Tumor Growth Instability and Its Implications for Chemotherapy

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Abstract

Optimal delivery of chemotherapy intensity is dependent on host- and tumor-specific characteristics. In this article, the chemotherapy late intensity schedule is revised to account for tumor growth instability, where a small tumor cell fraction emerges that exhibits a higher proliferation rate than the parent strain. Modeling this instability as simplified two-population dynamics, we find that: (a) if this instability precedes the onset of treatment, the slope of the linear increase of the drug concentration for the standard “Norton-Simon late intensity schedule” changes and the initial value of the dose strongly depends on the ratio of the two tumor cell populations and on their distinct growth rates; and (b) if the instability trails the initial treatment, the effective chemotherapeutic drug concentration changes as well. Both cases point toward testable potential refinements of the Norton-Simon late intensity schedule. [Cancer Res 2009;69(21):8507–15]

Major Findings

To investigate the influence of cancer heterogeneity on treatment impact, we study the case of an initially monoclonal tumor in which, at a given time, a secondary, faster replicating strain emerges from the parent tumor cell population. These “two-population dynamics” result in growth instability that impacts the current standard chemotherapeutic schedule for both situations, i.e., if the instability arises prior to or after the start of a nonspecific drug regimen. In both cases, the chemotherapeutic dose depends strongly on the balance of the two populations and on their specific growth rates, and consequently requires a nonlinear time modulation of the dose concentration of the “Norton-Simon late intensity” schedule. By comparing the model with available clinical data, it proved capable of predicting the different therapeutic response of breast cancers exhibiting an estrogen receptor (ER) positive subpopulation.

Introduction

Cancer therapy is aimed at targeting specific tumor (re)growth patterns to inhibit, or at least control, local tumor proliferation and metastatic dissemination. Mathematical modeling provides a promising tool by which to shed light onto these growth dynamics from a theoretical perspective, using both experimental and clinical data as input. Such works range from investigating various forms of exponential growth kinetics, e.g., (1, 2) to that of describing a Universal growth law (3), with intriguing implications of the latter to radiotherapy (4). For cytotoxic chemotherapy, a well-documented case in which theoretical reasoning had an impact on the therapeutic approach is that of breast cancer where, following the “Norton-Simon” hypothesis (5), standard treatment has been adjusted to a “dose dense” regimen; that is, the agent is given at a greater dose rate (i.e., multiple pulses of chemotherapy) to optimize efficacy, and to keep toxic side effects comparatively low. The underlying hypothesis is that, compared with the host organ, cancerous tissue exhibits a denser structure with higher fractal mass dimension, and is composed of smaller, relatively faster growing subseeds, all of which follow the Gompertz law (GL). Differing from the traditional “log-kill” concept (6), Norton and Simon then argued, based on a body of experimental and clinical studies on solid cancers, that “therapy results in a rate of regression proportional to the growth rate of an unperturbed neoplasm of that size” (7, 8).

This hypothesis has been tested in a phase III clinical study led by the Cancer and Leukemia Group B (CALGB 97–41; ref. 9). This large prospective randomized trial showed that shortening the time interval between each chemotherapy cycle while maintaining the same dose size resulted in significant improvements in disease-free and overall survival in patients with node-positive breast carcinoma. More recently, also a phase I study in patients with metastatic breast cancer confirmed the therapeutic results predicted by the Norton-Simon model (10). However, within the framework of the Norton-Simon model, the issue of drug resistance is only considered at the qualitative level, contrary to some leading work addressing this issue performed by Goldie and Coldman (11, 12). In a more recent theoretical work, Coldman and Murray (13) investigated a stochastic model of non–cell cycle phase–specific cancer chemotherapy for exponentially growing tumors including the development of drug resistance and the effect of chemotherapy on normal cells. The authors concluded that early intensification, a feature shared with the Norton Simon model, is a common aspect of successful regimes where drug resistance is likely. We note that the role of intratumor heterogeneity and its implications for the evolution of drug resistance and cell kinetics has already been considered by Gardner (14), who proposed a kinetically tailored treatment (KITT model). Finally, Monro and Gaffney (15) recently proposed a model aiming at a unifying description based on well-founded approximations, i.e., Gompertzian tumor growth, log cell kill by chemotherapy, and Luria-Delbruck mutation to resistance [this assumption was based on the Nobel Prize winning work of...
Quick Guide to Equations and Assumptions

Equation 1: \( \frac{1}{N} \frac{dN}{dt} = \alpha(t) - C(t) \)

This equation describes the "macroscopic" growth law for a cancer cell population under the effect of chemotherapy. \( N(t) \) is the cell number at time \( t \), \( \alpha(t) \) is the specific growth rate, and \( C(t) \) represents the effect (per cell) of a drug concentration. The specific growth rate can be written, in general, as a nonlinear function of \( N \) and, in particular, for the Gompertz growth: \( \alpha(t) = K \ln(\frac{\ln N}{\ln N^*}) \) where \( \ln N^* \) is the asymptotic value of the cell number and \( K \) is a constant. If \( N \) is a population composed of \( n \) subpopulations, each proliferating with a different specific growth rate and drug response function, the whole growth behavior and the chemotherapy results are different from the usual calculations based on a single Gompertzian dynamic. Within a simplified two-population model, it is then possible to evaluate the effects of tumor growth instability [where a small tumor cell fraction, \( N_1(t) \), emerges that exhibits a higher proliferation rate than the parent strain, \( N_0(t) \) on chemotherapeutic results.

Major assumptions: A cycle nonspecific drug response function is considered in Eq. (1) and in the corresponding equations (A.2.1 and A.2.2) for populations \( N_1(t) \), \( N_2(t) \).

Equation 2: \( N_j(t) = N_j(t^*) \exp(-\alpha_j(t)(t-t^*)) \)

Chemotherapeutic effects are described by a decrease in tumor cell (sub)population(s); in the previous equation, \( N_j(t^*) \) is the cell (sub)population(s) at the onset of treatment and \( \alpha_j(t) \) the corresponding rate of cell reduction. For a single Gompertzian growth, if \( \alpha(t) \) is constant, one obtains the Simon-Norton late intensity schedule, i.e., \( C(t) \) turns out (see Eq. 4) as a linear function of time. In other words, the requirement that the drug therapy reduces exponentially—with a constant rate—the tumor cell number implies, through Eq. 1, a linear increase of drug concentration. One can therefore evaluate how this behavior changes with tumor instability.

Major assumption: A constant \( \alpha(t) \) is, in general, an oversimplifying approximation if there are subpopulations with a different response to the drug therapy (see the next equation).

Equation 3: \( \omega_2(t) = \omega_1(t) \frac{1 - \exp(-K(t-t^*))}{K(t-t^*)} \)

By imposing that, due to chemotherapy, the subpopulation \( N_1(t) \) exponentially decreases with a constant rate \( \omega_1 \), whereas the subpopulation \( N_2(t) \) follows a time-dependent rate \( \omega_2(t) \) one obtains a differential equation for \( \omega_2(t) \). The previous equation is the solution of the differential equation with the boundary condition \( \omega(t^*) = \omega_1 - \beta \) [with \( \beta \) in equation (B.7)].

The solution of the equation for \( \omega_2(t) \) allows comparing the predicted evolution of the two different populations with experimental data.

Major assumption: by solving the equation, one obtains \( \omega_2(t) \) if \( \omega_1(t) \) is known, evaluating how the drug dose induces different reductions of the two subpopulations. The choice \( \omega_1 = \) constant has been made to simplify the calculations.

Equations 4 and 5: By imposing the exponential behavior in previous Eq. 2, the Gompertzian-specific rate \( \alpha(t) \) depends on the corresponding rates \( \omega(t) \). At least for short time, intervals after the end of chemotherapy, \( t_0 \), the patient's survival probability, \( P(t) \), should be proportional to the cell population regrowth rate at the end of the therapy: that is, a larger regrowth rate corresponds to smaller survival probability. Therefore one can write

\( P_i(t) = 1 - \alpha_i(t)(t-t_0) \)

which suggests an exponential form

\( P_i(t) = \exp(-\alpha_i(t)(t-t_0)) \)

The results obtained through the previous formulas are then compared with clinical data for two population dynamics corresponding to the survival probability for patients with ER+ (ER/PgR present) and ER-.

Luria and Delbruck in 1943 (16), who showed that bacterial cultures developed resistance to bacteriophages at random. They were able to show that palliative continuous chemotherapy achieves optimal results for intermediate (rather than high) dosage levels with later (rather than earlier) intervention, due to the effects of competition between resistant and sensitive cells. However, their approach focused only on the different tumor response to therapy and not on the different growth rate (both before and after therapy) that represents intrinsic tumor heterogeneity.

Here, we present evidence that cancer’s inherent clonal diversity results in nonhomogeneous growth patterns, which in turn—even for its simplest form of two-population dynamics—will require a modification of the “late intensification schedule” that has been put forward by Norton-Simon (17). The next section introduces the underlying growth instability model and its relevance for the current standard schedule for both situations, i.e., if the instability arises prior or after the regimen. We also propose an approach more closely related to clinical therapy and compare the results with data on survival probability.

Materials and Methods

Let us first recall that for an idealized homogeneous tumor system, the general growth law can be written as

\[ \frac{1}{N} \frac{dN}{dt} = \alpha(t) \] (1)

where \( N \) is the number of individual cancer cells at time \( t \) and \( \alpha \) is their specific growth rate (for a general classification of growth laws, see ref. 18). If \( N \) is a population composed of \( n \) subpopulations, \( N(t) = \sum_{i=1}^{n} N_i(t) \), each proliferating with a different specific growth rate \( \alpha_i \), \( i = 1, \ldots, n \),

\[ \frac{1}{N_i} \frac{dN_i}{dt} = \alpha_i(t) \] (2)

then Eq. 1 can be restored, provided \( \alpha \) is assumed to be the average of \( \alpha_i \), i.e., \( \alpha(t) = \frac{1}{N} \sum_{i=1}^{n} \alpha_i(t) N_i(t) \).

For simplicity, let us now consider a cancer system where, at time \( t_0 \), following a progression event, a small cancer cell subpopulation emerges, which grows at a distinctly different replication rate. At any time one can write \( N(t) = N_1(t) + N_2(t) \), where \( N_1(t) \) and \( N_2(t) \) are the number of cells belonging to the two subpopulations that we assume follow both the Gompertz growth law (GL; see Appendix A).

Therefore, the initial value is given by \( N(0) = N_1(0) \); at \( t = t_0 \), the cell population \( N_2(t) \) starts to grow, and because the instability emerges when a small portion of tumor cells is growing more rapidly, let us consider \( N_1(t_0) \gg N_2(t_0) \).
This translates into the idea that the same type of cancer, in its subclones $N_1(t)$ and $N_2(t)$, follows the same functional form of the growth law but with different values of the specific growth rate. In the simplest model of this instability (with $K = K_1^* = K_2^*$ in Eqs. A.0.1–A.0.3), the difference in the specific growth is related to the saturation value of the cell number $N(t \to \infty) = N^\infty$ given by $N^\infty = N_1^\infty + N_2^\infty$, and according to previous equations (A.0.2–A.0.3), different specific growth rates are obtained for $N_1^\infty 
eq N_2^\infty$. To evaluate the effects of the choices of the parameter values, various configurations will be considered.

1. Let us first show the effects of the delay, i.e., the onset of growth of $N_2$ at $t_\delta \neq 0$, on tumor growth assuming $N_1^\infty = N_2^\infty = N^\infty/(2)$, which is for equal
specific growth rates. Fig. 1A and B show the results for breast cancer with an asymptotic mass \( M^\infty = 640 \text{ grams} \) (the mass is assumed to be proportional to the cell number \( N \)) and an initial mass of \( M(0) = 6.4 \text{ grams} \). The delay \( t_0 \) equals 100 days in Fig. 1A and 500 days in Fig. 1B. The corresponding GL for a homogeneous cell population with the same initial and final mass is also plotted.

2. Let us now consider another case, in which the second cell population develops with the same delay of 500 days, but its specific replication rate (leading to a different fraction of the final tumor mass) is modified. In both cases, \( N_2 \) grows more rapidly than \( N_1 \), which is obtained by imposing \( N_1^* < N_2^* \). In Fig. 2A the result for \( M_1^* = 0.4M^\infty \) and \( M_2^* = 0.6M^\infty \) is depicted, whereas Fig. 2B shows the plot for \( M_1^* = 0.2M^\infty \) and \( M_2^* = 0.8M^\infty \), respectively.

3. Let us now clarify the effects of the size of the subsystem \( N_1 \) on tumor growth, at time delay \( t_0 \) with respect to the subsystem \( N_2 \). If the subclone \( N_2 \) contributes substantially to \( N^\infty \), its specific proliferation rate must strongly increase to reach a predefined fraction of the total final tumor mass, and such an increase is much larger depending on how, at \( t_0 \) its size compares to \( N_1 (t_0) \).

In Fig. 3A and B, we show, for the same set of parameters used in Fig. 2A, the dramatic impact of the unstable cancer system when \( N_1 (t_0) \) is \( 10^{-6} \) smaller than \( N_1 (t_0) \), for \( t_0 = 500 \) days. The effect of increasing the delay time is shown in Fig. 3B by considering the same set of values of the parameter as in Fig. 3A and \( t_0 = 1,000 \) days. Let us note that a similar analysis can be performed easily with other growth laws, for example the Universal law proposed by West and colleagues (19, 20). The results are expected to be qualitatively the same as those obtained using the GL, which is appropriately used here to relate the effects of tumor instability to chemotherapy regimen that still rely on GL.

To investigate the potential effects of such tumor instability on treatment, let us consider the "Norton-Simon" hypothesis with regards to its "late intensity" schedule for chemotherapy. Considering a monoclonal cell population that grows according to Eq. 1, and assuming that the dose-response per cell to a nonspecific chemotherapeutic cycle can be described by a function \( C(t) \), the time evolution of the system is given by the following equation:

\[
\frac{1}{N} \frac{dN}{dt} = \alpha(t) - C(t)
\]

That means, the tumor stops growing, and eventually regresses, when the therapeutic dose, delivered at time \( t^* \), is larger than a critical value given by \( C > C_{\text{cr}} = \alpha(t^*) \). Moreover, assuming that, due to therapeutic impact, the cancer cell number decreases exponentially with a rate \( \omega \), then \( N(t) = N(t^*) \exp [ -\omega (t - t^*)] \) and one obtains (see Appendix A.1) that \( C(t) \), in the case of Gompertzian growth rate, is a linearly increasing function of time:

\[
C^\infty (t) = A + B(t - t^*)
\]

where \( A = \alpha + \omega K^\infty \ln \left( \frac{N^\infty}{N(t^*)} \right) \) and \( B = \omega K^\infty \).

Therefore a linear increase of the chemotherapeutic drug dose with time can reduce the number of tumor cells exponentially. However, chemotherapeutic treatments based on the previous considerations assume single population dynamics.

Indeed, if one considers even the simplest case of heterogeneity, i.e., a system of two subpopulations with in general \( K^\infty_1 + K^\infty_2 \), because \( N(t) = N_1 (t) + N_2 (t) \), the exponential decrease gives \( N(t) = N_1 (t^*) \exp [ -\omega (t - t^*)] + N_2 (t^*) \exp [ -\omega (t - t^*)] \) and cancer populations \( N_1 \) and \( N_2 \) follow Gompertzian growth kinetics with the corresponding specific rates (see Eqs. A.2.1 and A.2.2) and drug concentrations.

We will now discuss which modification of the "late intensity schedule" can be relevant for an unstable, i.e., heterogeneous tumor exemplified by two-population dynamics according to the previous equations (for mathematical details see Appendix A.2).

**Onset of tumor instability after the beginning of chemotherapy.** Let us first assume that tumor instability starts after the beginning of the therapy treatment, i.e., \( t_0 > t^* \). In this case, for \( t^* < t < t_0 \), the time evolution is due to population \( N_1 (t) \) only and the corresponding drug concentration for a late intensity schedule, \( C_1 (t) \), is given by Eq. 4 with \( K^\infty_1 = K^\infty \).
Note that in this time interval, the growth rate of population $N_1(t)$ is that of the "one-population Gompertzian law" with saturation value $N_\infty$, because $N_1(t) = N(t)$.

By recalling that $N_1(t_\delta) = N_1(t_\delta)\exp(-\omega(t_\delta - t))$ and $M(t_\delta) = N_1(t_\delta) + M_2(t_\delta)$, therefore assuming that for $t > t_\delta$ the exponential decrease of the total tumor cell number through therapy is still effective, i.e., $N(t) = N(t_\delta)\exp(-\omega(t - t_\delta))$, it turns out

$$C(t) = A_d + \omega B_d(t - t_\delta)$$

with $A_d$ and $B_d$ in Eqs. A.2.10 and A.2.11.

Figure 4. Ratio $D = \frac{C(t)}{C(t_\delta)}$ when the instability starts at $t_\delta = 60$ d after the beginning of treatment. The parameters are the asymptotic mass, $M^e = 640$ g, $M(0) = 6.4$ g, $M_1(t_\delta) = M_1(t_\delta) 10^{-1}$, $M_2 = 0.5M^e$, $\omega = 0.1$ per month, and $K_g = 0.055$ per month. Different values for the ratio between $K_1$ and $K_2$ are depicted.

Figure 5. Ratio $D = \frac{C(t)}{C(t_\delta)}$ when the instability starts at $t_\delta = 60$ d before the beginning of treatment. $A$, $M_1^e = 0.1(M^e - M(0))$, $M_2^e = 0.5M^e$, $M_1(t_\delta) = M_1(t_\delta) 10^{-1}$; $B$, $M_1^e = 0.5M^e$, $M_2(t_\delta) = M_1(t_\delta) 10^{-1}$. The other parameter values are $M^e = 640$ g, $M(0) = 6.4$ g, $\omega = 0.1$ per month, and $K_g = 0.055$ per month. Different values for the ratio between $K_1$ and $K_2$ are depicted.
Table 1. Comparison with the experimental data for the linear interpolation as discussed in the text, Eqs. 8 and 9

<table>
<thead>
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<th>Time (y)</th>
<th>$P$ (Eq. 8)</th>
<th>$P$ (Eq. 9)</th>
<th>Exp. value</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>0.85</td>
<td>0.82</td>
<td>0.87</td>
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<tr>
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<td>0.70</td>
<td>0.67</td>
<td>0.65</td>
</tr>
<tr>
<td>3</td>
<td>0.55</td>
<td>0.54</td>
<td>0.5</td>
</tr>
<tr>
<td>4</td>
<td>0.40</td>
<td>0.45</td>
<td>0.45</td>
</tr>
<tr>
<td>5</td>
<td>0.25</td>
<td>0.38</td>
<td>0.45</td>
</tr>
</tbody>
</table>

**NOTE**: The experimental error is not included.

On the other hand, for a “one-population Gompertzian growth”, with saturation $N^\infty$, $C_1$ is given by Eq. 4 for $t^* = t_1$.

**Onset of tumor instability before the beginning of chemotherapy.**

Let us now assume that $t^* > t_0$, i.e., the treatment (delivered at time $t^*$) follows the onset of instability (at time $t_0$). Accordingly, the tumor grows following the “one-population GL” up until time $t_0$ [$N(t) = N_1(t)$ for $t < t_0$] when the population $N_2(t)$ emerges with a different specific proliferation rate.

Through Eq. A.2.5 in Appendix A, the drug concentration for $t < t^*$ turns out to be

$$C(t) = A_{2P} + \omega B_{2P}(t - t_0)$$

where $A_{2P}$ and $B_{2P}$ are given in Eq. A.2.8 and A.2.9.

The role of tumor instability is crucial for the time behavior of the drug concentration because it determines different populations, $N_1(t^*)$ and $N_2(t^*)$ at time $t^*$, and different slopes in the linear time dependence of $C(t)$. In fact, during the interval $t_0 < t < t^*$, the two populations, $N_1$ and $N_2$, follow a Gompertzian growth with different specific rates. As we shall see in the next section, the presence of instabilities, both before and after the time at which the therapy has been administered, postulates further improvements of the Norton-Simon late intensity schedule based on a uniform rate of regression, $\alpha$, of the number of cells according to $N(t) \rightarrow N(0)\exp(-\alpha(t-t^*))$. The previous equations (Eqs. 4, 5, and 6) show that, for the two population dynamics following Gompertzian growth, the average drug concentration required to obtain such a result is different from the drug concentration for a single tumor population exhibiting Gompertzian growth dynamics.

**Different drug effectiveness against cancer clones.** The previous general result is obtained by mimicking the differential response of the two cancer cell populations to the different drug concentrations $C_1$ and $C_2$ and the same $\alpha$. However, one can consider another approach to the problem that could, to some extent, be closer to the real setting, where a “cocktail” of drugs is given, which are expected to be effective against a particular tumor on the basis of previous empirical data. Indeed, in the therapy modeled according to the previous treatment, one expects that the drug concentration is uniformly delivered among cells and that $C_1 = C_2 = c$ but the “differential” effectiveness against a cancerous clones, i.e., the reduction (for unit time) of the tumor growth at different specific rates. As we shall see in the next section, the presence of instabilities, both before and after the time at which the therapy has been administered, postulates further improvements of the Norton-Simon late intensity schedule. For this reason, in the previous section, a more general approach with different rates of regression for populations $N_1$ and $N_2$ ($\omega_1 \neq \omega_2$) has been also considered. It can be applied to more realistic clinical cases. For example, it is well known that primary breast cancer cells can express a variety of surface receptors, a fact that correlates with clinical outcome. In particular, during their proliferative activity, breast cancer cell populations may start expressing receptors for hormones, mainly for estrogen and progesterone (21, 22). Let us then denote, in a breast cancer, the original cell population $N_1$, which fails to express estrogen receptor, as ER−, and the population $N_2$, which starts to express such receptors, as ER+. To be able to compare our model with clinical data, one has to relate the survival probability of a patient at time $t$, $P(t)$, after the end of chemotherapy (which occurred at time $\omega$), assuming a survival tumor cell fraction (at time $\omega$) computed according to the exponential decrease, i.e., with $\delta = \omega(t)$ and $\omega_2(t)$ in Eq. 7, in which $\omega_2 = 0$. Therefore, $\omega_1 = -\ln \left( \frac{N_1}{N_1} \right)$ and $\omega_2 = -\ln \left( \frac{N_2}{N_2} \right)$ (see Appendix B) and through a simple calculation $\omega_2(t_\omega) = \omega_1(t_\omega) + \beta \exp(-Kt_\omega)$.

where $K = K_1^2 = K_2^2$ and $P = K_1 \left( \frac{N_1}{N_2} \right)^{K_1}$, and $P = K_2 \left( \frac{N_2}{N_2} \right)^{K_2}$. This result allows us to compare the predicted evolution of the two different cancer cell populations with experimental data.

**Results**

Using the model described above, we now evaluate its effects on chemotherapy treatment (and thus its predictive power) by using medical data on breast cancer survival probability.

The ratio $\omega_1/\omega_2$ in Eqs. 4 and 5 describes the effects of the drug therapy with respect to the single Gompertzian growth when tumor instability starts after the beginning of the therapy, i.e., $t_0 > t^*$.

For $t^* < t < t_0$, this ratio is 1 and therefore one can set $\omega_2 = 0$. In Fig. 4, $D_{2P}$ is plotted for $t_0 = 60$ days and for $\omega = 0.5$ per month; different values of $\omega_1$ are considered. For a one-population Gompertzian growth, of course, $D_{2P} = 1$. With this set of parameters, $D_{2P}$ changes < 10%. Notice that, when $\omega_2 = 1$, the value of $D_{2P}$ can be less than one for some time intervals. This is due to the fact that at the onset of instability the total tumor mass splits in two subpopulations with different specific proliferation rates but also with different cell numbers at $t_0$. Therefore, a depletion of the large population $N_1(t_0)$ occurs and it requires some time for the growing instability, $N_2$, to compensate this initial negative step. Then, ratio $D = \frac{\omega_2}{\omega_1}$ in Eqs. 4 and 6 (which is, by definition, equal to 1 for $t < t_0$) describes the effects on onset of tumor instability before the beginning of chemotherapy. $D$ strongly depends on the specific proliferation rates of the two populations and on their relative fraction of the total cancer cell population at the onset of instability and the time of treatment. In Fig. 5A, the ratio $D$ is plotted as a function of time for $t^* = 365$ days and for $t_0 = 100$ days, for different values of $\omega_1$.

In Fig. 5B, $D$ is plotted for the same $t_0$ and $t^*$ values, and for the same ratios $\omega_1$, but changing the relative population $\frac{N_2}{N_1}$ at the onset of instability and at saturation (i.e., when $t \rightarrow \infty$). Depending on the value of $\omega_1$, the ratio $D$ increases from 20% to 70% (see figure captions for details).

We note that the values of the ratios $D$ and $D_{2P}$ depend on many parameters, which should be properly taken into account in a clinical context. On the other hand, the presence of instabilities, both before and after the time at which the therapy has been administered, postulates further improvements of the Norton-Simon late intensity schedule. For this reason, in the previous section, a more general approach with different rates of regression for populations $N_1$ and $N_2$ ($\omega_1 \neq \omega_2$) has been also considered. It can be applied to more realistic clinical cases. For example, it is well known that primary breast cancer cells can express a variety of surface receptors, a fact that correlates with clinical outcome. In particular, during their proliferative activity, breast cancer cell populations may start expressing receptors for hormones, mainly for estrogen and progesterone (21, 22). Let us then denote, in a breast cancer, the original cell population $N_1$, which fails to express estrogen receptor, as ER−, and the population $N_2$, which starts to express such receptors, as ER+. To be able to compare our model with clinical data, one has to relate the survival probability of a patient at time $t$, $P(t)$, after the end of chemotherapy (which occurred at time $t_\omega$), assuming a survival tumor cell fraction (at time $t_\omega$) computed according to the exponential decrease, i.e., with $\delta = \omega(t)$ and $\omega_2(t)$ in Eq. 7, in which $\omega_2 = 0$. Therefore, $\omega_1 = -\ln \left( \frac{N_1}{N_1} \right)$ and $\omega_2 = -\ln \left( \frac{N_2}{N_2} \right)$ (see Appendix B) and through a simple calculation $\omega_2(t_\omega) = \omega_1(t_\omega) + \beta \exp(-Kt_\omega)$.
At least for short time intervals after the end of chemotherapy, the patient survival probability should be proportional to the regrowth rate at the end of the therapy: that is, a larger regrowth rate corresponds to a smaller survival probability. Therefore, one can write
\[ P_i(t) = 1 - \alpha_i(t - t_e) \] (8)

For not too large \( t - t_e \), the value of parameter \( \alpha_i \) can be fitted by approximating the curve \( \text{ER} - \text{ER/PgR absent} \) in Fig. 1 of Colleoni and colleagues (23). This gives \( \alpha_i \sim 0.071 \) per year. To evaluate the survival probability with ER+, we use the corresponding formula with \( \alpha_i \) (note that a different \( t_e \) is simply a rescaling of \( \beta \)). By comparison with the corresponding clinical data, the parameter \( \beta \) can be estimated by trial and error methods and turns out to be \( \beta \sim 0.08 \) per year in the time range of 1 to 4 years. On the basis of such values, the survival probabilities can be estimated and the comparison with the corresponding clinical data are given in Table 1. These results are in good agreement with the clinical data reported in (23) for the curve ER+ (ER/PgR present) and suggest a generalization of Eq. 8 in exponential form,
\[ P_i(t) = \exp\left(-\alpha_i(t - t_e)\right) \] (9)

which gives the results reported in Table 1 by using \( \beta \sim 0.13 \) per year. One should note that the previous comparisons have been done by considering \( \alpha_i \) time independent and without any optimization of the parameter \( \beta \). This preliminary analysis does not show the saturation of the patient survival probability observed at long time intervals; however, it suggests a simple relationship between the drug dose required to obtain a given regression rate and the survival probability of the patient, which could be generalized in analogy to the so-called "linear-quadratic model" used in radiotherapy. We shall discuss this point in detail in a forthcoming article.

Discussion

In here, we propose a model aimed at gaining more insights into how tumors respond to chemotherapy, taking into account some features that are normally disregarded. In particular:

1. the well-known cancer characteristic of clonal heterogeneity and the growth instability it produces;
2. the analysis of the effects of such instabilities when occurring before and after the administration of chemotherapy;
3. their potential impact on the overall survival probability of the patient.

Although our method holds, in principle, also for the more realistic polyclonal tumors, for simplicity, we specifically investigated the case of an initially monoclonal tumor in which, at time \( t_e \), a secondary, faster replicating strain emerges from the parent tumor cell population. The resulting two-population dynamics are described in a way that is somewhat different from the usual interspecific competition of the two populations for survival and access to resources (prey-predator model, see, for example ref. 24) because the details about the dynamic evolution of the two distinct populations in the tumor required by such a more traditional approach would be unavailable, at least until the tumor is surgically approached and a biopsy taken for histologic evaluation. In our case, therefore, the competition between the two cell populations is encoded in the ratio \( \frac{N_1}{N} \) and in the boundary condition for long inter-

values: formally, the difference in specific growth is related to the saturation value of the cell number, \( N(t \to \infty) = N^\infty = N_1^\infty + N_2^\infty \). Because the maximum number of individuals is \( N^\infty \), the ratio \( \frac{N_1}{N} \) and the delay in the onset of growth between the two subpopulations results in different specific growth rates, a variety of distinct realistic situations can be described without necessity of other "ad hoc" assumptions. For instance, the occurrence of "dormancy" phases during tumor development [which has been simulated by Speer and colleagues (25), assuming that some parameters that describe the overall Gompertzian growth may experience stepwise variations, which in turn are responsible for the appearance of "plateaus" in the tumor growth curve] may be generated by proper choices of the delay time \( t_e \) (see Fig. 3B). We also investigated how the conventional "late-intensity" chemotherapy schedule proposed by Norton and Simon, based on the underlying assumption of single-population dynamics (with the parameters of a unique homogeneous Gompertzian growth), has to be modified when tumor time evolution is non uniform. We showed that in both case, i.e., if the instability starts before or after the onset of treatment the chemotherapeutic dose strongly depends on the balance of the two populations and on their specific growth rates.

Finally, by introducing a drug concentration nonlinear in time, the model was evaluated with some available clinical data, comparing its predictions for the subpopulation ER+ that emerges from the original breast cancer cells. Comparing the predicted survival probability following therapy, we conclude that the model is capable to prefigure the different responses on the basis of a small number of parameters, whose values can be easily fitted from clinical data. Most importantly, our simplified analysis indicates already that a global, average chemotherapeutic approach will not be successful if growth instabilities were to occur. It further argues that a better phenomenological and theoretical understanding of such therapeutic effects requires a more accurate description of these instabilities.

APPENDIX A

A.0. Gompertz growth follows a logarithmic-specific growth rate, i.e.,
\[ \frac{1}{N} \frac{dN}{dt} = \alpha(t) = K_0 \ln\left( \frac{N^\infty}{N} \right) \] (A.0.1)

where \( N^\infty \) is the asymptotic value for \( t \to \infty \) of the cell population and \( K_0 \) is a constant, which depends on specific properties of the population. Therefore, for the populations \( N_i(t) \) and \( N_2(t) \) one has
\[ \frac{1}{N_i} \frac{dN_i}{dt} = K_0 \ln\left( \frac{N_i^\infty}{N_i} \right) \] (A.0.2)

\[ \frac{1}{N_2} \frac{dN_2}{dt} = 0(t - t_e)K_0 \ln\left( \frac{N_2^\infty}{N_2} \right) \] (A.0.3)

where \( N_i^\infty, N_2^\infty \) are the asymptotic values for \( t \to \infty \) of the cell populations and \( \theta(t - t_e) \) is 1 for \( t \geq t_e \) and 0 for \( t < t_e \).

A.1. Assuming that
\[ N(t) = N(t^*) \exp\left(-\alpha(t - t^*)\right) \] (A.1.1)

By substituting
\[ \frac{1}{N} \frac{dN}{dt} = -\alpha \text{ in Eq. 3,} \]
\[ C(t) = \alpha(t) \left|_{N=N(t)} \right. \] (A.1.2)
i.e., the time-dependent dose, according to the Norton-Simon hypothesis, depends on the tumor growth rate \( \alpha(t) \) of a single population following GL:

\[
\alpha(t) \big|_{N=N(t)} = K\ln\left(\frac{N^*}{N}\right) \big|_{N=N(t)} \quad (A.1.3)
\]

By substituting for \( N \) the expression given in Eq. A.1.1 and collecting the constant and the time dependent terms, one obtains Eq. 4.

**A.2.** Through equations

\[
\frac{1}{N_1} \frac{dN_1}{dt} = \alpha_1(t) - C_1(t) \quad (A.2.1)
\]

\[
\frac{1}{N_2} \frac{dN_2}{dt} = \alpha_2(t) - C_2(t) \quad (A.2.2)
\]

for the time evolution of the total number of tumor cells, \( N(t) \), after onset of treatment, i.e., \( t > t^* \) one easily arrives at the following equation:

\[
\frac{1}{N} \frac{dN}{dt} = \frac{N_1(t)}{N(t)} \alpha_1(t) + \frac{N_2(t)}{N(t)} \alpha_2(t) - C(t) \quad (A.2.3)
\]

where

\[
\alpha(t) = \frac{N_1(t)}{N(t)} \alpha_1(t) + \frac{N_2(t)}{N(t)} \alpha_2(t) \quad (A.2.4)
\]

and

\[
C(t) = \frac{N_1(t)}{N(t)} C_1(t) + \frac{N_2(t)}{N(t)} C_2(t) \quad (A.2.5)
\]

Due to the exponential decrease of the populations, it turns out

\[
C_1 = K^g_1 \ln\left(\frac{N^*_1}{N_1(t^*)}\right) + \omega(t-t^*) \quad (A.2.6)
\]

\[
C_2 = K^g_2 \ln\left(\frac{N^*_2}{N_2(t^*)}\right) + \omega(t-t^*) \quad (A.2.7)
\]

And through substitution of \( C_1 \) and \( C_2 \) in Eq. A.2.5 and by taking into account that \( N(t) = N_1(t^*) \exp(-\omega(t-t^*)) + N_2(t^*) \exp(-\omega(t-t^*)) \), one obtains Eq. 6 with

\[
A_{d_2} = \omega + \frac{N_1(t^*)}{N(t^*)} K^g_1 \ln\left(\frac{N^*_1}{N_1(t^*)}\right) + \frac{N_2(t^*)}{N(t^*)} K^g_2 \ln\left(\frac{N^*_2}{N_2(t^*)}\right) \quad (A.2.8)
\]

and

\[
B_{d_2} = \frac{N_1(t^*)}{N(t^*)} K^g_1 + \frac{N_2(t^*)}{N(t^*)} K^g_2 \quad (A.2.9)
\]

By following the same procedure to evaluate \( C_{d_2} \) in Eq. 5 it turns out

\[
A_d = \omega + \frac{N_1(t_0)}{N(t_0)} K^g_1 \ln\left(\frac{N^*_1}{N_1(t_0)}\right) + \frac{N_2(t_0)}{N(t_0)} K^g_2 \ln\left(\frac{N^*_2}{N_2(t_0)}\right) \quad (A.2.10)
\]

and

\[
B_d = \frac{N_1(t_0)}{N(t_0)} K^g_1 + \frac{N_2(t_0)}{N(t_0)} K^g_2 \quad (A.2.11)
\]

**APPENDIX B**

From Eqs. A.0.2 and A.0.3 and Eqs. A.2.1 and A.2.2 with \( C(t) = C_2 \) \( t = C(t) \) for population \( N_1 \), it turns out that (one assumes for simplicity that \( K^g_1 = K^g_2 = K \))

\[
C(t) = \omega_1(1 + K(t-t^*)) + \ln\left(\frac{N^*_1}{N_1(t^*)}\right) \quad (B.1)
\]

which quantifies the linearly increasing drug concentration required for population \( N_1 \) to exhibit a decrease in time with constant rate \( \omega_1 \). Analogously, by assuming that \( \omega_2 \) is time independent, for population \( N_2 \) the required drug concentration turns out to be

\[
C(t) = \omega_2(1 + K(t-t^*)) + \ln\left(\frac{N^*_2}{N_2(t^*)}\right) \quad (B.2)
\]

In other terms, by requiring that the same dose \( C(t) \) can reduce the two populations with different constant rates, one obtains that, at any time, the following condition should be fulfilled:

\[
\omega_1(1 + K(t-t^*)) + \ln\left(\frac{N^*_1}{N_1(t^*)}\right) = \omega_2(1 + K(t-t^*)) + \ln\left(\frac{N^*_2}{N_2(t^*)}\right) \quad (B.3)
\]

Apart from the trivial solution \( \omega_1 = \omega_2 \) and

\[
\ln\left(\frac{N^*_1}{N_1(t^*)}\right) = \ln\left(\frac{N^*_2}{N_2(t^*)}\right) \quad (B.4)
\]

Eq. B.3 cannot be satisfied relaxing the requirement that the reduction rate is time independent, except one allows for some reduction rate to become time dependent. This implies that the exponential decrease for the effect of the drug concentration on population \( N_2 \), for example, must be modified with a time dependent \( \omega_2 \), i.e.,

\[
N_2(t) = N_2(t^*) \exp(-\omega_2(t)(t-t^*)) \quad (B.5)
\]

obtaining for \( \omega_2 \) the following linear differential equation:

\[
\frac{1}{N_2} \frac{dN_2}{dt} = -\frac{d\omega_2}{dt}(t-t^*) - \omega_2 = K\ln\left(\frac{N^*_2}{N_2(t^*)}\right) + \omega_2 K(t-t^*) - \omega_1 K(t-t^*) - K\ln\left(\frac{N^*_1}{N_1(t^*)}\right) \quad (B.6)
\]

where the last term is due to a decrease of cancer cell population \( N_1 \) with constant rate \( \omega_1 \). By defining the parameter

\[
\beta = K\ln\left(\frac{N^*_1 N_2(t^*)}{N_2 N_1(t^*)}\right) \quad (B.7)
\]

the solution of the previous equation can be written as

\[
\omega_2(t) = \omega_1 - \beta \ln\left(\frac{1-\exp(-K(t-t^*))}{K(t-t^*)}\right) \quad (B.8)
\]

with the boundary condition \( \omega_2(t^*) = \omega_1 \beta \).

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No potential conflicts of interest were disclosed.
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