

Early Start of Adjuvant Chemotherapy May Improve Treatment Outcome for Premenopausal Breast Cancer Patients With Tumors not Expressing Estrogen Receptors

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Purpose: The proper time to commence adjuvant chemotherapy after primary surgery for breast cancer is unknown. An analysis of the International (Ludwig) Breast Cancer Study Group (IBCSG) Trial V at a median follow-up of 11 years suggested that early initiation of adjuvant chemotherapy might improve outcome for premenopausal, node-positive patients whose tumors did not express any estrogen receptor (ER).

Patients and Methods: We investigated the relationship between early initiation of adjuvant chemotherapy, ER status, and prognosis in 1,788 premenopausal, node-positive patients treated on IBCSG trials I, II, and VI. The disease-free survival for 599 patients (84 with ER-absent tumors) who commenced adjuvant chemotherapy within 20 days (early initiation) was compared with the disease-free survival for 1,189 patients (142 with ER-absent tumors) who started chemotherapy 21 to 86 days after surgery (conventional initiation). The median follow-up was 7.7 years.

Results: Among patients with ER-absent tumors, the 10-year disease-free survival was 60% for the early

initiation group compared with 34% for the conventional initiation group (226 patients; hazard ratio [HR], 0.49; 95% confidence interval [CI], 0.33 to 0.72; $P = .0003$). This difference remained statistically significant in a Cox multiple regression analysis controlling for study group, number of positive nodes, tumor size, age, vessel invasion, and institution (HR, 0.60; 95% CI, 0.39 to 0.92; $P = .019$). Conversely, early initiation of chemotherapy did not significantly improve disease-free survival for patients with tumors expressing ER (1,562 patients; multiple regression HR, 0.93; 95% CI, 0.79 to 1.10; $P = .40$).

Conclusion: In premenopausal patients with ER-absent tumors, early initiation of systemic chemotherapy after primary surgery might improve outcome. Further confirmatory studies are required before any widespread modification of current clinical practice. In premenopausal patients with tumors expressing some ER, gains from early initiation are unlikely to be clinically significant.

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THE INITIATION Of adjuvant chemotherapy has typically been delayed a few weeks after surgery.¹⁻³ Surgical trauma and removal of the primary tumor may lead to an increased number of circulating tumor cells⁴ and to an accelerated growth of micrometastases.^{5,6} Tissue trauma is known to enhance biologic processes, which may stimulate both wound healing and tumor progression.^{7,8} In addition, a correlation between increase in neo-angiogenesis (in terms of vascular density) and circulating tumor cells has been observed after surgery.⁹ Moreover, the presence of increased vascular density was correlated with poor prognosis

for patients who received adjuvant chemotherapy.^{10,11} These data provide a plausible rationale for initiation of chemotherapy as close as possible to the surgical removal of macroscopic tumor.

Between 1981 and 1985, the International (Ludwig) Breast Cancer Study Group (IBCSG) conducted a randomized clinical trial (Trial V) to evaluate the timing and duration of adjuvant therapy for women with operable breast cancer.¹² Four hundred seventy-five premenopausal breast cancer patients with node-positive disease were randomized to receive either perioperative cyclophospha-

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Table 1. Study Designs of IBCSG Trials I, II, and VI

Trial		Accrual Years	No. of Eligible Patients	Treatment Groups	Median Follow-Up (years)
I	Premenopausal 1-3 N+	1978-1981	491	CMF × 12 v CMFp × 12	16
II	Premenopausal ≥ 4 N+	1978-1981	327*	CMFp × 12 v (Ox + CMFp × 12)	16
VI	Premenopausal N+	1986-1993	1476	CMF × 6 v CMF × 6 + reint v CMF × 3 v CMF × 3 + reint	7

Abbreviations: p, prednisone (7.5 mg/day orally, continuously); Ox, oophorectomy; reint, reintroduction of three additional single courses of CMF administered every third month after the completion of the initial block of CMF (ie, either months 9, 12, and 15, or months 6, 9, and 12).

*Only the 161 patients randomized to CMFp alone were considered for these analyses.

NOTE. CMF: cyclophosphamide, 100 mg/m² orally days 1 to 14 of each cycle; methotrexate, 40 mg/m² intravenously, days 1 and 8 of each cycle; and fluorouracil, 600 mg/m² intravenously, days 1 and 8 of each cycle.

midate, methotrexate, fluorouracil (CMF) chemotherapy followed by conventionally timed chemotherapy (CMF plus low-dose prednisone given for 6 months) or the conventionally timed chemotherapy alone. At a median follow-up of 11 years, among the 101 women whose tumors did not express any estrogen receptors (ER-absent), the 10-year disease-free survival rate was 48% for 46 patients who received perioperative CMF compared with 38% for 55 women who received conventionally timed chemotherapy alone (hazard ratio [HR], 0.80; 95% confidence interval [CI], 0.48 to 1.34; $P = .40$). Despite the small number of patients, the estimated HR of 0.80 (20% reduction in the risk of recurrence) provided motivation to investigate whether early initiation of chemotherapy might be beneficial for a subgroup of premenopausal patients. We, therefore, examined all other IBCSG trials of classical CMF chemotherapy for premenopausal patients (Trials I, II, and VI). The treatment outcome according to the timing of initiation of chemotherapy after primary surgery in these trials is the subject of this report.

PATIENTS AND METHODS

All trials have been previously described.¹³⁻¹⁵ The study designs are listed in Table 1. All trials used classical CMF.^{1,8} Briefly, between 1978 and 1981, Trial I¹³ accrued 491 eligible premenopausal women with one to three positive axillary lymph nodes who were randomized to receive 12 28-day cycles of adjuvant CMF chemotherapy or CMF plus continuous low-dose prednisone (CMFp). At a median follow-up of 16 years, there were no statistically significant differences in disease-free survival or overall survival between the treatment groups. Trial II also enrolled patients from 1978 to 1981 and included premenopausal women with four or more positive axillary lymph nodes who were randomized to receive either surgical oophorectomy followed by 12 28-day cycles of CMFp or CMFp alone.¹⁴ For the current analysis, only those women randomized to chemotherapy alone (without oophorectomy) were considered. Between 1986 and 1993, Trial VI enrolled 1,476 eligible premenopausal women with node-positive disease who were randomized to receive either three or six initial cycles of CMF therapy either alone or followed by three single cycles of reintroduction CMF delivered 3 months apart.¹⁵ At a median follow-up of 7 years, there was no statistically significant difference in disease-free survival or overall survival among the four treatment groups.

The data analyses presented in this report included 1,788 patients (84% of the 2,127 eligible patients enrolled onto Trials I, II, and VI) who received chemotherapy and had quantitative ER levels available. ER levels were determined by the dextran-coated charcoal assay¹⁶ performed in laboratories participating in regular quality control programs.¹⁷ Fourteen patients who never started chemotherapy (three in Trial I, two in Trial II, and nine in Trial VI) and 325 patients who did not have quantitative ER values (225 in Trial I, 70 in Trial II, and 30 in Trial VI) were not included in these analyses. The evaluated group, compared with the nonevaluated group, had more patients with four or more positive nodes (33% v 26%, respectively), fewer patients with vessel invasion (71% v 82%, respectively, among cases with known vessel invasion status), and more patients from one of the larger participating centers (institution A: 15% v 8%, respectively). Because more than 97% of the patients in Trial VI could be evaluated, almost all of the differences arise from the smaller earlier trials.

Patients were classified as receiving early initiation of adjuvant chemotherapy if treatment was started before 21 days from definitive primary surgery. The date of the most extensive surgery, which ordinarily included the axillary dissection, was taken as the definitive surgical procedure. Biopsies might have preceded this date. The cut point of 21 days was selected before starting data analysis primarily for two reasons: (1) the eligibility for enrollment was within 6 weeks of definitive surgery, so 21 days represented the middle of this period; and (2) only 6% of the patients in the conventionally timed chemotherapy arm of Trial V commenced their treatment before day 21 compared with 47% between days 21 and 27 and 47% after day 27.

Data available as of May 1998 were used for the analysis. Median follow-up was 7.7 years. Disease-free survival was defined as the interval from randomization to relapse, the appearance of a second primary cancer (including a contralateral breast cancer), or death, whichever occurred first. Disease-free survival curves were estimated using the Kaplan-Meier method.¹⁸ Cox proportional hazards regression model was used to test for differences between the disease-free survival curves, to provide analyses adjusted for a variety of covariates, and to test for interactions between the effect of timing of chemotherapy initiation and covariates.¹⁹ Covariates considered for the multiple regression models included ER-content group (ER-absent; ER-low: 1 to 9 fmol/mg cytosol protein; or ER-positive: ≥ 10 fmol/mg cytosol protein), number of positive axillary lymph nodes (one to three or four or more), tumor size (≤ 2 cm or > 2 cm), age (< 40 years old or ≥ 40 years old), and vessel invasion (absent; present; or not examined). An additional covariate was included for one large participating center in which early initiation of chemotherapy was more frequent than in the other centers (institution A). All analyses were stratified by trial. All P values were two-sided.

Table 2. Patients Characteristics

Characteristic	Commencement of Chemotherapy			
	< 21 Days		≥ 21 Days	
	No. of Patients	% of Patients	No. of Patients	% of Patients
Total no.	599	100	1189	100
Trial				
I	104	17	159	13
II	32	5	57	5
VI	463	77	973	82
ER-status				
Absent	84	14	142	12
1-9	141	24	238	20
10+	374	62	809	68
Institution				
A	222	37	51	4
Other	377	63	1138	96
No. of nodes				
1-3	403	67	797	67
4+	196	33	392	33
Tumor size*				
Size ≤ 2 cm	255	43	522	44
Size > 2 cm	344	57	667	56
Age				
< 40 years	155	26	237	20
≥ 40 years	444	74	952	80
Vessel invasion				
Absent	75	13	246	20
Present	306	51	472	40
Not examined	218	36	471	40
Comorbid condition†				
Absent	523	87	1034	87
Present	76	13	155	13

*Tumor size was based on pathologic measurement in all cases except for 36 in Trial VI, for whom clinical measurement was used. In three cases (one each from trials I, II, and VI), tumor size was unknown, and these patients were included in the smaller tumor category.

†In two cases (one each in trials I and VI), the question on comorbid condition was not answered, and these patients were included in the absent category.

RESULTS

A total of 599 patients (33.5%) commenced chemotherapy within 20 days from surgery, whereas 1,189 patients started chemotherapy 21 to 86 days after surgery. Chemotherapy was initiated within 2 weeks of surgery (≤ 13 days) for 10.1% of the patients, during week 3 after surgery for 23.4%, during week 4 for 29.4%, during weeks 5 or 6 for 32.6%, during weeks 7 or 8 for 4.4%, and beyond week 8 (56 to 86 days) for 0.4% (seven patients).

Table 2 lists the patient characteristics according to timing of the initiation of adjuvant chemotherapy. Characteristics were well-balanced between the early initiation and conventional initiation groups, especially with respect to nodal status and tumor size, features known to have prognostic relevance. In addition, the incidence of comorbid

conditions and treatment assignment were well-balanced across the different patient groups. The only exception was with respect to patients enrolled from institution A, more of whom started adjuvant chemotherapy early compared with the incidence of early initiation in other centers. The percentage of patients with ER-absent tumors was 12.6%.

Table 3 lists the results of disease-free survival analysis according to time of initiation of adjuvant chemotherapy. The 10-year disease-free survival among patients with ER-absent tumors was 60% for patients who started adjuvant chemotherapy within 20 days of surgery compared with 34% for those who started treatment 21 days or more after surgery (226 patients; HR, 0.49; 95% CI, 0.33 to 0.72; $P = .0003$). In contrast, the estimated magnitude of effect of early initiation was much smaller for patients with tumors expressing ER. This is displayed as disease-free survival curves in Fig 1A, 1B, and 1C according to timing of the initiation of chemotherapy for each of the three levels of ER in the primary tumor.

Table 4 lists the results of the multiple regression model. The difference in outcome according to timing of start of adjuvant chemotherapy remained statistically significant for the patients with ER-absent tumors (HR, 0.60; 95% CI, 0.39 to 0.92; $P = .019$). Early initiation of chemotherapy was associated with a 40% reduction in the risk of relapse compared with later initiation. Reductions in the risk of relapse were observed in all three trials (Trial I: HR, 0.18; 95% CI, 0.06 to 0.54; $P = .003$; Trial II: HR, 0.62; 95% CI, 0.18 to 2.08; $P = .44$; and Trial VI: HR, 0.62; 95% CI, 0.34 to 1.14; $P = .12$). Figure 2 shows the multiple regression HRs and 95% CIs comparing early versus later initiation of chemotherapy, both overall and separately according to ER content of the primary tumor. The solid vertical line in the figure indicates no difference between early and later commencement of chemotherapy (HR, 1.0), and the area of each black square is proportional to the amount of information (larger squares are associated with shorter CI). In contrast to the ER-absent group, early initiation of chemotherapy did not significantly improve disease-free survival for patients with tumors expressing ER (1,562 patients; multiple regression HR, 0.93; 95% CI, 0.79 to 1.10; $P = .40$). The improvement in disease-free survival associated with early initiation of chemotherapy for the ER-absent subgroup was significantly greater than the disease-free survival difference observed for patients with tumors expressing ER ($P = .0054$, multiple regression interaction term).

Even though disease risk factors and comorbid conditions were well-balanced between the early chemotherapy and later chemotherapy groups, it is possible that other postoperative factors may have influenced the ability of patients to start chemotherapy early. We noted, however, that among patients with ER-absent tumors, the percents with non-

Table 3. Univariate Results: Disease-Free Survival by ER Group

ER Group	Commencement of Chemotherapy										HR	95% CI	P*
	< 21 Days					≥ 21 Days							
	No. of Patients	5-Year DFS		10-Year DFS		No. of Patients	5-Year DFS		10-Year DFS				
Total	599	62	2	51	2	1189	57	1	42	2	0.83	0.71-0.95	.0095
ER-absent	84	64	5	60	5	142	45	4	34	4	0.49	0.33-0.72	.0003
ER 1-9	141	63	4	57	5	238	57	3	43	4	0.75	0.55-1.03	.072
ER 10+	374	60	3	46	3	809	59	2	43	2	0.95	0.80-1.13	.54

Abbreviation: DFS, disease-free survival.

*These *P* values are obtained from stratified (by trial) log-rank tests (single covariate Cox models).

breast-cancer-related events were 3.6% for the early chemotherapy group and 3.5% for the later chemotherapy group.

DISCUSSION

The optimal time to start adjuvant chemotherapy after primary surgery for early breast cancer is unknown. Previous retrospective studies suggested that early initiation might be beneficial. Improved disease-free survival was observed among breast cancer patients with one to three positive nodes who received adjuvant doxorubicin plus cyclophosphamide during the first 4 postoperative weeks versus patients who had received delayed chemotherapy.²⁰ Another small retrospective study demonstrated improved disease-free survival associated with chemotherapy initiation within 35 days of surgery compared with a later commencement.²¹ The Scandinavian Adjuvant Chemotherapy Study Group reported a significant increase in disease-free survival with perioperative cyclophosphamide (given immediately after surgery) compared with no adjuvant treatment but failed to show any benefit in a subgroup of patients treated in a single center where chemotherapy was delayed by 2 to 4 weeks because of radiation therapy.²²

The results from trials that compared a short duration of perioperative therapy with no adjuvant treatment might be useful for identifying subgroups of patients that could benefit the most from early initiation of adjuvant chemotherapy. In particular, a recent meta-analysis indicated that perioperative chemotherapy reduced the risk of relapse by 17% for women younger than 50 years of age.²³ Data on treatment effect according to ER status were not provided. In addition to the trials included in the above meta-analysis, the National Surgical Adjuvant Breast and Bowel Project demonstrated an advantage for perioperative thiotepa in terms of disease-free survival in premenopausal patients.²⁴

Sertoli et al²⁵ studied the impact of perioperative cyclophosphamide, epirubicin, and fluorouracil and concluded that ER-negative status was the most important predictor of the effect of perioperative treatment compared with no perioperative therapy. A statistically significant improve-

ment in disease-free survival was observed among patients with ER-negative disease, whereas no treatment effect was observed among those with ER-positive tumors.

Historically, patients were classified as having ER-negative (< 10 fmol/mg cytosol protein) and ER-positive (≥ 10 fmol/mg cytosol protein) tumors to facilitate prediction of response to endocrine therapies (eg, tamoxifen).²⁶ The relationship between ER and response to chemotherapy has received little attention. Two decades have passed since this relationship was debated with the presentation of conflicting reports.^{27,28}

The results of the present study show that premenopausal patients with ER-absent tumors and patients with tumors expressing some ER represent distinct populations with respect to responsiveness to early commencement of adjuvant chemotherapy. The magnitude of the effect of early initiation of chemotherapy was significantly greater for the ER-absent subpopulation compared with other patients (*P* = .0054). Although the classification of patients into an early initiation group based on commencement of chemotherapy before 21 days after definitive surgery was made before data analysis, this cut point was arbitrary and should not be considered as having special significance. In fact, many patients started chemotherapy during the weeks just preceding (23.4% of patients) and just after (29.2% of patients) the 21-day cutpoint.

Several models of tumor progression may provide biologic rationale for these results. A delay in the initiation of systemic therapy has been hypothesized to increase the probability of drug resistance of micrometastatic disease present at surgery.²⁹ Experimental kinetic data reported by Fisher et al^{30,31} support the hypothesis that administration of chemotherapy as close as possible to operation may improve patient outcome. In mice bearing both a large primary and a secondary tumor focus, the removal of the large primary tumor increased the labeling index of the distant focus compared with preoperative levels. In this animal model, cyclophosphamide was most effective against metastatic growth when the drug was administered either

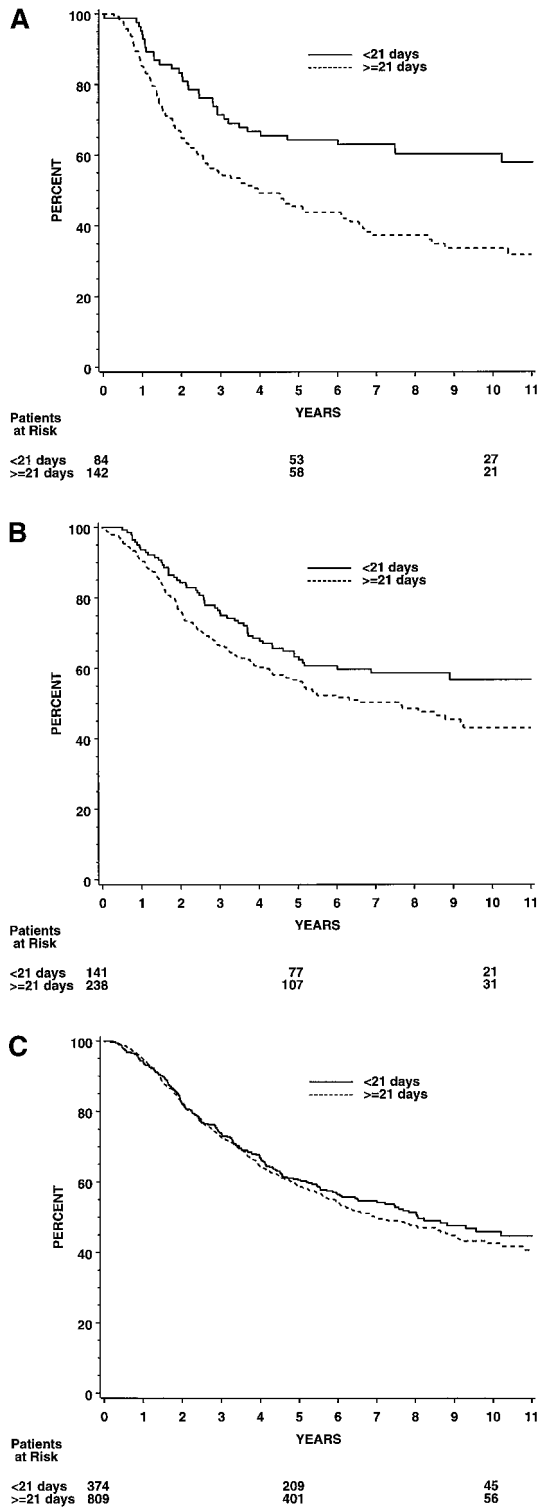


Fig 1. Kaplan-Meier curves for disease-free survival according to days from surgery to start of adjuvant chemotherapy (< 21 days or ≥ 21 days). Separate analyses are shown according to ER levels; (A) ER-absent; (B) ER-low; and (C) ER-positive. The median follow-up is 7.7 years.

Table 4. HRs From Multivariate Analyses: Disease-Free Survival According to the Timing of Commencement of Chemotherapy (< 21 days v ≥ 21 days)

ER Group	No. of Patients	HR	95% CI	P*
Total	1788	0.88	0.76-1.03	.121
ER-absent	226	0.60	0.39-0.92	.019
ER 1-9	379	0.92	0.63-1.33	.65
ER 10+	1183	0.91	0.76-1.10	.33

*Stratified by trial. Covariates include nodal status, tumor size, age, vessel invasion, and institution.

preoperatively or immediately after tumor removal compared with delayed administration.³⁰

Neo-angiogenesis is essential for growth of metastases, and only cells that induce the formation of new blood vessels can give rise to larger tumors. Removing the primary tumors in nude mice increased angiogenesis in the vascular bed surrounding metastases leading to their growth.³² The growth promoting effect on metastases of the removal of primary tumor seems related to the reduction of angiogenesis inhibitors such as angiostatin, which is a 38-kd fragment of plasminogen.^{6,32} Moreover, a significant correlation between detectable cancer cells in effluent venous blood during surgery for breast cancer and high vessel counts in the tumor⁹ suggest that increased angiogenesis is strongly involved in the metastatic process and that early start of systemic treatment may efficiently influence this process.

Several common anticancer agents have been shown to have anti-angiogenic activity. Low doses of methotrexate inhibited endothelial cell proliferation in vitro and inhibited neovascularisation by endothelial cell growth factor in the rabbit cornea assay.³³ Cyclophosphamide also has anti-angiogenic activity.³⁴ Therefore, it is possible that

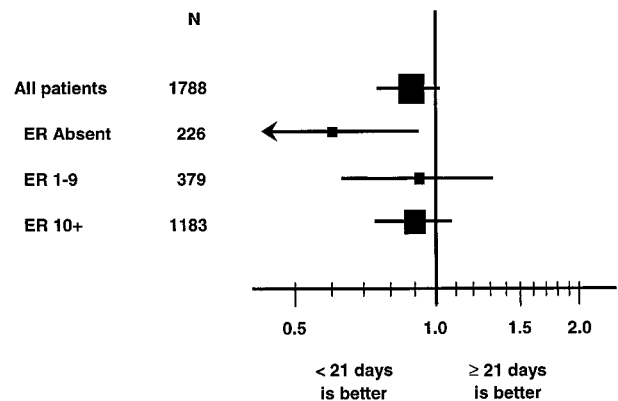


Fig 2. Multiple regression (Cox model) HR and 95% CI for disease-free survival comparing early (< 21 days) versus later (≥ 21 days) initiation of chemotherapy, both overall and separately according to ER content of the primary tumor.

conventional cytotoxic agents may exert a tumor suppressive effect partially through an anti-angiogenic mechanism and that early initiation of treatment may result in increased efficacy.

Tissue trauma caused by surgery is known to enhance tumor proliferation through the production of growth factors such as transforming growth factor alpha.^{8,35} Estrogens may amplify the effects of growth factors on tumor cells that are rapidly proliferating, such as those that do not contain ER.³⁶ Early initiation of chemotherapy may, therefore, be particularly relevant to inhibit the growth of tumors that are not susceptible to the effects of endocrine therapies because of lack of ER.

It is well known that chemotherapy exerts some of its effect via an endocrine mechanism in premenopausal women with ER-positive tumors.³⁷ The benefits of early initiation of chemotherapy may not be important for these patients because endocrine mechanisms are available to interfere with tumor cell growth. In contrast, patients with ER-absent tumors benefit exclusively from the cytotoxic mechanisms of chemotherapy. Early initiation of chemotherapy, therefore, may be relevant especially for this subpopulation of patients.

The results of this analysis are in apparent contradiction with those of the National Surgical Adjuvant Breast and Bowel Project Trial B-18, which demonstrated comparable disease-free survival for patients who received preoperative chemotherapy or postoperative chemotherapy.³⁸ Unfortunately, no analyses are available concerning treatment effects within the subpopulation of premenopausal patients with ER-absent tumors. In addition, the timing of initiation of postoperative chemotherapy relative to definitive surgery has not been presented.

The method used for ER assessment (dextran-coated charcoal assay)¹⁷ in the IBCSG studies may have influenced the results. More recently, the immunohistochemical (IHC) staining method has gained increasing popularity because it is suitable for use on fixed paraffin-embedded tissue and is

less expensive.^{39,40} Despite some controversies over the accuracy of IHC staining compared with that of dextran-coated charcoal assays in ER determination, IHC staining provides reasonable information on ER content and on the degree of heterogeneity of its distribution in the tumor tissue.⁴¹ In fact, there is significant correlation between the two methods in the majority of studies,³⁹⁻⁴² suggesting that the conclusions that are presented here may be extended also when an IHC analysis is performed. Additional research related to response to cytotoxics and heterogeneity of receptor distribution in the tumor might be useful.

The efficacy of adjuvant systemic therapy for early breast cancer depends on features of the tumor, the patient, and the treatment. Among treatment-related factors we have previously drawn attention to duration⁴³ and schedule.⁴⁴ The present results indicate that timing of initiation of chemotherapy might be important for premenopausal patients with tumors not expressing ER. Despite the impressive magnitude of the observed effect associated with early initiation of chemotherapy in this subgroup of patients, the compelling biologic explanations for this effect and the balance between groups with respect to prognostic factors, comorbid conditions, and non-breast-cancer-related mortality, the potential for bias still exists because of the retrospective nature of the evaluation. Further studies designed to confirm the importance of early initiation of chemotherapy for premenopausal patients with ER-absent tumors are required before widespread modification of current clinical practice.

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