Original article

Breast cancer recurrence dynamics following adjuvant CMF is consistent with tumor dormancy and mastectomy-driven acceleration of the metastatic process

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Purpose: The aim of this study was to better understand human breast cancer biology by studying how the timing of metastasis following primary resection is affected by adjuvant CMF (cyclophoshamide, methotrexate, 5-fluorouracil) chemotherapy.

Patients and methods: Discrete hazards of recurrence and recurrence risk reductions for treated patients relative to controls were analyzed for all patients enrolled in two separate randomized clinical trials [study 1 (386 women): no further treatment versus 12 cycles of CMF; study 2 (459 women): six versus 12 cycles of CMF] and a historical group (396 women: surgery alone) of axillary node-positive patients undergoing mastectomy.

Results: (i) Nearly all CMF benefit occurs during the first 4 years following resection/chemotherapy. (ii) The CMF recurrence rate reduction is largely restricted to two specific spans. These temporally separate recurrence clusters occur during the first and third year of follow-up, while the second-year recurrences are weakly affected. (iii) Prolonging adjuvant treatment from 6 to 12 months partially alters this recurrence timing, without appreciably affecting the overall recurrence rate. (iv) These effects upon the dynamics of post-resection occurrence are menopausal status-independent.

Conclusions: At least two different therapeutically vulnerable proliferative events, resulting in clinical appearance of two metastasis temporally distinct clusters of post-resection cancer recurrence, apparently occur during the administration of adjuvant chemotherapy. Metastases that transpire outside of these temporal windows are refractory to adjuvant therapy. The dynamics of both post-treatment recurrence risk and CMF effectiveness are similar for both pre- and postmenopausal women, suggesting that post-resection mechanisms by which chemotherapy prevents metastases are similar, but of different magnitude in pre- and postmenopausal women. These findings are consistent with a metastasis model that includes tumor dormancy in specific micrometastatic phases (single cells and avascular foci) and with the acceleration of the metastatic process by the surgical resection of the primary breast cancer.

Key words: adjuvant chemotherapy, breast cancer, growth model, tumor dormancy, recurrence

Introduction

Adjuvant systemic therapy was developed to increase cure frequency. Its success is usually gauged by assessment of the 10-, 15- or 20-year disease-free and overall survivals. This analysis is focused on cumulative event-free survival. The dynamics of post-surgical breast cancer resection (the timing of recurrences) also holds substantial and useful clinical

information. In this paper, we study the recurrence pattern following a probe to reveal biological features of the host–disease balance, as reflected by the post-surgical timing of metastatic cancer spread (recurrence dynamics). This conceptual approach is analogous to using X-rays or accelerated particles as probes to investigate the atomic structure. The probes are CMF (cyclophosphamide, methotrexate, 5-fluorouracil) adjuvant chemotherapy given after resection for either 6 or 12 months. The effect of these probes is examined in both pre- and postmenopausal breast cancer patients.

The hazard function reflecting the recurrence risk in a given year of follow-up [1] provides interesting details describing the biological behavior of metastases. The shape of the recurrence

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risk over time provides robust information about the interaction of cancer host and treatment. The hazard functions for local-regional recurrence and for distant metastasis following mastectomy alone demonstrate a double-peaked shape [2]. A detailed analysis of the first major peak [3, 4] shows that early metastatic cancer spread is correlated to menopausal status. Indeed, during the first 4 years after surgery, node-positive premenopausal patients show a two-peaked hazard function surge, while postmenopausal patients display a single peak. The timing and shape of these prominent early recurrence peaks suggest the occurrence of some triggering event, correlated to primary tumor surgical removal (in patients not undergoing surgery, disease outcome dynamics is intrinsically different [5]), resulting in recurrence synchronization. This effect is conspicuous among premenopausal node-positive patients. A metastasis development model incorporating tumor dormancy, stochastic transitions between specific micrometastatic phases and sudden acceleration of the metastatic process due to surgery [6, 7] is most consistent with these findings.

Overall, adjuvant CMF has a salutary effect. The recurrence risk of CMF-treated patients, which displays a double peaked pattern [8], is lower than the corresponding risk of patients undergoing only surgery. Most of the difference reflects the modulation of the early recurrences (first peak following resection) [8]. Therefore, we now investigate the effect of adjuvant CMF for either 6 or 12 months on breast cancer recurrence. Recent reports suggest that the impact of chemotherapy may depend on hormone receptor status [9, 10]. Unfortunately, patients cannot be segregated by hormone receptor status, because when the analyzed trials were performed, this parameter was not routinely assessed. Therefore, results from our analysis by menopausal status could reflect effects related to hormone receptor status.

The analysis of the recurrence timing during the first 4 years following mastectomy was performed among node-positive patients recruited in two randomized clinical trials on the effectiveness and optimal duration of adjuvant CMF, as well as an historical series of similar patients not receiving any adjuvant treatment.

Patients and methods

Two randomized clinical trials on the effectiveness and optimal duration of adjuvant chemotherapy carried out at the Istituto Nazionale Tumori of Milan, for which detailed information is reported elsewhere [11, 12], were analyzed. Moreover, data for all axillary node-positive patients undergoing mastectomy alone as primary treatment for operable breast cancer, who from 1964 to 1980 entered into different clinical trials, were extracted from the database of each individual trial, and compounded into a historical control group. The main patient characteristics are reported in Table 1.

Trials

The first randomized trial (study 1) involved 386 node-positive women who were randomly assigned to receive either no further treatment after mastectomy (control) or 12 monthly cycles of adjuvant combination chemotherapy (CMF12a). Adjuvant treatment consisted of cyclic administration of CMF (cyclophosphamide $100\,\text{mg/m}^2$ orally from day 1 to 14; methotrexate $40\,\text{mg/m}^2$ intravenously on days 1 and 8; 5-fluorouracil $600\,\text{mg/m}^2$ intravenously on days 1 and 8; followed by a 2-week rest period from days 15–28). In patients older than 60 years of age doses were reduced (methotrexate to $30\,\text{mg/m}^2$ and 5-fluorouracil to $400\,\text{mg/m}^2$). Chemotherapy was started 2–4 weeks after mastectomy.

The second randomized trial (study 2) involved 459 evaluable node-positive women who were randomly assigned to receive either six (CMF6) or 12 (CMF12b) monthly cycles of adjuvant CMF after mastectomy. Eighteen patients older than 65 years (CMF12b, 12 patients; CMF6, six patients) were started on the same dose reduction reported for study 1. Studies 1 and 2 were identical as regards all other study modalities.

Table 1. Main patient characteristics

	Study 1[a]		Study 2[b]		Historical control	Historical plus study 1 control
	Control	CMF12a	CMF6	CMF12b		
Total number	179	207	216	243	396	575
Size						
T1	95	103	102	119	50	145
T2-T3	84	104	114	124	346	430
Nodes						
1–3	126	140	131	149	216	342
>3	53	67	85	94	180	233
Menopausal status						
Premenopausal	87	104	163	160	164	251
Postmenopausal	92	103	53	83	232	324

[a]Study 1 involved 386 node-positive women who were randomly assigned to receive either no further treatment after mastectomy (control) or 12 monthly cycles of adjuvant combination chemotherapy (CMF12a).
[b]Study 2 involved 459 evaluable node-positive women who were randomly assigned to receive either six (CMF6) or 12 (CMF12b) monthly cycles of adjuvant CMF after mastectomy.
CMF, cyclophosphamide, methotrexate, 5-fluorouracil.

Primary tumor was treated by radical or modified radical mastectomy and no patient received postoperative radiotherapy. For both trials and the historical series, follow-up was carried out as follows: physical examination, biochemical tests and chest X-ray every 4–6 months and skeletal survey every 6–8 months during the first 5 years and once a year thereafter; and mammography once a year. A strict clustering of follow-up visits around fixed times from surgery did not occur and no special work-up was performed for any fixed term. Most of the recurrences were documented in patients requiring physical or radiographic examination because of symptom appearance between visits, regardless of the scheduled follow-up. In the presence of controversial clinical findings, examinations were performed more often than originally planned. Appropriate radiological, radioisotopic and surgical investigations were carried out whenever recurrence was suspected or clinically evident. Particular attention was paid to assess the recurrence time by carefully reviewing clinical, radiological and laboratory documentation.

Treatment failure (recurrence) was defined as the first clinically documented evidence of new disease manifestation(s) in either local-regional area(s) (i.e. chest wall, axilla and/or ipsilateral supraclavicular region) or distant site(s), or any combination of these sites (classified as distant). Contralateral tumors were considered second primaries [13]. Menopausal status, which was collected and recorded at the time of primary tumor diagnosis, was defined as postmenopausal if 1 year had elapsed since the last menstrual period.

Statistical methods

Recurrence-free survival (RFS) was considered as the time elapsed from the date of surgery to the first documented evidence of treatment failure; the time was censored at the date of last clinical evaluation, or at the date of death without recurrence or second primary cancer, contralateral breast cancer included. The RFS curves were estimated using the Kaplan–Meier method; comparison between the curves was performed by the log-rank test.

A Cox regression model was used to explore the possible variation during time of the hazard ratio of recurrence for CMF-treated patients versus controls. The model covariates were treatment and the interaction between treatment and a time-dependent binary covariate, categorized as ≤4 years or >4 years. Two models were fitted, one on the study 1 case series and another on the overall series.

The timing of the recurrence risk within 4 years from surgery was studied by estimating with the life-table method the recurrence hazard rate (RH), i.e. the conditional probability of manifesting recurrence in a time interval, given that the patient is clinically free of any recurrence at the beginning of the interval. We applied a discretization of the time axis in 3-month units (tmu). To descriptively evaluate treatment effectiveness, the recurrence relative risk reduction (rRRR) was calculated at each tmu, with $rRRR = (RHc - RHt)/RHc$, where RHc is the RH of control patients and RHt is the corresponding value of treated patients. Since both RH and rRRR display some instability owing to random variation, a Kernel-like smoothing procedure [14] was adopted to aid the reader in observing the underlying pattern, and the smoothed curves were represented graphically. Different time intervals were utilized in a preliminary smoothing analysis which showed that the 3-month interval is a good compromise between smoothing data and displaying the underlying structure.

To compare statistically the timing of recurrence in the groups of treated and control patients, we followed a similar strategy to that reported in a previous analysis [8]. Briefly, following Efron [15] the RHs were fitted by logistic regression models. The model predictor included: treatment, a three-knots cubic spline of time (expressed as mid-point of each 3-month interval) and the interaction term of treatment × time (for testing

the discrepancy between the RHs in the two groups). The significance of the interaction terms was tested by means of the likelihood ratio test.

All statistical tests performed were two-sided and the conventional 5% significance level was adopted. We used SAS, version 6 (SAS Institute Inc., Cary, NC, USA) and the S-PLUS 2000 Guide to Statistics, Vol. 1 and 2 (Data Analysis Products Division, MathSoft, Seattle, WA, USA) to perform modeling and statistical calculations.

Results

The RFS curves of all analyzed groups are reported in Figure 1. All subsets of patients receiving similar treatment show similar behaviors, in spite of some differences among population traits (Table 1). In particular, RFS curves of patients given 12 CMF cycles (CMF12a and CMF12b) are superposed, the control arm of study 1 and the historical series display very similar RFS curves, and the recurrence rates at 4 and 15 years are remarkably similar (50% and 54% at 4 years and 70% and 71% at 15 years).

The RFS curves for treated and untreated patients display early divergence, while the pattern becomes fairly parallel after ∼4 years. A Cox regression model was utilized to explore the possible variation during the time of hazard ratio of recurrence for treated versus control patients. Adjuvant treatment did not significantly influence recurrences beyond the fourth year, as shown in Table 2; indeed, the hazard ratio associated with a time >4 years approached the value of 1, and the respective confidence interval included the value of 1. On the contrary, a significant treatment effect was observed in the interval ≤4 years. Similar findings resulted from the analysis of the randomized study 1 only (CMF12a versus control) and from the whole series of patients (CMF12a + CMF12b + CMF6 versus historical + study 1 controls).

For study 1, the RH pattern during the first 4 years following surgery is reported in Figure 2, upper panel. The control
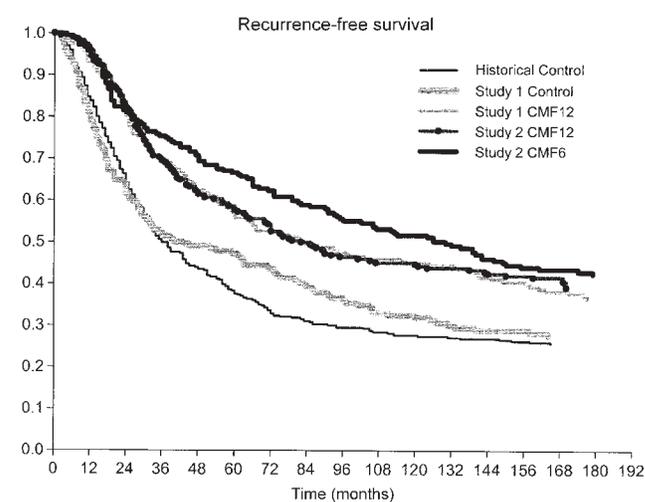

Figure 1. Recurrence-free survival curves for patients undergoing mastectomy alone or mastectomy plus adjuvant CMF (cyclophosphamide, methotrexate, 5-fluorouracil). Data are from the historical control series and from study 1 (no adjuvant treatment versus adjuvant CMF × 12 cycles) and study 2 (adjuvant CMF: six versus 12 cycles).

Table 2. Hazard ratios of recurrence (95% confidence intervals) for adjuvant CMF (cyclophosphamide, methotrexate, 5-fluorouracil)-treated patients versus controls estimated by Cox models

	Model including treatment only	Model including treatment and its interaction with the time-dependent covariate[a]	
	Years 1–15	Years 1–4	Years 5–15
Study 1	0.69 (0.54–0.88)	0.60 (0.44–0.82)	0.90 (0.43–1.88)
All patients	0.62 (0.54–0.71)	0.53 (0.45–0.62)	0.85 (0.56–1.29)

[a]See Patients and methods for explanation.

arm displays an asymmetrical curve with an early, quite steep rise, reaching the maximum recurrence risk at the end of the first year, followed by a slight slope lasting ~ 1.5 years and a successive definite lessening until the end of the fourth year. A similar pattern was reported for patients undergoing mas-

Figure 2. Hazard rate for recurrence for the two examined randomized studies. Upper panel: study 1 [control versus adjuvant CMF (cyclo phosphamide, methotrexate, 5-fluorouracil) × 12 cycles]. Lower panel: study 2 (CMF × 12 cycles versus CMF × six cycles).

tectomy alone [3], suggesting that even in the present series the recurrence risk curve may result, like in that study, from the superposition of differently timed peaks. By contrast, CMF12a patients show a more regular recurrence risk distribution, peaking at $\sim 25-27$ months.

In study 2, the CMF12b arm results in a RH curve remarkably similar to the corresponding curve of the study 1 patients, with a single peak at 26–29 months (Figure 2, lower panel). Even the recurrence risk of patients receiving six CMF cycles displays a single peak, which, however, is quite symmetrical and reaches a maximum at 17–20 months. Even if the RFS curves were not significantly different ($P = 0.363$, log-rank test), the RHs fitted in the two groups by the logistic model showed a significantly different pattern (likelihood ratio test P value for testing the interaction treatment × time $= 0.045$), suggesting that the risk peaks occur at different times.

As the recurrence risk for pre- and postmenopausal women not receiving adjuvant treatments proved to display a different time pattern, while tumor size and nodal status did not influence recurrence timing [3], the patients were analyzed according to menopausal status. This analysis may even obviate some unbalances in menopausal status distributions between groups (Table 1). The control arm of study 1 yielded hazard rate patterns similar to the corresponding patterns of the historical control, there being a few lesser differences compatible with the limited number of randomized patients (87 premenopausal, 92 postmenopausal) (data not shown). Therefore, to obtain a more suitable sized group of untreated patients, data from the control group of study 1 and from the historical series were merged in further analyses. For the same reason, data from the CMF12 arm of premenopausal patients from both studies were merged. For postmenopausal patients, comparisons were limited to study 2 women, as the merging of treated patients was precluded by the fact that in study 1 postmenopausal patients received a systematically lower dose than in study 2. Figure 3 reports the hazard rate curves by menopausal status and by treatment (CMF12, CMF6, control). In contrast to the different menopausal status-related recurrence risk patterns for control patients, premenopausal and postmenopausal women receiving adjuvant CMF are characterized by quite similar RH curve patterns. Moreover, for both menopausal groups the single observable peak occurs at the same time, which nevertheless is related to the treatment duration; it can be observed at 27–30 months for the 12-month treatment group and at 17–20 months for the 6-month treatment group.

To evaluate the time dependence of the ability of adjuvant chemotherapy to reduce the recurrence risk, the rRRR was assessed, and is graphically depicted in Figure 4, according to treatment and menopausal status. The curves are remarkably consistent within the groups, and this finding is a little surprising for postmenopausal patients, for whom high variability, owing to the low number of both population and events, was anticipated. Figures show that CMF6 patients of both menopausal statuses obtained a risk reduction for recurrences in the first and third year, while second-year recurrences were little or not at all affected by treatment (Figure 4, left lower panel).
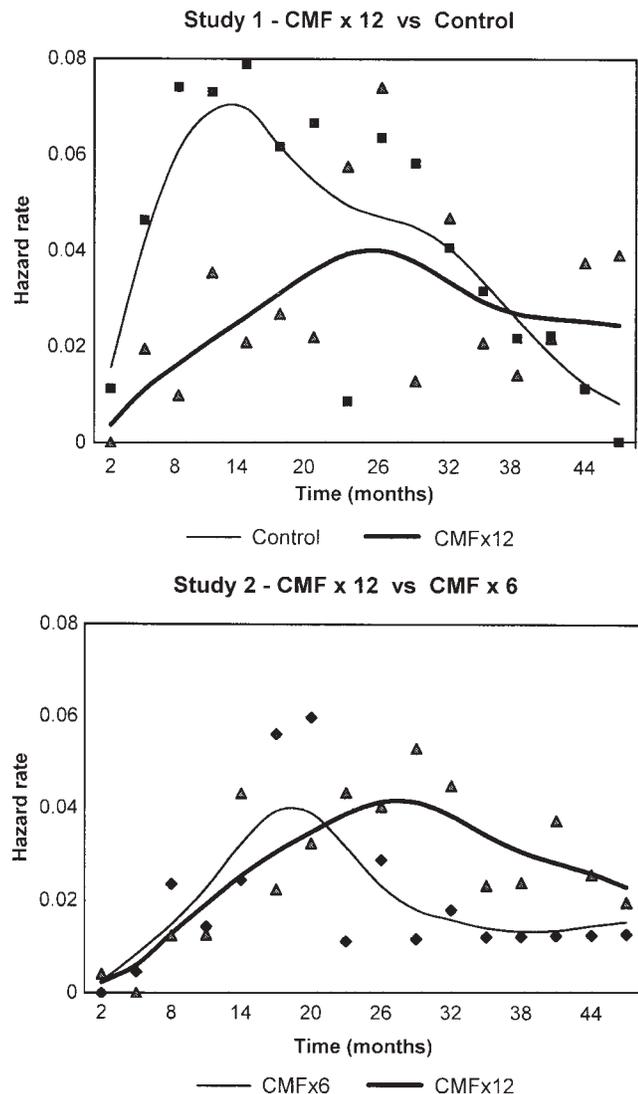
Premenopause - CMF x 12 vs CMF x 6 vs Control

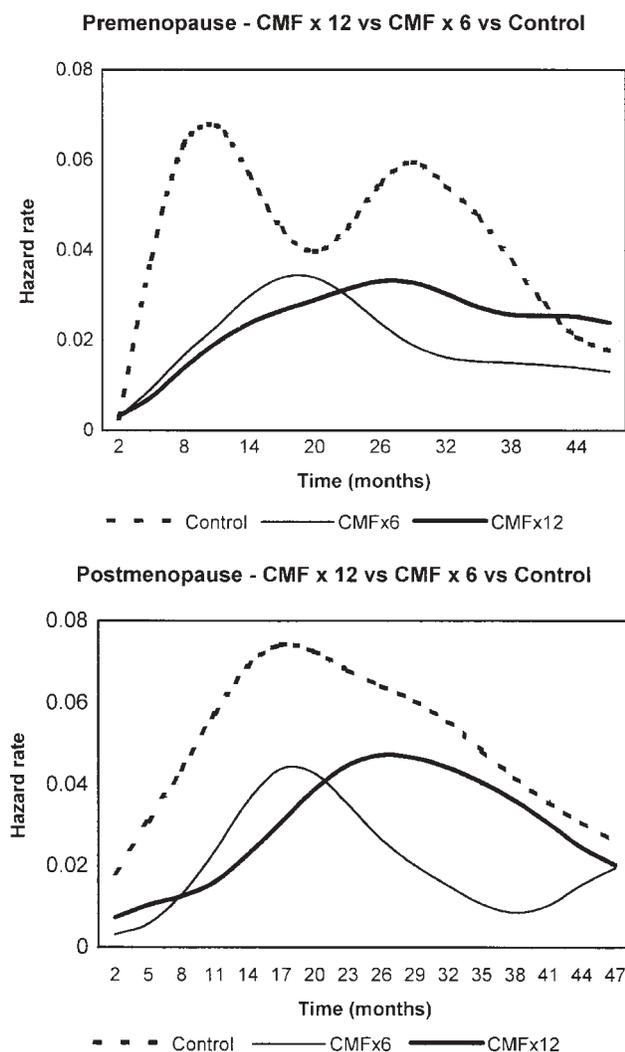**Postmenopause - CMF x 12 vs CMF x 6 vs Control**

Figure 3. Hazard rate for recurrence for premenopausal (upper panel) and postmenopausal (lower panel) patients. Premenopausal patients: 251 (historical plus study 1) controls; 264 (study 1 plus study 2) treated with CMF (cyclophosphamide, methotrexate, 5-fluorouracil) × 12 cycles; 163 (study 2) treated with CMF × six cycles. Postmenopausal patients: 324 (historical plus study 1) controls; 83 (study 2) treated with CMF × 12 cycles; 53 (study 2) treated with CMF × six cycles.

In contrast, prolonging adjuvant treatment until 12 months resulted in similar early results followed, however, by better outcome during the intermediate time and a worse course afterwards. This different pattern is more evident for premenopausal than for postmenopausal patients (Figure 4, left upper panel).

Discussion

The 15-year recurrence risk pattern for node-positive breast cancer patients undergoing mastectomy without or with adjuvant CMF was formerly investigated [2, 8], showing that the recurrence risk reduction due to CMF was restricted to early years following mastectomy [8]. In the present investigation, the analysis of the time dependence of the recurrence

hazard ratio for treated versus control patients in both randomized study 1 and the whole series provides evidence that adjuvant CMF does not appreciably influence recurrences beyond the fourth year. Therefore, most of the consequences of adjuvant treatment administration on recurrence rate and recurrence timing may be usefully investigated in this early time interval.

A preliminary observation needs emphasis: findings from different arms of the two studies are remarkably coherent and, in particular, the recurrence risk peak position remains unchanged when different subsets of similar patients are considered (e.g. CMF12a, CMF12b). Therefore, the possibility that peaks may occur by chance, i.e. resulting from some random fluctuation of the recurrence timing, should be considered very unlikely, although the finding needs confirmation to be considered unquestionable. This coherence strengthens the concept that different peak positions should be regarded as having biological significance and supports the biological and clinical reliability of comparisons between groups of patients from all the examined series, even between non-randomized groups.

For premenopausal women, 6-month CMF administration resulted in considerable rRRR during the first and the third year, corresponding to the two hazard rate peaks of untreated patients, while the second-year recurrences were virtually unaffected (Figure 3, upper panel; Figure 4, left lower panel). Therefore, our pharmacological probe, mainly acting on proliferating cells, reveals that for these patients at least two near contemporary early proliferative events should be assumed, resulting afterwards in the clinical appearance of two differently delayed and temporally separate (<1 versus ~2.5 years) metastasis clusters. Moreover, metastases clinically diagnosed at the intermediate times (~18 months), little or not at all affected by adjuvant treatment, indicate that they were in a rather refractory status during therapy administration. Furthermore, for premenopausal patients, the CMF6 and CMF12 arms display recurrence risk peaks ~10 months apart (17–20 versus 27–30 months) (Figure 3, upper panel), despite the observation that the longer treatment was equally as effective as the shorter one. Both findings, considered together, suggest that the shifted recurrence peak of the CMF12 arm may result from a delay in metastasis appearance due to the influence of treatment during the second 6-month period of therapy. This explanation fits well with the rRRR pattern reported in Figure 4, left upper panel, where the different time distributions of treatment effectiveness is quite evident. It is also in agreement with the previously suggested occurrence of a poorly responsive subclinical metastasis condition, resulting in clinical recurrence during the second year after surgery.

The study of data from postmenopausal patients is limited by the above-reported features of the examined trials that forced us to analyze quite small populations with a reduced number of events. Only data from study 2 patients were utilized, as CMF doses adopted in study 1 proved to be suboptimal [16]. In spite of this, results from older women are consistent with the corresponding findings from younger ones (Figure 3, lower panel;
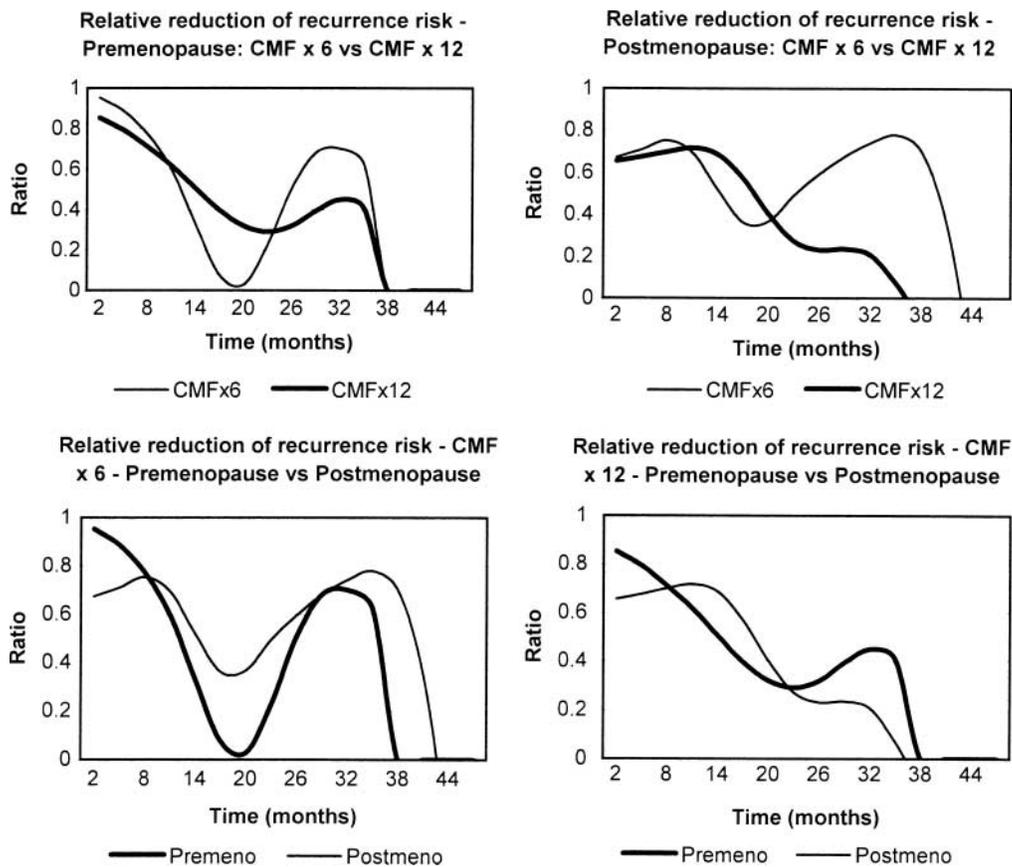
Figure 4. Relative reduction of the recurrence risk defined as rRRR = (RHc − RHt)/RHc, where RHc is the hazard rate for recurrence of control patients and RHt is the corresponding value of treated patients. Calculated values according to treatment and menopausal status.

Figure 4, lower panels). Even for postmenopausal patients, the 6-month treatment results in high rRRR during first and third year, while second-year recurrences are less affected (Figure 3, lower panel; Figure 4, left lower panel). In addition, the recurrence risk pattern of postmenopausal patients receiving the 12-month treatment is rather similar to the corresponding pattern for younger women (Figure 3, lower panel). The analysis of the rRRR provides a curve (Figure 4, right lower panel) that, even though it is less suggestive than that from premenopausal patients, displays a definite enough shape to be persuading, especially when one takes into account the fact that the dose-intensity administered in this group was lower than that delivered in the CMF6 group [17].

These findings add strong support to a previously proposed model for breast cancer metastasis development [6, 7], that is very briefly summarized here. Metastatic cells may reside in two dormant states: (i) single cells or nests containing a few cells, where most of them are non-dividing [18, 19]; and (ii) non-angiogenic micrometastases (and angiogenic ones in the presence of anti-angiogenic factors) that cannot grow more than 1–2 mm avascular foci [20]. Orderly transitions between these two dormant states eventually result in progressive appearance of clinical metastases. Also, surgical removal of the primary tumor may stimulate tumor cells to proliferate and/or may elicit angiogenesis [21, 22], thus resulting in sudden acceleration of the metastatic process. Indeed,

the transition rate between the first and the second dormant state may be induced by growth stimulating factor(s) [23] (the so-called Fisher effect) or some other mechanism [24], and the escape from the second dormant state may by accelerated by switching micrometastatic foci to active angiogenesis. This last phenomenon could have a double mechanism: the direct shift of the balance of positive and negative angiogenic factors by withdrawal of angiogenesis inhibitor(s) [25] (the so-called Folkman effect), or even changes in the steady-state dynamics of dormant micrometastases, may eventually result in switching of a subset of tumor cells to the angiogenic phenotype [26].

For patients not given adjuvant chemotherapy the model reasonably explains the observed recurrence risk pattern (Figure 3) [3]. The early peak of premenopausal patients could be ascribed to a considerable Folkman effect causing sudden escape from the second, avascular dormant state, while this effect would be very modest for postmenopausal patients. Other peaks may result from the Fisher effect, followed by the micrometastases 'spontaneous' switching to the angiogenic phenotype. The differences between premenopausal and postmenopausal patients may be explained by peculiar conditions relevant to breast cancer development, in particular conditions acting on angiogenesis, dependent on the host hormone milieu [27–30].

Our adjuvant chemotherapy probe now gives support to the main structure of the model. Indeed, at the time of treatment

administration two surgery-driven highly proliferative processes are underway, according to the model. Some just vascularized (via the Folkman effect) micrometastases are growing and will result in early (<1 year) clinical recurrence, and some formerly dormant single cells are actively proliferating (via the Fisher effect), fated to add new, temporarily avascular micrometastases to the second dormant state, eventually resulting in clinical metastasis appearance after a further tumor dormancy phase. Therefore, following effective cytotoxic chemotherapy targeting proliferating cells, recurrence rate reduction at different, not consecutive, times should be expected, as in fact occurs. The model assumes, moreover, that during treatment administration other metastases, such as those not engaged into transition processes between dormant states, display minor proliferative activity and may be not at all (for single cells [31, 32]) or little (for avascular micrometastases) responsive to cytotoxic drugs, thus escaping important treatment effects. The second-year recurrences, in fact poorly affected by adjuvant therapy, could arise by this mechanism.

Even the observed increased delay of recurrences for patients receiving 12-month CMF supports the model structure. It should be taken into account, indeed, that the growth time of metastases, reaching clinical size after dormancy interruption, has been estimated to be $\sim 6-8$ months [3, 33], and that the time needed for single cells to grow to avascular micrometastases ($\sim 10^5-10^6$ cells) may be considered quite shorter. Therefore, according to the model, during the second 6 months of CMF12 treatment administration almost all definitely chemosensitive events have been concluded. In these conditions, the major outcome of the second 6 months of adjuvant CMF treatment may reasonably be considered to be only tumor growth delay.

In both studies 1 and 2, postmenopausal patients showed less benefit from adjuvant CMF than premenopausal patients, and the difference was attributed to the lower dose level of administered drugs [16, 17], undoubtedly an important factor. However, findings from other randomized trials and the overview of the Early Breast Cancer Trialists' Collaborative Group [34] support the concept that older patients show less chemoresponsiveness to adjuvant chemotherapy than their younger counterparts. The model provides a biological reason for this finding. Indeed, even if the metastatic process is similar for both menopausal statuses, both the Fisher and Folkman effects may definitely be larger in younger women, resulting in higher rate of chemosensitive tumor foci for these patients, while for postmenopausal patients the different hormone milieu may result in higher load of micrometastases not sensitive to the accelerating effect of primary surgical removal.

Some other trials have been conducted to investigate the optimal duration of adjuvant non-anthracycline-based chemotherapy for node-positive patients. A meta-analysis of CMF trials confirmed that 6-month treatment is as effective as a longer duration therapy [34]. A single course of perioperative chemotherapy was reported to have some, but inadequate, effectiveness [35, 36], while it did not improve six cycle standard postoperative CMF [36]; three courses of adjuvant CMF

were inferior or similar to six courses, depending on age and estrogen receptor status [37]. Similar results were obtained for anthracycline-based treatments [38, 39], a suitable duration of which was suggested to be, for the AC (doxorubicin plus cyclophosphamide) combination, four cycles [40]. The model provides a fairly persuading rationale explaining all these findings. Among others, the two trials from the International Breast Cancer Study Group (IBCSG trial VI and VII) testing early versus late versus early plus late chemotherapy in node-positive patients [41, 42] deserve a particular mention. Indeed, as the temporal pattern of risk for breast cancer spread after primary breast cancer resection is very different for postmenopausal and premenopausal women, we should expect that the timing of adjuvant chemotherapy *vis à vis* the resection should have different impact for young and older women. This is precisely what has been found in IBCSG trials. Reintroduction of (likely suboptimal) chemotherapy in the second 6 months following surgery resulted in additional benefit only for premenopausal patients receiving three initial cycles in comparison with patients given six initial courses. Three early cycles of chemotherapy resulted in significant disease-free survival improvement for postmenopausal women receiving tamoxifen, while delayed chemotherapy did not. Both findings are in keeping with the model.

At least one main therapeutically relevant suggestion may be taken from this investigation. In CMF-treated patients, early refractory metastases represent a cluster clinically peaking at $17-20$ months, following a growth time that may be estimated to be $6-8$ months [3, 30]. Therefore, clinical trials could be designed for high-risk patients, testing the effectiveness of the administration of low-toxicity metronomic chemotherapy targeting both tumor cells and tumor endothelial proliferating cells, following standard adjuvant chemotherapy. This strategy, overlaid with multitargeted inhibition of platelet-derived growth factor receptor and vascular endothelial growth factor receptor, gave highly impressive results in a well known animal model [43].

Conclusions

The main results of this investigation are (i) the recurrence reduction by adjuvant CMF of specific, temporally separate recurrence clusters at the first and third year, and (ii) the finding that prolonging adjuvant treatment from 6 to 12 months partially changes the recurrence timing by delaying some recurrences.

These findings suggest that the recurrence pattern following mastectomy may result from the superposition of three metastatic clusters peaking at $\sim 7-10$, $17-20$ and $27-33$ months, respectively (Figure 5). The first and the third cluster are chemosensitive and are nearly obliterated by 6 months of CMF, while the intermediate cluster is relatively refractory to the administered treatment. The cluster sizes are quite different for pre- and postmenopausal patients. Young women display prominent peaks of chemosensitive recurrences at the first and third year, obscuring the second peak, while for older
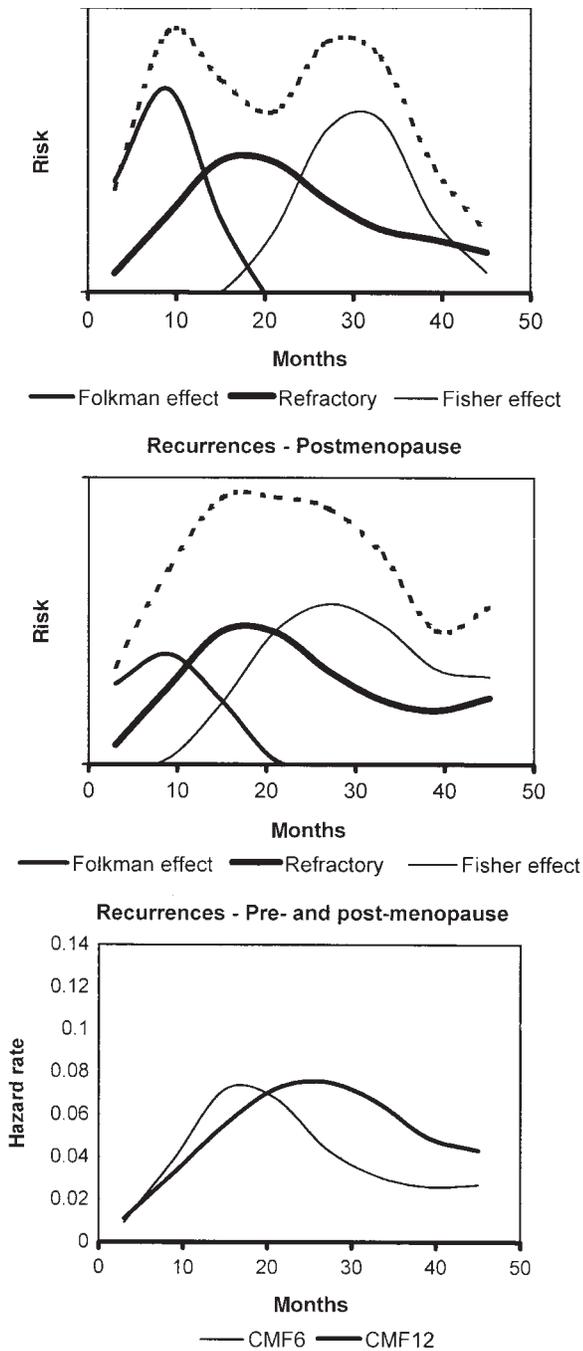
Recurrences - Postmenopause

Recurrences - Pre- and post-menopause

Figure 5. Outline of the pattern of CMF (cyclophosphamide, methotrexate, 5-fluorouracil) sensitive and refractory recurrences occurring in the first 4 years following mastectomy. The recurrence risk curve for premenopausal (upper panel) and postmenopausal (middle panel) patients is resolved in early, intermediate and late composing peaks. In both panels, the upper curve for patients not given adjuvant chemotherapy may be considered as the sum of a first peak resulting from the Folkman effect (see text), a second peak resulting from micrometastases nearly quiescent at the time of CMF administration and a third peak resulting from the Fisher effect (see text). Adjuvant CMF (6 months) obliterates the risk for early and late recurrences. The intermediate peak is shifted by ~ 10 months when an extra 6 months of CMF are administered (lower panel).

patients, by contrast, these two peaks are obscured by the dominance of the intermediate one.

Six months of adjuvant CMF chemotherapy is a probe that reveals the underlying structure of the recurrence pattern of patients undergoing surgery alone. An extra 6 months of CMF does not result in lower recurrence risk, but rather delays the appearance of the therapy-refractory risk peak identically in both pre- and postmenopausal women.

This picture is in agreement with the proposed metastasis development model that includes tumor dormancy in specific micrometastatic phases (single cells and avascular foci) and with the acceleration of the metastatic process by the surgical resection of the primary breast cancer. The model provides explanations for why adjuvant CMF is especially effective for premenopausal patients and why prolonging treatment duration from 6 to 12 months does not improve outcome. The model may be considered a conceptual framework within which established factors and mechanisms influencing adjuvant chemotherapy, such as optimal dose level and drug resistance, as well as other biological phenomena, may be fully integrated.

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