined approach. Third, an interaction between the treatments themselves may result in an adverse, or at least less than maximally additive, effect on tumor cell reduction.

The effect of hormonal therapy on breast cancer cell kinetics suggests that hormonal therapy may antagonize the effects of cytotoxic chemotherapeutic agents by blocking tumor cells in an unfavorable position in the cell cycle. After treatment with tamoxifen, a progressively larger fraction of tumor cells accumulates in the G_1 phase of the cell cycle [8]. Most cytotoxic chemotherapeutic agents are not maximally effective on cells in the G_1 phase. Thus, combined hormonal therapy and cytotoxic chemotherapy may not be additive and may be less efficacious than the two modalities used separately.

Other published studies of the use of combined chemohormonal therapy in postmenopausal women are not easily compared to the present study because of the use of differing types of chemotherapy or hormonal therapy, failure to stratify by ER status, small numbers of patients enrolled, or the use of an historical, rather than concomitant, control group. Cocconi *et al.* performed a similar study on 133 postmenopausal patients using CMF with or without tamoxifen [9]. It should be noted that 42% of the patients in this study had received prior endocrine therapy. Response rates were greater in the group receiving tamoxifen, but neither the duration of response nor overall survival were prolonged. Mouridsen *et al.* reported a similar trial of CMF with or without tamoxifen in 150 patients and also reported a significant advantage to the combined therapy group [10]. The use of the less effective CMF chemotherapy combination may have permitted any effect of tamoxifen to become apparent. Conversely, our use of the more potent CAF therapy may have obliterated any effect of the tamoxifen.

Two additional observations of the present study are of particular interest. First, no difference in response rates to T-CAF or to CAF were noted between premenopausal and postmenopausal patients. Second, patients who had completed adjuvant chemotherapy more than 6 months prior to entry on protocol responded well to CAF despite the fact that almost all patients in this group had received prior therapy with CMF, i.e. they had previously received at least two of the agents. Moreover, the response rate of these patients to CAF was essentially the same as those patients who had not been previously treated (43 vs 52%). This response rate is markedly better

than the 20–25% expected for Adriamycin alone in previously treated patients [11]. This implies that patients who develop recurrent disease after completion of adjuvant chemotherapy may do so because of kinetic reasons rather than intrinsic drug resistance.

In conclusion, this study shows that no benefit accrued from adding the antiestrogen, tamoxifen, to CAF. Specifically, there was no difference in CR or PR or in length of remission duration. This lack of additional benefit was seen in all sites of dominant disease, even in patients with positive ER assays. The failure of this combination to be synergistic may reflect an effect on tamoxifen on tumor cell kinetics that interferes with the activity of the chemotherapeutic agents.

REFERENCES

1. Greene G. L. and Jensen E. V.: The use of monoclonal antibodies in estrogen receptor assays (Abstract). *Proc. 13th Intl. Cancer Congr.*, Seattle, Washington, September 8–15 (1984) p. 573.
2. Mercer W. D., Lippman M. E., Wahl T. M., Carlson C. A., Wahl D. A., Legotte D. and Teague P. O.: The use of immunocytochemical techniques for the detection of steroid hormones in breast cancer cells. *Cancer* **46** (1980) 2859–2868.
3. Aisner J., Weinberg V., Perloff M., Weiss R., Raich P., Perry M. and Wiernik P. H.: Chemoimmunotherapy for advanced breast cancer: A randomized comparison of six combinations (CMF, CAF vs CAFVP) each with or without MER immunotherapy. A CALGB study. (Abstract) *Am. Soc. clin. Onc.* **22** (1981) 443.
4. Ingle J. N., Ahmann D. L., Green S. J., Edmonson J. H., Bisel H. F., Kvols L. K., Nichols W. C., Creagan E. T., Hahn R. G., Rubin J. and Frytak S.: Randomized clinical trial of diethylstilbestrol versus tamoxifen in post-menopausal women with advanced breast cancer. *New Engl. J. Med.* **304** (1981) 16–21.
5. Perry M. C., Kardinal C. G., Weinberg V., Ginsberg S. and Wood W.: *Chemotherapy Compared to Chemotherapy Plus Hormonal Therapy in the Treatment of Postmenopausal Women with Advanced Breast Cancer: An interim report. Current Controversies in Breast Cancer.* (Edited by F. C. Ames, G. R. Belimenschein and E. D. Montague). University of Texas Press, Austin (1984) pp. 477–486.
6. Kardinal C. G., Perry M. C., Weinberg V., Wood W., Ginsberg S. and Raju R. N.: Chemoendocrine therapy therapy alone for advanced breast cancer in postmenopausal women: Preliminary report of a randomized study. *Breast Cancer Res. Treat.* **3** (1983) 365–371.
7. Hilf R., Witliff J. L., Rector W. D., Savlov E. D., Hall T. C. and Orlando R. A.: Studies on certain cytoplasmic enzymes and specific estrogen receptors in human breast cancer and in nonmalignant disease of the breast. *Cancer Res.* **33** (1973) 2054–2062.
8. Osborne C. K.: Combined chemo-hormonal therapy in breast cancer: A hypothesis. *Breast Cancer Res. Treat.* **1** (1981) 121–123.
9. Cocconi G., De Lisi V., Mori P., Malacarne P., Amadori D. and Giovannelli E.: Chemotherapy versus combination of chemotherapy and endocrine therapy in advanced breast cancer: A randomized study. *Cancer* **51** (1983) 581–588.
10. Mouridsen H. T., Palshof T., Engelsman E. and Sylvester R.: CMF versus CMF plus tamoxifen in advanced breast cancer in postmenopausal women. An E.O.R.T.C. trial. In *Breast Cancer—Experimental and Clinical Aspects* (Edited by H. T. Mouridsen and T. Palshof). Pergamon Press, Oxford (1980) pp. 119–123.
11. Kardinal C. G.: Chemotherapy. In *Cancer of the Breast* (Edited by W. L. Donegan and J. S. Spratt). W. B. Saunders, Philadelphia (1979) pp. 405–447.