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Minimizing Long-Term Tumor Burden: The Logic for Metronomic Chemotherapeutic Dosing and its Antiangiogenic Basis

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The general utility of the maximum tolerated dose (MTD) paradigm, a strategy aimed at optimizing the chance of total tumor cell eradication, is here questioned. Evidence to date suggests that for many tumors the potential for eradication is in fact remote, with patients consistently demonstrating tumor cell presence subsequent to MTD treatments having eradicative intent. The failure to eradicate is attributed largely to the heterogeneous nature of the tumor. Heterogeneous cell populations demonstrate short-term refractoriness to up-front dose delivery, but "resensitize" as part of dose recovery, showing increased overall susceptibility to a given series of doses when delivered more evenly spaced. It is demonstrated: (1) that the minimization of total tumor burden, rather than complete eradication, may often be the more practical objective; and (2) that regularly spaced, "metronomic" dosing is the best way to achieve it. As a corollary, it is found that the more efficient ability of the tumor endothelial cells to resensitize following dosing predicts a targeting bias towards the endothelial compartment of a tumor when metronomic dosing is employed. This lends theoretical support to recent empirical studies showing that regularly spaced dosing schedules with no extended rest periods act more antiangiogenically, thereby delaying or avoiding the onset of acquired resistance.

Introduction

To date, the goal of chemotherapy has been complete tumor kill. Dosing by "front-loading" has thus become the mainstay of treatment, owing largely to theoretical and laboratory studies over the past 40 years, which demonstrate that the probability of tumor cell extinction is optimized by up-front administration of the maximum tolerated dose (MTD) (Skipper *et al.*, 1964; Skipper, 1965). With the exception of lymphoid, germ cell, and some pediatric cancers, however, consistent tumor eradication has been elusive. More commonly, in spite of dosing to MTD, impressive initial regressions or remissions are followed by regrowth or recurrence. Even when patients are considered to be in complete remission, evidence that full eradication is not being achieved comes from PCR

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studies demonstrating the presence of circulating tumor cells (Baldi *et al.*, 2000). But despite the failures, it was thought until recently that if the dose could be further escalated beyond MTD, the last tumor clonogen could be killed. Yet, high-dose trials with metastatic breast cancer using lethally intensive dosings coupled with autologous bone marrow rescue (Stadtmauer *et al.*, 2000) demonstrate survival outcomes proving to be no better and sometimes even worse than standard MTD despite higher complete response rates. In the transplant setting, tumor cells have been found to persist, whether patients are treated in overt relapse (considerable tumor burden) or in remission (small tumor cell burden) (Norton, 1997). At present, neither clinical experience (Baldi *et al.*, 2000; Kasimir-Bauer *et al.*, 2001; Kohda *et al.*, 2001; Kruger *et al.*, 2001) nor recent laboratory studies (Browder *et al.*, 2000) support the notion that all tumor cells can be reliably eliminated by up-front dosing schemes.

It may now be time to recognize the limitations of aiming for complete tumor cell kill, a goal that has proven both unrealistic and unnecessary for patient viability. Patient survival is not incompatible with tumor presence. In fact, people live asymptomatically with significant tumor burden for considerable periods prior to cancer presentation. Data collected from autopsy cases show that the prevalence of microscopic dormant tumors in organs such as breast, prostate, and thyroid greatly exceeds that of overt neoplasms within these organs (Black & Welch, 1993). Additionally, insight into the angiogenic control of tumor growth supports the idea of the existence of viable yet dormant tumor populations, both in the pre- and post-vascularization phases (Holmgren *et al.*, 1995; Hahnfeldt *et al.*, 1999). It appears that large numbers of apparently healthy individuals in fact have tumor burden, emphasizing that complete tumor cell eradication is by no means a prerequisite to host viability. An alternative therapeutic goal is therefore suggested — the minimization of total tumor burden over the long term. Managing cancer as a chronic disease, with tumor burden held at the lowest achievable level, may prove to be a more realistic and satisfactory therapeutic strategy than striving for complete eradication.

Interestingly, there is increasing evidence that maintaining a minimum tumor burden is accomplished not by ongoing MTD delivery, but instead, by comparatively mild regular or continuous dosing (Braunschweiger & Schiffer, 1980; Hansen *et al.*, 1996; Gabra *et al.*, 1996; Wolmark *et al.*, 1998; Seidman *et al.*, 1998). The basis for the dose-rate effect transcends standard drug pharmacokinetic classifications, as evidenced by observed benefits to dose-rate reductions using both type-1 drugs (e.g. cyclophosphamide) and type-2 drugs (e.g. 5-fluorouracil) (Browder *et al.*, 2000; Gabra *et al.*, 1996; Fuse *et al.*, 1995). Analysis points instead to the overall heterogeneity found within and among the various cell subpopulations comprising a tumor as the driving force. Heterogeneous cell populations are found to present increased refractoriness immediately following intense dose deliveries. This may be explained by the presence of cells having differing sensitivities to a therapeutic agent, and the ability of those cells to shift among the different sensitivity compartments over time following treatment. Accompanying the compensatory shifting of cells following dosing is a ''resensitization'' effect (Hahnfeldt & Hlatky, 1996; 1998; Hahnfeldt & Sachs, 1997) that can best be exploited by applying doses metronomically, i.e. with uniform spacing.

In addition, it is now recognized that tumor endothelial cells play a pivotal role in sustaining and thereby controlling tumor mass (Folkman, 1971; Boehm *et al.*, 1997), and that these cells are also targets of cytotoxic therapy (Browder *et al.*, 2000; Klement *et al.*, 2000). Since endothelial cells contribute to overall tumor heterogeneity, we considered that they may play a role in modulating the tumor resensitization effect rationalizing the low and regular dosing approach. Indeed, our analysis of resensitization kinetics confirms that the tumor endothelial cell population itself should become a greater percentage of the total therapeutic target when metronomic dosing is employed.

Cell Heterogeneity and MTD

Diversity in drug sensitivity stems from the genetic and epigenetic variations among

subpopulations of tumor cells in addition to temporal sensitivity differences between genetically similar cells due, for example, to asynchronous cycling. At the time the classic dosing models were first formulated, intratumor heterogeneity was not well-appreciated. All cells of a given tumor were thought to be clonogens having the same drug sensitivity. Under those assumptions the argument proved quite plausible that, at least in principle, every clonogen within a sufficiently small tumor could indeed be eliminated if a drug was delivered at the maximum tolerated rate. Additionally, the preclinical tumor models used in these early investigations, notably the L1210 leukemia (Venditti *et al.*, 1959), were models that expressed particularly low heterogeneity and were thus more amenable to full eradication. Although much has been recognized about the cure-limiting aspect of tumor heterogeneity (Skipper & Schnabel, 1984) since the inception of the up-front MTD paradigm, theoretical study of the ramifications of tumor heterogeneity in dose-schedule design has only marginally been undertaken, with the exception of radiotherapy (Hahnfeldt & Hlatky, 1996; Dillehay, 1990; Zaider & Minerbo, 1993; Sachs *et al.*, 1997; Brenner *et al.*, 1998).

An important connection between population heterogeneity and chemotherapeutic dose scheduling may be appreciated by considering that a heterogeneous population will, at some sufficiently fast rate of drug delivery, begin to show increased refractoriness as the later portions of that dose are delivered. Such a point of "diminishing returns" with increased rapidity of drug delivery is realized because, as the agent is administered, the sensitive variants in the population are preferentially depleted, leaving behind a transiently refractory population that can better withstand the final increments of that dose administration (Hahnfeldt & Hlatky, 1996; 1998; Hahnfeldt & Sachs, 1997). Slower drug delivery, on the other hand, provides more time for tumor cells to re-occupy the depleted sensitive states, permitting the overall sensitivity of the population to remain closer to its pretreatment level throughout the dosing period. It follows that, where overall population suppression (vs. immediate eradication) is concerned, optimum dosing schedules arise that significantly depart from acute drug delivery. Given that growth suppression, or "maintenance", is already the *de facto* goal in the treatment of many types of cancer, applying more continuous or regular dosing schedules in these instances stands to offer greater efficacy than up-front dosing.

It remains to understand this effect in a more precise form amenable to treatment planning. To this end, a simple theoretical construct is devised that captures the relevant dynamics in a way that allows formal comparison of alternative dosing schedules and drug combinations to determine best efficacy.

Analytic Description of the Heterogeneous Tumor Population

Although tumor cell populations are composed of cells possessing a wide range of sensitivities, it is sufficient for understanding the influence of sensitivity variation to analytically treat a tumor population as partitionable into two sensitivity groups. Each cell of the tumor population, then, is here considered to have one of two sensitivities, α or β, to a given chemotherapeutic agent. By "sensitivity", it is meant that a cell exposed to a fixed concentration c_0 of that agent for a time t_0 would theoretically have a survival probability $\exp(-\alpha c_0 t_0)$ or $\exp(-\beta c_0 t_0)$, in accordance with standard pharmacokinetic theory (Fuse *et al.*, 1995). Cells of sensitivity α comprise "subpopulation 1" and cells of sensitivity β comprise "subpopulation 2". Because each subpopulation is also assumed to be large (consistent with a low tumor eradication probability), the survival probabilities also represent subpopulation survival fractions following drug exposure.

The sizes of subpopulations 1 and 2 at time t are $p(t)$ and $q(t)$, respectively. Apart from drug exposure, the sizes of these subpopulations are presumed to be influenced by just two other factors — the respective net proliferation rates λ_1 and λ_2, and the transition rates reflecting the rates at which cells in each subpopulation undergo a transition to the other subpopulation (e.g. cells moving from a non-mitotic compartment to a mitotic one, and vice versa). The

transition rate of cells from subpopulation 1 to 2 is γ_1 and that from 2 to 1 is γ_2 (Fig. 1). The inclusion of transition rates between states of different sensitivity captures a critical property of real heterogeneous cell populations — a tendency for their various subpopulations whose relative proportions are altered by a toxic insult to return to their former equilibrium proportions once the insult ceases (Fig. 2). To illustrate this restorative tendency using the cell cycle analogy, if cells in mitosis are harvested preferentially from an asynchronous cell culture, e.g. by the well-known "mitotic shake-off" technique, and are replated under the original conditions, the cells will eventually redistribute among the cycle phases in the same relative proportions as existed before the shake-off. Another example is the observed restoration over time of the pre-treatment proportion between radiosensitive normoxic cells and radioresistant hypoxic cells following a dose of radiation that preferentially kills the oxygenated subpopulation (Hall, 1988).

Population Response to General Treatment Regimens

If the drug concentration seen by the cell population is taken to be an arbitrary function of time, say $c(t)$, the complete time dependence of the sizes of the two subpopulations in response to the dosing becomes

$$\frac{dp(t)}{dt} = \big(\lambda_1 - \gamma_1 - \alpha c(t)\big)p(t) + \gamma_2 q(t), \qquad (1a)$$

$$\frac{dq(t)}{dt} = \gamma_1 p(t) + \big(\lambda_2 - \gamma_2 - \beta c(t)\big)q(t). \qquad (1b)$$

These subpopulation dynamics are illustrated in Fig. 1.

A general solution to eqns 1 for arbitrary $c(t)$ is not available, but for combinations of bolus doses, solutions are easy to come by based on the straightforward solution to the dose-free case $c(t) \equiv 0$. The three cases that together cover the range of dosing situations relevant to therapy are here considered: (I) a pair of spaced bolus doses; (II) a repeating pattern of equal bolus doses with different spacings; and (III) a repeating pattern of unequal bolus doses. Significant generalizations for optimum dosing follow.

Solution for Patterns of Bolus Doses

The term "bolus" formally refers to a very short-lived, impulse exposure of the target cell population to a drug concentration, and "dose" refers to the area under the concentration curve. Expressions for $p(t)$ and $q(t)$ due to a general series of bolus doses are sought to determine which regimens show superior suppression for a fixed total amount of dose. To this end, the strategy is to move forward in time from time zero to the time of the first dose using the solution to the dose-free equations, then calculate the instantaneous change to p and q due to that dose, at which point the procedure is repeated.

The solution to the dose-free version of eqns (1a,b) is

$$p_{free}(t) = A\exp(r_1 t) + B\exp(r_2 t), \qquad (2a)$$

$$q_{free}(t) = uA\exp(r_1 t) + vB\exp(r_2 t), \qquad (2b)$$

where

$$u = \frac{\xi}{\gamma_2},\ v = \frac{-\eta}{\gamma_2},\ r_1 = \xi + \lambda_1 - \gamma_1,$$
$$r_2 = -\eta + \lambda_1 - \gamma_1 \qquad (2c)$$

and

$\xi(>0)$ and $-\eta(<0)$ are roots of

$$z^2 + ((\lambda_1 - \gamma_1) - (\lambda_2 - \gamma_2))z - \gamma_1\gamma_2 = 0. \qquad (2d)$$

It follows that if we let $p_0(t)$ and $q_0(t)$ represent $p(t)$ and $q(t)$ for times $t \geqslant \tau_0 (= 0)$ up to the first bolus dose at τ_1, and $p_n(t)$ and $q_n(t)$ represent $p(t)$ and $q(t)$ for times t between the n-th bolus dose of size d_n at time τ_n and the $(n+1)$-th bolus dose of size d_{n+1} at time $\tau_{n+1} (n \geqslant 1)$, then it is possible to solve for constants A_i and B_i such that

$$p_i(t) = A_i\exp(r_1 t) + B_i\exp(r_2 t), \qquad (3a)$$

$$q_i(t) = uA_i\exp(r_1 t) + vB_i\exp(r_2 t) \qquad (3b)$$

for

$$\tau_i \leqslant t \leqslant \tau_{i+1},\ i \geqslant 0,\ \tau_0 = 0.$$

and since the effect of the n-th bolus dose is to apply the factors $\exp(-\alpha d_n)$ and $\exp(-\beta d_n)$ at

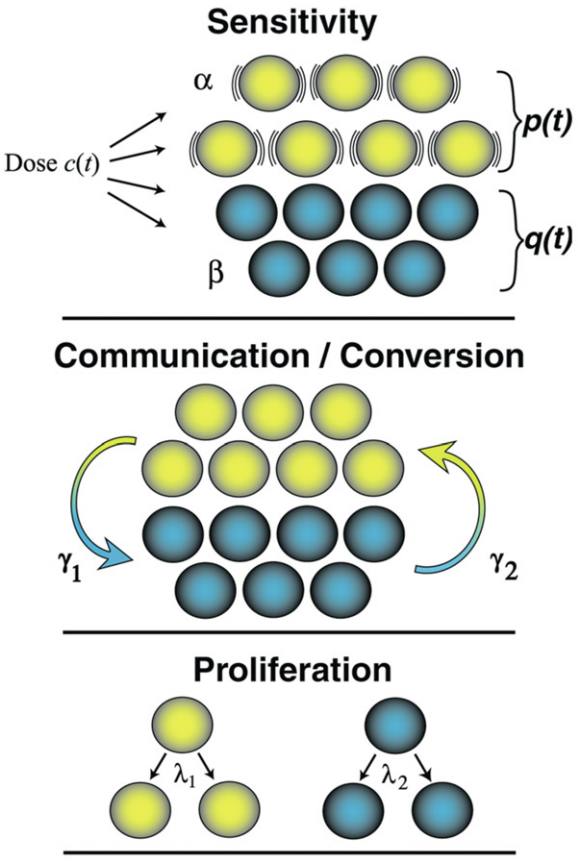

FIG. 1. Heterogeneous target population. The cell population subject to treatment is in general not uniform, consisting instead of sensitive and resistant subpopulations. This population is represented theoretically as one having two sensitivity compartments, one of size $p(t)$ (subpopulation 1) whose cells have a sensitivity α, and one of size $q(t)$ (subpopulation 2) whose cells have a sensitivity β. Through cell cycle progression and other effects, cells naturally undergo sensitivity changes, here reflected by "movements" between these compartments; γ_1 and γ_2 represent the "flow" rates from Compartments 1 to 2 and 2 to 1, respectively. Cells in each compartment are assumed to proliferate at rates λ_1 and λ_2.

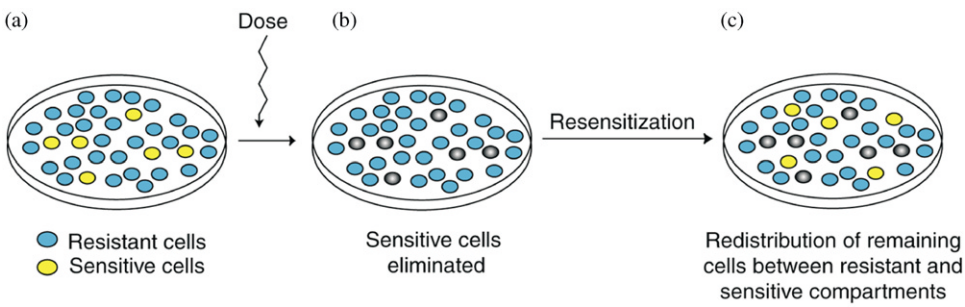

FIG. 2. Resensitization effect. A cell population composed of two or more mutually influential or interconvertible subpopulations will display a resensitization effect when exposed to chemotherapy. (a) A hypothetical asynchronous cell population where yellow cells are highly sensitive and blue cells are highly resistant. These two cell states will maintain a fixed proportion (here, 30 : 6 or 5 : 1) if left undisturbed. A bolus dose of chemotherapy kills the sensitive cells [shown as dead gray cells in b], leaving behind the resistant ones. As time progresses without dosing, the resistant cells eventually refill the sensitive void so as to re-establish the natural 5 : 1 proportion (c). This action has the effect of resensitizing the population as a whole. At this point, as before dosing, the number of cells entering the sensitive state has come into balance with the number leaving. Cell proliferation is neglected here, but under fairly broad circumstances, a tumor population will be optimally suppressed if dosing is applied in regular intervals to allow for the general phenomenon of resensitization.

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time τ_n to $p(t)$ and $q(t)$,

$$p_{i+1}(\tau_{i+1}) = p_i(\tau_{i+1})\exp(-\alpha d_{i+1}), \qquad (3c)$$

$$q_{i+1}(\tau_{i+1}) = q_i(\tau_{i+1})\exp(-\beta d_{i+1}), \qquad (3d)$$

Using eqns (3a–d), $p_i(t)$ and $q_i(t)$ may be solved for $i = 0,1,2,\ldots$ by solving for the unknown constants A_i and B_i in turn. Condensing this iterative procedure into a simple matrix operation, the values of A_n and B_n may be written in terms of A_{n-1} and B_{n-1} ($n \geqslant 1$) as

$$\begin{pmatrix} A_n\omega_n \\ B_n\psi_n \end{pmatrix} = M_n \begin{pmatrix} A_{n-1}\omega_{n-1} \\ B_{n-1}\psi_{n-1} \end{pmatrix} =$$

$$\begin{pmatrix} \dfrac{(\eta x_n + \xi y_n)\omega_n}{(\xi+\eta)\omega_{n-1}} & \dfrac{\eta(x_n - y_n)\psi_n}{(\xi+\eta)\psi_{n-1}} \\ \dfrac{\xi(x_n - y_n)\omega_n}{(\xi+\eta)\omega_{n-1}} & \dfrac{(\xi x_n + \eta y_n)\psi_n}{(\xi+\eta)\psi_{n-1}} \end{pmatrix} \begin{pmatrix} A_{n-1}\omega_{n-1} \\ \\ B_{n-1}\psi_{n-1} \end{pmatrix} \qquad (4)$$

where $x_n = \exp(-\alpha d_n)$, $y_n = \exp(-\beta d_n)$, $\omega_n = \exp(r_1\tau_n)$, $\psi_n = \exp(r_2\tau_n)$, $A_0 = C$, $B_0 = 0$. Equation (4), coupled with eqns (3a, b), allow assessment of the effect of arbitrary administrations of bolus doses on the size of the heterogeneous model population. Together, the functions $p_i(t)$ and $q_i(t)$ describe the dose–response of $p(t)$ and $q(t)$, and thus the total population size $p(t)+q(t)$, for all times t.

Case I: Population response to fixed total dose delivered as two spaced boli. Considered first is the result of delivering a total dose D as two spaced boli instead of just one bolus. The two bolus doses of sizes d_1 and d_2 are delivered at times $\tau_1 = 0$ and $\tau_2 = T$, respectively, with $d_1 + d_2 = D$.

The effect of the two doses on the long-term population growth is reflected in the coefficient $(1+\mu)A_2$ of the faster growing exponential $\exp(r_1 t)$ in $p_2(t)+q_2(t)$ (that $r_1 > r_2$ follows from the definitions in eqns (2c,d)). The factor by which this coefficient is reduced over what it would have been with no dosing ($x_n = y_n = 1$) determines dosing efficacy. This factor R is A_2/A_0, or:

$$R = \frac{1}{(\xi+\eta)^2}((\eta x_1 + \xi y_1)(\eta x_2 + \xi y_2) + \xi\eta e^{-(\xi+\eta)T}(x_1 - y_1)(x_2 - y_2)). \qquad (5)$$

It is readily seen that, as the spacing T gets larger, the ratio decreases and dose–response improves (Fig. 3). The quantity $\xi+\eta$ is the effective resensitization rate.

Case II: Repeated delivery of a fixed pattern of N equal bolus doses. A general chemotherapeutic dosing regimen involves delivering a fixed pattern of equal doses, separated by "rest periods" to allow stem cell recovery. This complete dosing cycle is repeated indefinitely. The effect of this continuing regimen on tumor growth may be described quantitatively. If the first of the repeating cycles is represented by N equal bolus doses of size d, delivered at the N times $\tau_1,\ldots,\tau_N$, ($\tau_N = T$, the fixed "width" of the repeating cycle), then the next cycle would begin at time T and its N doses would be delivered at the times $\tau_{N+n} = T + \tau_n$ for $n = 1,\ldots,N$ (Fig. 4). In eqns (3a, b), k such cycles delivered in succession will produce the new coefficients A_{kN} and B_{kN}. Using eqn (4), a simple way of expressing A_{kN} and B_{kN} in terms of A_0 and B_0 is

$$\begin{pmatrix} A_{kN}\omega_N^k \\ B_{kN}\psi_N^k \end{pmatrix} = Q^k \begin{pmatrix} A_0 \\ B_0 \end{pmatrix} = (M_N M_{N-1}\cdots M_1)^k \begin{pmatrix} A_0 \\ B_0 \end{pmatrix} \qquad (6)$$

where in the matrices, $x_1 = x_2 = \cdots = \exp(-\alpha d)$ and $y_1 = y_2 = \cdots = \exp(-\beta d)$.

Because Q depends only on dose spacings rather than absolute times, the effect of each new cycle of N doses is represented by another multiplication by the same matrix Q. According to matrix theory, however, repeated multiplication by a matrix eventually has the effect of multiplying by δ_1, its largest eigenvalue. Biologically, this value represents the ultimate factor suppression (or growth) of the population between the end of one cycle and the end of the next (Fig. 4). It follows that the regimen whose dose spacings are such as to minimize δ_1 will be the regimen with optimum suppressive

ability over the long term. Because the product of the two eigenvalues δ_1 and δ_2 of Q is $(xy)^N \exp((r_1+r_2)T)$, a constant independent of the individual dose spacings, δ_1 is minimized precisely when $\delta_1+\delta_2$ is minimized. But $\delta_1+\delta_2$ is the sum of the two diagonal elements of Q, which can be shown to be a polynomial function $P(\phi_1, \phi_2,\cdots, \phi_N)$ [where $\phi_n=\exp(-(\xi+\eta)(\tau_n-\tau_{n-1}))$] having all positive coefficients and possessing the property that $P(\phi_1, \phi_2,\cdots, \phi_N)=P(\phi_2, \phi_3,\cdots, \phi_N, \phi_1)=P(\phi_3, \phi_4,\cdots, \phi_N, \phi_1, \phi_2)=\cdots=P(\phi_N, \phi_1, \phi_2,\cdots,\phi_{N-1})$. These properties are sufficient to establish that P is minimized when $\phi_1=\phi_2=\cdots=\phi_N$. The optimum dose spacing for any regimen involving repeated delivery of a prescribed cycle of equal bolus doses must therefore be uniform, a pattern that is now referred to as "metronomic" (Fig. 5) (Hanahan *et al.*, 2000).

Case III: Repeated delivery of a fixed pattern of N different bolus doses with various spacings. It was shown that among all ways of repeatedly delivering a prescribed cycle of equal doses, optimum tumor suppression is attained when all dose spacings are equal. The argument may now be extended to the case where the doses are not equal in the following manner. The doses d_1, d_2, $\cdots$, d_N comprising one entire dosing cycle may be each thought of as integer multiples of the same very small dose ε, i.e. $d_1\approx m_1\varepsilon$, $d_2\approx m_2\varepsilon$, and so on. From this perspective, the dosing pattern technically involves the delivery of equal doses ε (albeit with many of these being clustered on top of one another at specific times). But in Case II it was demonstrated that equal doses are best delivered equally spaced. Applying this rule here, the result is a large number of very small doses ε spaced very closely but uniformly. As $\varepsilon\to 0$, this regimen in fact becomes a continuous dosing scheme where delivery over each cycle time T is at the constant dose intensity $c(t)=(d_1+d_2+\cdots+d_N)/T$. Since this is the only scheme that cannot be improved upon by the dose-splitting procedure, continuous dosing represents the optimum form of dose delivery overall, barring certain threshold effects.

Drug Threshold Effects

A phenomenon that may limit the advantages of low-dose metronomic or continuous dose delivery is a threshold effect for drug activity. Specifically, there may exist a minimum concentration of drug below which no tumor inhibition takes place. Because the approach to metronomic or continuous dose delivery away from acute delivery involves lowering the peak drug concentration experienced by the tumor, it is possible that the initial benefits to protraction could give way to a loss of effect as the peak concentration falls below the minimum threshold for effect. The result would be a roughly U-shaped dependence of drug effectiveness as a function of dose spreading.

The Clearance Rate Effect

To this point, dose delivery has referred to the form of the concentration curve $c(t)$ at the site of the target population, and not to the rate, say $e(t)$, at which drug is actually administered to the patient. The importance of the distinction comes from considering that the objective here has been to optimize population suppression subject to the total dose D [area under $c(t)$ for one cycle] being fixed. However, the goal in practice is to optimize suppression subject to the total amount of administered drug E [area under $e(t)$ for one cycle] being fixed. Fortunately, the constraints are equivalent since $E=rD$, where r is the clearance rate.

A related concern comes from the fact that a bolus dose delivery to the population site may not even be possible if clearance is too slow. This concern is alleviated as well when it is considered that the drug clearance rate is usually fast (on the order of minutes to hours) compared to the subpopulation proliferation rates λ_1 and λ_2 and flow rates γ_1 and γ_2 (on the order of hours to days or longer), so that a bolus drug administration leads to an effectively bolus dose experience at the tumor site. Under these conditions, the foregoing conclusions about bolus dose deliveries carry over intact to the actual timings of bolus drug administrations to the patient.

In any event, under cyclic dosing, because continuous dosing at the tumor site is obtained only when dose is administered continuously, and because a continuous dose experience at the tumor site is shown to be superior to all others, it

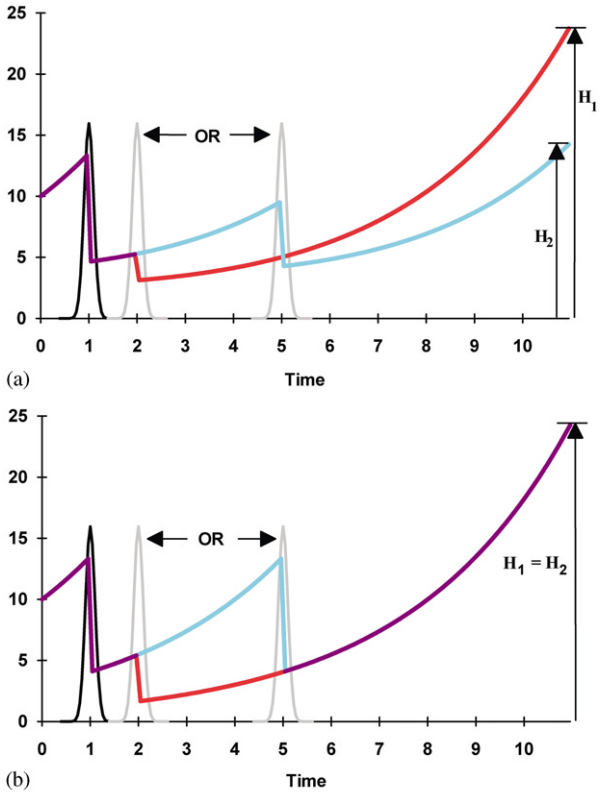

FIG. 3. Dose separation effect. The effect of spacing two equal bolus doses with "area-under-the-curve" strength $d=4$ is examined (units not important). The vertical axis refers to the instantaneous dose concentration for the indicated bolus doses, or the population size in the case of the colored curves. The first dose is at time 1 and the second is at either 2 or 5. The red curve represents the response to doses at 1 and 2, and the blue curve the response to doses at 1 and 5. The purple segments indicate where the two curves overlap. In (a), the relevant parameters are $\lambda_1=0.5$, $\lambda_2=0.1$, $\gamma_1=0.3$, $\gamma_2=0.1$, $\alpha=1.0$, $\beta=0.1$. In (b), sensitivities are assumed to be equal: $\alpha=\beta=0.3$. A sensitivity difference underlies the advantage to greater dose spacing (a); without it, there is no effect (b). The suppression factor R [eqn (5)] achieved in each case is the limiting ratio H_2/H_1 of population sizes.

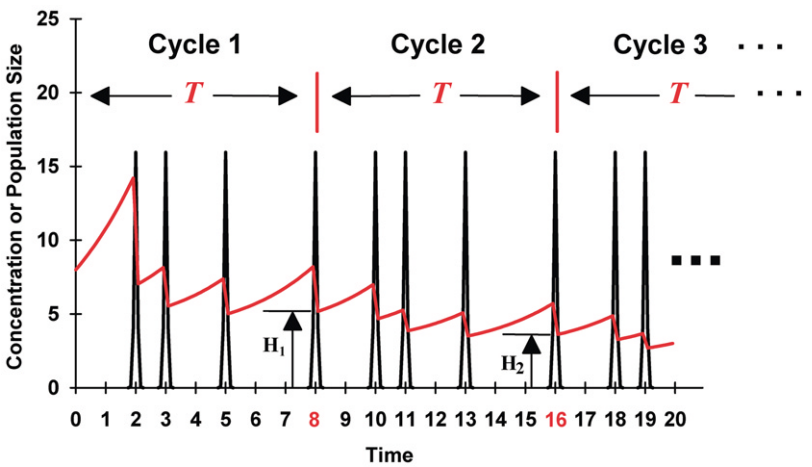

FIG. 4. Cyclic dose delivery. Doses are usually delivered in repeating cycles of width T. Here, $N=4$ doses of strengths $d=2$ are delivered at times $\tau_1=2$, $\tau_2=3$, $\tau_3=5$, and $\tau_4=T=8$, with the spacings repeated for each successive cycle. The parameters used here are $\lambda_1=0.5$, $\lambda_2=0.1$, $\gamma_1=0.3$, $\gamma_2=0.1$, $\alpha=1.0$, $\beta=0.1$. The measure of dose cycle effectiveness for ongoing dosing differs from the finite dosing case (Fig. 3). Here, it is the limiting ratio of the population size H_2 at the end of a cycle to the population size H_1 at the end of the previous cycle. This limiting ratio is the largest eigenvalue δ_1 of matrix Q [eqn (6)].

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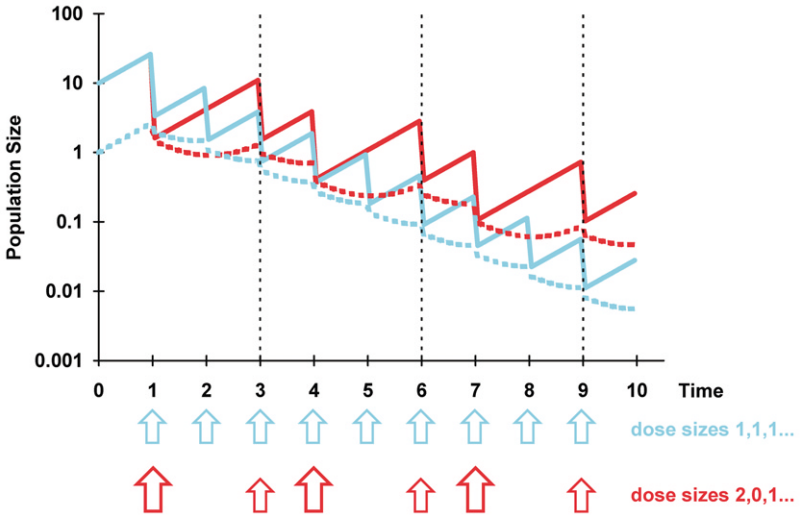

FIG. 5. Response to uneven vs. metronomic dose delivery. Shown are the responses to an irregular ($d=6$ at $\tau_1=1$; $d=0$ at $\tau_2=2$; $d=3$ at $\tau_2=3$; red lines), and a metronomic ($d=3$ at each of $\tau_1=1$, $\tau_2=2$, $\tau_3=3$; blue lines) dosing regimen, where the total dose delivered over the cycle in each case is fixed at 9. The parameters here are $\lambda_1=1.0$, $\lambda_2=1.0$, $\gamma_1=0.2$, $\gamma_2=1.8$, $\alpha=1.0$, $\beta=0.1$. The solid line in each case is the total population response and the dotted line the response of the more resistant subpopulation. Although the more up-front schedule is competitive early on, metronomic delivery provides better long-term suppression.

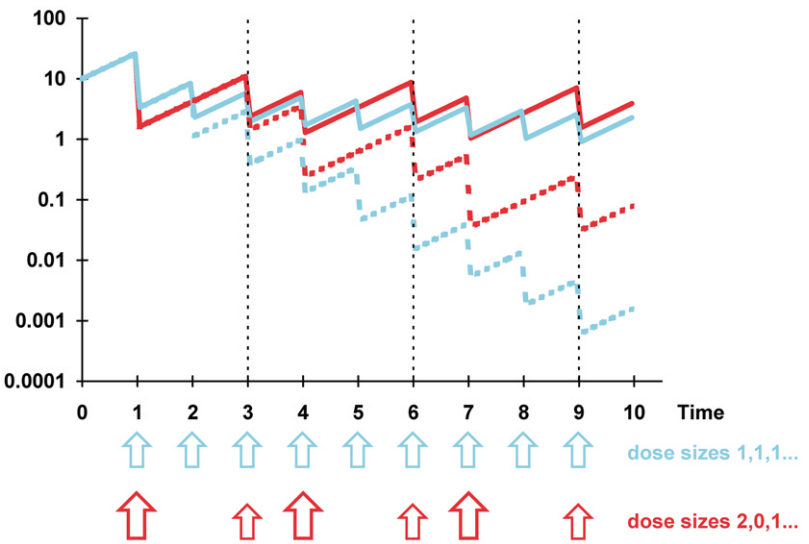

FIG. 6. Resensitization rate effects on dose response. The rate at which the two subpopulations comprising a heterogeneous tumor population attempt to re-establish their natural proportions following a dose experience may in itself affect how effective a shift to metronomic delivery may be. Two populations are considered. Both have the same parameters $\lambda_1=1.0$, $\lambda_2=1.0$, $\alpha=1.0$, $\beta=0.1$, but in one case, there is slow resensitization ($\gamma_1=0.1$, $\gamma_2=0.9$), while in the second the resensitization rate is four times faster ($\gamma_1=0.4$, $\gamma_1=3.6$). The differential response between irregular (solid red line) and metronomic (solid blue line) dosing is small in the slowly resensitizing case, while the differential is very large in the quickly resensitizing case (dotted red and blue lines). The former case may be more representative of the tumor cell population with its broad cycling range and inefficient resensitization, and the latter more representative of the endothelial cell population with its characteristically tighter cycling distribution and more efficient resensitization. This could explain the observed increased therapeutic gain against endothelium under metronomic dosing.

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may be concluded generally that a continuous dose administration is superior to other forms of dose administration, irrespective of the clearance rate.

Problems with Up-front Dosing—Clusters and Gaps

Recently, there has been considerable attention paid to how more continuous dosing schemes with antiangiogenic agents may better target the tumor endothelium (Hahnfeldt *et al.*, 1999; Drixler *et al.*, 2000; Fidler & Ellis, 2000; Kamen *et al.*, 2000), and how chemotherapy may be delivered so as to act more antiangiogenically, circumventing the problem of tumor cell resistance (Browder *et al.*, 2000; Kerbel, 1991). In particular, Browder *et al.* demonstrated that Lewis lung carcinoma previously made resistant to the chemotherapeutic cyclophosphamide by exposure to cycles of conventional up-front dosing with subsequent rest periods could still be suppressed by that agent by shifting to a metronomic delivery schedule free of extended rest periods. The more uniform schedule was also found to be more antiangiogenic.

As one explanation for the need to avoid extended gaps in treatment, a sublethal damage repair mechanism has been suggested (Ellis *et al.*, 2001). Sublethal damage repair by definition, involves the repairing of "wounded" cells before the next dose renders those cells lethally damaged. In radiobiology, however, it is already well-established that sublethal damage repair, where operative, underlies the greater potency of intense, up-front dosing, and that fractionation, the radiobiological equivalent of metronomic dosing, actually minimizes repair-related cell killing. Indeed, the idea of fractionation arose from the need to spare normal tissues (Hall, 1988). Its killing effect stems not from repair disruption, but from the capacity of a tissue to resensitize between fractions. It would seem, then, that if sublethal injury is somehow involved in the benefits of metronomic dosing in chemotherapy, it would have to be in the form of a delayed onset of such damage after drug exposure. In this case, though, the time to onset would constitute a sensitization period for the cells analogous to natural resensitization. On the subject of resensitization-type effects, it is worth pointing out that, although we focused here on a paradigm of direct toxicity in our description of what amounts to a saturation effect when doses are applied too quickly, this same saturation effect may also apply, for example, to secondary toxic processes elicited by dosing that contribute to suppression. In this case as well, the same overall resensitization kinetics would be demonstrated.

As for the concluded need to avoid large gaps (Ellis *et al.*, 2001), it could be argued that the problem with extensive gaps within a predefined dosing cycle period is not with the large gaps *per se*, but with the relative clustering of doses elsewhere in the cycle implied by those gaps. Tighter dose clusters can start to resemble up-front doses and take on the same inefficiencies from the maintenance standpoint.

Antiangiogenic Nature of Metronomic Dosing

As it turns out, differences in resensitization kinetics of endothelial vs. tumor cell populations following a dose exposure can explain a shift in targeting towards endothelium as dose is delivered more continuously or steadily. A diverse population such as a tumor tends to be less sensitive to dosing overall than its calculated cell-averaged sensitivity would suggest. This effect has been traced to the upward curvature of the exponential curve that relates dose to survival (Hahnfeldt & Hlatky, 1996). By an extension of this logic, any population exhibiting diversity in resensitization rate amongst its subpopulations should exhibit a lower overall resensitization rate with respect to dose response than a calculated average of those resensitization rates would suggest. The upshot is that, even if their calculated average resensitization rates are equal, heterogeneously resensitizing populations such as tumors will resensitize more slowly overall than will more uniformly resensitizing populations such as tumor endothelium. But a slower overall resensitization rate means a poorer response to metronomic dosing, as illustrated by eqn (5). The benefit of continuous or steady dosing is, therefore, expected to be more dramatic for the endothelium than for the tumor population, especially given that tumor

cells in fact have a lower calculated average resensitization rate than endothelial cells. All this may be demonstrated formally using a four-compartment version of the above model, consisting of two pairs of subpopulations that resensitize at different rates.

The consequence is a targeting bias towards the endothelial compartment as dose is applied more continuously or steadily (Fig. 6). Treatments with specific agents may even exacerbate this bias by selectively eliminating the more sensitive tumor cell clones over time. The result would be a tumor cell population that becomes comparatively more refractory to treatment while the therapeutic responsiveness of endothelium remains unchanged.

Conclusions

Given the all-too-common clinical scenario of having to chronically treat a recurrent tumor, treatment schedules that naturally anticipate the need for chronic suppression may be desirable over MTD strategies that continue to pursue eradication. In such instances, suppressive dosing strategies should outperform those having eradicative intent. Suppressive strategies are characterized by lower, more even dose delivery, in contrast to the high dose intensities seen with the MTD approach. Because residual cell sparing is inherent to the maintenance concept, low and even dose delivery should also present a reduced threat to the bone marrow and other at-risk stem cell compartments. This may be exploitable through the administration of slightly higher average dose intensities. By contrast, typical eradicative strategies have difficulty confining their objective to the tumor alone.

The prospect of having to forego an up-front eradication objective in favor of a more modest tumor suppression and maintenance approach is not without precedent. Suppressive therapy is already employed to treat such common diseases as herpes, malaria, and HIV. The occasions warranting the maintenance approach would seem to have some features in common. The invading population in each instance shows a remarkable resistance to obliteration owing to a significant diversity of survival response to deleterious agents. With few exceptions (e.g. tumors of the blood and germ cell variety), tumor cell populations prove to be extreme even in this regard. Their entropically driven, "fractalizing" diversity places them at the top of the list of target populations having the poorest prospects for up-front elimination (Folkman *et al.*, 2000), requiring instead a shift to the more chronic strategy of metronomic or continuous dosing.

A pivotal consequence of metronomic dosing derives from differences in resensitization kinetics between the tumor tissue and its associated endothelium. Because unlike tumor cells, endothelial cells are genetically stable (Folkman *et al.*, 2000; Anderson *et al.*, 2001), they display less diversity with respect to resensitization times following dosing. This implies a heightened overall resensitization rate for endothelium, and thus a higher relative susceptibility to metronomic dosing. This natural connection between metronomic dosing and antiangiogenic effect offers a theoretical rationale for a growing number of empirical observations (Browder *et al.*, 2000; Hainsworth & Greco, 1995; Gately & Kerbel, 2001; Mross, 2000; Soffer *et al.*, 2001), and because endothelium does not become resistant (Boehm *et al.*, 1997), reinforces the notion that long-term suppression may be had with reduced risk of tumor escape.

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