Rapidly acting antitumoral antiangiogenic therapies

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We deal with a biophysical description of antitumor antiangiogenic therapies. In particular, by means of some simple models, we study the possible effects of the delay between the drug consumption by endothelial cells and their death on the outcome of the therapy. We have found that this time lag implies an increase in the minimal dose guaranteeing tumor eradication and, if the delay is greater than a meaningful threshold, it may preclude the total regression. These results might be of interest in better understanding the causes underlying the contradictory literature on the clinical trials of antiangiogenic therapies.

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I. INTRODUCTION

Solid tumors, in their first phase of growth, are small aggregates of proliferating cells that receive oxygen and nutrients only through diffusion from external blood vessels. In order to grow beyond $1-2$ mm^3 [1], the formation of new blood vessels inside the tumor mass is required. Poorly nourished tumor cells start producing a series of molecular factors that stimulate (and also control) the formation of an internal vascular network [2]. This process, called angiogenesis, is sustained by a variety of mechanisms [2], such as the cooptation of existing vessels and the formation of new vessels from preexisting ones. As far as tumor-driven control of the growth is concerned, endogenous antiangiogenic factors have been both evidenced experimentally [3] and studied theoretically [4]. It has to be remarked that tumor vasculature may be not fully adequate to supply nutrients to all tumor cells, and thus the cells most remote from vessels may undergo necrosis. An excellent recent reference on this intricate topic is the biophysically oriented review paper [5].

Coming to therapeutic applications, there is compelling experimental evidence that inhibiting angiogenesis may induce tumor regression or sometimes cure [4]. Drugs having such inhibiting properties are called antiangiogenic drugs. Moreover, targeting tumor vasculature has been regarded as a means to overcome acquired drug resistance, since endothelial cells are considerably more genetically stable than the continuously mutating tumor cells [6]. In fact, antiangiogenic therapy provides "a mean to control an exceptionally heterogeneous, unconstrained tumor population via a relatively homogeneous and constrained endothelial population" [7], reducing the tumor capability to resist to the therapy. Most angiogenesis inhibitors are cytostatic agents that inhibit the formation of new blood vessels, but some direct inhibitors may have cytotoxic action, inducing rapid destruction of existing blood vessels [8]. Their effectiveness in the control and, in some cases, in the permanent remission of experimental tumors has been demonstrated [4,7], and the potentiality of antiangiogenic therapy in humans is currently investigated [4,6,9] with conflicting outcomes despite some encouraging results [4,9].

Among the factors that influence the clinical effectiveness of angiogenesis inhibitors, the administration schedule appears to be particularly relevant [9–11]. Antiangiogenic therapy has always been proposed as uninterrupted, long-term treatment, to obtain effective tumor growth control [8]. Although this concept has pervaded the clinical development of antiangiogenic drugs, a deeper insight into the relationships between drug pharmacokinetics and antivascular activity could be useful to improve clinical results.

Biophysical models of the interaction between tumor growth and the development of its vascular network, as well as of the action of angiogenesis inhibitors, could help in planning more effective antiangiogenic therapies. A number of quite complex mathematical models of the transition from the avascular to the vascular phase have been published in recent years [12,13], and interesting computational approaches which explicitly model some relevant aspects of therapies are described in Refs. [12,14].

A simple mathematical model that emphasizes the concept that tumor growth is a process strictly controlled by the development of vasculature has been proposed by Hahnfeldt *et al.* [7]. Focusing on tumor eradication, its potential for clinical applications was further exploited [10,11] under regimens of continuous infusion or periodic antiangiogenic therapy. The model [7] assumes that tumor cells produce two families of factors exerting a stimulatory and, respectively, an inhibitory effect on the vascular network. The model provides a framework to portray the effects of antiangiogenic therapies, and it was successful in fitting experimental data of the growth and the response to different antiangiogenic drugs of Lewis lung carcinomas implanted in mice.

The above model is based on the notion of the carrying capacity of the vasculature, $K(t)$, defined as the tumor volume potentially sustainable by the vessels. The carrying capacity will be proportional to the extent of effective vasculature. As a consequence, the dynamics of the tumor volume may be described by the following equation:

$$V' = VF\left(\frac{V}{K}\right), \tag{1}$$

where $F'(u)<0$, $F(1)=0$ and $F''(u)>0$ [11].

To model the regulatory action of stimulatory and inhibitory molecules, it has been assumed in Ref. [7] that (i) the

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system is in a regime of quasi stationary diffusion, (ii) the clearance rate of the inhibitory molecules is small, and (iii) the clearance rate of the stimulatory molecules is very high. The above assumptions imply that the concentration of the inhibitory factors inside the tumor is approximately proportional to the square of the tumor radius (i.e., to $V^{2/3}$), whereas the concentration of the stimulatory factors is roughly independent of the tumor size. Drugs are assumed to induce loss of the neo-formed vasculature according to a rate constant proportional to the drug concentration in blood. Summarizing, the following equation describing the dynamics of K was proposed [7]:

$$K' = bV - dV^{2/3}K - \mu K - \eta g(t)K, \tag{2}$$

where $g(t) \geqslant 0$ denotes the drug concentration in blood, b is a proportionality parameter for the "stimulatory capacity of tumor upon the inducible vasculature" [7], d is a proportionality parameter for the "endogenous inhibition of previously generated vasculature" [7], μ is the spontaneous loss rate constant of the vasculature, and η is a proportionality factor for the drug-induced loss rate of vessels. Note that the drugs examined in Ref. [7] (and in our work) act exclusively on the vessels, without direct antitumor effects, which implies that Eq. (1) remains unchanged. Note also that the possible insurgence of genetic resistance to the antiangiogenic therapy is disregarded in Refs. [7,10–14] and will be disregarded here.

The main aim of the present work is to help medical oncologists in identifying the basic mechanisms leading to the success or failure of clinical trials of antiangiogenic therapies. A central problem in the introduction of a new therapy based on a new drug is the identification of the minimal drug concentration in blood leading to remission from the disease. In the framework of the model [7], we have previously shown [10] that, in the case of continuous infusion therapy, this minimal concentration G_{cr}^{o} is simply related to the tumor production of factors stimulating the birth of new vessels:

$$G_{cr}^{o} = \frac{b-\mu}{\eta}. \tag{3}$$

Moreover, to explain some experimental findings, we stressed that the success of antiangiogenic therapies is strictly related to the clearance rate of the drug: drugs having excessively high values of this parameter are subjected to strong constraints on their schedulings; otherwise, they are ineffective. In this way we tried to explain why, in many clinical trials, continuous infusion therapy seems to be more effective than the boli-based therapy [9,15]. In Ref. [11], we rooted biologically these theoretical observations by relating them to some microscopic mechanisms such as intercell inhibition between tumor cells and tumor-vasculature cooperation. Another point of interest that we stressed in Refs. [10,11] is the fact that, in order to achieve tumor eradication, antiangiogenic drugs with purely cytostatic effect need a remarkable base-line loss rate of vasculature, which, on the contrary, seems to be, in some cases, small or zero [7].

In this paper, we focus on a new potential cause of failure of antiangiogenic drugs: the noninstantaneous death of endothelial cell (ECs) after drug consumption.

In the framework of chemotherapy, where it is not simple to assess the relation between drug concentration and cell death because of the complex pattern of drug effects on tumor cells [16], the relevance of the delay between drug uptake into the cell and their death has been discussed and modeled in Refs. [17,18]. In the framework of antiangiogenic therapy, where the targets of drugs are complex structures such as the blood vessels, it is very likely that the time between the drug uptake by endothelial cells and the loss of functionalityof vessels may be considerable. Moreover, the growth of blood vessels seems to be governed by two time scales of very different magnitude order. In fact, the dynamics of the ECs is by far more rapid than the characteristic growth times of the tumor, as also suggested by the values estimated in Ref. [7], where the characteristic tumor-driven time of ECs for Lewis' lung carcinoma in mice is $b^{-1} \approx 4.1$ h. Thus, the characteristic time of the dynamics of drug effects after the consumption by ECs may likely be a significant fraction of b^{-1}, or even be comparable with b^{-1}. Note also that in case of boli-based therapies, there is a third time scale to be taken into the account: the time scale of the drug concentration profile. For this reason, the delay in the death of ECs should be investigated by explicitly including it in the mathematical model.

II. MODELING THE DELAYED ECS DEATH

In order to model the effect of the time lags, we set the following simplifying assumptions: (i) immediately after the drug consumption the ECs remains alive, but because of the impairing of their internal functions, they are no longer under the influence of the pro- and antiangiogenic chemicals produced by the tumor (we will relax later this assumption); (ii) the vessels continue to carry the nutrients up to their death; (iii) the ECs enter in a state P (="poisoned") and remain there for a time t_P, after which they die. The time t_P is a random variable of which we know the probability distribution $\rho(t_P)$ and, as a consequence, also the cumulative probability distribution function $F(t_P) = \int_0^{t_P} \rho(u)du$ and the mean time T_P (mean time necessary for the blood vessels to die after the drug uptake). We shall model all this by adding to the model [7] a third compartment of "poisoned" vessels, and we shall denote by $K_P(t)$ the carrying capacity sustained by the poisoned vessels. As a consequence of the above assumptions, the equation ruling the dynamics of V has to be changed as follows:

$$V' = VF\left(\frac{V}{K+K_p}\right). \tag{4}$$

The quantity $K_p(t)$ obeys an integral equation obtained by summing for each past instant τ the vessels that, due to drug consumption, entered the P compartment and survived up to time t:

$$K_P(t) = \int_0^t S(t-\tau)\,\eta g(\tau) K(\tau) d\tau, \tag{5}$$

where S is the survival function. Equations similar to Eq. (5) arise in many compartmental models with delays—e.g., in

epidemic theory [19]. The survival function is defined as follows:

$$S(\theta) = \text{Prob}\{t_P > \theta\} = 1 - \text{Prob}\{t_P \leqslant \theta\} = 1 - F(\theta). \quad (6)$$

It is interesting to note that the function S is such that its integral is equal to the mean value of t_P:

$$\int_0^{+\infty} S(u)du = \int_0^{+\infty} u\rho(u)du = T_P.$$

Note also that the survival of poisoned cells to the spontaneous ECs loss (with rate μ) is "embedded" in $S(\theta)$. Assuming that the greater probability of death is for small times, followed by rapid decay, a good choice to approximate $\rho(t_P)$ is the exponential distribution. Otherwise, in the case of "bell-shaped distributions" (e.g., characterized by a well-defined mean time with a more or less strict range of variance), a good choice is the Erlang-Gamma distribution. Finally, the equation for K remains unchanged.

Under continuous infusion therapy the blood drug concentration profile is assumed to be approximately constant: $g(t)=G$. At the steady state all state variables will be constant and such that

$$K_P^e = \eta G K^e \int_0^{+\infty} S(u)du = \eta G T_P K^e, \quad (7)$$

$$V^e = K^e + K_P^e = (1 + \eta G T_P)K^e, \quad (8)$$

and imposing $K'=0$

$$d(V^e)^{2/3} = b(1 + \eta G T_P) - \mu - \eta G = b - \mu + \eta(bT_P - 1)G. \quad (9)$$

From Eq. (9) it follows that if

$$T_P > \frac{1}{b}, \quad (10)$$

i.e., if the mean killing time is greater than the mean characteristic time of the tumor-stimulated growth of vessels, there cannot be eradication. Eradication is possible only if $bT_P - 1 < 0$ and

$$G > G_{cr} = \frac{1}{\eta}\frac{b-\mu}{1-bT_P} > G_{cr}^0, \quad (11)$$

where G_{cr}^0 indicates the eradication threshold in the case of instantaneous death of vessels and G_{cr} is the minimal eradicative drug concentration under continuous infusion therapy in the presence of delays and absence of sensitivity, in poisoned vessels, to tumor-released chemical messengers. Formula (11) shows that not only must bT_P be less than 1, but also that bT_P has to be significantly smaller than 1. In fact, for bT_P slightly smaller than 1 G_{cr} is very large: for example, if $bT_P=0.5$, then $G_{cr}=2G_{cr}^0$, which might exceed the maximum tolerated dose for the host organisms.

The biological interpretation of the above relationships is straightforward: if the death of ECs is slower than the process of tumor-stimulated growth of vessels, there cannot be vessel elimination and, as a consequence, there cannot be tumor eradication. If the death process is only slightly faster, then a drug concentration markedly greater than that required in the base-line case of instantaneous vessel elimination is needed.

Moreover, if the death process induced by the drug is slower than the birth process, there is an increase of the carrying capacity, which is evident from Eq. (9): if $bT_P > 1$, the dependence of $V_e^{2/3}$ on the variable G is linear with positive slope; i.e., the steady-state tumor volume in the presence of therapy is greater than in the case of absence of therapy.

We remark here that the above model is minimal, since it is based on the highly idealized assumption (i), which we shall relax in the next section. However, this extreme idealization allowed us to infer, in a very general case, an interesting and biologically sound result.

III. SENSITIVITY TO THE TUMOR-RELEASED CHEMICAL MESSENGERS

The phenomenon of an increasing steady-state volume, in the case of long time lags, depends on the assumption that the poisoned vessels are not sensitive to the tumor action. If we suppose that they are sensitive to the release of anti angiogenic factors and that they might have a reduced or null sensitivity to the pro angiogenic factors and if, for the sake of simplicity, we assume that the distribution of t_P is exponential, instead of Eq. (5) we have the following equation:

$$K_P' = \eta G K + \lambda b V - d V^{2/3} K_P - (\mu + q)K_P, \quad (12)$$

where

$$0 \leqslant \lambda \leqslant 1$$

is the reduction factor for the stimulatory capacity of tumor and q is the loss rate constant of poisoned vessels in the case of the exponentially distributed "time to death."

At the steady state, after some simple algebra, it is easy to see that the equilibria are given by the intersection of the following two curves:

$$K_P = \frac{V}{q}[(1+\lambda)b - \mu - dV^{2/3}] \quad (13)$$

and

$$K_P = \frac{(\lambda b + \eta G)V}{\eta G + \mu + q + dV^{2/3}}. \quad (14)$$

Considering that for $G >> 1$ the second curve gives $K_P \approx V$, one finds that $(1+\lambda)b - \mu > q$ implies the impossibility of tumor eradication. Since in this case the average time to death for the poisoned vessels is $T_P = (\mu+q)^{-1}$, then

$$(1+\lambda)bT_P > 1 \quad (15)$$

implies that eradication is impossible: the null equilibrium is unstable and there is another noneradicative equilibrium $V^* \in (0, V_e^0)$. Note that (i) if $\lambda=0$, we recover the rule $bT_P > 1$; (ii) if $\lambda \in (0,1)$, one gets that if

$$T_P > \frac{b^{-1}}{1+\lambda} \in (0.5b^{-1}, b^{-1}),$$

then there is no eradication, so we obtained a stricter constraint. Biologically this is due to the fact that if $\lambda > 0$, then there is tumor-stimulated growth of poisoned endothelial cells.

Setting $Z = dV^{2/3}$ and solving the resulting second-degree algebraic equation

$$T_P Z^2 + \{[G\eta - b(\lambda+1) + \mu]T_P + 1\}Z + \mu + G\eta \times [1 - b(\lambda+1)T_P] - b(\lambda\mu T_P + 1) = 0, \quad (16)$$

condition (15) is recovered and the eradication condition becomes

$$G > G_{min}(T_P, \lambda) = \frac{1}{\eta}\frac{b(\lambda\mu T_P + 1) - \mu}{1 - b(\lambda+1)T_P}, \quad (17)$$

where $G_{min}(T_P, \lambda)$ is defined as the minimal eradicative drug concentration under continuous infusion therapy in the presence of both delays and sensitivity, in poisoned vessels, tumor-released chemical messengers.

Observe that $\lim_{T_P \to 0^+} G_{\min}(T_P, \lambda) = G^0_{cr}$ and that for $\lambda = 0$ it yields

$$G_{min}(T_P, 0) = \frac{G^0_{cr}}{1 - bT_p}. \quad (18)$$

Moreover, when $\lambda = 0$, $b < q$, and $G < G_{min}(T_P)$, rewriting Eq. (16) in the form

$$Z = b - \mu - \eta G \frac{q}{q + \mu + \eta G + Z}, \quad (19)$$

it is easy to notice that its positive solution is such that $Z \in (b - \mu - \eta G, b - \mu)$; i.e., the equilibrium volume is smaller than that reached in the absence of therapy, but it is greater than the volume reachable in the case of an instantaneous effect of the drug ($q = \infty$). If $q < b$, Eq. (19) indicates that also for extremely elevated doses, the minimum reachable volume (in the case of very high G) is $V_{min} = [(b - \mu - q)/d]^{3/2}$. Finally, we notice here that if $\lambda = 0$, then the non eradication condition $bT_P > 1$ may be recovered also for probability distributions more general than the exponential one [20].

IV. LOCAL STABILITY

For the case $\lambda = 0$, the local asymptotic stability of the non-null equilibrium (when it exists) can be studied analytically. By defining the new variable $\sigma = K + K_p$, we can study the equivalent system (V, σ, K_p). By means of Mathematica, we obtained the characteristic polynomial at the equilibrium point, which yielded the following Routh-Hurwitz condition:

$$(q + \mu - b + P_2)\alpha^2 + \left[P_1 + \left(\mu + \frac{5}{3}z\right)(q + \mu - b)\right]\alpha + P_0 > 0, \quad (20)$$

where $\alpha = -F'(1) > 0$ and $P_2 > 0$, $P_1 > 0$, and $P_0 > 0$ denote three polynomial positive functions of the parameters of the system, including G. Clearly, if $q + \mu > b$ [and there exists the equilibrium point $G < G_{min}(q)$], then inequality (20) is satisfied and there is local asymptotic stability. If $q + \mu = b$, then the equilibrium point is, of course, locally stable as well. In the case $q + \mu < b$, a careful analysis of P_2, P_1, and P_0 [and of the properties of Eq. (16)] allowed one to verify that the Routh-Hurwitz condition holds. This fact and extensive simulations suggested that the equilibrium should also be globally stable. Other simulations seem to indicate that, when there is eradication, it is globally stable as well. We plan to demonstrate analytically these properties.

V. INFLUENCE OF THE "TIME FROM POISONING"

A more detailed description of the poisoned vessels is needed in order to stress the influence of the time from the drug consumption id est, more informally speaking, the time τ elapsed from the vessel "poisoning" (TFP). In fact, the greater the TFP is, the greater is the rate of death and the smaller is the stimulatory effect of the proangiogenic chemicals produced by the tumor on the poisoned vessels. Thus we define the function $\varphi(t, \tau)$ such that $\varphi(t, \tau)d\tau$ measures, at time t, the tumor volume sustainable by the vessels that were poisoned between $\tau + d\tau$ and τ time units before. Furthermore, we assume that (i) the death rate of poisoned vessels $q(\tau) > 0$ is a non decreasing function of the TFP: $q'(\tau) \geqslant 0$; (ii) the sensitivity of the poisoned vessels, $\lambda(\tau) \in [0, 1]$, is a decreasing (or identically null) function of TFP: $\lambda'(\tau) \leqslant 0$. Based on the above assumptions, we obtain the following infinite-dimensional model:

$$V' = VF\left(\frac{V}{K + \int_0^{+\infty} \varphi(t, \tau)d\tau}\right), \quad (21)$$

$$\frac{\partial \varphi}{\partial t} + \frac{\partial \varphi}{\partial \tau} = \lambda(\tau)bV - [\mu + q(\tau)]\varphi, \quad (22)$$

$$\rho(t, 0) = \eta G K(t), \quad (23)$$

whereas the dynamics of K is ruled by Eq. (2). The equilibrium distribution of φ is

$$\varphi_e(\tau) = e^{-\mu\tau - dV_e^{2/3}\tau - Q(\tau)} \times \left(bV_e \int_0^{\tau} be^{\mu U + dV_e^{2/3}U + Q(U)}\lambda(U)dU + G\eta K_e\right) = \eta G K_e r_1(\tau; V_e, Q(\cdot)) + V_e r_2(\tau; V_e, \lambda(\cdot), Q(\cdot)),$$

with $Q(\tau) = \int_0^{\tau} q(w)dw$. As far as the steady-state tumor volume V_e is concerned, we must have

$$V_e = K_e + \int_0^{+\infty} \varphi_e(\tau)d\tau = K_e[1 + \eta G R_1(V_e, Q(\cdot))] + bV_e R_2(V_e, \lambda(\cdot), Q(\cdot)), \quad (24)$$

where R_1 and R_2 are the integrals for $\tau \in [0, +\infty)$ of r_1 and r_2, respectively, which leads to

$$K_e = \frac{1 - bR_2(V_e, \lambda(\cdot), Q(\cdot))}{1 + \eta G R_1(V_e, Q(\cdot))} V_e. \tag{25}$$

From Eq. (2) it must be

$$K_e = \frac{bV_e}{dV_e^{2/3} + \mu + \eta G}. \tag{26}$$

Thus, it follows that the eradication condition is given by

$$\frac{1 - bR_2(0, \lambda(\cdot), Q(\cdot))}{1 + \eta G R_1(0, Q(\cdot))} > \frac{b}{\mu + \eta G},$$

that is

$$G > G^{\infty}(\lambda(\cdot), Q(\cdot)) = \frac{b[1 + \mu R_2(0, \lambda(\cdot), Q(\cdot)) - \mu]}{1 - b[R_1(0, Q(\cdot)) + R_2(0, \lambda(\cdot), Q(\cdot))]}, \tag{27}$$

again obtaining an upper limit for b. In particular, for $\lambda(\tau) = 0$ we have the constraint

$$R_1(0, Q(\cdot)) < b^{-1}. \tag{28}$$

Finally, it is easy to verify that R_1 is decreasing for increasing $q(\cdot)$, id est:

$$q_l(\tau) < q_h(\tau) \text{ for all } \tau \in [0, +\infty) \Rightarrow R_1(0, Q_h(\cdot)) < R_1(0, Q_l(\cdot)),$$

with evident biological meaning: kill quickly is better.

VI. CYTOSTATIC AND CYTOTOXIC DRUGS

Let us now suppose that the drug has both cytotoxic and cytostatic effects. In such a case, the equation ruling the dynamics of K is as follows:

$$K' = b\frac{a}{a + g(t - w)} V - dV^{2/3} - \mu K - \eta g(t) K, \tag{29}$$

where the effect of the cytostatic antiangiogenic drug, as in Refs. [10,11], is roughly summarized by means of a reduction of the constant rate b and w is a time lag that models, in a very simple way, the delay between the drug consumption and their effect in reducing b. The constant a is the halving constant, since for $G=a$ the "stimulatory capacity of tumor upon the inducible vasculature" [7] is halved. In order to find the minimal eradicating dose under continuous infusion therapy (CIT), proceeding as in the previous section, we obtain the equation

$$(1 + \eta T_P G) b \frac{a}{a + G} = \mu + \eta G. \tag{30}$$

Equation (30) has a straightforward geometrical interpretation: of finding the intersection between an hyperbole and a straight line. Note that in the absence of delay, the minimal dose eradicating G_{CS}^{o} is anyway smaller than G_{cr}^{o}, due to the synergy between the two effects. For $T_P > 0$, since $(1 + \eta T_P G) b[a/(a+G)] > b[a/(a+G)]$, the minimal eradicating dose G_{CS} is such that $G_{CS} > G_{CS}^{o}$. Furthermore, if the hyperbole $(1 + \eta T_P G) b[a/(a+G)]$ is increasing—i.e., if $(\eta a) T_P > 1$—then, $G_{CS} > G_{cr}^{o}$. Biologically this means that for $0 < T_P < (\eta a)^{-1}$ the presence of the delay partially compensates for the beneficial effects obtained with the cumulative cytostatic+cytotoxic action; if $T_P \geqslant (\eta a)^{-1}$, eradication is reached with a drug concentration greater than that needed for eradication in the absence of cytostatic effects and time lags.

Note that also in this case the non-null equilibrium point is locally asymptotically stable. In fact, it is possible to repeat the analysis done in Sec. IV, provided that, instead of b, we use the parameter $b^* = b[a/(a+G)]$.

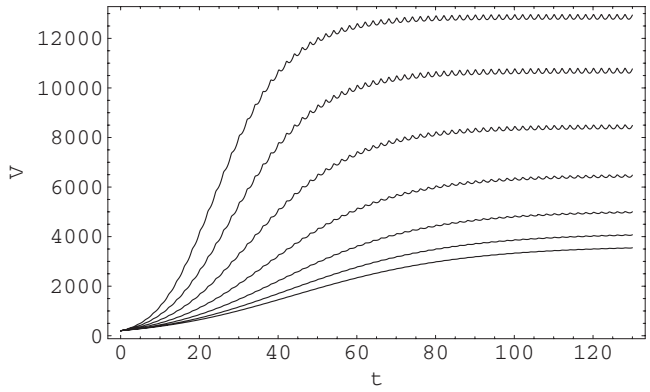

FIG. 1. Simulations of the time course of the tumor volume under boli-based therapy with $q < b$ and increasing values of the dose (from top to bottom, $D = 20, 40, 80, 160, 320, 640, 1280$ mg/kg). Simulated drug: endostatin, $c = 1.7$ day^{-1}. Tumor volume V (mm^3), time t (days). $F(u) = \alpha \ln(u)$, $\alpha = 0.192$ day^{-1}, $T = 2$ days, $b = 5.85$ day^{-1}, $d = 0.00873$ day^{-1} mm^{-2}, $\eta = 0.66$ day^{-1} kg/mg.

VII. NUMERICAL ANALYSIS

We performed some computer simulations with Mathematica in order to assess the behavior of the models under boli-based therapy. We assumed a monoexponential dynamics for the drugs [7,10,11]:

$$g(t) = \frac{D}{1 - \exp(-cT)} \exp[-c \bmod(t, T)], \tag{31}$$

where c is the clearance rate constant of the drug, T is the time interval between two boli [the period of $g(t)$], and D is the dose per kilogram. In all simulations we used $F(u) = \alpha \ln(u)$ [7] and the numerical values of the parameters estimated in Ref. [7].

In Fig. 1 we show the behavior of V for $q < b$ under various increasing values of the dose D. As in the CIT, tumor eradication is never reached and the asymptotic behavior results in being a small oscillation around a mean value close to the minimum value predicted under CIT for $G \gg 1$. Figure 1 reports simulation results for the drug endostatin, whose clearance is $c = 1.7$ day^{-1}. In Refs. [10,11], we stressed that drugs having high clearance rates need to have mean values $\langle g(t) \rangle$ considerably higher than the CIT minimal drug concentration G_{min} in order to achieved tumor eradication under

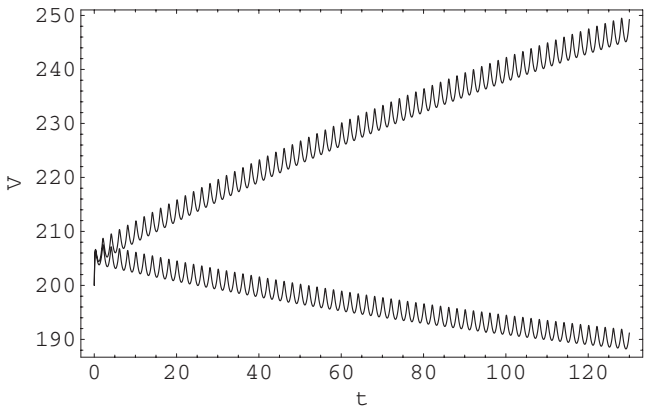

FIG. 2. Simulations of the time course of the tumor volume under boli-based therapy with angiostatin ($c=0.38$ day^{-1}, $\eta=0.66$ day^{-1} kg/mg) with $q=3b>b$ and two values of the dose: $D=42$ mg/kg$<D_{min}^{Boli}$ (upper curve: no eradication) and $D=43$ mg/kg$>D_{min}^{Boli}$ (lower curve: eradication). Other parameters values as in Fig. 1. Tumor volume V (mm^3), time t (days).

periodic therapy. In our simulations we observed a similar phenomenon concerning drugs with high clearances (e.g., TNP 740, with $c=10.1$ day^{-1}): the convergence towards V_{min} is by far slower for drugs having high c. Note anyway that excessively high values of G are not compatible with tolerable toxicity in patients.

Being $\langle g(t)\rangle=D/(cT)$, if there were a "one-to-one" correspondence between CIT and periodic therapy, the eradication condition would be $D>D_{min}(q;c,T)=cTG_{min}(q)$. In the case of an instantaneous effect of the drug, we find in Refs. [10,11] that in reality the minimal eradicating dose is, roughly speaking, a more than linearly increasing function of cT. In the numerical simulations we performed, we recovered the same phenomenon. E.g., for $q=3b$ (i.e., $T_P\approx 82$ min) and drug angiostatin with $c=0.38$ day^{-1}, the correspondence with the CIT would imply an eradication threshold $D_{min}^{CIT}\approx 22.23$ mg/kg. Note that in absence of delay the threshold would have been 7.65 mg/kg.

Figure 2 shows that for angiostatin the value of the effective threshold under periodic therapy is $D_{min}^{Boli}\approx 2D_{min}^{CIT}$. Moreover, increasing cT, our simulations indicate that an increasingly higher threshold has to be exceeded in order to achieve the eradication, exactly as in the case of absence of delays. For TNP 740, with $c=10.1$ day^{-1}, for which $D_{th}(q)=68.2$ mg/kg (with no delay: 45mg/kg) our simulations show a remarkable increase in the effective threshold. Finally, our simulations did not stress new effects due to interferences between q and the c.

VIII. FINAL REMARKS

We have developed here a mathematical model for the tumor-vasculature interaction and the antiangiogenesis therapy that takes into account a delay between the consumption of a drug by endothelial cells and their death. Our aim was to assess the possible influence of the noninstantaneous death of vessels on the outcome of this type of antitumoral therapies and, as a consequence, on the dosing of the drugs. In the case of continuous infusion therapy, which was revealed to be the most effective way to administer antiangiogenic therapies, our models indicate that a mean delay greater than the mean characteristic time of the tumor-stimulated growth of vessels impedes the tumor eradication, which is not attained even in the theoretical case of an infinite concentration of the drug. More in general, our models showed that the presence of the delay implies that the administered dose must be larger than in the case of instantaneous death, in order to achieve remission from the disease. Numerical simulations seem to indicate that for periodical boli-based schedulings similar results hold. Quite interestingly, all the eradication conditions (under CIT) given here and in Refs. [10,11] are independent of the parameters ruling the dynamics of the tumor volume.

Note that in Ref. [7] and in the present work "a vast array of spatial… details of tumor cell expression" [7] has been disregarded, though the ordinary differential equation (ODE) models of Ref. [7], which we extended here, were derived starting from a simple spatial partial differential equation (PDE) model by applying the assumptions (i)–(iii) of Sec. I.

We would like to stress that this lack of an explicit spatial description is a strong limitation, since it implies that our models are more explanatory and qualitative than predictive and quantitative. On the contrary, it would be important to deepen our analysis by including in detail spatial effects (which we hope to do in the near future). In particular, for a fair quantitative analysis, the spatial discrepancies between blood flow and perfusion must be properly taken into account. In fact, the carrying capacity "is a measure of actual tumor sustenance and thus ignores that portion of the microcirculation that may be dysfunctional for a variety of reasons" [7]. Furthermore, a spatial modeling might allow one to describe in detail the phenomena of "physical resistance," id est of resistance unrelated to genetic instability (the endothelial cells being far more stable than the tumor cells), since they are due to more subtle phenomena [21], such as problems related to the interaction between some drugs and the surface of target cells. Many of these phenomena have been pointed out in chemotherapy, and some of them (or others) might be present in antiangiogenic therapy. However, from a qualitative point of view, including all these spatial phenomena should not lead to biological results significantly different from those illustrated here—i.e., that one of the main possible causes of failure of the therapeutic experiments in antiangiogenic therapy might be the delay in the response to drugs after the drug consumption by the ECs. These findings might have some relevance in clinical applications. We hope that our theoretical analysis might trigger experimental and biostatistical investigations aimed at assessing the vital dynamics of endothelial cells after the administration of antiangiogenic drugs and their influence on the survival of patients.

Finally, we note that we considered here purely antiangiogenic drugs, because we were interested in stressing the effect of time delays in EC death. However, it is also of interest in the study [22] of therapies using drugs having both direct antitumor effects and antiangiogenic effects (or, as in Ref. [14], of a combination of chemo therapy and antiangiogenic therapy), which would lead to modify Eq. (1) by adding a therapy term as follows:

$$V' = VF\left(\frac{V}{K}\right) - \gamma h(t)V \tag{32}$$

[with $h(t)=g(t)$ for therapies having double effect] and appropriate modifications to model the resistance to chemotherapy. The main results obtained in Ref. [22], under the hypothesis that the cytotoxic effect on tumor cells alone is not effective in eradicating the disease, are the following:

(i) In the absence of delays and of resistance to chemotherapy, the presence of the cytotoxic action on tumor cells reduces the minimal dose required to eradicate the tumor. This is an obvious consequence of the synergy between the direct chemotherapeutic effect and the antiangiogenic effect.

(ii) Introducing the delay but not the resistance, one obtains, proceeding as in the previous sections, that the minimal dose for eradication must be increased.

(iii) In the presence of the resistance to chemotherapy, the subpopulation of tumor cells that are sensitive to the therapy becomes extinct. Thus the model of Ref. [22] reduces to that illustrated here, and antiangiogenic therapy may induce tumor remission, of course provided that condition (17) holds.

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[20] In fact, in such a case, in the steady state, we may embed the influence of the tumor-produced inhibiting factors in the survival S and obtain a relationship of the form $K_P=\eta G(V-K_P)\int_0^{+\infty}S(\tau;V)d\tau$, which for $G\gg 1$ reads $K_P\approx V$. By using Eq. (13), one easily recovers the noneradication condition $bT_P>1$.

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