

## Proposal for a new model of breast cancer metastatic development

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### Summary

**Background:** The commonly accepted theory of breast cancer metastatic development assumes continuous tumor growth from tumor seeding until documentation of clinical recurrence. In particular, Gompertzian growth kinetics is currently the theoretical cornerstone of the natural history of breast cancer, and has been widely utilized for planning treatments.

**Materials and methods:** To verify agreement between findings and the implications of the continuous growth model, several published papers about the natural history of breast cancer after removal of the primary tumor were reviewed. Also, findings from animal models concerning metastasis biology were considered.

**Results:** The continuous growth model failed in important ways upon this critical reappraisal. As an alternative, the tumor dormancy hypothesis was considered to provide a more

reasonable description of tumor recurrence. Moreover, primary tumor removal was revealed as a potentially perturbing factor for metastasis development.

**Conclusions:** A new general outline of metastatic development of breast cancer incorporating tumor dormancy in specific micrometastatic phases, stochastic transitions between them, and start signals from surgery for micrometastatic growth was designed. The proposed model suggests new views concerning scheduling of current chemotherapy, new treatment approaches aimed at keeping micrometastases in a dormant state for the patient's entire life, and the careful reappraisal of the timing of surgery within the multimodal treatment of operable breast cancer.

**Key words:** Gompertzian kinetics, stochastic model, surgery signals, tumor dormancy, tumor growth

### Introduction

It should be recognized that both the long-term risk of recurrence – the appearance of metastasis even more than 30 years after curative primary tumor removal [1] – as well as the protean clinical behaviour following disease recurrence at any time are good reasons for the difficulty in composing a simple outline of breast cancer's natural history. It should also be recognised that current beliefs about this subject, which provide the conceptual basis for tumor growth modelling, are at best inadequate. Indeed, models and the biological concepts underlying them remain insufficient as foundations on which to develop broadly curative systemic treatments, and consequently, current strategies have not turned out to be breakthroughs. In addition, it can be verified that continuous Gompertzian growth kinetics, the cornerstone of the current theory of the natural history of breast cancer, fails in important ways when subjected to critical reappraisal. Here we will discuss findings from clinical and laboratory studies which should be considered relevant for modelling metastatic development in patients undergoing surgery for early breast cancer. The discussion will outline a novel view of this phenomenon, suggesting a new paradigm.

### Continuous growth crisis

The commonly accepted theory of metastatic development assumes that neoplastic growth begins with tumor seeding and continues until clinical recurrence is documented. The timing of local and distant recurrences and patient survival after disease relapse are usually explained by interpatient variability of continuous tumor growth. In particular, Gompertzian growth kinetics, i.e., near-regular exponential growth at small cell numbers with decelerated growth at larger cell numbers, has been widely utilized for planning treatment [2]. Continuous tumor growth, however, conflicts with several findings.

According to continuous growth, the later that tumors recur the more slowly they will have grown and the longer will be the survival after their recurrence. This relationship was examined by several clinical investigators. Some studies failed to find any statistical correlation between recurrence-free survival (RFS) and survival after recurrence (SAR) [3–7]. Other reports, which concluded that SAR correlates with RFS, limited their investigation to univariate analysis [8, 9]. The correlation was confirmed by multivariate analysis only for selected patient populations [10, 11], and when adjuvant chemotherapy had been administered to most [12] or all [13] patients. The latter finding is not surprising: it is well documented that most patients relapsing soon after

adjuvant chemotherapy are resistant to further systemic treatments and have poor prognoses [14]. Although the finding is of clinical importance, it cannot be generalised to explain the natural history of breast cancer, since adjuvant chemotherapy is a perturbing selective factor. Finally, a notable report on a large series of patients showed that when RFS was treated as a continuous variable in a multifactorial analysis, it failed to reach prognostic importance, while in a discrete categorisation RFS longer than two years favourably affected SAR [15]. Of note is the fact that all authors claiming a correlation between RFS and SAR used the two-year cut-off, a very short time in comparison to the long natural history of breast cancer. In summary, these studies do not support continuous growth, and at most, lead to no firm conclusion.

Recently, the continuous growth model was placed in serious question by the results of an investigation that some of us performed on local recurrences in 122 breast cancer patients undergoing mastectomy-alone without systemic therapy [16]. The study tested whether the continuous growth model was able to fit the 'all-or-nothing' phenomenon resulting from the regular patient follow-up of well-conducted clinical trials. Indeed, in this setting detection of local recurrence is preceded by a series of physical examinations during which no tumor is detected (i.e., clinically negative). The study provided evidence that the hypothesis of uninterrupted constant growth implies a statistically significant departure from observed data. Indeed, continuous growth yielded tumor sizes too large to be missed at the preceding negative physical examinations, and required growth rates significantly lower than those consistent with clinical data.

In addition, continuous growth was unable to explain the time distribution of first-treatment failure in 1173 breast cancer patients undergoing mastectomy alone [17]. Indeed, the cause-specific hazard function for local-regional recurrences and distant metastases presented an early peak at approximately 18 months after surgery, a second peak at approximately 60 months, and a plateau-like tail extending out to 15 years, i.e., the maximum period available for the analysis. This finding was confirmed by a similar analysis on 877 node-positive patients receiving adjuvant CMF [18]. The multi-peak hazard curve suggests that the process resulting in overt clinical (local or distant) recurrences has discrete features, and does not seem to be explicable by uninterrupted tumor growth. Tumor growth rates would need to be clustered around two or more mean values. To our knowledge, no data supporting such a discrete pattern have been published.

Lastly, the concept that clinical outcome is determined by tumor growth kinetics was challenged by the finding that in randomized studies of breast cancer screening, interval cancers showed the same fatality rate as tumors detected independently of screening [19, 20].

In conclusion, these findings cast serious doubt on the current understanding of breast cancer, which is based on Gompertzian growth kinetics.

### The tumor dormancy hypothesis

As an alternative to continuous tumor growth, the tumor dormancy hypothesis has been advocated to describe the development of breast cancer metastasis [21]. This hypothesis assumes that for some patients during the pre-clinical phase, micrometastases do not grow for a given period of time, depending on tumor and/or host factors. The immune system was considered the most likely of various possible sources of this phenomenon.

More recently, tumor dormancy was directly documented in animal tumor models [22–26] as well as in humans [27]. Furthermore, tumor dormancy fits well with current knowledge of metastatic development as a highly selective sequential step process involving multiple host-tumor interactions [28–31]. In particular, micrometastatic foci have been directly observed and studied [26, 27, 32, 33]. Initial tumor cell microfoci in mouse lungs consisted of one to thirty cells, most of which, if not all, were in G0 or early G1 phases of the cell cycle. A more advanced phase of lung micrometastasis consisted of avascular deposits forming cuffs around pre-existent vessels, thin layers on the pleural surface or spheroidal colonies of about  $10^3$ – $10^5$  cells, depending on tumor type. Avascular micrometastases were in a steady state, with a high proliferation index, high apoptotic index and no necrosis. These metastases exhibited rapid growth, temporally correlated with the onset of blood vessel formation, when inhibition of angiogenesis was removed. Therefore, an angiogenesis-based mechanism of tumor dormancy was proposed [26].

The above-cited clinical studies on local and distant recurrences after 'curative' surgery [16, 17] give further support to the tumor dormancy hypothesis. The findings of the study on local recurrences suggest that the assumption of partial or total growth interruption followed by a fairly fast growth phase could provide an alternative and more reasonable description of tumor recurrence than continuous tumor growth. Furthermore, tumor dormancy may satisfactorily fit the discrete metastatic development suggested by the multi-peak hazard function. Indeed, it may be assumed that the simultaneous micrometastatic wake-up which occurs after surgery is not unrelated to this local therapeutic procedure.

### Primary tumor removal and metastasis growth

The early debate about the role of surgery in breast cancer metastasis development was focused on tumor cell shedding during operating manoeuvres. Lately it has been realized that primary tumor removal can affect metastasis much more profoundly than was previously believed.

The perturbing effect of surgery on tumor growth kinetics has been documented in animal models. Partial [34] and total [35] tumor removal resulted in stimulation of cell proliferation in macrometastatic foci. The

thymidine labelling index (TLI) increase was temporary (a few days), and resulted in an upward shift of the growth curve, but did not seem to affect the general growth pattern. This phenomenon was caused by a growth-stimulating factor that was found in the serum of animals after tumor removal. Prior effective systemic treatment of tumor-bearing mice completely suppressed the TLI increase [36]. Only a few tumors failed to show any proliferation change, suggesting that the surgery-related proliferative impulse is likely to be a common phenomenon.

Recently, by more sophisticated laboratory techniques which allow the direct study of micrometastases, it was shown that some experimental tumors produced angiogenesis inhibitor factors that detained distant micrometastases in an avascular phase. Also, primary tumor removal caused a switch of micrometastatic foci to the angiogenic phenotype. The most important finding related to kinetics was the considerable apoptosis reduction temporally correlated with the onset of blood vessel formation. This single change, without any proliferative index modification, resulted in the growth of metastases [26].

All these findings suggest that surgery may be considered a major perturbing factor for metastasis development. It is likely that the natural history of untreated breast cancer differs considerably from current concepts of it, and that some metastatic processes are initiated at primary tumor removal.

### A new tumor growth model

There have been attempts to address some of the shortcomings of Gompertzian growth kinetics. For instance, irregular growth kinetics was assumed in the stochastic numerical Speer–Retsky model [37]. This unorthodox model was strongly criticized by Gompertzian growth kinetic advocates [2]. At present, however, enough new findings have accumulated to support a picture of the natural history of operable breast cancer, incorporating tumor dormancy in given micrometastatic phases [16, 26, 27, 32, 33], stochastic transitions between them [17] and start signals from surgery for micrometastatic growth [26, 34–36]. In the following, we propose a description of metastatic development, with the minimum of assumptions, to fit current knowledge.

Having passed through all early metastatic phases, breast tumor cells lodge within seeded tissues and become organised as single cells or nests containing a few cells [27, 32], a first biological state that may be designated S1. Most S1 tumor cells may be non-dividing [27, 32]. This quiescent state, corresponding to a first dormant state, lasts until tumor cell or seeded tissue changes induce cell proliferation. Like most phenomena involved in the metastatic process [28, 30], some of these changes may occur by genetic mechanisms, while others, involving extrinsic factors, may result from mostly unpredictable alterations of the complex local milieu.

Therefore, the transition from resting to growing tumor may be considered a stochastic process, and to occur with a given probability.

The micrometastatic growth phase following S1 can have different outcomes, depending on the ability to induce angiogenesis. Indeed, only a subset of primary tumor cells (as few as 4% to 10%) [34, 38], and presumably only a subset of metastatic cells, have the angiogenic phenotype. Therefore, non-angiogenic micrometastases (and angiogenic ones in the presence of anti-angiogenic factors) result in avascular foci, the estimated size of which is in a range of  $2 \times 10^3$  to  $1.5 \times 10^5$  cells [26, 33, 38]. This size corresponds to a second dormant state, S2. The growth pattern during this transition phase is unknown. It may be assumed, however, that growing tumor behaves like an integrated, organ-like entity, and that, like organs, it follows a Gompertzian growth.

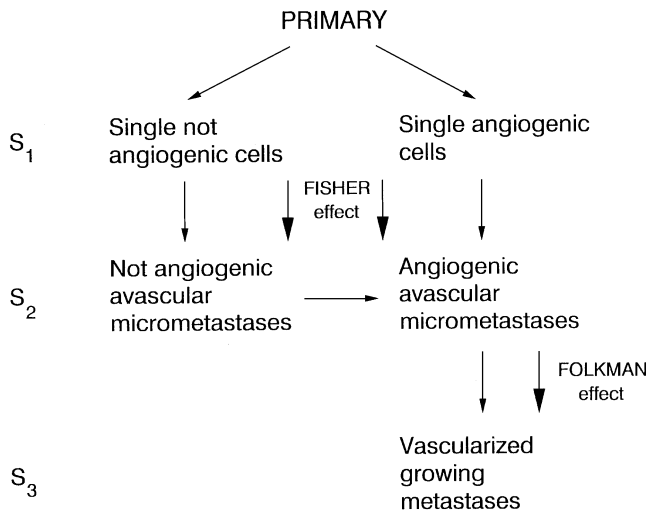
Micrometastases may escape dormancy by at least two mechanisms: 1) the removal of an angiogenesis inhibitor may release those already capable of inducing neovascularization, or 2) a subset of tumor cells within the micrometastases may switch to an angiogenic phenotype [26]. At least the latter process has a stochastic nature. Following the beginning of the vascular phase, Gompertzian growth may be assumed [40] until overt clinical recurrence. This growing phase may be designated S3.

This outline of the metastatic development of unperturbed breast cancer evolution may be summarized as a sequential passage through different biological states, namely S1, S2, and S3, with stochastic transitions from one to the next state. This orderly process may be perturbed by surgery. Indeed, primary tumor removal could stimulate S1 cells to proliferate, probably via the conversion of non-cycling G0 cells [35, 36], and/or remove the angiogenic inhibition that detains S2 cells in the avascular phase [26, 38]. Therefore, tumor removal will result in sudden acceleration of the metastatic process by significantly increasing the transition probability between S1 and S2 (we call this the Fisher effect), and between S2 and S3 (we call this the Folkman effect). We speculate that the early peak of the hazard function for local and distant recurrences in resected breast cancer patients [17] is generated by the Fisher and Folkman effects, joining to the ‘regular’ metastatic development of unperturbed disease. An outline of the proposed model is shown in Figure 1.

### Some model predictions

New paradigms lead us to reconsider the information we have, and to modify our approach to actuality. We will utilize the proposed model to reinterpret certain clinical findings. This effort can produce no more than an outline, since some aspects of it (e.g., the effect of chemotherapy on avascular micrometastases) are still unknown. Nevertheless, the exercise yields interesting results.

According to the model, a non-negligible number of



**Figure 1.** Breast tumor cells lodge within seeded tissues and are organized as single cells or nests containing a few cells, a first biological state named S1. Most S1 tumor cells may be non-dividing, and only a subset of them has the angiogenic phenotype. This quiescent state lasts until tumor cell or seeded tissue changes induce cell proliferation. The transition from resting to growing tumor is assumed to be stochastic and to occur with a given probability. Non-angiogenic micrometastases (and angiogenic ones in the presence of antiangiogenic factors) result in avascular foci, corresponding to a second dormant state, S2. Micrometastases may escape S2 dormancy: 1) the removal of an angiogenesis inhibitor may release those already capable of inducing neovascularization, or 2) a subset of tumor cells within the micrometastases may switch to an angiogenic phenotype. In the absence of angiogenesis inhibitors, micrometastases from S1 cells with angiogenic phenotype can induce vascularization. Following the beginning of the vascular phase, the micrometastases grow until overt clinical recurrence. This growing phase is designated S3. This orderly process may be perturbed by surgery. Primary tumor removal will result in sudden acceleration of the metastatic process by significantly increasing the transition probability between S1 and S2 (Fisher effect), and between S2 and S3 (Folkman effect).

micrometastases are quiescent after surgery, while others have been induced to actively grow, but at a progressively decreasing rate. Therefore, we can anticipate that the effectiveness of adjuvant chemotherapy will be restricted to a given subset of patients and to a limited time period after surgery. Furthermore, later recurrences caused by the growth of micrometastases that were quiescent during adjuvant chemotherapy should behave as in previously untreated patients, both in timing of the metastasis appearance and chemosensitivity. In fact, data in good agreement with such model predictions have been published. The effect of adjuvant chemotherapy on DFS and S, while significant was no more than modest, and prolonging drug administration beyond four to six months did not improve results [41]. It was also reported that a single chemotherapy course just after surgery produced a long-lasting reduction in recurrences, at least for some subsets of patients [42, 43]. Moreover, adjuvant chemotherapy was able to lower the probability of early relapse, with little or no effect on late events [18]. Lastly, patients recurring more than one year after the end of adjuvant CMF benefited from the same chemotherapy, analogous to previously untreated patients [44].

Unlike early-stage breast cancer, metastatic breast cancer is commonly believed to be incurable, and the extended survival of some long-term survivors is attributed to the indolent nature of their disease, which is usually related to tumor growth kinetics, as previously mentioned. The model, however, suggests a quite different picture. Indeed, should treatments be able to deplete the S3 level in some patients with metastatic breast cancer, the same conditions which occur for early stage will be resumed. Therefore, for some patients achieving complete remission (CR) the recurrence pattern could be determined by the transition probabilities between S1, S2 and S3, while for others it should result from metastatic tumor growth rates. Consequently, the model anticipates a recurrence risk curve presenting a long-lasting tail after an early high level, with long-term CRs, and yet susceptible to late failure. Data supporting model expectations were recently published in a report on 1581 patients treated for metastatic breast cancer at the M.D. Anderson Cancer Center [45]. The disease-free status after treatment was studied in 263 patients achieving CR, with a minimum follow-up period of more than 10 years. The analysis showed that after the initial five years there was a substantial decrease in disease recurrence and a change in the DFS curve. A few late failures (> 10 years) occurred, not explainable by classical kinetic arguments (median pretreatment disease-free interval: 19 months for all 1581 patients and 18 months for patients in CR > 5 years).

The model imputes most adjuvant chemotherapy failures to kinetics rather than to genetic drug resistance, which could have a moderate effect. Therefore, attempts to overcome genetic drug resistance, for instance by high-dose chemotherapy, are expected to achieve only modest improvements, and it will be interesting to learn the results of ongoing clinical trials on adjuvant high-dose treatments.

Following this new picture of breast cancer's natural history, current chemotherapy may be utilized with new schedules that could be more effective than standard administrations, at least in the adjuvant setting. For instance, reinduction chemotherapy (e.g., by reintroduction of the same regimen) could be considered; a report on this subject was recently published [46]. CMF reintroduction after regular adjuvant administration of three or six cycles to node-positive patients showed a trend toward therapeutic effect. This was statistically significant for premenopausal patients older than 40 ( $P = 0.04$ ). We regard this finding as investigationally significant, since the choice of single cycles of reintroduction chemotherapy administered three months apart may not be the most effective approach [46]. Computer-based simulations following the proposed model could be very useful in this area to suggest potentially optimal designs for clinical trials, which remain the testing ground for all hypotheses.

Finally, the model suggests more definite departures from the current tumor cell killing paradigm. Indeed, as microscopic dormant metastases may be analogous to

infection without disease (e.g., herpes zoster), ways to keep patients safely alive with dormant breast cancer microfoci should be considered. From this perspective, we believe that it will be important to determine, for instance, whether antiangiogenic therapy can prolong dormancy.

### Concluding remarks

A new model is proposed here for metastatic development in breast cancer. We also hypothesize that the concepts of tumor dormancy in specific micrometastatic phases, stochastic transitions between them and start signals from surgery for micrometastatic growth could be applied to different neoplasms. In particular, malignant melanoma, for which data supporting possible tumor dormancy have been reported [47], seems a good candidate.

The new paradigm forces us to reconsider our knowledge and to modify our traditional approach to research and treatment. Most importantly, the model suggests that new therapeutic approaches aimed at keeping micrometastases in the dormant state for the patient's entire life (e.g., with angiogenesis inhibitors) could be considerably more effective. Also, the model indicates that the timing of surgery within the multimodal treatment of operable breast cancer should be carefully reconsidered because of the potentially profound influence of primary tumor removal on metastatic development. Therefore, we believe that a treatment strategy based on the optimal combining and sequencing of surgery, chemotherapy and 'tumor sleeping therapy' should be seen as a way to achieve substantial improvements in breast cancer 'cure'.

However, models are not reality, but only means for understanding it. As such, they are most successful when, in the face of new evidence, their limits are discerned.

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