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TUMOUR ERADICATION BY ANTIANGIOGENIC THERAPY: ANALYSIS AND EXTENSIONS OF THE MODEL BY HAHNFELDT ET AL. (1999)

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Abstract

The model proposed by Hahnfeldt *et al.* (1999) describes the growth of a tumour assuming that tumour growth is strictly controlled by the evolution of the vascular network that supplies oxygen and nutrients to tumour cells. Consequently, it provides a framework to represent the effects of antiangiogenic therapies. In this paper, some possible modifications of that model are proposed, and conditions that guarantee the eradication of the tumour under a regimen of periodic antiangiogenic therapy are derived. The model variants considered assume the potential doubling time of the vasculature to be constant, and subdivide the endothelial cell pool, which is involved in angiogenesis, in resting and proliferating cells allowing for a more detailed description of drug effects.

Key words: Tumour growth models, Angiogenesis, Periodic anticancer therapy, Global stability, Forced oscillators

1. Introduction

During the progression of tumours, the development of a vascular network inside the tumour mass becomes necessary to support the tumour growth [1]. This phase (vascular phase of growth) may be preceded by an avascular phase in which the tumour is a small mass of proliferating cells that receive oxygen and nutrients only through diffusion from external blood vessels. Multicellular spheroids growing *in vitro* [2] can be considered good experimental models of the avascular phase, and the growth of multicellular spheroids has been extensively investigated by mathematical models (see the review by Adam [3], and more recently: Byrne [4], Ward and King [5,6]). The complex process which leads to the formation of new blood vessels is named *angiogenesis* and is stimulated and controlled by molecular factors released by the tumour itself [1,7,8]. The tumour vasculature, however, may be inadequate to supply all the tumour cells, and consequently the cells most remote from vessels may undergo necrosis. Histological studies have indeed evidenced the presence of large regions of necrosis into solid tumours. Mathematical models of the process of formation of new vessels under the angiogenic stimulus are presented by Chaplain [9] and Anderson and Chaplain [10].

Models aimed at describing the growth of tumours in the vascular phase including also the development of the vasculature are rather few. A simple mathematical model that emphasizes the concept that the tumour growth is a process strictly controlled by the development of the vasculature has been proposed by Hahnfeldt *et al.* [11]. This model assumes that tumour cells produce two family of factors exerting a stimulatory and, respectively, an inhibitory effect on the vascular network. Endogenous antiangiogenic factors have been evidenced [12,13], and the control of tumour growth by the balance of proangiogenic and antiangiogenic signals has also been considered by Ramanujan *et al.* [14]. More complex PDE models with spatial structure, that include tumour cells, vascular endothelial cells, angiogenesis stimulatory factors, and possibly the cell nutrient, have been proposed by De Angelis and Preziosi [15] and Jackson [16], whereas in Breward *et al.* [17] angiogenesis is introduced in the framework of a mechanical model of tumour growth. Models that distinguish between immature and mature vessels and describe vessel maturation and destabilization, have been presented in Arakelyan *et al.* [18,19].

The model in [11] can easily represent the effect of antiangiogenic drugs, and the predictions of the model have been successfully compared with the volume response of an experimental subcutaneous tumour (a Lewis lung carcinoma implanted in mice) treated with different drugs. A modification of this model has been recently adopted by Ergun *et al.* [20] in a theoretical study of optimal antiangiogenic therapy. The effectiveness of antiangiogenic drugs in the control, and in some case in the permanent remission, of experimental tumours has been demonstrated [21], and the potentiality of antiangiogenic therapy in humans is currently investigated. Antiangiogenic therapy should not be impaired by the capability of tumour cells to evolve toward resistant phenotypes, since the therapy is directed against non-transformed, genetically stable endothelial cells, and this fact is a strong point favouring it [22]. The antiangiogenic potential of conventional chemotherapeutic drugs has also been shown [23,24].

In this paper, the model by Hahnfeldt *et al.* [11] is reconsidered with the aim of deriving conditions that guarantee the eradication of the tumour under a regimen of periodic antiangiogenic therapy. Some possible modifications of the model are proposed. The model variants we consider assume the potential doubling time of the vasculature (*i.e.* the doubling time in the absence of vasculature loss) to be constant, and subdivide the endothelial cell pool which is involved in the angiogenesis and originates the vessels into resting and proliferating cells. This latter modification could make it possible a more detailed description of drug effects.

2. The model by Hahnfeldt et al. (1999)

2.1 The original model

The model proposed by Hahnfeldt *et al.* [11] describes the growth of an experimental tumour under the main assumption that tumour growth is strictly controlled by the evolution of the vascular network. To account for this control, the authors introduce the concept of *carrying capacity* of the vasculature, defined as the tumour volume potentially sustainable by it. The carrying capacity will be proportional to the effective vasculature extent, and will be a time dependent variable. The model has been used to describe both the unperturbed growth and the tumour response to regimens of antiangiogenic drugs.

The dynamics of the tumour volume $V(t)$ is described by

$$V' = \alpha V f\left(\frac{V}{K}\right), \tag{2.1}$$

where $K(t)$ is the carrying capacity of the vasculature at time t, and f is a smooth function of V/K decreasing on $(0, \infty)$ and equal to zero for $V/K = 1$ [25]. In particular, in [11] the following form was chosen

$$V' = -\alpha V \ln\left(\frac{V}{K}\right). \tag{2.2}$$

It can be noted that the Gompertz form of this equation is not to be related to the possible Gompertzian growth of the overall tumour, since the time behaviour of V, according to Eq. (2.2), is ultimately determined by the behaviour of K as a function of time. In fact, if $K(t)$ were exponentially growing with a given rate constant, $V(t)$ would exhibit asymptotically an exponential growth with the same rate constant of K. What Eq. (2.2) actually says is that the tumour volume would grow according to a Gompertz curve towards a constant value, if the vasculature extent were constant in time. In the following we will consider for V also the equation

$$V' = \alpha V \left(1 - \frac{V}{K}\right), \tag{2.3}$$

in which the r.h.s. has the logistic form. We notice that when $V < K$, V'/V has an upper bound given by α according to Eq. (2.3), whereas it is unbounded according to Eq. (2.2). Since the doubling time of a tumour, even in the case of an optimal vascular support, is bounded by the mean cell cycle time of tumour cells, equation (2.3) appears more sound on a biological basis. When K is lower than V, Eq. (2.1) predicts a decrease of V, that is the tumour regression.

Concerning the development of the tumour vascular network, the authors assume that angiogenesis is regulated by the action of stimulatory and inhibitory molecules, secreted by the tumour itself. Moreover, they assumed that: (*i*) the tumour has spherical symmetry; (*ii*) the secretion rates of factors within the tumour mass are constant in space and time; (*iii*) the transport of molecules is due to diffusion (and diffusion is quasi-stationary); (*iv*) the clearance rate of the inhibitory molecules is much smaller than D/R^2, D being the diffusion coefficient and R the tumour radius; (*v*) the clearance rate of the stimulatory molecules is much greater than D/R^2. From these assumptions it is possible to show that the concentration of the inhibitory factor inside the tumour will be approximately proportional to the square of the tumour radius, whereas the concentration of the stimulatory factor will be relatively independent of the tumour size. On the basis of this result, the presence of a vasculature loss, induced by the inhibitory factors,

having a rate constant proportional to $V^{2/3}$ has been hypothesized. Moreover, to represent the tumour response to treatments by antiangiogenic drugs, drugs are assumed to induce loss of the neo-formed vasculature according to a rate constant proportional to the drug concentration in blood. In view of these assumptions, Hahnfeldt et al. proposed the following equation for K:

$$K' = bV - (\mu + dV^{2/3})K - \eta g(t)K, \tag{2.4}$$

where $g(t) \geq 0$ denotes the drug concentration. The term $-\mu K$ represent a spontaneous vasculature loss independent of the inhibitory signal generated by the tumour. In the unperturbed growth ($g(t) = 0$), the negative term in (2.4) proportional to $V^{2/3}$ is of fundamental importance since it determines the saturating behaviour of tumour growth. If it were absent, Eqs. (2.1),(2.4) would admit an exponentially growing solution.

It can be easily seen that system (2.1),(2.4) has the set $\mathbb{R}^2_+$ (the positive quadrant of $\mathbb{R}^2$) as the positively invariant set, and in the absence of therapy if $b > \mu$ it has there a unique equilibrium point (V_e, K_e), with

$$K_e = V_e = \left(\frac{b-\mu}{d}\right)^{3/2}. \tag{2.5}$$

Note that $(0,0)$ is not an admissible point.

2.2 Constant potential doubling time of the vasculature

According to equation (2.4), the ratio K'/K has bV/K as upper limit, so that the potential doubling time of the carrying capacity (defined by the value of $\ln 2/(K'/K)$ in the absence of vasculature loss) is not constant in time, and can become very short when $K < V$, *i.e.* during the therapy. Since in such case the overall loss term in (2.4) may be small because V can decrease and $g(t)$ can be close to zero at some times, for instance at the end of the time intervals between successive bolus administrations of a rapidly cleared drug, the actual growth rate of K may result so fast that could hardly meet the limitations imposed by the proliferation kinetics of the vessel endothelial cells. This behaviour can be seen in one of the simulations reported in [11] (fig. 3b of that paper). In a more detailed modelling, the growth term of K should take into account the proliferation of endothelial cells within the tumour, their migration from the peritumoral region into the tumour, and the possible contribution of circulating endothelial precursors. To account for the proliferation kinetics of endothelial cells, in a simplified view the growth term in the equation for K may be assumed proportional to K with a rate constant independent of the tumour volume (according to the hypothesis of a concentration of stimulatory factors independent of the tumour size). Thus, we will consider the following equation for K, as an alternative to Eq. (2.4):

$$K' = bK - (\mu + dV^{2/3})K - \eta g(t)K. \tag{2.6}$$

A dynamics for the carrying capacity independent of V has been proposed in [20].

Numerical simulations have shown that model (2.2),(2.6) describes the unperturbed growth with a shape comparable to that of model (2.2),(2.4). Figure 1 gives an example of growth curves (having the same equilibrium point) produced by the two models. The system (2.1),(2.6) has the point defined by (2.5) as equilibrium point.
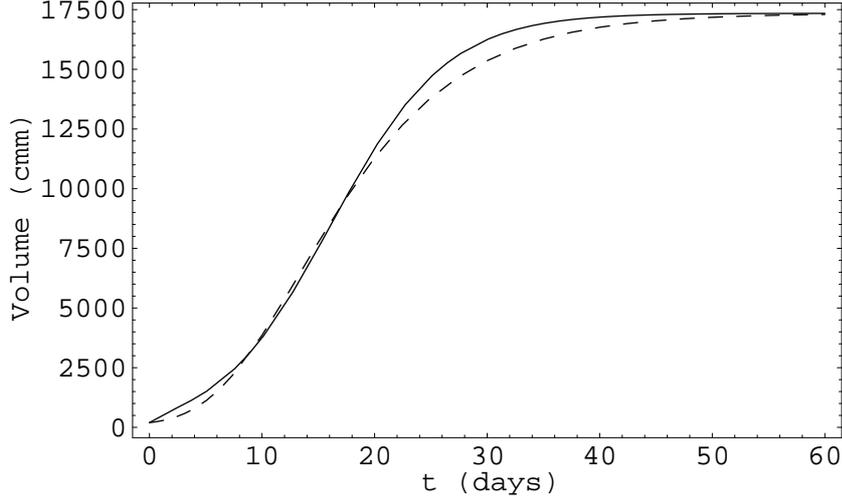
6.

Fig. 1. Volume growth of unperturbed tumours. Growth according to Eqs. (2.2),(2.4) (dashed line): $\alpha = 0.192$ day^{-1}, $b = 5.85$ day^{-1}, $\mu = 0$, $d = 8.73 \cdot 10^{-3}$ day^{-1}mm^{-2}, $V(0) = 200$ mm^3, $K(0) = 625$ mm^3 (reference values, as in Hahnfeldt et al. 1999). Growth according to Eqs. (2.2),(2.6) (solid line): $\alpha = 1.08$ day^{-1}, $b = 0.243$ day^{-1}, $\mu = 0$, $d = 3.63 \cdot 10^{-4}$ day^{-1}mm^{-2}, initial condition unchanged.

2.3 Stability of the equilibrium point

We will consider here the behaviour of the tumour in the absence of therapy, that is when $g(t) = 0$. By means of the variable and parameter transformations

$$\bar{t} = \alpha t \tag{2.7}$$

$$(\bar{b}, \bar{\mu}, \bar{d}) = \frac{1}{\alpha}(b, \mu, d) \tag{2.8}$$

$$x = \ln\left(\frac{V}{V_e}\right) \tag{2.9}$$

$$y = \ln\left(\frac{K}{K_e}\right), \tag{2.10}$$

from Eqs. (2.2),(2.4), (2.3),(2.4), (2.2),(2.6) and (2.3),(2.6) we obtain, respectively, the following bidimensional systems:

$$x' = -x + y, \quad y' = \bar{b}e^{x-y} - \bar{\mu} - (\bar{b} - \bar{\mu})e^{\frac{2}{3}x} \tag{s1}$$

$$x' = 1 - e^{x-y}, \quad y' = \bar{b}e^{x-y} - \bar{\mu} - (\bar{b} - \bar{\mu})e^{\frac{2}{3}x} \tag{s2}$$

$$x' = -x + y, \quad y' = (\bar{b} - \bar{\mu})(1 - e^{\frac{2}{3}x}) \tag{s3}$$

$$x' = 1 - e^{x-y}, \quad y' = (\bar{b} - \bar{\mu})(1 - e^{\frac{2}{3}x}), \tag{s4}$$

For the above systems, $\mathbb{R}^2$ is the feasible set and $(0,0)$ the unique equilibrium point. The eigenvalues of the Jacobian matrix computed at the equilibrium point for the systems (s1) and (s2) are real and negative, whereas, in the case of systems (s3) and (s4), they have negative real part, and are real if and only if $(b - \mu)/\alpha \leq 3/8$. Thus the equilibrium point is locally asymptotically stable. Concerning the global stability, we have the following:

Proposition 1. *Let us consider the systems given by Eqs. (2.2),(2.4) and (2.3),(2.4) with* $g(t) = 0$ *and* $b > \mu$. *The equilibrium point (2.5) is globally asymptotically stable in* $\mathbb{R}^2_+$.

Proof. We will prove this property for the transformed systems (s1) and (s2). Since in both the cases the divergence of the vector fields associated to these systems is negative, limit cycles (or homoclinic orbits) are excluded thanks to the Dulac's theorem. In order to demonstrate the global asymptotic stability (GAS) of the origin, we need to show that any semiorbit is contained in a compact set containing the point $(0,0)$. Therefore we have to find for each system a family of adsorbing curves, i.e. a family of closed curves around the origin such that the flux is never directed outwards, and such that for every points in $\mathbb{R}^2$ there exists a curve containing it in its interior. For both the systems, the nullclines $y = x$ and $y = \gamma(x)$, where

$$\gamma(x) = x - \ln\left(\frac{e^{\frac{2}{3}x}(\bar{b} - \bar{\mu}) + \bar{\mu}}{\bar{b}}\right), \tag{2.11}$$

divide the plane in 4 open sets:

$$Q_1 = \left\{(x,y)|\ x < 0, x < y < \gamma(x)\right\}$$

$$Q_2 = \left\{(x,y)|\ y > \max(x, \gamma(x))\right\}$$

$$Q_3 = \left\{(x,y)|\ x > 0, \gamma(x) < y < x\right\}$$

$$Q_4 = \left\{(x,y)|\ y < \min(x, \gamma(x))\right\}.$$

It is easy to see that all the rectangles parallel to the axes having vertices A_1, A_2, A_3, A_4 such that $A_1 \in Q_1$, $A_2 \in Q_2$, $A_3 \in Q_3$, $A_4 \in Q_4$, are adsorbing and contain $(0,0)$. Thus $(0,0)$ is GAS. ∎

We can state also:

Proposition 2. *Let us consider the systems given by Eqs. (2.2),(2.6) and (2.3),(2.6) with* $g(t) = 0$ *and* $b > \mu$. *The equilibrium point (2.5) is globally asymptotically stable in* $\mathbb{R}^2_+$.

Proof. Let us consider the transformed systems (s3) and (s4). By setting $z = -x + y$, system (s3) can be rewritten as

$$x' = z \tag{2.12}$$

$$z' = -z - (\bar{b} - \bar{\mu})(e^{\frac{2}{3}x} - 1). \tag{2.13}$$

For system (2.12),(2.13), which has the point $(0,0)$ as the unique equilibrium point, the "total energy":

$$L(x,z) = \frac{1}{2}z^2 + (\bar{b} - \bar{\mu})\int_0^x (e^{\frac{2}{3}s} - 1)ds, \tag{2.14}$$

is a Lyapunov function, and it is easy to verify that $L' = -z^2 \leq 0$. Therefore, from the LaSalle's invariance theorem it holds that $(0,0)$ is GAS in $\mathbb{R}^2$. In the case of system (s4), by setting $z = 1 - e^{x-y}$, we have $x' = z$, $y = x - \ln(1-z)$, and $y' = z + z'/(1-z)$. Thus (s4) can be rewritten as

$$x' = z \tag{2.15}$$

$$z' = (1-z)\left(-z + (\bar{b} - \bar{\mu})(1 - e^{\frac{2}{3}x})\right). \tag{2.16}$$

8.

System (2.15),(2.16) has the invariant set $\Xi = \{(x, z) | \ -\infty < x < +\infty, \ z < 1\}$, whose unique equilibrium point is $(0, 0)$. By defining the following Lyapunov function:

$$L(x, z) = -z - \ln(1 - z) + (\bar{b} - \bar{\mu})\left(\frac{3}{2}\mathrm{e}^{\frac{2}{3}x} - x\right), \tag{2.17}$$

we obtain

$$L' = \frac{zz'}{1 - z} + z(\bar{b} - \bar{\mu})(\mathrm{e}^{\frac{2}{3}x} - 1) = -z^2 \leq 0. \tag{2.18}$$

Therefore, $(0, 0)$ is GAS in Ξ. ∎

Moreover, we have:

Proposition 3. *If $b \leq \mu$, $V(t)$ and $K(t)$, solutions of Eqs. (2.2),(2.4), (2.3),(2.4), (2.2),(2.6) and (2.3),(2.6) with $g(t) = 0$, for any initial condition in $\mathbb{R}_+^2$ tend to zero as $t \to \infty$.*

Proof. The nullclines of systems (2.2),(2.4) and (2.3),(2.4) are $K = V$ and $K = \gamma(V)$, where

$$\gamma(V) = \frac{bV}{\mu + dV^{\frac{2}{3}}}. \tag{2.19}$$

These curves subdivide the positive quadrant of the (V, K) plane in three regions: $K \geq V$, $V > 0$ (P_1); $\gamma(V) < K < V$, $V > 0$ (P_2); $0 < K < \gamma(V)$, $V > 0$ (P_3). It is easy to see that starting from P_1 or P_3 any trajectory enter P_2, whereas starting from P_2 it is not possible to enter P_1 or P_3, and that in P_2 it is $V' < 0$ and $K' < 0$. Since in P_2 there is no equilibrium point, any trajectory will tend to $(0, 0)$. When K' is given by Eq. (2.6), $\mathbb{R}_+^2$ can be subdivided in two sets P_1 and P_2, with P_2 such that $0 < K < V$, having the above stated properties, and thus the previous argument still holds. ∎

Figure 2 shows an example of the phase portrait for systems (2.2),(2.4) and (2.2),(2.6), both for $b > \mu$ and $b < \mu$.

3. Periodic antiangiogenic therapy

Let us suppose that an antiangiogenc drug be administered, so that $g(t) > 0$ for some $t > 0$. We will focus on the *periodic therapy*, that is we will suppose $g(t)$ to be a periodic non-negative function with period T, which is bounded and piece-wise continuous with a finite number of discontinuities in any finite interval. The periodic therapy idealizes actual drug schedules, such as the drug regimens experimentally tested in [11] in which antiangiogenic drugs were administered to the animal bearing the tumour as a sequence of boluses at equispaced times. In this section we will establish conditions for the tumour eradication, i.e. conditions for obtaining

$$\lim_{t \to \infty} V(t) = 0,$$

for any initial condition in $\mathbb{R}_+^2$. Let us define

$$\langle g \rangle = \frac{1}{T} \int_0^T g(t)dt, \tag{3.1}$$

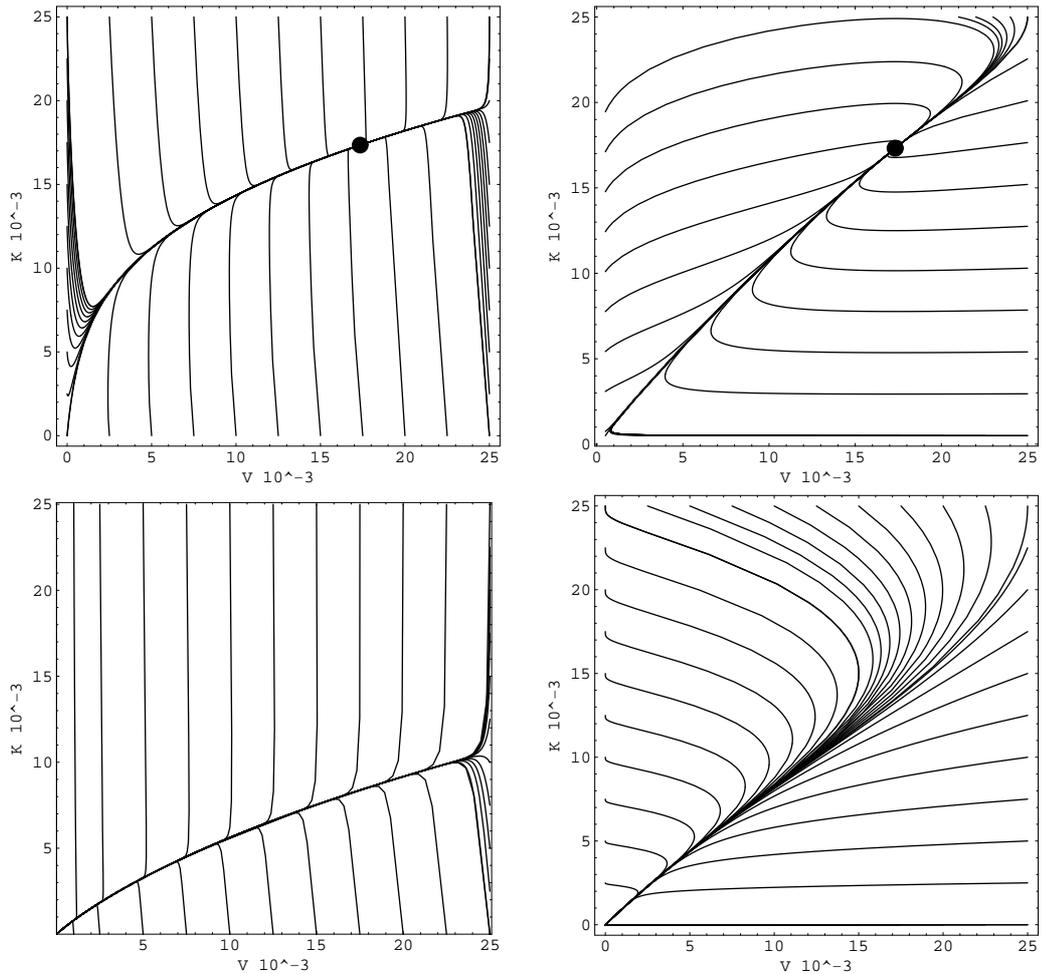$$a = b - \mu - \eta \langle g \rangle, \tag{3.2}$$

Fig. 2. Phase portraits of systems (2.2),(2.4) (left column), and (2.2),(2.6) (right column). Parameters values as in Fig. 1, except that for the lower panels in which $\mu > b$ ($\mu = 1.1b$, left panel; $\mu = 2.0b$, right panel).

and

$$F(t) = -\eta g(t) + \eta \langle g \rangle, \qquad (3.3)$$

$F(t)$ being thus a bounded periodic function with mean equal to zero.

The case of a constant infusion of drug is represented by $g(t) = constant = G$, and it corresponds in the model to an exogenous factor which increases the natural death rate μ to a value $\mu + \eta G$. Thus, in view of Propositions 1, 2 and 3, it easy to see that the condition

$$a \leq 0, \qquad (3.4)$$

that is $\eta G \geq b - \mu$, is necessary and sufficient for tumour eradication in the case of constant therapy. Moreover, when $a < 0$, it can be seen that asymptotically V decreases to 0 in an exponential way. In the case of model (2.2),(2.4) indeed, recalling the variable and parameters transformations (2.7)-(2.10), we obtain for the variable $x = \ln(V/V_e)$ the second order equation:

$$x'' + x' - \bar{b}e^{-x'} + (\bar{b} - \bar{\mu})e^{\frac{2}{3}x} + \bar{b} - \bar{a} = 0, \qquad (3.5)$$

where $\bar{a} = a/\alpha$. Since $x \to -\infty$ as $\bar{t} \to \infty$, equation (3.5), after setting $w = x'$, is asymptotically equivalent to the equation

$$w' = -w + \bar{b}\mathrm{e}^{-w} - \bar{b} + \bar{a}, \tag{3.6}$$

which has a negative and globally attractive equilibrium point $\hat{w}$. Therefore, $V'/V = x' \to \hat{w}$, with $\hat{w} < 0$. Similar arguments can be followed in the case of models (2.3),(2.4), (2.2),(2.6) and (2.3),(2.6).

We state now the following Lemma:

Lemma 1. *Let $K(t)$ be an assigned positive, bounded and piece-wise continuous function on $[0, \infty)$. If $\lim_{t \to \infty} K(t) = 0$, the solution $V(t)$ of Eq. (2.2) or Eq. (2.3) for any positive initial condition tends to zero for $t \to \infty$. Moreover, $\lim_{t \to \infty} \min_{\tau \in [0,t]} K(\tau) = 0$ is necessary to have $\min_{\tau \in [0,t]} V(\tau) \to 0$.*

Proof. By the variable transformation $X = \ln V$, Eq. (2.2) can be rewritten as the forced linear equation

$$X' = -\alpha X + \alpha \ln K(t), \tag{3.7}$$

which yields $\lim_{t \to \infty} X(t) = -\infty$ if $K(t) \to 0$. In the case of Eq. (2.3), by setting $Z = V^{-1}$ and rewriting (2.3) as

$$Z' = -\alpha Z + \alpha K^{-1}(t), \tag{3.8}$$

the same result is obtained.

Since $\min_{\tau \in [0,t]} K(\tau)$ is a positive and non-increasing function of t, it will tend to a non-negative limit $\tilde{k}$. It is easy to see, by writing explicitly the solution of the linear equations (3.7) and (3.8), that if this limit is positive $X(t)$ and $Z(t)$ will be bounded. Thus, $\tilde{k} = 0$ is necessary for $\min_{\tau \in [0,t]} V(\tau) \to 0$. ∎

3.1 Periodic therapy: models (2.2),(2.6) and (2.3),(2.6)

In the case in which the dynamics of the carrying capacity of the vasculature is described by Eq. (2.6), a necessary and sufficient condition for tumour eradication can be easily obtained, thanks to the fact that when V is approaching 0, the differential equation for K becomes substantially a linear one.

Proposition 4. *Given the models (2.2),(2.6) and (2.3),(2.6), the condition $a \leq 0$ is necessary and sufficient for the eradication of the tumour under a periodic therapy.*

Proof. Since $\mathbb{R}_+^2$ is invariant, we have from Eq. (2.6)

$$K' \leq (b - \mu - \eta g(t))K, \tag{3.9}$$

so that it is $K(t) \leq \bar{K}(t)$ for $t \geq 0$, where $\bar{K}(t)$ is the solution of

$$\bar{K}' = (b - \mu - \eta g(t))\bar{K}, \qquad \bar{K}(0) = K(0). \tag{3.10}$$

By writing explicitly the solution of (3.10), we have

$$K(t) \leq K(0)\mathrm{e}^{(b - \mu - \eta \langle g \rangle)t} \, \mathrm{e}^{\int_0^t F(s)ds}. \tag{3.11}$$

Since the integral of F is bounded for any t, the condition $b - \mu - \eta\langle g\rangle < 0$, i.e. $a < 0$, implies $\lim_{t\to\infty} K(t) = 0$. In view of Lemma 1, this implies the eradication of the tumour both in the cases in which V' is given by Eq. (2.2) and by Eq. (2.3). If $a = 0$, equation (2.6) becomes

$$K' = (-dV^{2/3} + F(t))K, \tag{3.12}$$

and we have:

$$K(t) = K(0)\mathrm{e}^{-d\int_0^t V(s)^{2/3}ds}\,\mathrm{e}^{\int_0^t F(s)ds}. \tag{3.13}$$

Since $V(t)$ is positive, $\chi(t) = \int_0^t V^{\frac{2}{3}}(s)ds$ is a non-decreasing function of t, and it may be either $\chi(t) \to +\infty$ or $\chi(t) \to A < +\infty$, $A > 0$. If $\chi(t) \to +\infty$ we have from (3.13) that $K(t)$ must tend to 0 that implies V vanishing from Lemma 1. If $\chi(t) \to A$, since it would be

$$K(t) \geq K(0)\phi_m\,\mathrm{e}^{-dA}, \tag{3.14}$$

where $\phi_m = \min\exp(\int_0^t F(s)ds)$, we would have that $\lim\min_{\tau\in[0,t]}K(\tau) > 0$. Thus, following Lemma 1, it should be necessarily $\lim\min_{\tau\in[0,t]}V(\tau) > 0$, because $\lim\min_{\tau\in[0,t]}V(\tau) = 0$ implies $\lim\min_{\tau\in[0,t]}K(\tau) = 0$, in contradiction with χ tending to a finite limit. Therefore we have $V(t) \to 0$ also for $a = 0$.

Let us suppose now that $V(t) \to 0$ and $a > 0$. A time t_a will exist such that for $t > t_a$ it is $dV^{2/3} < a/2$. For $t > t_a$ we have

$$b - \mu - \eta\langle g\rangle - dV(t)^{2/3} > \frac{a}{2}, \tag{3.15}$$

that implies

$$K(t) > K(t_a)\mathrm{e}^{\frac{a}{2}(t-t_a)}\mathrm{e}^{\int_0^t F(s)ds}, \tag{3.16}$$

and $K(t) \to +\infty$, in contradiction with $V(t) \to 0$. Thus $a \leq 0$ is necessary to have tumour eradication. ∎

We note that the model (2.2), (2.6) can be formulated as a forced nonlinear oscillator with linear damping. Recalling that the variable and parameter transformations (2.7)-(2.10) lead to system (s3), we may obtain for $x = \ln(V/V_e)$ the following second-order differential equation:

$$x'' + x' + \phi(x) = F(\bar{t}), \tag{3.17}$$

where

$$\phi(x) = (\bar{b} - \bar{\mu})\mathrm{e}^{\frac{2}{3}x} - \bar{a}. \tag{3.18}$$

The function ϕ is thus increasing with x, and $\lim_{x\to-\infty}\phi(x) = -\bar{a}$ (negative when $a > 0$) and $\lim_{x\to+\infty}\phi(x) = +\infty$. Therefore, when $g(t)$ is continuous, we can apply a result by Ahmad (Theorem 3.1 in [26]) and recognize that $a > 0$, is necessary and sufficient to have a bounded solution of (3.17)-(3.18) on $[0,\infty)$. Thus we find again that $a \leq 0$ is necessary to have tumour eradication. This necessity is physically intuitive: if $a > 0$ (i.e. $\langle g\rangle$ enough small), the potential energy $E_{pot}(x) = \int_0^x \phi(s)ds$ associated to the damped oscillator (3.17) has a unique absolute minimum, and $\lim_{x\to-\infty} E_{pot}(x) = \lim_{x\to+\infty} E_{pot}(x) = +\infty$. In this case the external bounded zero-mean "force" F may make rich the dynamics of the oscillator around the minimum of the potential energy, but it cannot unstabilize the damped motion.

Similar results can be obtained if Eq. (2.6) is generalized as follows. Let the growth rate constant of the vasculature be a sufficently smooth non-decreasing or non-increasing bounded function of V, and let the effect of the inhibitors be increasing with V:

$$K' = b(V)K - (\mu + d(V))K - \eta g(t)K. \tag{3.19}$$

In Eq. (3.19) we assume $\lim_{V \to \infty} b(V) = b_\infty < \infty$, $d(0) = 0$, $d'(V) > 0$ and $\lim_{V \to \infty} d(V) = d_\infty \le \infty$. We also suppose that $b(0) > \mu$ and $b_\infty < \mu + d_\infty$, so that the system in the absence of therapy has one equilibrium point, determined by the equation $b(V) = \mu + d(V)$. In the presence of therapy, we have

$$K' \le (b_{max} - \mu - \eta\langle g\rangle + F(t))K, \tag{3.20}$$

where $b_{max} = \max[b(0), b_\infty]$, and, if $b_{max} - \mu - \eta\langle g\rangle = 0$,

$$K' = (b(V) - b_{max} - d(V) + F(t))K$$

$$\le (-d(V) + F(t))K. \tag{3.21}$$

Thus, by means of the arguments seen in the above proof, we obtain that

$$b_{max} - \mu - \eta\langle g\rangle \le 0 \tag{3.22}$$

is sufficient, and

$$b(0) - \mu - \eta\langle g\rangle \le 0 \tag{3.23}$$

is necessary for tumour eradication.

3.2 Pulsed periodic therapy: models (2.2),(2.6) and (2.3),(2.6)

A largely diffused method to model anti-cancer therapy is to assume that the effect of drug be instantaneous. This kind of ideal therapy is called "pulsed therapy" [27,28]. Therefore, if we suppose to administer the antiangiogenic drug with periodicity T, we may describe the behaviour of the tumour subjected to therapy by Eqs. (2.1) and (2.6) with $g(t) = 0$, and under the infinite initial conditions

$$V(nT^+) = V(nT^-) \tag{3.24}$$

$$K(nT^+) = (1 - p)K(nT^-), \qquad n = 0, 1, \cdots, \tag{3.25}$$

where $p \in (0, 1)$. From Eq. (2.6) and taking into account (3.25), we can then write

$$K(nT^-) = K(0^-) \prod_{i=0}^{n-1} (1 - p)e^{(b-\mu)T}\psi_i, \qquad n = 1, 2, \cdots, \tag{3.26}$$

where

$$\psi_i = e^{-d \int_{iT}^{(i+1)T} V(s)^{2/3} ds}, \qquad i = 0, 1, \cdots. \tag{3.27}$$

Note that $\psi_i < 1$, and that

$$\prod_{i=0}^{n-1} \psi_i = e^{-d \int_0^{nT} V(s)^{2/3} ds}. \tag{3.28}$$

Moreover, the sequence ψ_i tends to 1 if V tends to zero. By suitably adapting the arguments that lead to Proposition 4, we easily obtain the following:

Proposition 5. *Given the models (2.2),(2.6) and (2.3),(2.6), with $g(t) = 0$ and the infinite initial conditions (3.24) and (3.25), the condition*

$$(1 - p)\mathrm{e}^{(b-\mu)T} \leq 1, \tag{3.29}$$

is necessary and sufficient for the eradication of the tumour.

3.3 Periodic therapy: models (2.2),(2.4) and (2.3),(2.4)

When the dynamics of the carrying capacity of the vasculature is described by Eq. (2.4), we can prove the following:

Proposition 6. *Given the models (2.2),(2.4) and (2.3),(2.4), the condition $a \leq 0$ is necessary for the eradication of the tumour under a periodic therapy.*

Proof. Recalling (3.3), equation (2.4) can be rewritten as

$$K' = \left(b\frac{V}{K} - \mu - dV^{\frac{2}{3}} - \eta\langle g \rangle + F(t)\right)K, \tag{3.30}$$

from which we have

$$\frac{K'}{K} + b(1 - \frac{V}{K}) = b - \mu - dV^{\frac{2}{3}} - \eta\langle g \rangle + F(t). \tag{3.31}$$

If the tumour volume is governed by Eq. (2.2), $\ln(V/K)$ is equal to $-V'/(\alpha V)$, and because $\ln x \leq x - 1$ we get $1 - V/K \leq V'/(\alpha V)$. Then it follows

$$\frac{K'}{K} + b(1 - \frac{V}{K}) = a - dV^{\frac{2}{3}} + F(t) \leq \frac{K'}{K} + \bar{b}\frac{V'}{V}, \tag{3.32}$$

that is:

$$z' \geq a - dV^{\frac{2}{3}} + F(t), \tag{3.33}$$

where

$$z(t) = \ln K(t) + \bar{b}\ln V(t). \tag{3.34}$$

In the case of Eq. (2.3) it is $1 - V/K = V'/(\alpha V)$, and we obtain

$$z' = a - dV^{\frac{2}{3}} + F(t). \tag{3.35}$$

Thus, in both the cases of Eq. (2.2) and Eq. (2.3) we obtain

$$z(t) \geq z(0) + \int_0^t (a - dV(s)^{\frac{2}{3}})ds + \int_0^t F(s)ds. \tag{3.36}$$

If we suppose $V(t) \to 0$ and $a > 0$, a time t_a will exist such that for $t > t_a$ it is $a - dV^{2/3} > a/2$. For $t > t_a$ we will have

$$z(t) > z(t_a) + \frac{a}{2}(t - t_a) + \int_0^t F(s)ds, \tag{3.37}$$

that implies $\lim_{t\to+\infty} z(t) = +\infty$, that is $KV^{\bar{b}} \to +\infty$. If $V(t) \to 0$, it must be $K(t) \to +\infty$, in contradiction (in view of Lemma 1) with the hypothesis of V tending to 0. ∎

In the case of model (2.2),(2.4) we are in a physical framework similar to what we have seen during the analysis of model (2.2),(2.6): a damped oscillator excited by a bounded periodical external force. At variance of that model, the damping term is now nonlinear. By means of the variable transformations (2.7)-(2.10), we can indeed rewrite Eqs. (2.2),(2.4) as the second-order equation in the unknown $x = \ln(V/V_e)$:

$$x'' + C(x')x' + \phi(x) = F(\bar{t}), \tag{3.38}$$

where

$$C(x') = \begin{cases} 1 + \bar{b}\frac{1-\mathrm{e}^{-x'}}{x'} & \text{if } x' \neq 0 \\ 1 + \bar{b} & \text{if } x' = 0, \end{cases} \tag{3.39}$$

and $\phi(x)$ is still given by (3.18). Note that $C(x') > 1$ and $\lim_{x'\to-\infty} C(x') = +\infty$, $\lim_{x'\to+\infty} C(x') = 1$, thus equation (3.38) represents a damped oscillator whose associated potential energy $E_{pot}(x)$ has a minimum for $a > 0$, forced by a zero-mean force. We were not able to find an extension of the Ahmad's theorem to nonlinearly damped oscillators to proof that the solution of (3.38) remains bounded for $a > 0$, but also in this case such a property appears physically intuitive.

As a consequence, in analogy with model (2.2),(2.6), one could expect that $a < 0$ is sufficient for eradication, since the potential energy becomes such that $\lim_{x\to-\infty} E_{pot}(x) = -\infty$. As we are going to see, this does not appear to be true when the therapy is really periodic (i.e. when $F(t) \neq 0$). Depending on the characteristics of the waveform $g(t)$, eradication may not occur for values of $\langle g \rangle$ that guarantee the eradication in the case of constant therapy. Numerical simulations have shown that when the amplitude of the oscillation of $g(t)$ around the mean value is too large, $V(t)$ does not tend to 0 even if $a < 0$, but asymptotically oscillates around a positive value smaller than the global equilibrium in absence of therapy.

To assess the influence of the shape of $g(t)$ we used the square waveform $g(t) = G_1 - (G_1 - G_2)\mathrm{H}(\mathrm{Mod}(t,T) - t_1)$, with $G_1 > G_2 \geq 0$ and $0 < t_1 \leq T/2$, $\mathrm{H}(s)$ being the Heaviside function. By introducing the ratio $\sigma = S/\langle g \rangle T$, where S denotes the area of $g(t)$ above the mean value over the interval $[0, T]$, and $\tau_1 = t_1/T$, we can rewrite $g(t)$ as

$$g(t) = \langle g \rangle \left[1 + \frac{\sigma}{\tau_1} - \sigma \left(\frac{1}{\tau_1} + \frac{1}{1 - \tau_1} \right) \mathrm{H}(\mathrm{Mod}(t,T) - \tau_1 T) \right], \tag{3.40}$$

with the constraint $0 < \sigma < 1 - \tau_1$. The result of simulations performed keeping $\langle g \rangle$ fixed to a value which yields $a < 0$, is that the behaviour of the system depends critically on σ: if σ is below a certain threshold (≈ 0.42, in the cases reported when $\tau_1 = 0.49$) there is eradication, whereas eradication does not occur above the threshold (Fig. 3). This threshold increases as the value of $\langle g \rangle$ increases (data not shown), and decreases as τ_1 becomes smaller. Fig. 4 shows in fact that for $\sigma = 0.4$ there is no regression of V when $\tau_1 = 0.05$ (in this case the drug concentration profile presents very high but very short periodic spikes), and eradication when $S = 0.3$.

To illustrate further the effect of the shape of $g(t)$, we also assumed the expression:

$$g(t) = \frac{g^\star}{1 - \mathrm{e}^{-cT}} \mathrm{e}^{-c\mathrm{Mod}(t,T)}. \tag{3.41}$$

The function (3.41) describes the asymptotic time-behaviour of the drug concentration when drug is administered as a sequence of boluses with period T, in the case of a first-order drug pharmacokinetics. In (3.41) $g^\star$ denotes the ratio between the single dose administered and the
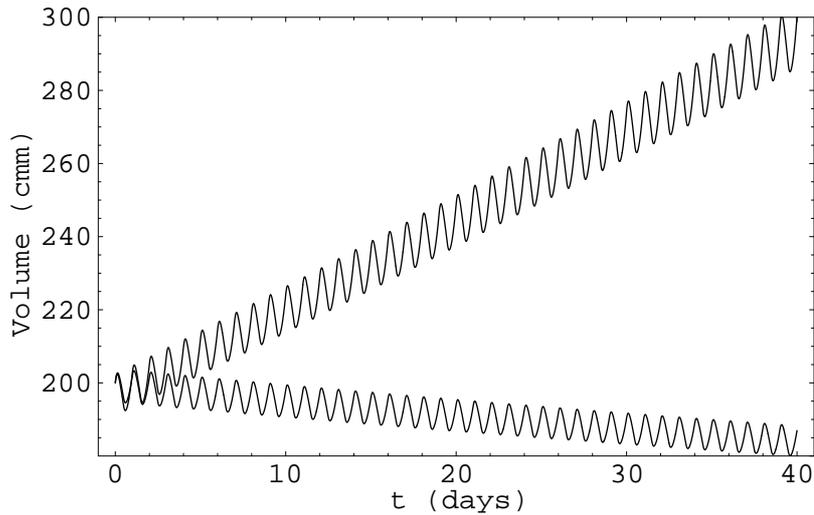
Fig. 3. Evolution of tumour volume during periodic therapy (according to Eqs. (2.2),(2.4)). Model parameters as in Fig.1 (reference values), $\eta = 1.3$ day^{-1}conc^{-1}. $g(t)$ given by Eq. (3.40) with $T = 1.0$ day, $\langle g \rangle$ such that $a = -0.585$, $\tau_1 = 0.49$, $\sigma = 0.5$ (upper curve), $\sigma = 0.4$ (lower curve).
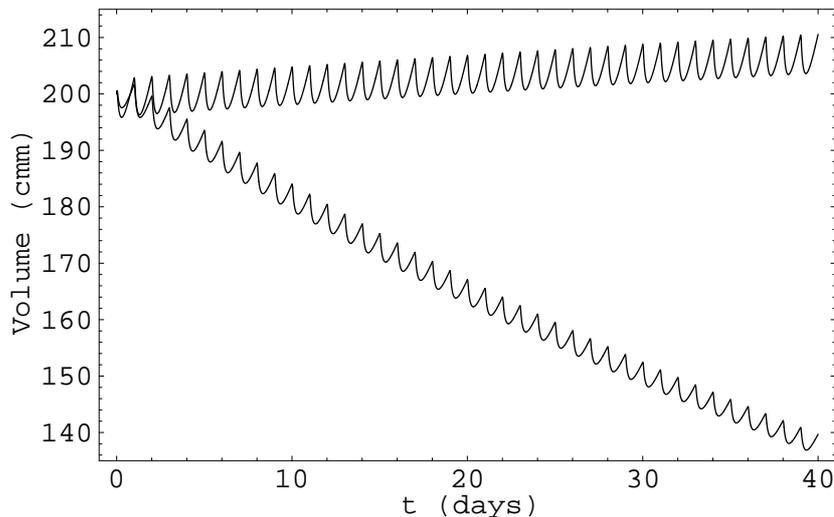

Fig. 4. Evolution of tumour volume during periodic therapy (according to Eqs. (2.2),(2.4)). Model parameters as in Fig.1 (reference values), $\eta = 1.3$ day^{-1}conc^{-1}. $g(t)$ given by Eq. (3.40) with $T = 1.0$ day, $\langle g \rangle$ such that $a = -0.585$, $\tau_1 = 0.05$, $\sigma = 0.4$ (upper curve), $\sigma = 0.1$ (lower curve).

distribution volume of the recipient, and c is the rate constant of drug disappearance. We have $\langle g \rangle = g^\star/cT$, so that for $g^\star > g^\star_{min}(c)$, $g^\star_{min}(c) = cT(b - \mu)/\eta$, it is $a < 0$. Fig. 5 shows the time-course of V for three different values of c, assuming $g^\star = 1.1 g^\star_{min}(c)$ (so that in all the three simulations the values of $\langle g \rangle$ and a are the same). As c increases, and the drug concentration tends to be a sequence of shorter spikes (see Fig. 6), the capability of inducing tumour regression is lost, although the area under the curve of $g(t)$ does not change. This phenomenon was found also when Eq. (2.2) is replaced by a generalized-logistic equation with the esponent of V/K less
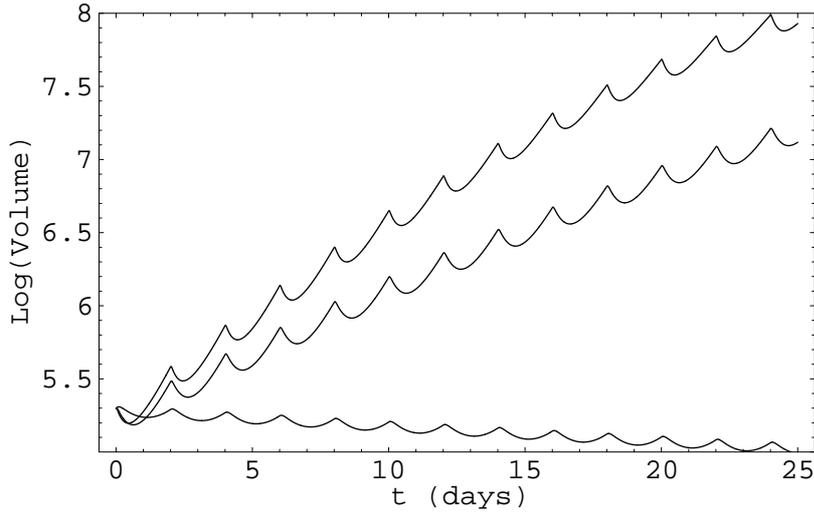
16.

Fig. 5. Evolution of tumour volume during periodic therapy (according to Eqs. (2.2),(2.4)). Model parameters as in Fig.1 (reference values), $\eta = 1.3$ day^{-1}conc^{-1}. $g(t)$ given by Eq. (3.41) with $T = 2.0$ day, $c = 10.0$ day^{-1} (upper curve), $c = 5.0$ day^{-1} (middle curve), $c = 1.0$ day^{-1} (lower curve), $g^\star = 1.1 \cdot b/cT\eta$.

than 1 (not shown).

When Eq. (2.2) is replaced by the logistic equation (2.3), we have instead:

Proposition 7. *Given the model (2.3),(2.4), the condition $a \leq 0$ is sufficient for the eradication of the tumour under a periodic therapy.*

Proof. From Eq. (3.35) we have

$$z' \leq a + F(t), \tag{3.42}$$

and

$$z(t) \leq z(0) + at + \int_0^t F(s)ds, \tag{3.43}$$

i.e., by using the original variables:

$$KV^{\bar{b}} \leq q(t) := K(0)V(0)^{\bar{b}} \, e^{at + \int_0^t F(s)ds}. \tag{3.44}$$

Inequality (3.44) implies (remember that $K > 0$)

$$-\frac{1}{K} \leq -\frac{V^{\bar{b}}}{q(t)} \tag{3.45}$$

which yields:

$$V' = \alpha V\left(1 - \frac{V}{K}\right) \leq \alpha V\left(1 - \frac{V^{\bar{b}+1}}{q(t)}\right). \tag{3.46}$$

When $a < 0$, it is $q(t) \to 0$ as $t \to +\infty$, and then by arguing as in the proof of Lemma 1 we have that the solution $\bar{V}$ of

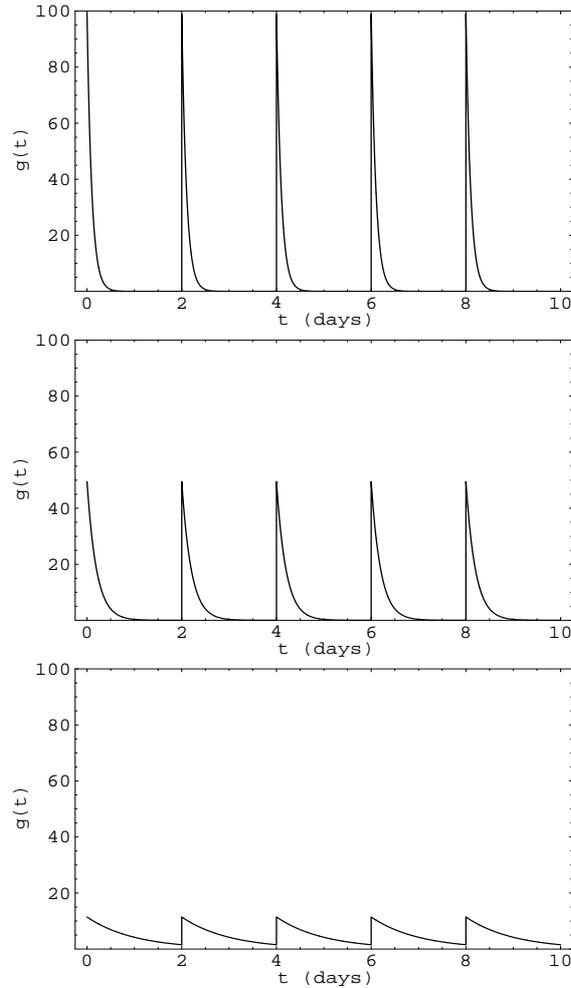$$\bar{V}' = \alpha\bar{V}\left(1 - \frac{\bar{V}^{\bar{b}+1}}{q(t)}\right) \tag{3.47}$$

Fig. 6. Drug concentration profiles assumed in the simulations of Fig. 5. $c = 10.0$ day^{-1} (upper panel), $c = 5.0$ day^{-1} (middle panel), $c = 1.0$ day^{-1} (lower panel).

will tend to 0, implying $V(t) \to 0$ (note that (3.47) can be rewritten as a linear equation by setting $Z = V^{-(\bar{b}+1)}$). When $a = 0$, Eq. (3.35) reduces to

$$z' = -dV^{\frac{2}{3}} + F(t), \tag{3.48}$$

from which

$$z(t) = z(0) - d\int_0^t V(s)^{\frac{2}{3}} ds + \int_0^t F(s) ds. \tag{3.49}$$

Thus we obtain

$$KV^{\bar{b}} = q(t) := K(0)V(0)^{\bar{b}} e^{\chi(t) + \int_0^t F(s) ds}, \tag{3.50}$$

where $\chi(t) = \int_0^t V^{\frac{2}{3}}(s) ds$, and

$$V' = \alpha V\left(1 - \frac{V^{\bar{b}+1}}{q(t)}\right). \tag{3.51}$$

Since $\chi(t)$ is a non-decreasing function of t, it may be either $\chi(t) \to +\infty$ or $\chi(t) \to A < +\infty$, $A > 0$. If $\chi(t) \to +\infty$, we have from (3.50) that $q(t)$ must tend to 0 that implies, taking into

account (3.51), that V tends to 0. If $\chi(t) \to A$, since it would be

$$q(t) \geq q(0)\phi_m\, e^{-dA}, \tag{3.52}$$

where $\phi_m = \min \exp(\int_0^t F(s)ds)$, we would have that $\lim \min_{\tau \in [0,t]} q(\tau) > 0$. Thus, following Lemma 1 extended to Eq. (3.51), it should be necessarily $\lim \min_{\tau \in [0,t]} V(\tau) > 0$, because $\lim \min_{\tau \in [0,t]} V(\tau) = 0$ implies $\lim \min_{\tau \in [0,t]} q(\tau) = 0$, in contradiction with χ tending to a finite limit. Therefore we have $V(t) \to 0$ also for $a = 0$. Furthermore, since it is

$$K' \leq bV - (\mu + \eta g_{min})K, \tag{3.53}$$

g_{min} denoting $\min g(t)$, from $V(t) \to 0$ we obtain also that $K(t) \to 0$. ∎

From the above results in the case of Eq. (2.4), it can be conjectured that when the function $f(V/K)$ in Eq. (2.1) is such that $|V'/\alpha V| < V/K - 1$ for $V > K$, the knowledge of the averaged value of $g(t)$ is not sufficient to decide if eradication will occur, whereas it is enough if $|V'/\alpha V| \geq V/K - 1$.

4. A model incorporating stimulation and proliferation of endothelial cells

In Hahnfeldt *et al.* [11], the antiangiogenic drug is assumed to induce a direct loss of the tumour vasculature. Another possible effect of the antiangiogenic agents is the inhibition of stimulation and/or of proliferation of the cells involved in angiogenesis, causing in this way a global impairment of vasculature growth. This effects could be accounted for in the previous models by making the growth rate bV in Eq. (2.4) (or the term bK in (2.6)) dependent on the drug concentration. More accurately, the stimulation of proliferation and the proliferation of the endothelial cells forming the tumour vessels may be taken separately into account in the model, allowing for a greater detail in the description of the action of drugs. We propose here to represent the vascular network as a pool of endothelial cells subdivided into quiescent (Q) and cycling (P) cells. Q cells can be stimulated to enter the cycle by angiogenic factors and P cells are assumed to reenter the quiescent state after division. As in [11], the concentration of the stimulatory factor is assumed to be constant, independently of tumour volume, thus the rate constant of the stimulation of endothelial cells is taken independent of V. The P and Q subpopulations are subjected to loss with a rate constant proportional to $V^{2/3}$, again in accordance with [11]. The carrying capacity of the vasculature is assumed proportional, according to a coefficient m, to the size of the Q cell population, since functional vessels are likely to be formed by resting cells.

Therefore, for the unperturbed tumour growth the following equations can be written

$$V' = \alpha V f\left(\frac{V}{mQ}\right) \tag{4.1}$$

$$P' = bQ - \beta P - (\mu_1 + d_1 V^{2/3})P \tag{4.2}$$

$$Q' = 2\beta P - bQ - (\mu_2 + d_2 V^{2/3})Q, \tag{4.3}$$

where f in (4.1) must be read either as in Eq. (2.2) or in (2.3), bQ is the number of quiescent endothelial cells recruited into the cell cycle in the unit time, and βP is the number of endothelial cells dividing in the unit time. The above system is defined on the set $\mathcal{D} = \{V > 0,\ P \geq 0,\ Q >$

0}, and it is easy to recognize that $\mathcal{D}$ is invariant. By defining $X = mP$ and $Y = mQ$, Eqs. (4.1)-(4.3) become

$$V' = \alpha V f\left(\frac{V}{Y}\right) \tag{4.4}$$

$$X' = bY - \beta X - (\mu_1 + d_1 V^{2/3})X \tag{4.5}$$

$$Y' = 2\beta X - bY - (\mu_2 + d_2 V^{2/3})Y, \tag{4.6}$$

where now Y denotes the carrying capacity of the vasculature. Note that β is related to the duration of the cell cycle of endothelial cells, so that information on the possible value of this parameter is available [29-31]. Some algebra shows that a (unique) positive equilibrium point exists if and only if:

$$\mu_1\mu_2 + \mu_1\beta + \mu_2 b - b\beta < 0. \tag{4.7}$$

Note that condition (4.7) guarantees that the endothelial cell pool is exponentially growing in the absence of the inhibition driven by $V^{2/3}$.

In the above model the action of antiangiogenic drugs can be represented by an exogeneous loss that affects P and Q cells, possibly with different rate constants. Thus, during the treatment, X and Y will obey the following equations

$$X' = bY - \beta X - (\mu_1 + d_1 V^{2/3})X - \eta_1 g(t)X \tag{4.8}$$

$$Y' = 2\beta X - bY - (\mu_2 + d_2 V^{2/3})Y - \eta_2 g(t)Y. \tag{4.9}$$

Some drugs, for instance conventional chemotherapeutic agents used as antiangiogenic compounds [23], can induce apoptosis mainly of proliferating cells (and it will be $\eta_1 > \eta_2$ in Eqs.(4.8),(4.9)). The possible cell-cycle-phase specificity of these drugs is here implicitly disregarded. Preferential loss of resting endothelial cells could be induced by factors, such as angiopoietin-2, that destabilize mature tumour vessels [8].

If the drug instead reduces the mitotic rate of P cells and/or reduces the rate of stimulation of Q cells, we can write

$$X' = b\frac{k_1}{k_1 + g(t)}Y - \beta\frac{k_2}{k_2 + g(t)}X - (\mu_1 + d_1 V^{2/3})X \tag{4.10}$$

$$Y' = 2\beta\frac{k_2}{k_2 + g(t)}X - b\frac{k_1}{k_1 + g(t)}Y - (\mu_2 + d_2 V^{2/3})Y. \tag{4.11}$$

Conventional chemotherapeutic drugs are known to produce blocks of cell proliferation. On the contrary, inhibitors of the vascular endothelial growth factor (VEGF) and competitors for VEGF receptors, or inhibitors of metalloproteases, will affect the stimulation of resting endothelial cells (and their effect can be represented by setting $k_2 >> k_1$).

In the following, we focus our analysis on model (4.4),(4.8),(4.9). Results that parallel those we will illustrate can be obtained for model (4.4),(4.10),(4.11) by means of similar arguments.

20.

4.1 Asymptotic equivalence to a 2-D system

If $d_1 = d_2 = d$, in the case of a *symmetric* therapy, that is when the drug affects uniformly the loss of quiescent and proliferating endothelial cells, or the stimulation and the cell proliferation (*i.e.* $\eta_1 = \eta_2$ or $k_1 = k_2$), it can be shown that for t large the behaviour of the tumour volume predicted by the preceding models tends to be that described by a 2-dimensional model. With reference to Eqs. (4.8),(4.9), after setting $\mathbf{x} = (X\ Y)^T$, we may observe that these equations can be rewritten as

$$\mathbf{x}' = A\mathbf{x} - (dV^{\frac{2}{3}} + \eta g(t))\mathbf{x}, \tag{4.12}$$

where $\eta = \eta_1 = \eta_2$. The matrix A has two real eigenvalues:

$$\lambda_+ = \frac{1}{2}(-b - \beta - \mu_1 - \mu_2 + \sqrt{(b + \beta + \mu_1 + \mu_2)^2 - 4(\mu_1\mu_2 + \mu_1\beta + \mu_2 b - b\beta)}) \tag{4.13}$$

$$\lambda_- = \frac{1}{2}(-b - \beta - \mu_1 - \mu_2 - \sqrt{(b + \beta + \mu_1 + \mu_2)^2 - 4(\mu_1\mu_2 + \mu_1\beta + \mu_2 b - b\beta)}), \tag{4.14}$$

λ_- being negative, whereas under the condition (4.7) the eigenvalue λ_+ is positive. Let H be a diagonalizing matrix for A, i.e. $HAH^{-1} = Diag(\lambda_+,\ \lambda_-)$, and let us define the new variables $(U\ W)^T = H\mathbf{x}$. We thus obtain easily a decoupling between the variables U e W:

$$U' = (\lambda_+ - dV^{\frac{2}{3}} - \eta g(t))U \tag{4.15}$$

$$W' = (\lambda_- - dV^{\frac{2}{3}} - \eta g(t))W. \tag{4.16}$$

Choosing H such that the second row of H^{-1} is equal to (1 1), we have $Y = U + W$ and

$$V' = \alpha V f\left(\frac{V}{U + W}\right). \tag{4.17}$$

Furthermore, by means of some algebra it is easy to see that the invariant set $\mathbb{R}^2_+$ is mapped by the transformation $(U\ W)^T = H\mathbf{x}$ onto the set:

$$\Omega = \left\{(U, W)|\ U + W > 0,\ U > 0,\ W < -\frac{\lambda_- + \beta + \mu_1}{\lambda_+ + \beta + \mu_1}U\right\}. \tag{4.18}$$

Since from (4.16) it is $\lim_{t \to +\infty} W(t) = 0$, and

$$\frac{W(t)}{U(t)} = \frac{W(0)}{U(0)}e^{(\lambda_- - \lambda_+)t}, \tag{4.19}$$

we obtain

$$V' = \alpha V f\left(\frac{V}{U + W}\right)$$
$$= \alpha V f\left(\frac{V}{U(1 + \frac{W(0)}{U(0)}\exp[(\lambda_- - \lambda_+)t])}\right) \to \alpha V f\left(\frac{V}{U}\right), \tag{4.20}$$

i.e. the equations for the variables (V, U) become asymptotically independent of $W(t)$. Thus, in the case of $g(t) = 0$ or of periodic symmetric therapy, model (4.4),(4.8),(4.9) is asymptotically equivalent to model (2.1),(2.6) (see [32]).

In the unperturbed growth, the system (4.15)-(4.17) has in $\mathbb{R}^3_+$ the unique equilibrium point

$$V_e = \left(\frac{\lambda_+}{d}\right)^{\frac{3}{2}}, \qquad U_e = V_e, \qquad W_e = 0. \tag{4.21}$$

From the above result of equivalence, recalling Proposition 1, the global asymptotic stability of the equilibrium point easily follows.

Recalling the conditions found of Sections 3.1 and 3.2, it is easy to obtain sufficient (and necessary) conditions for the tumour eradication by simmetric therapy. In the case in which $g(t)$ is periodic, $d_1 = d_2$, and the drug induces endothelial cell loss, from Eq. (4.15) we get the following necessary and sufficient condition to have $\lim_{t \to +\infty}(V(t), U(t)) = (0,0)$:

$$\lambda_+ - \eta\langle g \rangle \leq 0. \tag{4.22}$$

4.2 Asymmetric therapy

Since the vasculature has been subdivided in two different subpopulations of cells, it is of interest to analyze the effect of drugs which do not have the same effect on both.

In the case of model (4.4),(4.8),(4.9), we note that equations (4.8),(4.9) define a system which is positive and cooperative, *i.e*

$$\frac{\partial X'}{\partial Y} = b > 0, \qquad \frac{\partial Y'}{\partial X} = 2\beta > 0, \tag{4.23}$$

and it is

$$X' = bY - \beta X - (\mu_1 + d_1 V^{2/3} + \eta_1 g(t))X \leq bY - \beta X - (\mu_1 + \eta_1 g(t))X \tag{4.24}$$

$$Y' = 2\beta X - bY - (\mu_2 + d_2 V^{2/3} + \eta_2 g(t))Y \leq 2\beta X - bY - (\mu_2 + \eta_2 g(t))Y. \tag{4.25}$$

By applying the Kamke's theorem (see [33]), we have $0 \leq X(t) \leq \bar{X}(t)$ and $0 < Y(t) \leq \bar{Y}(t)$, where $\bar{X}(t)$ and $\bar{Y}(t)$ are the solutions of

$$\bar{X}' = b\bar{Y} - \beta\bar{X} - (\mu_1 + \eta_1 g(t))\bar{X}, \qquad \bar{X}(0) = X(0), \tag{4.26}$$

$$\bar{Y}' = 2\beta\bar{X} - b\bar{Y} - (\mu_2 + \eta_2 g(t))\bar{Y}, \qquad \bar{Y}(0) = Y(0), \tag{4.27}$$

with $X(0) \geq 0$ and $Y(0) > 0$. Therefore, if $g(t)$ is a periodic function and M is the Floquet's matrix of the linear time-periodic differential system (4.26)-(4.27), we have that the condition

$$\sigma(M) \subset \{w \in \mathcal{C} : |w| < 1\} \tag{4.28}$$

implies $(\bar{X}, \bar{Y}) \to (0,0)$ for $t \to +\infty$, and then, taking also into account Lemma 1, $(V, X, Y) \to (0,0,0)$.

In the special case of constant therapy $(g(t) = G)$, the linear system (4.26),(4.27) becomes time-independent, and we have that eradication is achieved if both the eigenvalues have negative real parts. These eigenvalues are indeed real, and are both negative if and only if

$$\eta_1\eta_2 G^2 + (b\eta_1 + \beta\eta_2 + \mu_1\eta_2 + \mu_2\eta_1)G > -(\mu_1\mu_2 + \mu_1\beta + \mu_2 b - b\beta). \tag{4.29}$$

Thus, since the condition (4.7) is assumed to be satisfied, a threshold for G above which eradication occurs there exists and can be easily derived. In particular, if $\eta_2 = 0$ this sufficient condition becomes

$$\eta_1 G > \frac{b\beta - \mu_1\mu_2 - \mu_1\beta - \mu_2 b}{b + \mu_2}, \tag{4.30}$$

and, if $\eta_1 = 0$,

$$\eta_2 G > \frac{b\beta - \mu_1\mu_2 - \mu_1\beta - \mu_2 b}{\beta + \mu_1}. \tag{4.31}$$

The meaning of the conditions (4.30) and (4.31) appears more clear when $\mu_1 = \mu_2 = 0$: if the drug kills proliferating endothelial cells only, it must be $\eta_1 G > \beta$; if the target cells are the quiescent ones, it must be $\eta_2 G > b$.

If the effect of drug may be considered as a pulse of cell loss, Eqs. (4.5), (4.6) will be subjected to the infinite conditions

$$X(nT^+) = (1 - p_x)X(nT^-), \quad Y(nT^+) = (1 - p_y)Y(nT^-), \quad n = 0, 1, \dots \tag{4.32}$$

with, in general, $p_x \neq p_y$. Since system (4.5),(4.6) is positive and cooperative, by applying the Kamke's theorem in each subinterval $(nT, (n+1)T)$, it is easy to see that $0 \leq X(t) \leq \bar{X}(t)$ and $0 < Y(t) \leq \bar{Y}(t)$, where $(\bar{X}\ \bar{Y})^T = \bar{\mathbf{x}}$ is now the solution of

$$\bar{\mathbf{x}}' = A\bar{\mathbf{x}} \tag{4.33}$$

$$\bar{\mathbf{x}}(nT^+) = Diag\big((1 - p_x), (1 - p_y)\big)\bar{\mathbf{x}}(nT^-), \tag{4.34}$$

$$\bar{\mathbf{x}}(0^-) = \mathbf{x}(0^-). \tag{4.35}$$

By setting $\bar{\mathbf{z}} = H\bar{\mathbf{x}}$, H being a diagonalizing matrix for A, we have

$$\bar{\mathbf{z}}' = Diag\big(\lambda_+, \lambda_-\big)\bar{\mathbf{z}} \tag{4.36}$$

$$\bar{\mathbf{z}}(nT^+) = H\,Diag\big((1 - p_x), (1 - p_y)\big)H^{-1}\bar{\mathbf{z}}(nT^-), \tag{4.37}$$

from which

$$\bar{\mathbf{z}}((n+1)T^+) = H\,Diag\big((1 - p_x), (1 - p_y)\big)H^{-1}Diag\big(e^{\lambda_+ T}, e^{\lambda_- T}\big)\bar{\mathbf{z}}(nT^+). \tag{4.38}$$

Thus, defining

$$E = H\,Diag\big((1 - p_x), (1 - p_y)\big)H^{-1}Diag\big(e^{\lambda_+ T}, e^{\lambda_- T}\big), \tag{4.39}$$

we have that the condition

$$\sigma(E) \subset \{w \in \mathcal{C} : \ |w| < 1\}, \tag{4.40}$$

where $\sigma(E)$ is the spectrum of the matrix E, implies $\lim_{t \to +\infty} \bar{\mathbf{z}}(t) = \mathbf{0}$ and then $(V, X, Y) \to (0, 0, 0)$. When the effect of therapy is symmetric, i.e. $p_x = p_y = p$, condition (4.40) becomes simply

$$(1 - p)e^{\lambda_+ T} < 1. \tag{4.41}$$

According to the above results, tumour eradication can be achieved in the case of model (4.4),(4.8),(4.9) even if $\mu_1 = \mu_2 = 0$. This is expected to be not true for model (4.4),(4.10),(4.11). The use of Kamke's theorem allows us to recognize that for that model the presence of *spontaneous* vasculature loss, i.e. $\mu_1 + \mu_2 > 0$, is necessary for eradication when the therapy is constant.

If we suppose in fact $V \to 0$, for any $\epsilon > 0$ a time t_ϵ will exist such that $d_1 V^{2/3}, d_2 V^{2/3} < \epsilon$ for $t > t_\epsilon$. Then, for $t > t_\epsilon$, we have that $\mathbf{x} = (X, \ Y)^T$ as given by (4.4), (4.10),(4.11) is componentwise greater than, or equal to, the solution $\hat{\mathbf{x}}$ of the time-invariant system

$$\hat{\mathbf{x}}' = (A_G - \epsilon I)\hat{\mathbf{x}}, \quad \hat{\mathbf{x}}(t_\epsilon) = \mathbf{x}(t_\epsilon), \tag{4.42}$$

in which the matrix A_G is obtained from (4.10),(4.11) with $g(t) = G$. Therefore, for ϵ small enough, $\mu_1 = \mu_2 = 0$ would imply the existence of a positive eigenvalue of $A_G - \epsilon I$, in contradiction with the hypothesis.

5. Concluding remarks

The mathematical model proposed by Hahnfeldt *et al.* [11], fully embodies the concept that tumour growth is controlled ultimately by the evolution of the blood vessel network supplying oxygen and nutrients to tumour cells, and consequently that tumour regression can be achieved by inducing regression of the vasculature. Despite its simplifications, this model therefore seemed to us worthy to be furtherly investigated as a possible model for antiangiogenic therapy. The model is formulated in a very simple way, by introducing the carrying capacity of the vasculature as the control variable, and writing a single differential equation for describing its evolution. In the present paper simple variants have been proposed, such as different expressions for the dynamics of the tumour volume and of the carrying capacity, and the subdivision of the pool of vessel cells into proliferating and resting cells, without altering some important features of the original model.

For all these resulting models the effect of a periodic antiangiogenic therapy has been studied, and conditions for tumour eradication have been derived. When the dynamics of the carrying capacity is that proposed in [11], apparently similar alternatives in the dynamics of tumour volume have been found to be not equivalent with respect to the eventual effectiveness of therapy. In the comparison of different drug schedules, periodic schedules characterized by the same average values of the drug concentration gave the same final outcome (eradication of tumour or not) if the tumour volume obeys an equation of logistic type, whereas if the tumour is governed by an equation of Gompertz type the outcome may be different, depending on the shape of the drug concentration time-course. Concentration profiles having short (and high) peaks were less effective than time-courses not too far from a constant concentration. This finding might be of interest in the current debate on the effectiveness of mild, dense in time, dosing of drugs [34].

We stress that the results concerning the eradication here presented refer to periodic therapies without any constraint on the overall duration and amount of drug, and thus differ qualitatively from results on optimal scheduling of antiangiogenic therapy as those obtained by Ergun *et al.* [20] under the constraint of a fixed total amount of available drug. Since the model adopted in [20] is derived from model (2.2),(2.4) and has some similarities with model (2.2),(2.6), some general features of the optimal scheduling found in [20] could be expected to hold also for the above models. A study of the optimal dosing for these models, even under different constraints, would be certainly of interest. We also note that a periodic therapy that mainly targets proliferating endothelial cells could be affected by "resonance" effects [35-37], that is by a reduced efficacy of treatment when its period is equal to (or is a multiple of) the mean cell cycle transit time, if the drug is specific for a particular phase of the cell cycle. In this case, the condition for eradication should not be independent of the period, and its assesment would require a model in which the compartment of proliferating endothelial cells is structured with respect to the cell cycle.

24.

A more detailed and realistic description of the relationship between the tumour and the vasculature evolution appears very complex and is still a challenging task. To account for the decrease of the specific growth rate observed in the growth of experimental tumours, other explanations are possible than the progressive accumulation inside the tumour of a factor responsible of the regression of vessels, in the presence of a constant level of pro-angiogenic factors [11]. The angiogenesis process involves a delicate interplay among different molecular factors [7,8], such as VEGF and angiopoietins produced by tumour cells and endothelial cells, and goes through the formation of immature vessels that evolve into more stable (mature) ones. This latter transition, together with the time delay between the first stimulation of angiogenesis and the assessment of an effective vasculature, has been considered in [18,19]. It has been observed that angiogenesis is more active in the peripheral (and peritumoral) region of the tumour in comparison with the central region, where massive necrosis is often present. This spatial pattern of the neo-vascularization could be relevant to shape the law of the overall tumour growth. It must also be noted that in tumours different from sub-cutaneously implanted tumours, the cooption of preexisting host vessels [38] might be of importance in assessing the carrying capacity of the vasculature. Moreover, the carrying capacity is not only related to the geometric extent of the vasculature, but it is related to the amount of oxygen and nutrients that are available in the unit time for the transport to tumour cells, and then to the total volumetric blood flow within the tumour. Thus the repeated branching of exchange vessels inside the tumour due to angiogenesis should lead to vessels in which the flow is progressively slower, and exchange less efficient, if the number of supporting arteries does not change. As the tumour grows, it can be hypothesized that the arteries involved do not increase in number at a rate sufficient to guarantee a suitable blood flow in the core of the tumour, and so the overall growth could be impaired.

Finally, we note that in [11], and in all the models here studied, the tumour volume is implicitly assumed proportional to the size of the tumour cell population, this population being the direct target of the control exerted by the extent of the effective vasculature. This view appears to be reasonable during the tumour growth phase, but it may be less adequate during the tumour regression, when the volume dynamics may be influenced by the removal of the abundant necrotic material produced by cell death. This phenomenon leads to a time-shift between the volume response and the cell response to treatments [39], and it might be of importance in the analysis of experimental volumetric data collected after therapy.

Acknowledgments

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