

## Hypothesis

# Computer simulation of a breast cancer metastasis model

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## Summary

Recent analysis of relapse data from 1173 untreated early stage breast cancer patients with 16–20 year follow-up shows that the frequency of relapse has a double peaked distribution. There is a sharp peak at 18 months, a nadir at 50 months and a broad peak at 60 months. Patients with larger tumors more frequently relapse in the first peak while those with smaller tumors relapse equally in both peaks.

No existing theory of tumor growth predicts this effect. To help understand this phenomenon, a model of metastatic growth has been proposed consisting of three distinct phases: a single cell, an avascular growth, and a vascularized lesion. Computer simulation of this model shows that the second relapse peak can be explained by a steady stochastic progression from one phase to the next phase. However, to account for the first relapse peak, a sudden perturbation of that development at the time of surgery is necessary.

Model simulations predict that patients who relapse in the second peak would have micrometastases in states of relatively low chemosensitivity when adjuvant therapy is normally administered. The simulation predicts that 15% of T1, 39% of T2, and 51% of T3 staged patients benefit from adjuvant chemotherapy, partially offsetting the advantage of early detection. This suggests that early detection and adjuvant chemotherapy may not be symbiotic strategies. New therapies are needed to benefit patients who would relapse in the second peak.

## Introduction

While benefit has been clearly demonstrated from adjuvant systemic chemotherapy and hormone therapy in clinical trials [1], survival gain of treated patients in comparison with controls is at most modest. This observation requires both a careful assessment of the progress that has been made thus far in breast cancer treatment, and a critical examination of the rationale of current therapies and experi-

mental treatment strategies. These issues have been recently and extensively discussed by Abeloff, Armitage, Lichter and Neiderhuber [2] and by Holmberg and Baum [3].

Abeloff et al. note the lack of agreement as to which of several tumor growth models is most useful to design chemotherapy regimens. However, it is clear to them that growth models provide a structure with which to formulate questions and to test chemotherapy strategies. They add that the future

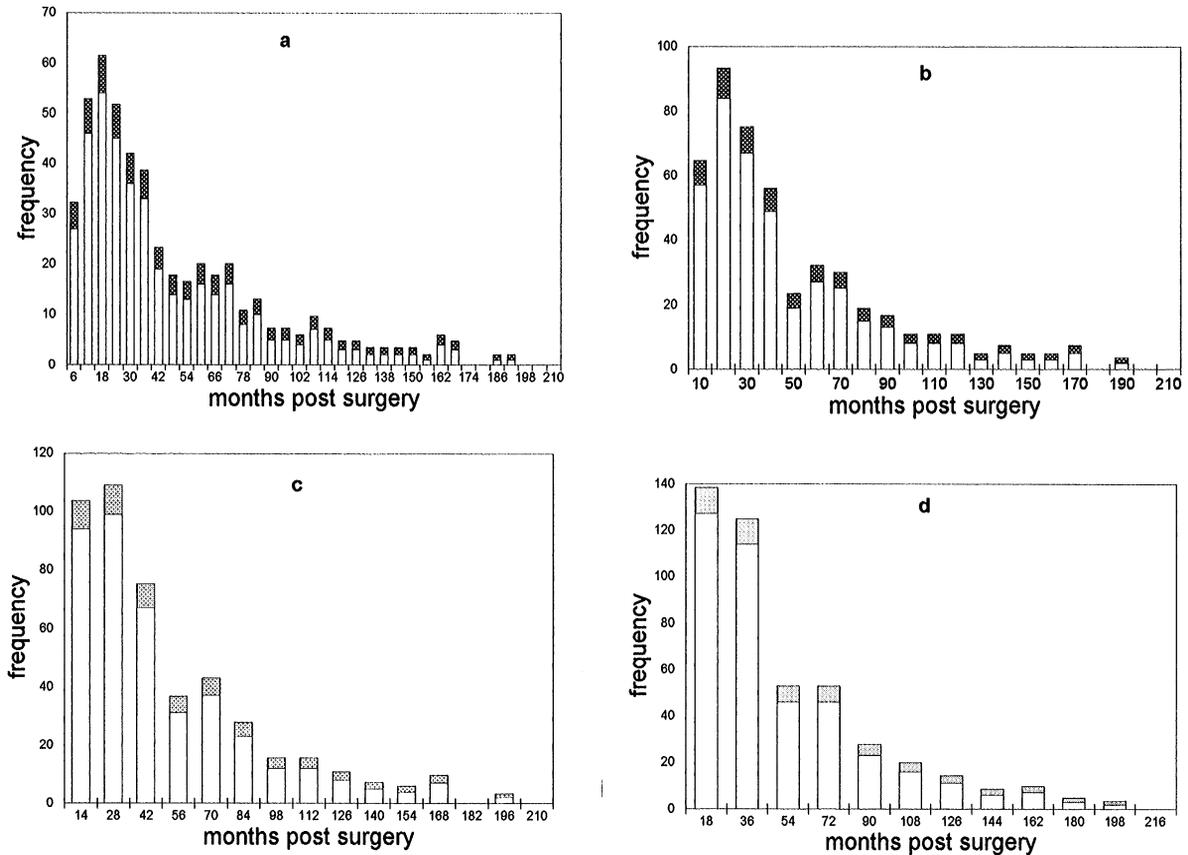


Figure 1. Frequencies of distant relapse data are shown with various size bins of grouping from 6 months (a), 10 months (b), 14 months (c), and 18 months (d). Each bin contains relapses above the previous lower grouping. For example, the 50 months bin in Figure 1b contains all relapses  $> 40$  months and  $\leq 50$  months. Shaded areas are standard deviations.

refinement of the clinical approach to chemotherapy will require attention to rigorously derived models in addition to the clinical empiricism that has to date characterized the approach.

Holmberg and Baum observe that the theory of breast cancer as a systemic disease from the inception with a window of opportunity when metastases are few and small has not resolved the problem of how to treat breast cancer. To make further progress, they propose that new theories are needed accompanied by distinct testable predictions. Data are cited showing an extraordinary polyphasic pattern in relapse hazard, suggesting that the act of surgery somehow initiates an initial wave of metastases. They note it may be necessary to regard pre-existing metastases not in a state of autonomous growth with kinetics predetermined by the primary

tumor, but as a complex organism existing in a state of dynamic equilibrium near chaos.

New clues to understanding breast cancer kinetics may be found in the unexpected structure of relapse hazard. Similar data had previously been reported by Demicheli et al. who examined Milan National Cancer Institute clinical data of relapse for patients treated by surgery only [4]. The Milan data show that the hazard of relapse has a statistically significant bimodal distribution for distant and local relapse but not for contralateral breast cancer. The Milan group conjectured that each peak would require a different biological explanation. Preliminary speculation was that the first peak corresponds to patients with actively growing metastatic disease and the second peak corresponds to patients with temporarily dormant disease at the time of mastectomy.

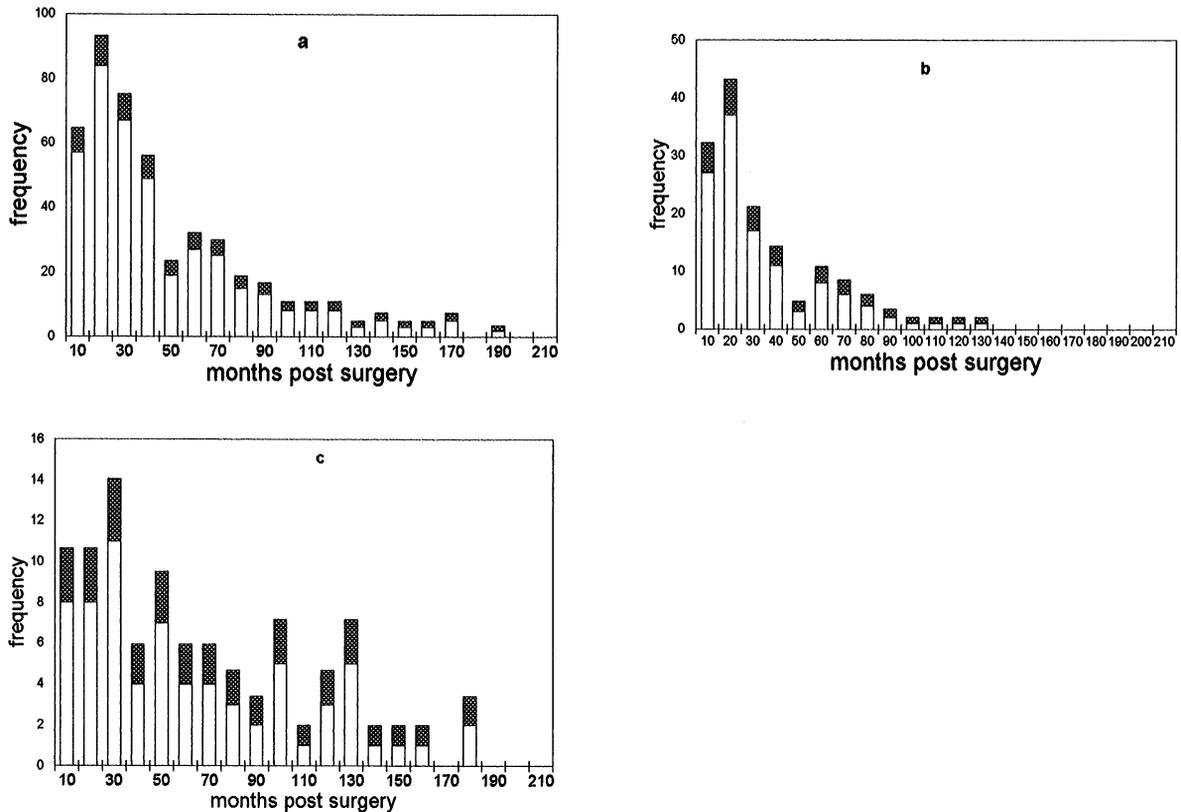


Figure 2. Frequency of distant (a), local (b), and contralateral (c) relapse. Shaded areas are standard deviations. Figure 2a is identical to Figure 1b and is duplicated to show the full series.

The Holmberg and Baum analysis and the Demicheli et al. analysis are consistent with one another, and challenge interpretation by any existing kinetic hypothesis. Because significant percentages of patients relapse in each of the two peaks, it is important to understand both. Clearly new theories are needed.

The intention of this study is to numerically simulate disease development from the detection and removal of the primary tumor to possible relapse based upon a proposed simple biological model. This model is developed to fit the Milan bimodal data. It is proposed that quantitative understanding of these processes will lead to insights concerning the distinctive biology of each peak of the polyphasic pattern seen, and this in turn will lead to new ideas concerning therapy. Our goal is not to develop an abstract mathematical construct that will artificially grow tumors to match relapse data. Instead,

we report a computer simulation attaching quantitative values to establish insights into biological processes guided by biology and clinical data.

### Patients

The data used to develop the simulation were provided by the Milan National Cancer Institute. All patients from January 1964 through 1980 who entered into three different clinical trials at the Cancer Institute, with mastectomy alone as the primary treatment for their operable breast cancer, were retrospectively evaluated. Before surgery all patients underwent complete physical examination, X-ray study of chest, skull, spine, and pelvis, bilateral mammography, ECG, complete hemogram, and routine biochemical tests. The primary tumor was treated by radical or modified radical mastectomy.

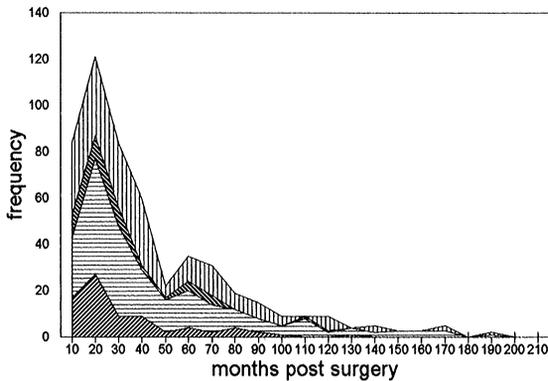


Figure 3. Frequency of distant and local relapses for premenopausal and postmenopausal patients (stacked). Bottom: postmenopausal local, next to bottom: postmenopausal distant, next to top: premenopausal local, top: premenopausal distant.

No patient received post operative radiotherapy or chemotherapy. After surgery, follow-up consisted of physical examination, biochemical tests, and a chest X-ray every 6–8 months during the first three years and once a year, thereafter plus skeletal survey and mammography once a year. 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. No special work-up was performed at the 5 year follow-up examination.

Treatment failure 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 contralateral breast. Relapse-free survival was considered as the time elapsed from the date of surgery to the first documented evidence of treatment failure or, for continuously disease-free patients, the date of last clinical evaluation.

1173 patients were included, and of these, 520 relapsed. Median age was 52 years with a range of 23 to 82. The tumor size distribution was T1: 459, T2: 628, T3: 86. There were 598 node negative and 575 node positive patients. Of the node positive patients, 342 had 1–3 and 233 had > 3 positive nodes. There were 516 premenopausal and 657 postmenopausal patients.

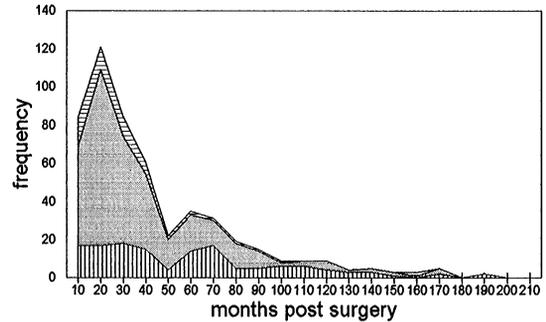


Figure 4. Frequency of relapses (distant plus local) for patients with T1, T2, T3 size tumors (stacked). Bottom: T1, middle: T2, top: T3.

**Results and discussion**

*Relapse data*

Usually, treatment failure data in cancer have been presented by way of disease-free survival models. However, since there are suggestions of structure in relapse sequence, there are better means of data presentation that more clearly show this. Demicheli et al. and Baum [5] have used a hazard model. Grouped frequency of relapse, a way of presenting raw treatment failure data with a minimum of computational manipulation, will be used here.

If the group bins are too small, there is little visual insight provided in graphical presentation. If the

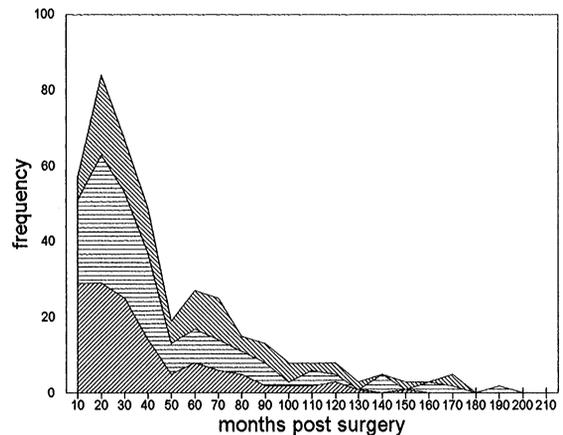


Figure 5. Frequency of distant relapses grouped by nodes positive (stacked). Bottom: > 3, middle: 1–3, top: zero. There are a similar number of relapse events among the three groups. That results from the combination of more node negative patients in the data base and lower relapse rate for node negative patients compared to node positive patients.

bins are too large, grouping will excessively smooth and can mask structure in data. Groupings of distant relapse events with bins of 6 months, 10 months, 14 months, and 18 months are shown in Figures 1a–1d. It would appear that there is structure in relapse patterns and also that a bin size of 10 months seems a good compromise between smoothing data and displaying structure. Thus, a bin size of 10 months was used.

Distant relapse frequency and local relapse frequency are shown in Figures 2a and 2b. There is a peak at approximately 18 months and another peak at 60 months but with a long tail. A nadir at approximately 50 months separates the two peaks. For distant relapse, the second peak includes 33% of relapses and is 32% the height of the first peak. For local relapse, the second peak includes 21% of relapses and is 22% the height of the first peak. Although there are fewer events, there seems to be no obvious multipeak pattern in the contralateral relapse data shown in Figure 2c.

This multipeak pattern shows no distinct dependence on menopausal status ( $p > 0.05$ ) or whether relapse is distant or local ( $p > 0.05$ ) as shown in Figure 3. Tumor size, however, significantly affects the ratio of first peak relapses to second peak relapses as indicated in Figure 4. For T1 tumors 50% of relapses are in the first peak, for T2 tumors 75% of relapses are first peak, and for T3 tumors 83% are first peak relapses ( $p < 0.05$ ).

As expected there are relatively more distant relapses as the number of positive nodes increases. Figure 5 shows dependence of distant relapse frequency on nodal status. There was a higher than expected incidence of distant relapse in the first 10 month period for patients with more positive nodes. The ratio of first 10 month distant relapses to second 10 month distant relapses significantly depends on the number of nodes positive. That ratio is 0.35 for node negative, 0.65 for 1–3 nodes positive, 1.0 for  $> 3$  positive nodes, and 0.69 for all nodal states ( $p < 0.05$ ). No other obvious difference in relapse pattern with nodal number has been found. In particular, local relapse had no detectable dependence on nodal status.

Figure 6 shows distant plus local relapse data presented both in disease-free survival and in frequen-

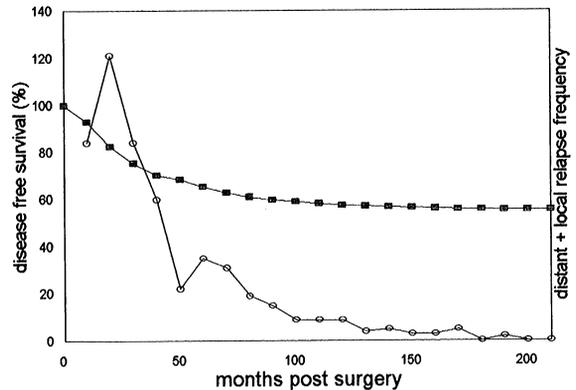
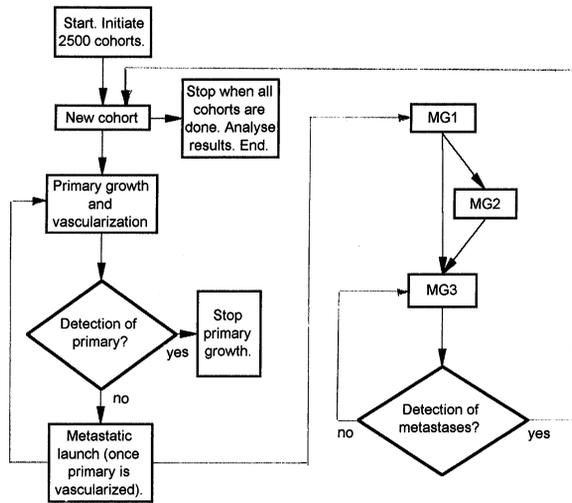


Figure 6. Milan National Cancer Institute data showing both frequency of distant plus local relapse (open circles) and population disease-free survival (solid squares). The 50 month nadir in relapse frequency corresponds to a subtle flattening in disease-free survival curve.

cy of relapse formats. This demonstrates that the double peaked structure would be easily missed if the data were only plotted as disease-free survival. The nadir at 50 months in frequency of relapse is seen as only a subtle flattening of the disease-free survival curve at 50 months.

Demicheli et al. have addressed whether these data are possibly an artifact of the follow-up protocol [6]. While it is difficult to quantify the influence of follow-up schedule on relapse frequency, there are several observations that can be made. If the follow-up protocol had a closely grouped schedule up to 4 years, then a gap in scheduled examinations followed in turn with a continuation of examinations, there would be reason to suspect that bimodality of relapse could be an artifact of follow-up. Likewise, if there was a special intensive work-up at 5 or 6 years, that could also account for a bimodal distribution of relapse. As described earlier, there were no such patterns in follow-up that would provoke such suspicion. Also, approximately 85% of recurrences were documented in patients requiring physical or radiological examination because of symptom appearance, regardless of the scheduled follow-up. In addition, since contralateral disease is likely a separate disease not biologically linked in time to the first primary [7–9], it provides a built-in control to test if bimodality is an artifact of follow-up. Contralateral breast cancer does not demonstrate a bimodal pattern. Considering this informa-



**Figure 7.** Flow chart of the computer simulation. 2500 cohorts are used to obtain acceptable repeatability of results. Once vascularized and continuing until detection and removal, the growing primary releases metastatic cells into single cell phase MG1. Metastatic development continues to avascular phase MG2 and then to vascularized phase MG3 until detected. Transitions are stochastic processes with probability augmented temporarily at the time of surgery.

tion, it is difficult to postulate how the bimodal relapse distribution could be an artifact of follow-up.

### Proposed model

To our knowledge, the bimodal distribution in breast cancer relapse has not been predicted by any existing models. It may result from some previously unknown processes that may reveal important biological activity. Thus we have proposed a simple model of metastatic growth [10] and now report computer simulation results meant to determine compatibility of that model with these bimodal data.

Based on animal and human data and theories of metastasis development [11–14], we have postulated that there need be at least three distinct phases of metastatic tumor growth with stochastic transitions from one phase to the next. The first phase is a single metastatic cell that has been shed from a primary tumor and has undergone the sequence of events required for it to arrive at a viable location where it may temporarily exist in an arrested

growth state or resting state. The second phase, well documented by angiogenesis studies [14–17], is the transient avascular metastable viable tumor limited in size to 0.1 to 0.5 mm in diameter. Subsequent re-growth after vascularization to the point of detection of relapse is the third and final phase. It is unknown what equations best describe the second and third growth phases. However, as a first approximation, Gompertzian or damped exponential growth has been assumed.

This basic metastatic growth simulation thus incorporates three distinct phases. Once the primary has achieved a minimum size corresponding to onset of vascularization, single metastatic cells are shed stochastically as the primary grows [15]. The first metastatic growth phase, called MG1, is a cell existing in a metastable state as a temporarily non-dividing single cell. A cell can escape from MG1 by a stochastic process. The second phase, called MG2, is avascular growth and is assumed to be Gompertzian. An MG2 deposit has maximum size of a few times  $10^5$  cells. Tumor density is conventionally taken as  $10^9$  cells per cc. Transitions from MG2 are also allowed as stochastic events. MG3, the final phase, is after a deposit becomes vascularized and growth occurs in a Gompertzian fashion until the deposit is detected. The simulation flow chart shown in Figure 7 indicates the various phases of the disease process and the relationship to the primary growth and detection.

Growth rate of the primary in this simulation is taken from a previously developed computer model of primary (not metastatic) growth [18]. The primary growth rate is consistent with doubling time data. For the present study, the role of the primary growth model is to numerically establish the metastasis pattern at diagnosis and surgery.

The metastatic launch algorithm in the model is stochastic. A large primary tumor grows for a longer time and will shed more metastatic cells than a smaller primary tumor. A tumor with accompanying positive nodes will have higher probability of metastatic launch.

Stochastic transitions are allowed between MG1 and MG2 and this probability is labelled P12 ('P one two'). Likewise, transitions are allowed between MG2 and MG3 and the probability of this transition

is labelled P23. Also, transitions are allowed directly from state MG1 to state MG3 with transition probability P13. Metastatic tumor growth rates were based upon data from the literature on animal and human tumors [19–23].

### Results of simulations

The model as outlined above with a stochastic progression from one phase to the next was capable of generating the second peak but not capable of generating the first peak. The first peak was too narrow and sharp to be fit with the model.

The most likely way to produce the first peak would be some precipitating event at or near the time of surgery to induce phase transitions. There is evidence that at the time of surgery, such enhanced temporary probabilities of transitions can occur. Gunduz et al. [24] and De Wys [25] have reported that tumor removal in animal models resulted in a stimulation of cell proliferation in metastatic foci. The labelling index was increased for a period of a few days resulting in an upward shift of the general growth pattern. This phenomenon was caused by a growth stimulating factor that may be found in serum of animals submitted to tumor removal. According to the authors, it is probably the result of the conversion of noncycling  $G_0$  cells into proliferating cells. A few tumors studied did not show any proliferation change, however. Prior effective treatment of tumor bearing mice completely suppressed labelling index increase [26]. Holmgren et al. report that some experimental tumors produce angiogenesis inhibitor factors that maintain distant micrometastases in the avascular phase [16]. Primary tumor removal in these tumors caused a switch of micrometastatic foci to the angiogenic phenotype. The most relevant kinetic finding in growing deposits was the considerable apoptosis reduction, without any proliferative index modification. In the computer model, these surgery related phase transitions are allowed in the immediate few days after surgery and are respectively labelled PS12 and PS23. If both events occur together the term is labelled PS13.

These terms allowed the model to include en-

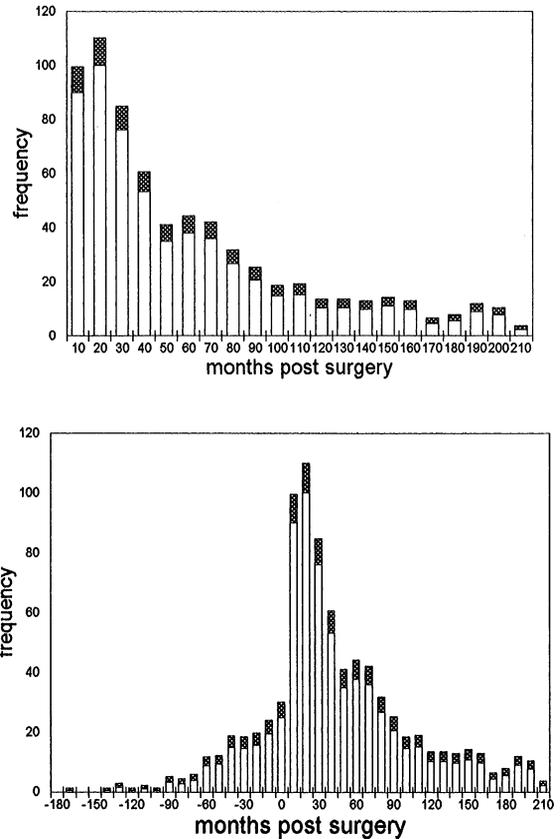


Figure 8. Simulation results shown in Figure 8a in same scale as Figures 2a and 2b. Metastatic disease detection in the years prior to detection and removal of primary disease is shown in Figure 8b.

hanced transition probabilities at surgery. As a result, the simulation was able to generate double peaked relapse histograms reasonably similar to the Milan data. The resulting numerical stochastic and surgery induced transition values that best fit all Milan data are:  $P12 = 0.0093$ ,  $P23 = 0.005$ ,  $P13 = 0.00007$ ,  $PS12 = 0.65$ ,  $PS23 = 0.1$ , and  $PS13 = 0.05$ . The probabilities for the  $Pij$  terms are per 5 day period and the  $PSij$  terms are only applicable immediately post surgery. The half-lives for  $Pij$  transitions are 345 days ( $\approx 1$  year) for P12, 691 days ( $\approx 2$  years) for P23, and 49,500 days ( $>$  patient lifetime) for P13.

The simulation results are shown in Figure 8a in the same format as Figures 2a and 2b to ease comparison. While the correspondence is less than perfect, several of the characteristics of this model are similar to the Milan data. There are two peaks. The

first peak is sharp and extends from 10 to 40 months with the top at 10–20 months. The second peak is at 50 to 70 months and has a long tail extending out to 250 months. The height of the second peak is 40% that of the first peak. The second peak includes 42% of relapses. The nadir between the peaks is not as deep and distinct in Figure 8a as it is in Figures 2a and 2b.

In the simulation, there are some cohorts that demonstrate detectable metastases before the primary is detectable. Figure 8b shows the simulation results including the period of time before surgery. Some metastatic events precede primary detection by ten years.

The second peak in Figure 8b is part of the continuum of spontaneous transitions  $MG1 \rightarrow MG2 \rightarrow MG3$  governed by P12, P23, and P13. This continuum begins well before detection of the primary and continues out to 20 years post surgery. The benefit of surgical removal of the primary is seen in Figure 8b. The sequence  $MG1 \rightarrow MG2 \rightarrow MG3 \rightarrow$  eventual relapse is analogous to a long pipeline. Surgery stops the launch of metastatic cells into MG1 or shuts off the spigot at the pipeline entrance. However, the pipeline is so long that the flow at the output does not begin to decrease until 5 years later. The beginning of this decrease marks the top of the second peak. Without surgery, relapses would continue until all patients eventually succumb [27]. In other words, instead of decreasing after 5 years, the relapse rate would continue to rise if the primary had not been removed. Also evident from Figure 8b is how patients who initially present with metastatic disease or some patients who present with cancer of unknown origin fit the model [15]. Those patient groups are seen to be early segments of the second peak continuum.

Figure 8b shows why it was not possible to simulate the first peak until the surgery associated enhanced transition terms (PS12, PS23 and PS13) were added. Detection of stage IV disease well before surgery indicates that the metastatic launch window has been open in excess of 10 years prior to surgery. Without a precipitating event at or near surgery, it is not likely possible to compress the over 10 year long metastatic launch window to fit the 4 year long first relapse peak.

The surprisingly high value of PS12 shows unanticipated metastatic tumor growth just after surgery. The first peak is associated with metastases that were in MG1 and MG2 at the time of surgery and stimulated to progress to MG2 and MG3 (governed by PS12, PS13, and PS23) at that time. The  $MG1$  and  $MG2 \rightarrow MG3$  at surgery (via PS13 and PS23) show up in large measure as the earliest relapses in the first peak, i.e., within the first ten months after surgery, and increase with positive nodes similar to data shown in Figure 5. The majority of remaining first peak relapses are due to transitions from MG1 to MG2 at surgery (PS12) and subsequent spontaneous transitions of MG2 to MG3 (P23).

### *Implications*

Campos [28] reported a ‘hump’ in survival of breast cancer patients treated with radical mastectomy between 1940 and 1955. That was a slight deviation between the third and fifth year from a fitted exponentially decaying survival rate. Other than that vague report, of the thousands of studies of relapse in breast cancer published in past years, no reports of bimodality have been made until Demicheli et al. and Holmberg and Baum. The demonstration in Figure 6 that the bimodal relapse pattern can be quite subtle if presented in disease-free survival format may explain that. Other large and mature patient data bases should be examined for confirmation of those findings.

While we have shown that our proposed model is consistent with relapse frequency of patients treated with mastectomy, we have not shown that our model is the best model. The stochastic model fits the various subsets of the Milan data reasonably well but before any accuracy is cited it will be more meaningful to test the model with a different data base that was not used in its development. On the basis of the computer simulations, we believe that angiogenesis and the removal of angiogenesis factors at primary surgery play a very important role in producing the bimodal relapse pattern but also realize that other biological factors may need to be considered.

As mentioned, it was initially speculated that the first peak in relapse frequency corresponds to patients with actively growing metastatic disease and the second peak corresponds to patients with temporarily dormant disease at the time of mastectomy. However, the model suggests that the situation may be more complex. There could be a slowly evolving orderly cascade of metastatic development from a single cell to avascular clumps to actively growing deposits. Coincident with surgery, approximately 65% of micrometastases in the single cell state and approximately 10% of micrometastases in the avascular growth state undergo sudden induced phase transitions. These surgery induced transitions result in the first relapse peak. The micrometastases that were unperturbed at the time of surgery continue to develop eventually producing the broad second peak. Our results agree with Holmberg and Baum that surgery disturbs the state of dynamic equilibrium of the complex organism described as micrometastases.

Adjuvant therapy is commonly recommended for either positive nodes or large tumor size since each are known markers for poor prognosis. This study suggests that metastatic growth activity is quite different in each case. On one hand, many positive nodes indicates that very frequently metastatic tumors are induced into MG3 from MG2 at the time of surgery and will relapse within the first year. Thus during the time frame when adjuvant chemotherapy is administered, i.e., the first six or so months after surgery, micrometastatic disease for patients with many positive nodes will be actively growing MG3 deposits. On the other hand, a different situation is predicted for patients with large primaries, i.e., many metastatic tumors are newly in MG2 immediately after surgery. The transient chemosensitivity and curability of tumors in MG2 and MG3 will likely differ. Thus the model predicts different treatment results for patients with poor prognosis due to large primary tumors compared to patients with poor prognosis due to many positive nodes. It is further predicted that there are different optimum therapies for each of those situations.

The observation that tumor size has a strong influence on the ratio of first to second peak relapses has important implications. Metastatic tumors that

without systemic treatment would be detected under the second peak would have been in states of relatively low chemosensitivity such as MG1 and mature MG2 at the time of neoadjuvant or adjuvant chemotherapy. Thus the benefit of adjuvant therapy may be confined to those patients who without treatment would have relapsed under the first peak. Modern emphasis on screening to detect smaller tumors would therefore inadvertently result in a gradual decline in the impact of adjuvant therapy. This may partially explain why there has been a plateau in recent years in mortality from breast cancer despite aggressive screening for early detection.

To illustrate by comparing T1 and T2 staged patients, 139/459 or 30.3% of T1 patients and 328/628 or 52.2% of T2 patients have relapsed in the Milan data. Therefore untreated T1 patients are 30.3/52.2 or 58% as likely as untreated T2 patients to ultimately relapse which is in agreement with common expectations. However, partially offsetting that advantage for T1 patients, adjuvant therapy only benefits 15% of T1 patients while it benefits 39% of T2 patients. For T3 tumors, similar analysis indicates that adjuvant therapy benefits 51% of patients.

This conclusion is ironic and disturbing. Adjuvant chemotherapy and early detection may not be symbiotic strategies. Adjuvant chemotherapy may benefit only those patients who were not cured by local therapy and who would relapse under the first peak. Smaller tumors, considered a good prognostic factor, tend to relapse under the second peak. Therefore as mammography and breast-self-examination find smaller and smaller tumors with improved long range prognosis, therapies become less and less effective, tending to reduce the overall gains of early detection.

New therapies will have to be developed to benefit patients who have significant probability to relapse under the second peak, such as those with small tumors and a few positive lymph nodes. Perhaps the class of angiogenesis-inhibiting drugs currently under development [17] or reintroduction of chemotherapy [29] will fill this need.

Unexpected tumor activity immediately after surgery results in first peak relapses. It should be possible to take advantage of this short duration in-

tense growth period to more effectively treat patients [30].

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