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Breast Cancer Dormancy Can Be Maintained by Small Numbers of Micrometastases

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Abstract

Late relapse of breast cancer can occur more than 25 years after primary diagnosis. During the intervening years between initial treatment and relapse, occult cancers are maintained in an apparent state of dormancy that is poorly understood. In this study, we applied a probabilistic mathematical model to long-term follow-up studies of postresection patients to investigate the factors involved in mediating breast cancer dormancy. Our results suggest that long-term dormancy is maintained most often by just one growth-restricted dangerous micrometastasis. Analysis of the empirical data by Approximate Bayesian Computation indicated that patients in dormancy have between 1 and 5 micrometastases at 10 years postresection, when they escape growth restriction with a half-life of <69 years and are >0.4 mm in diameter. Before resection, primary tumors seed at most an average of 6 dangerous micrometastases that escape from growth restriction with a half-life of at least 12 years. Our findings suggest that effective preventive treatments will need to eliminate these small numbers of micrometastases, which may be preangiogenic and nonvascularized until they switch to growth due to one oncogenic mutation or tumor suppressor gene inactivation. In summary, breast cancer dormancy seems to be maintained by small numbers of sizeable micrometastases that escape from growth restriction with a half-life exceeding 12 years. *Cancer Res; 70(11); 4310–7. ©2010 AACR.*

Introduction

The practice of administering systematic therapy (e.g., chemotherapy, endocrine therapy, or treatment such as trastuzumab) to breast cancer patients after apparently curative surgery is designed to eradicate their unseen micrometastases. Yet these therapies are not wholly effective.

Late relapse is well documented in breast cancer. In many cases, metastases develop without evidence of local recurrence, suggesting that the initial metastatic event occurred before the original treatment (e.g., in ref. 1, after 5 years postresection, 91% of primary recurrences are at distant sites). Further, the long duration between resection and relapse is thought inexplicable from continual growth of secondary cancers (1–5). Thus, the unseen micrometastases must undergo a phase of growth restriction where there is a balance between cell proliferation, cell death, and cell migration.

Dormancy in cancer is a period of growth restriction of patients' unseen micrometastases. It has been investigated for some time with the aim of designing optimal systematic therapies (e.g., refs. 6–11). Dormancy is a common phenomenon in breast cancer, yet the associated biological mechanisms are unknown.

Meng and colleagues (12) showed that at least one in three of 36 breast cancer patients examined 8 to 22 years postresection, who were clinically regarded as cured and who showed no other signs of disease recurrence, had circulating tumor cells (CTC) in their blood. The CTCs were viable but not proliferating, and died with a half-life of approximately 3 hours (12). Second and third blood tests, between 2 months and 2 years later, suggested that CTCs remained at a steady concentration (1–2 cells per 12 mL) in every patient; patients seemed to harbor micrometastases in a growth-restricted phase, which contain proliferating cells with the capacity to replenish populations of CTCs. Experimental models of metastasis have shown that dormancy may arise via at least three mechanisms: solitary "dormant" cells (13, 14), which may persist in a quiescent state for months or possibly years postresection (15); nonvascularized nonangiogenic micrometastases (16–20), restricted to a size of 1 to 2 mm in diameter (16); and vascularized micrometastases that are held at an equilibrium size by action of the immune system (21–23).

In this study, by investigating a probabilistic mathematical model and applying it to empirical data, we aim to

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identify clinically significant aspects of disease course in breast cancer dormancy. Our model features as its variable the number of unseen micrometastases in a postresection breast cancer patient. The micrometastases are subject to (*a*) growth event causing escape from growth restriction, for example, via new genetic or epigenetic mutations inducing switch to the angiogenic phenotype or escape from immune system surveillance; (*b*) disappearance, often attributed to random effects of high rate of cell apoptosis, immune attack, or the effects of systematic therapy; and (*c*) metastasis of disseminated cells to seed new micrometastases. (*c*) is plausible because of the number of CTCs in patients' blood and because metastases are often observed at multiple sites on late relapse, suggesting that residual cancer cells had the capacity to metastasize before the growth event. These events, all intrinsically random, shape the dynamics of disease progression.

Mathematical analysis and computer simulations led us to a hypothesis that explains the steady numbers of CTCs observed by Meng and colleagues: small numbers of micrometastases maintain dormancy in most breast cancer patients from approximately 8 years postresection. We used our model to establish quantitatively when data from two long-term follow-up studies of postresection breast cancer patients (12, 24) support this hypothesis.

Materials and Methods

(i) Model

Patients postresection can be in one of four states: all metastases in growth restriction as micrometastases ("dormancy"); one or more growing metastases ("growth"); detectable metastases ("relapse"); or no residual cancer ("clearance"). A patient without detectable or growing cancers at the time of resection progresses between states in the following manner.

At time t postresection, the number of micrometastases in the patient is $n(t)$. At time of resection ($t = 0$), there are n_B micrometastases, where n_B is the number of micrometastases seeded by the primary tumor taking nonnegative integer values with probabilities determined by a Poisson distribution,[8] mean $E[n_B]$. If $n_B > 0$, the patient has dormancy. As time increases, micrometastases undergo growth events [$n(t) \to$ growth] at steady probabilistic rate κ per micrometastasis per year. Once a growth event occurs, a micrometastasis transforms into a growing metastasis; then, it is only a matter of time until the growing metastasis becomes detectable and the patient relapses (growth $\to$ relapse in time τ; the size of the micrometastasis in growth restriction phase determines τ). Micrometastases each disappear [$n(t) \to n(t) - 1$] at steady stochastic rate μ per year, so a patient with dormancy may reach cancer clearance [$n(t) \to 0$ = clearance] whereupon there is no further risk to the patient.

In addition, suppose that the disseminated cells of micrometastases themselves metastasize [$n(t) \to n(t) + 1$] at steady probabilistic rate λ per micrometastasis per year. If $\lambda > 0$, the number of micrometastases may increase, which renders relapse more likely as there are more opportunities for growth events.

The capacity of cancer cells to metastasize can be altered in a reversible fashion depending on the microenvironment from which they originate; for example, bone marrow–derived human mesenchymal stem cells, when mixed with breast cancer cells, transiently increase their metastatic ability (25). The parameter that accounts for this is p_M, the frequency with which micrometastases are independently seeded in such environments. For a summary of parameters, see Table 1. For mathematical descriptions, see Supplementary Materials.

Supposing that μ and λ are constant is a parsimonious assumption in the absence of further information. Micrometastases are assumed independent: they do not influence one another's progression [although the primary tumor may inhibit growth of metastases (13), the 1,000-fold smaller size of micrometastases makes mutual inhibition among them improbable]. If the growth event results from genetic alterations, κ may increase with time either because the proliferation rate of cells within micrometastases gradually increases by selection or because the growth event requires an accumulation of genetic alterations; cases for which the assumption κ constant is valid are addressed in Section (iii) of Results. The time between growth and relapse (τ) was set at 3 years; the median doubling time in 199 cases of growing primary and metastatic breast cancer was 3.5 months (26), which gives an estimated time of 3 years for micrometastases to grow from 1 mm in diameter to a detectable size; study (5) suggests that metastasis growth time is only 9 months.

(ii) Summary statistics for fitting the model by Approximate Bayesian Computation

We used Approximate Bayesian Computation (refs. 27, 28; for review, see ref. 29) to identify regions of the parameter space of $\{\lambda, \mu, \kappa, p_M, E[n_B]\}$ that fit the following summary statistics from empirical data simultaneously: (*a*) The cumulative frequency of relapse from 5 years postresection from the Early Breast Cancer Trialists' Collaborative Group (24) to within a total squared deviation of ε (data used from EBCTCG was a 15-year follow-up study of 11,796 women ages 50 to 69 y; most high-grade recurrences occur in younger women and within 5 y). (*b*) The prevalence of dormancy (defined as the fraction of nonrelapse patients with micrometastases) exceeds 36% at 13 years postresection (to agree with the average time of patient examination in ref. 12 and the fraction of CTC-positive patients in ref. 12). An additional hypothetical summary statistic was also investigated: (*c*) The frequency of cancer clearance among nonrelapse patients exceeds 15% at 13 years postresection. (*c*) is related to the accuracy with which the assay in ref. 12 detects dormancy in patients; for example, if the accuracy is such that between 35% and

[8] L. Willis, T.A. Graham, T. Alarcón, I.P.M. Tomlinson, K.M. Page. Predictions for therapy from a probabilistic model of cancer dormancy. In preparation. Available upon request.

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Table 1. Parameter meanings and information related to their empirical values in breast cancer

Parameter	Meaning	Information from literature
$E[n_B]$	Average number of micrometastases at or soon after resection	The number of micrometastases on resection n_B is Poisson distributed. This assumes the n_B micrometastases are seeded by the primary tumor.
λ	Metastasis rate of cells of micrometastases (per year)	$<10^6$ cells from cancer cell lines are administered to mice to initiate tumors (19, 39). In breast cancer dormancy, patient data suggest that at least 1.5×10^6 CTCs are disseminated per year (see NOTE). Then CTCs may be of a number large enough to seed micrometastases at a consequential rate per year.
μ	Disappearance rate of micrometastases (per year)	Spontaneous regression of tumors is well documented (40–44): In a large study of benign colon polyps, 18% disappeared within 5 y (45), a rate of 4% per year (here $\mu > 0.04$). In early years, disappearance may be due to therapy.
κ	Growth event rate of micrometastases (per year)	Mechanism unknown. Possibly due to genetic alterations causing angiogenesis (16) or escape from immune control [ref. 23; Section (iii) of Results].
p_M	Frequency with which micrometastases are seeded in environments that confer metastatic ability to their cells	The metastatic ability of breast cancer cells is transiently increased when mixed with human mesenchymal stem cells derived from bone marrow (25). Therefore, $p_M < 1$.

NOTE: Calculated from the lower bound on CTCs of 500 and the CTC average death rate of <3 h (16), the number of CTCs disseminated per year exceeds $500/3 \times 24 \times 365 = 1.46 \times 10^6$ [see Section (iv) of Results].

100% of patients with dormancy test positive for CTCs, then we expect cancer clearance among nonrelapse patients to exceed approximately 15% at 13 years postresection.

By using data from these studies, we made two important assumptions about the empirical data: (*a*) that the CTC count of Meng and colleagues (12) is representative of dormancy (it remains to be proved that these CTCs are shed from metastases that are derived from the primary tumor or are eventually responsible for relapse) and (*b*) that relapses >5 years postresection are due mostly to metastases that have undergone growth restriction phase, rather than to new independently initiated tumors.

(iii) Materials

The model was run numerically in two ways: (*a*) by the Gillespie algorithm (ref. 30; a method for simulating stochastic models that is exact in the sense that it avoids finite step approximations) and (*b*) by numerically solving appropriate differential equations (available on request).

Approximate Bayesian Computation proceeded as follows. Points (2×10^8) were sampled from a uniform prior distribution over the parameter space $\{\lambda, \mu, \kappa, p_M, E[n_B]\} < [0, 0.3] \times [0, 0.3] \times [0, 0.1] \times [0, 1] \times [0, 50]$. For each sample from parameter space, the summary statistics were simulated by running the model 8,847 times (8,847 is the approximate number of patients remaining in the EBCTCG study after $\tau = 3$ years, by which time we supposed all patients with growing metastases on resection to have relapsed). If the simulated summary statistics fitted (*a*) and (*b*) of Section (ii), the point was "accepted"; otherwise, it was "rejected." Frequency plots of accepted points approximate the posterior distribution of parameters given the prior distribution and data (*a*) and (*b*).

The following estimates of $P(D, f)$, the number of cells that proliferate per hour in a micrometastasis of diameter D (mm), where f is the fraction of cancer cells that are viable (i.e., able to proliferate) in the vascularized region of micrometastases, when micrometastases are (*a*) vascularized or (*b*) nonvascularized, were used in Sections (iii) and (iv) of Results. If a micrometastasis is approximately spherical of diameter D (mm), the volume of its vascularized region is $\pi D^3/6$ when fully vascularized, and $\pi(D^3 - (D - 0.2)^3)/6$ when nonvascularized (it is well documented that only cells within 0.1 mm of a blood supply are sufficiently supplied by oxygen and nutrients to proliferate). The vascularized region of a micrometastasis is assumed to be packed with cancer cells, as supported by the high proportion of Ki67+ cells (a proliferative maker) in lymph node micrometastases from breast cancer patients (see Supplementary Materials). Each cancer cell occupies a spherical space of diameter 20 μm and the viable fraction f proliferate (*i*) once per 24 hours (31) giving a high estimate of $P(D, f)$, or (*ii*) once per 5 days giving a medium estimate of $P(D, f)$. [Cell proliferation rates in nonvascularized tumors are comparable to proliferation rates in vascularized tumors (16, 19), although there is evidence that the proliferation rate is lower if the micrometastasis is growth restricted by the immune system (23).] A high estimate of $P(D, f)$ is therefore $1/24 \times 1/(4\pi 10^{-6}/3) \times \pi D^3/6 \times f < 5{,}000 f D^3$ [(*a*)—vascularized] or $10^4 \times \pi(D^3 - (D - 0.2)^3)/6 \times f < 5{,}000 f(D^3 - (D - 0.2)^3)$ [(*b*)—nonvascularized]. Similarly, a medium estimate of $P(D, f)$ is $1{,}000 f D^3$ [(*a*)—vascularized] or $1{,}000 f (D^3 - (D - 0.2)^3)$ [(*b*)—nonvascularized].

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Results

(i) The number of micrometastases tends to 1 after long time periods

Early mathematical analysis and computer simulations show that the frequency distribution of the number of micrometastases n among dormancy patients converges to a distribution that no longer changes with time. Further, for the whole volume of parameter space, this limiting distribution is peaked at $n = 1$ micrometastasis (detailed in Supplementary Materials and ref. 32). The question that determines the validity of our hypothesis is "Do parameters that fit the summary statistics (*a*) and (*b*) of Section (ii) of Materials and Methods also give convergence to a sharply peaked stationary distribution within approximately 8 years of resection?"

(ii) Long-term dormancy is maintained by 1 to 5 micrometastases when the growth event occurs with a half-life of less than 69 years

The model's fit to relapse data (*a*) in Section (ii) of Materials and Methods is shown in Supplementary Material for the total squared deviation $\varepsilon = 0.001$ and $\varepsilon = 0.0005$. The following results are for $\varepsilon = 0.001$; they are only marginally perturbed for $\varepsilon = 0.0005$ and $\varepsilon = 0.00025$ (results not shown).

Shaded regions of Fig. 1 show, for all accepted values of κ, the frequencies with which dormancy patients have less than or equal to 3, 5, or 10 micrometastases at 10 and 20 years postresection; the final column shows the frequencies with which patients have more than 20 micrometastases at 10 and 20 years postresection. We see that for any accepted value of κ exceeding 0.01 (equivalently, thinking of growth restriction as "life" and escape from growth restriction as "death," micrometastases escape from growth restriction with a half-life of 69 years or less), dormancy patients surviving beyond 10 years postresection have less than or equal to 5 micrometastases with a probability of at least 60%. For any accepted value of κ exceeding 0.03 (micrometastases escape from growth restriction with a half-life of 23 years or less), more than 80% of dormancy patients have between 1 and 3 micrometastases from 10 years postresection. For any accepted value of $\kappa < 0.003$ (micrometastases escape from growth restriction with a half-life >230 years), dormancy patient have more than 20 micrometastases at 10 years postresection with probability >20%.

There is a reciprocal relationship between accepted values of κ and $E[n_B]$ (see Fig. 2, $\kappa \times E[n_B] \approx 0.06$). κ is greater than 0.01 (or 0.03) if and only if $E[n_B]$ is less than or equal to 6 (or 3 respectively), and κ is less than 0.003 if and only if $E[n_B]$ is greater than 20. This suggests a second way of validating or invalidating the hypothesis: by finding the average number of dangerous micrometastases among patients who do not have detectable or growing metastases on resection.

The medians and the ranges of the marginal posterior distributions generated by Approximate Bayesian Computation are in Table 2 (see Supplementary Materials for plots of the marginal posteriors). The range of the marginal posterior for κ is $[9 \times 10^{-4}, 0.06]$, meaning micrometastases escape from growth restriction with a half-life exceeding 12 years. The lower bound is determined by the upper bound (50) of the prior distribution over $E[n_B]$; as the true upper bound for $E[n_B]$ is unknown, calculating a Bayes factor to assess whether $\kappa > 0.01$ would be uninformative. However, if statistic (*c*) of Section (ii) of Materials and Methods is added to summary statistics (*a*) and (*b*), the range of κ is dramatically refined to [0.005, 0.06] (see Fig. 2) and then the following statement can be made: In excess of 40% of patients with dormancy who survive beyond 10 years postresection have less than 5 micrometastases (see Fig. 1). Information not only from dormancy-detecting assays, but further on the accuracy of these assays, would allow a more informative statistical analysis.

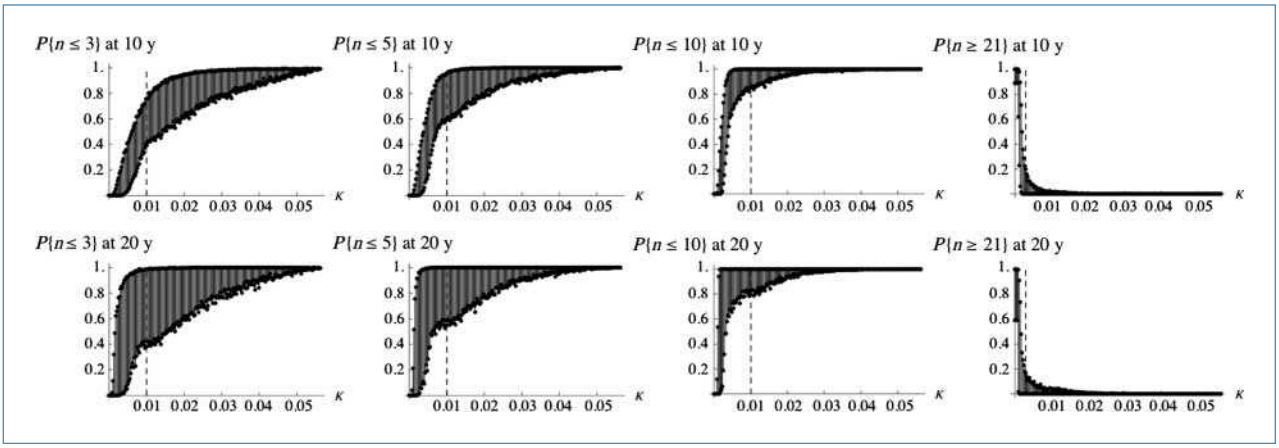

Figure 1. Probabilities associated with the number of micrometastases in dormancy patients. The shaded regions correspond to probabilities that the number of micrometastases in dormancy patients are less than or equal to 3 ($P\{n \leq 3\}$), 5 ($P\{n \leq 5\}$), 10 ($P\{n \leq 10\}$), or greater than 20 ($P\{n \geq 21\}$) at 10 y postresection (top row) and 20 y postresection (bottom row), for all accepted parameters generated by an Approximate Bayesian Computation using summary statistics (*a*) and (*b*) of Section (ii) of Materials and Methods.

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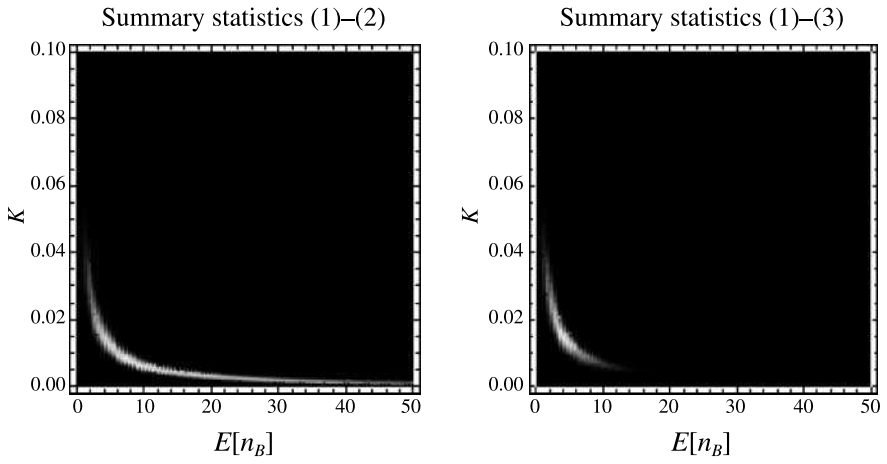

Figure 2. Reciprocal relationship between κ and $E[n_B]$. Gray-scale maps (small values are black, large values are white) of the joint marginal posteriors of κ and $E[n_B]$ of accepted parameters using summary statistics (*a*) and (*b*) (left) and summary statistics (*a*) to (*c*) (right)—see Section (ii) of Materials and Methods. In both plots, there is a reciprocal relationship between κ and $E[n_B]$ such that $\kappa \times E[n_B] \approx 0.06$, and accepted values of κ are restricted to the region $\kappa < 0.06$. The addition of summary statistic (*c*) restricts κ to the region $0.005 < \kappa < 0.06$.

(iii) Mutation rates are in a plausible range

Suppose that growth events are triggered by genetic mutations in the dividing cells of micrometastases. In two cases, the rate of growth-phenotype mutations m per cell division in a micrometastasis of diameter D (mm) and fraction f of cancer cells that are viable in vascularized regions, along with estimates of the number of cells that proliferate per hour, $P(D, f)$, can be used to estimate κ. The cases are as follows:

1. Switch to the growth phenotype requires 1 mutation (1 hit). This could correspond to 1 oncogenic mutation. Then, $\kappa = m \times [365 \times 24 \times P(D, f)]$.
2. Switch to growth phenotype requires 2 mutations (2 hits) and the size of the micrometastasis satisfies certain constraints (see Supplementary Materials). This could correspond to the inactivation of two alleles in a tumor suppressor gene. For example, when m_1 and m_2 are the rates of the first and second mutations per cell division, respectively, when the first mutation is neutral, and when the total number of viable cells in the micrometastasis exceeds $1/\sqrt{m_2}$ and is less than $1/m_1$, then $\kappa = m_1 \times \sqrt{m_2} \times [365 \times 24 \times P(D, f)]$ (refs. 33, 34).

Figure 3 shows how estimates of κ depend on the mutation rate m for cases 1 and 2 when micrometastases are (*a*) vascularized (thick lines) and (*b*) nonvascularized (thin lines). The solid lines are upper bounds on κ [from the high estimate of $P(D, f)$] for different values of D and f. The dashed lines are estimates of κ [from the medium estimate of $P(D, f)$]. In Supplementary Materials, serial sections of lymph node micrometastases from breast cancer patients show that at least 20% of cancer cells are proliferating, so at least 20% are viable and $f > 0.2$. Experimentally determined point mutation rates in various normal cell types (corresponding to the 1-hit case) are around 10^{-9} per basepair per cell division (35–37). The rate of mutation of an allele (the 2-hit case) is normally assumed to be 10^{-7} per allele per cell division in mathematical models. The range for κ identified in Section (ii) of Results of [0.01, 0.06] places mutation rates close to experimentally determined mutation rates for $D = 2$ mm, $0.2 \le f \le 0.8$; however, the same is true when κ is in the range [0.003, 0.01].

Table 2. Statistics of the marginal posterior distributions of accepted values generated by Approximate Bayesian Computation using summary statistics (*a*) and (*b*) of Section (ii) of Materials and Methods

Parameter	Median	Range
κ	0.0063	9.2×10^{-4} to 0.058
$E[n_B]$	10	1.0–50

(iv) Estimates suggest that a single micrometastasis must have diameter exceeding 0.4 mm to account for the CTCs

More than a decade postresection, results of Meng and colleagues (12) suggest at least 500 CTCs are in the blood of dormancy patients.[9] From measurements of the decline in a patient's CTC count on the removal of their primary breast cancer, the average life span of a CTC was roughly estimated as 1.5 to 3 hours (12). Thus, the total number entering the blood is at least 500/3 or approximately 200 per hour (100% of CTCs were detected) or 5,000/3 or approximately 2,000 per hour (10% of CTCs were detected).

The total number of cancer cells that proliferate per hour in each dormancy patient must exceed the number of CTCs entering the blood per hour; otherwise, the residual cancer would disappear rapidly. The total number of cancer cells

[9] The assay (12) extracted 1-2 CTCs from 12.5mL of blood. As there is approximately 6 liters of blood in the human body, a lower bound on the total number of CTCs in the blood is 6,000/12.5 or approximately 500 CTCs.

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that proliferate per hour in a patient with n micrometastases of diameter D is $n \times P(D, f)$. This specifies lower bounds on D given f in a patient with n micrometastases as shown in Fig. 4, for high and medium estimates of $P(D, f)$, for four cases: (*i*) the assay sensitivity was high (100% of CTCs were detected); (*ii*) the assay sensitivity was medium (10% of CTCs were detected); (*a*) micrometastases are vascularized and (*b*) micrometastases are nonvascularized.

When only a single micrometastasis persists, the lower bounds suggest its minimum diameter is 0.4 mm [100% of CTCs were detected, high estimates of $P(D, f)$, f = 0.8]; more realistic lower bounds are 1 to 2.1 mm [10% of CTCs were detected, medium estimates of $P(D, f)$, f = 0.8]. The minimum diameter of 0.4 mm does not change much when, rather than 1 micrometastasis, up to 5 micrometastases persist.

Figure 4 indicates that a single nonvascularized micrometastasis of diameter 1 to 2 mm can account for the CTCs in patients' blood unless (*a*) the sensitivity of the CTC assay is medium-low (less than 10% of CTCs are detected), the proliferation rate $P(D, f)$ is medium-low (viable cancer cells divide less often than once per 5 days), and $f < 0.8$ (less than 80% of cancer cells in vascularized regions are viable); or (*b*) the sensitivity of the CTC assay is medium-low and $f < 0.2$; or (*c*) the proliferation rate $P(D, f)$ is medium-low and $f < 0.1$. These estimates could be improved if the sensitivity of the assay detecting CTCs and the exit rate of CTCs from the blood were known. Supplementary Materials contains a table of the minimum number of micrometastases necessary to account for the CTCs when D = 2 or 3 mm.

Discussion

Breast cancer is infamous for long gaps between primary treatment and recurrence. Improvements in systematic therapy are increasing the frequency with which patients survive 5 years postdiagnosis (24) while breast cancer is already the most frequently diagnosed cancer in women in the Western world. Yet, current systematic therapies improve prognosis beyond 5 years only marginally (24, 38): elucidating the causes of dormancy and subsequent relapse is becoming a priority problem.

Analyses of our model led to the following hypothesis: long-term breast cancer dormancy is maintained by a small number, between 1 and 3—most frequently just 1, of dangerous micrometastases (dangerous micrometastases are those which can cause relapse). The hypothesis would account for the steady, similar number of CTCs observed by Meng and colleagues over time and in different patients from approximately 8 years postresection, subject to the condition that the persisting undetectable micrometastases are sizeable, that is, their diameters exceed 0.4 mm considerably. This condition is met if the micrometastases are preangiogenic and nonvascularized, for then their limiting size is 1 to 2 mm (16–18). However, the lower threshold of the diameter may vary upward above 2 mm depending on the density and proliferation rate of cancer cells in micrometastases and the sensitivity of the assay of Meng and colleagues; thus, this condition ought to be checked when more empirical data related to these variables are available.

The validity of the hypothesis depends strongly on the time scale of growth restriction of dormancy maintaining

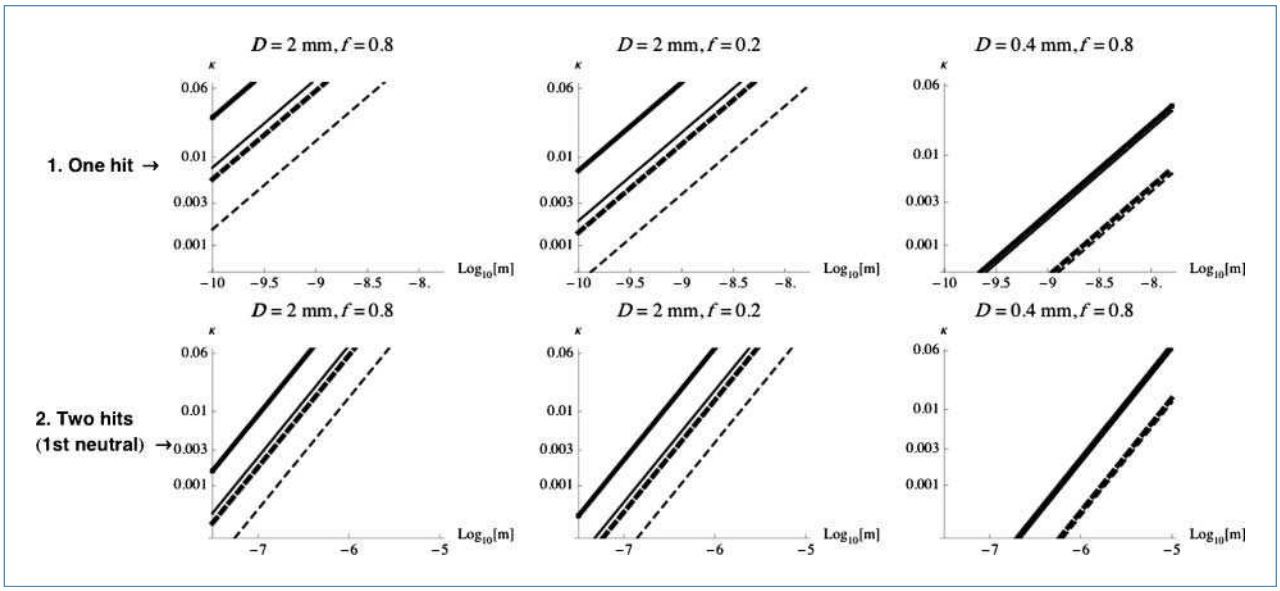

Figure 3. Mutation rates are in a plausible range. Plots illustrate how estimates for κ depend on the mutation rate per cell division m, supposing that the switch to growth phenotype is due to one oncogenic mutation (1 hit) or two mutations in a tumor suppressor gene (2 hits, 1st neutral)—see Section (iii) of Results. Thick/thin lines are for (*a*) vascularized/(*b*) nonvascularized micrometastases. Solid/dashed lines are for upper bounds/estimates of κ using high/medium estimates of $P(D, f)$ [see Section (iii) of Materials and Methods], where values of D (micrometastasis diameter) and values of f (fraction of cancer cells that are viable in vascularized regions) are indicated on each plot. Thick and thin lines coincide in the graphs of the right-hand column.

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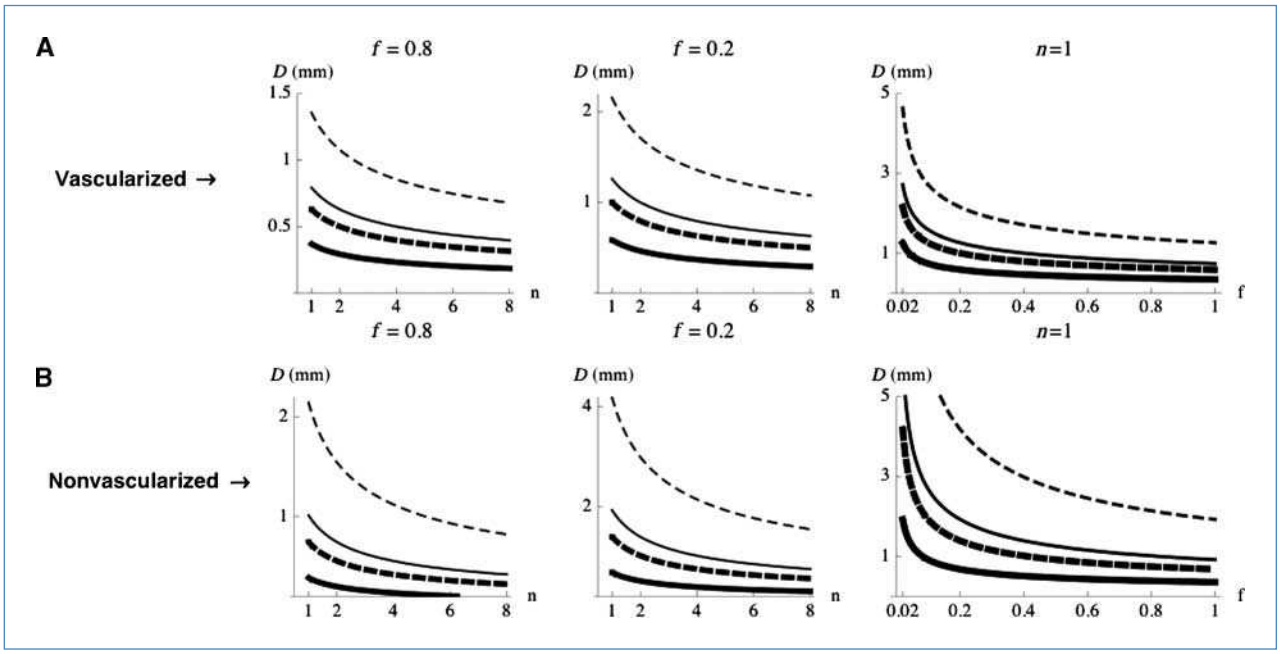

Figure 4. The hypothesis implies that the diameter of micrometastases exceeds 0.4 mm. The plots illustrate how the minimum diameter of micrometastases *D* (mm) depends on both the number of micrometastases *n* in a patient from approximately 8 y postresection and the fraction *f* of the vascularized tissue of micrometastases that is occupied by cancer cells, when micrometastases are (*a*) vascularized and (*b*) nonvascularized. This lower bound on *D* is estimated from patients' CTC counts [see Section (iv) of Results]. Thin/thick lines are for (*i*) medium/(*ii*) high sensitivity of CTC detection assay; solid/dashed lines are for high/medium estimates of $P(D, f)$ [see Section (iii) of Materials and Methods].

micrometastases in humans. Assuming micrometastases disappear and their cells metastasize to seed new micrometastases slowly over years or decades, our analysis showed that micrometastases escape growth restriction with a half-life exceeding 12 years; if, further, the half-life is found to be less than 23 years, this will provide firm evidence for our hypothesis, with the additional consequence that the average number of dangerous micrometastases on resection among patients without detectable or growing metastases is 3 or less. If it were possible to watch a sample of human micrometastases switching to growth phenotype, the half-life could be measured. Perhaps more instructive is the following finding: If the half-life of escape from growth restriction is found to be less than 69 years, the majority of dormancy patients have between 1 and 5 micrometastases from 10 years postresection, and the average number of dangerous micrometastases on resection among patients without detectable or growing metastases is 6 or less. Switch to growth phenotype on these time scales agrees roughly with experimentally measured mutation rates supposing that either one oncogenic mutation or mutations in two alleles (presumably in tumor suppressor genes) cause the switch.

We urge caution in treating the bounds stated here as inflexible: they should be treated as approximations to be refined with further experimental data. Of particular use for this and a more informative statistical analysis would be the results of Meng and colleagues independently corroborated with a system where either the specificity for detecting CTCs in the blood or the effectiveness for detecting dormancy in a patient was known. Our exposition makes it clear how the quantitative bounds can be refined.

On the basis of our study, we predict that effective preventive treatments for patients with long-term dormancy will be those targeted at eradicating the proliferating cells of small numbers of micrometastases or preventing the growth of these micrometastases. These micrometastases may not have formed at time of early systematic therapy. Screening patients for dormancy and testing effectiveness of treatment could be achieved by measuring patients' CTC counts or by measuring disseminated tumor cell counts in bone marrow aspirates.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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