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A. dOnofrio, A. Gandolfi

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A. dOnofrio – Department of Experimental Oncology, European Institute of Oncology, Via Ripamonti 435, 20141 Milano, Italy. e-mail: alberto.donofrio@ieo-ifom-campus.it.

A. Gandolfi – Istituto di Analisi dei Sistemi ed Informatica "A. Ruberti" - CNR, Viale Manzoni 30, 00185 Roma, Italy. e-mail: gandolfi@iasi.cnr.it.

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Istituto di Analisi dei Sistemi ed Informatica “Antonio Ruberti”
Consiglio Nazionale delle Ricerche

viale Manzoni 30, 00185 ROMA, Italy

tel. ++39-06-77161
fax ++39-06-7716461
email: iasi@iasi.cnr.it
URL: http://www.iasi.cnr.it

Chemotherapy of vascularised tumours: role of vessel density and the effect of vascular "pruning"

Alberto d'Onofrio [1], Alberto Gandolfi [2]

[1] Department of Experimental Oncology, European Institute of Oncology,
Via Ripamonti 435, 20141 Milano, Italy.
e-mail: *alberto.donofrio@ieo-ifom-campus.it*, phone: +390257489819

[2]Istituto di Analisi dei Sistemi ed Informatica "A. Ruberti" - CNR,
Viale Manzoni 30, 00185 Roma, Italy.
e-mail: *gandolfi@iasi.cnr.it*

Abstract

In this work we propose to model chemotherapy taking into account the mutual interaction between tumour growth and the development of tumour vasculature. By adopting a simple model for this interaction, and assuming that the efficacy of a drug can be modulated by the vessel density, we study the constant continuous therapy, the periodic bolus-based therapy, and combined therapy in which a chemoterapic drug is associated with an anti-angiogenic agent. The model allows to represent the vessel-disrupting activity of some standard chemotherapic drugs, and shows, in the case of constant continuous drug administration, the possibility of multiple stable equilibria. The multistability suggests an explanation for some sudden losses of control observed during therapy, and for the beneficial effect of vascular "pruning" exherted by anti-angiogenic agents in combined therapy. Moreover, in case of periodic therapies in which the drug amount administered per unit time is constant ("metronomic" delivery), the model predicts a response, as function of the bolus frequency, significantly influenced by the extent of the anti-angiogenic activity of the chemotherapic drug and by the dependence of the drug efficacy on the vessel density.

Keywords: Tumour, angiogenesis, chemotherapy, pruning, multistability.

1 Introduction

The use of chemotherapies in oncology has been one of the major steps forward in the so called war against cancer (Peckham et al. 1995, Boyle et al. 2003). Chemotherapy has a clinical role so important that in the common usage of many languages the word chemotherapy nowadays uniquely denotes *anti-tumour chemotherapy.* A

huge *corpus* of experimental and clinical literature has been produced in the last 60 years, but chemotherapy also triggered the production of a large amount of theoretical researches due to its apparently simple translation into mathematical models (Skipper 1986, Wheldon 1988, Cojocaru and Agur 1992, Afenya 1996, Swierniak et al. 1996, Panetta 1997, Ledzewicz and Schättler 2006, Simeoni et al. 2004, Ribba et al. 2005). Quite interestingly, and unlike from other fields of biomedicine, here there has been a limited but important interplay between theoretical and experimental-clinical scientists (Goldie and Coldman 1979, Norton and Simon 1986, Panetta et al. 2008, Ubezio and Cameron 2008).

Since their first applications, it has been plain that a number of serious side effects are related to the use of cytotoxic chemicals to cure tumors, for the simple reason that these agents, which never are perfectly selective, kill a more or less wide range of proliferating cells. However, even when the amount of side effects was relatively limited, a number of failures related to the emergence of resistance of tumour cells to the drug were noticed. Progresses in molecular biology allowed to explain this phenomenon: tumour cells are strongly characterized by a mutation rate significantly higher than that of healthy cells (Schimke 1984, Goldie 2001). Thus, the response of tumour cells to chemotherapy is characterized by a considerable evolutionary ability to enhance the cell survival in an environment becoming hostile. Moreover, small subpopulations of cells intrinsically unsensitive to the treatment may be present *ab initio* in the tumour and, after some time, these cells may become the dominant population leading to the failure of therapy. Considerable research efforts have been and are devoted in finding means to overcome drug resistance (Frame 2007).

Chemotherapy aims at targeting the main peculiar characteristic of tumour cells, i.e. their proliferative derangement. However, tumour cells show a vast array of genetic and epigenetic events, and of microscopic and macroscopic interactions with other cellular populations. As a consequence, the study of these phenomena may open the way for the creation of new therapies. J. Folkman (Folkman 1972, Folkman 1975) stressed in early seventies that during the progression of tumours the development of a vascular network inside the tumour mass becomes necessary to support the tumour growth, and he named this process neo-angiogenesis. The tumour angiogenesis is a very complex phenomenon, driven by *pro-angiogenic* factors released by the tumour cells lacking a full level of nutrients. Interestingly enough, tumour cells also release *anti-angiogenic* chemicals to modulate the growth of the vessel network. In such a way, a solid tumour deploys a sophisticated strategy to control its own growth. However, Folkman did not only study the neo-angiogenesis. He also had the idea that controlling the development of the tumoral vessel network could be a powerful way to control, in turn, the neoplastic growth via the reduction of nutrients supply, and he termed this new kind of therapy 'anti-angiogenic therapy'. *Per se*, this new way of controlling the tumor burden appears intriguing. Moreover, it has another fascinating advantage respect to conventional chemotherapy: endothelial cells forming blood vessels are far more genetically stable than tumour cells, so that the anti-angiogenic agents should be less subject to resistance phenomena.

It is worth noting that tumours differ from normal tissues also in density, topology

and functionality of their vessel network, which is characterized by a remarkable degree of intricacy as well as by a variety of disfunctionalities. The neovessel network that brings nutrients to the tumour also is the route to deliver chemotherapic drugs. As a consequence, the poor functionality of the network itself may be expected to affect the efficacy of chemotherapy negatively. Moreover, since in the angiogenesis process endothelial cells proliferate, chemotherapic drugs have been found to exert an anti-angiogenic action (Browder et al. 2000). Summarizing these observations, one may infer that:

- the theoretical characterization (e.g. by mathematical models) of chemotherapy must take into account the existence of neovessels and their dynamics;
- neovessels can be destroyed by chemotherapy;
- conversely, the excessive reduction of the vessel network by an anti-angiogenic agent might be detrimental for chemotherapy.

However, it has been observed that an anti-angiogenic agent has not necessarily a detrimental effect on the efficacy of a chemotherapic drug: on the contrary, better results have been obtained by combined therapy (Browder et al. 2000, Klement et al. 2000, Siemann and Rojiani 2002). To explain these findings, R.K. Jain hypothesized that the preliminary delivering of such an agent, by 'pruning' the vessel network, may regularize it with beneficial consequences for a succesive therapy with cytotoxic anti-tumour drugs (Jain 2001, Jain and Munn 2007).

A full description of all these phenomena to create a comprehensive model of chemotherapy and of the combination of chemotherapy and anti-angiogenic therapy could require a multi-scale approach. Instead, we aim at assessing whether a relevant part of the interplay between a given chemotherapy, the neovessels and a possible anti-angiogenic therapy may simply be explained by ecological interaction between the two involved cellular populations: tumour celles and endothelial cells. Elementary models of this interaction, indeed, have been proved able to reproduce the main features of tumour growth and of anti-angiogenic therapies (Hahnfeldt et al. 1999, d'Onofrio et al. 2009, Swierniak 2009).

The present paper is organized as follows: after a brief introduction of some basic concepts (Section 2), we summarize in Section 3 recent results on modelling the growth of a tumour and of its vasculature. In Section 4, we introduce our model of chemotherapy that assumes the dependence of the drug efficacy on the vessel density, and includes the possible vessel disrupting action of the chemotherapeutic drug. In Section 5, we study the effects of a constant uninterrupted chemotherapy, by stressing a number of phenomena linked to the possible multistability of the model. In the next Section 6, we study by simulations effects arising in the case of periodic boli-based therapy. In Section 7, we estend our model by allowing the co-presence of an anti-angiogenic therapy, and after studying the case of continuous constant therapy, we focus on the role of vascular "pruning". Final remarks conclude the work.

2 Preliminary concepts

The most simple model of chemotherapy can be written by coupling an empirical law for the unperturbed growth of the tumour, with a loss term depending on the drug concentration according to the so called "log-kill" hypothesis (Skipper 1986). If the insurgence of the drug resistance is disregarded, and a logistic law is assumed for the unperturbed growth, we can write:

$$N' = \alpha\Big(1 - \frac{N(t)}{N_\infty}\Big)N(t) - \gamma c(t)N(t), \tag{1}$$

where $N(t)$ denotes the tumour size, N_∞ the (fixed) carrying capacity of the logistic growth, and α the proliferation rate constant. The coefficient γ represents the sensitivity of the cells to the drug, and $c(t)$ is the drug concentration in blood.

In case of $c(t) = C$ constant, which is the case of a continuous constant infusion treatment, it is easy to see from (1) that if

$$C > \frac{\alpha}{\gamma},$$

N will tend to zero for any positive initial condition, i.e. asymptotic tumour eradication is achieved. Conversely, if $C < \alpha/\gamma$ there will exist a positive equilibrium smaller than N_∞, precisely $N_e = (1 - \gamma C/\alpha)N_\infty$. Note that the eradication would *not* be possible, whatever the magnitude of the drug concentration may be, if the growth law in (1) were assumed Gompertzian (d'Onofrio 2005). This fact is due to the unboundedness of the relative growth rate of the Gompertz law, $N'/N \sim \ln(N_\infty/N)$, for N going to zero. Such an unboundedness clearly contradicts the physical basis of cell kinetics, since in any cell populations the relative growth rate has a finite upper bound because the cell cycle duration has a finite lower bound. This pathology was recognized by Wheldon (Wheldon 1988), who proposed the so-called Gomp-exp growth law, in which the growth is exponential for $N < \bar{N}$, $\bar{N}$ given, and for $N \geq \bar{N}$ it obeys to the Gompertz law. In the following, therefore, we will consider only tumour growth laws with *bounded* relative growth rate.

From the point of view of the cure, the really relevant measure of the tumour size is the number of viable cells, whereas when measurements on solid tumours are performed, usually the tumour volume is recorded. The two quantities have different dynamics (Bertuzzi et al. 2003, Simeoni et al. 2004, Ubezio and Cameron 2008), since in the evolution of the tumour volume the delay in the dead cells degradation and in the reabsorption of waste fluids play an important role. In this paper, however, we will view for simplicity the number of viable tumour cells and the tumour volume as *proportional* quantities, and identify the tumour size with the tumour volume, $V(t)$.

The appreciation of the role of angiogenesis in tumour development has led to the concept of a varying carrying capacity, defined as the tumour size potentially sustainable by the vascular network existing at a given time (Hahnfeldt et al. 1999). The carrying capacity may be assumed proportional to the extent of the actual tumour vasculature (Hahnfeldt et al. 1999). By introducing in Eq. (1) the variable carrying

capacity, $K(t)$, and by describing its dynamics according to Hahnfeldt et al. (1999), we may obtain the model (see (d'Onofrio 2007, d'Onofrio et al. 2009)):

$$V' = \alpha\Big(1 - \frac{V(t)}{K(t)}\Big)V(t) - \gamma c(t)V(t), \tag{2}$$

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

where b, d, and μ (with $b > \mu$) are constants related to the stimulation, inibition and natural loss of the vasculature respectively. In the case of a costant infusion therapy, the behaviour predicted by model (2),(3) is not substantially different from that of model (1). It can be easily derived that there exists a critical value for C above which there is eradication,

$$C > \frac{\alpha}{\gamma}\frac{b-\mu}{b},$$

and below which a positive equilibrium is established. Note that now the critical value of the drug concentration depends also on the parameters characterizing the dynamics of the vasculature. However, as we will see in the following, model (2),(3) can be a useful starting point towards extensions aimed at describing the possible dependence of the efficacy of the cytotoxic drug on the "quality" of the vascular network, the anti-angiogenic effect of some chemotherapeutic drugs, and the synergy between vessel targeting and tumour-cell targeting drugs.

3 A family of models for the tumour growth

To describe the interplay between the tumour and its vasculature, we adopt a family of models previously proposed (d'Onofrio and Gandolfi 2009), which includes as particular cases the models in (Hahnfeldt et al. 1999, Sachs et al. 2001, d'Onofrio and Gandolfi 2004). In this family of models, we assume that (*i*) the carrying capacity of the tumour vasculature is simply proportional to the amount of vessels, and (*ii*) the specific growth rate of the tumour, V'/V, and the specific "birth" rate of vessels depend on the ratio between the carrying capacity and the tumour size. Since the ratio K/V may be interpreted as proportional to the tumour vessel density, assumption (*ii*) agrees with the model proposed by Agur et al. (2004)(see also (Forys et al. 2005)). Following Hahnfeldt et al. (1999), the growth of the neo-vasculature will be antagonized by endogenous factors and we can write:

$$V' = VF(\frac{K}{V}) \tag{4}$$

$$K' = K\Big(\beta(\frac{K}{V}) - \psi(V) - \mu\Big) \tag{5}$$

where:

- $F'(u) > 0$, $F(1) = 0$, $0 < \lim_{u\to+\infty} F(u) < +\infty$, and $0 > \lim_{u\to 0^+} F(u) \geq -\infty$;

- $\beta(+\infty) = 0$, $\beta'(u) < 0$ and $\lim_{u\to 0^+} \beta(u) = \beta_0 \leq +\infty$;
- $\psi(0) = 0$, $\psi'(u) > 0$ and $\lim_{u\to+\infty} \psi(u) = +\infty$;
- $\beta(1) > \mu$.

In Eq. (5), $\psi(V)$ represents the vessels loss due to the possible accumulation into the tumour of an endogenous inhibitory factor secreted by the tumour cells, and μ represents the natural loss of vessels.

The assumption that $F(+\infty) < +\infty$ is the only restriction we set on the family of models proposed in (d'Onofrio and Gandolfi 2009), and it means that V'/V will have a finite upper bound. Thus the Gompertz law is not included in F. As an example of possible expressions for $F(u)$ we may consider the generalised-logistic:

$$F(u) = \alpha(1 - u^{-\nu}), \qquad \nu > 0. \tag{6}$$

We mention here that a generalised-logistic growth with $\nu = 2/3$ can be predicted for spherical tumours supplied by diffusible nutrients at the periphery (Bodnar and Forys 2007), assuming the cell proliferation rate proportional to nutrient concentration and a uniform cell death.

The function $\beta(u)$ includes power laws

$$\beta(u) = bu^{-\delta}, \qquad \delta > 0, \tag{7}$$

so that it may be $\lim_{u\to 0^+} \beta(u) = +\infty$, functions such as

$$\beta(u) = \beta_M \frac{1}{1 + ku^n}, \qquad n \geq 1,$$

which have $\lim_{u\to 0^+} \beta(u)$ finite and correspond to Hill functions in the variable u^{-1}, and combinations of the above two expressions as:

$$\beta(u) = \beta_1 \frac{1}{1 + ku^n} + \beta_2 \frac{1}{u}. \tag{8}$$

The expression (7) with $\delta = 1$ yields $K\beta(K/V) = bV$, as proposed by Hahnfeldt et al. (1999), whereas (8) yields

$$K\beta(\frac{K}{V}) = \beta_1 \big[1 + k(\frac{K}{V})^n\big]^{-1} K + \beta_2 V.$$

The above expression for the growth term of the tumour vasculature distinguishes the contribution of the endothelial cell proliferation, which is assumed depending on the vessel density, and the input of new endothelial cells due to their migration from the peritumoral regions or to the influx of circulating endothelial progenitors (Rafii et al. 2002). The latter contribution is simply taken proportional to the tumour size. **On the other hand, the prescribed properties of the function β exclude those modifications of the Hahnfeldt's model proposed in (Ergun et al. 2003) and**

(d'Onofrio and Gandolfi 2004) that disregard the role of the tumour mass in the proangiogenic activity.

Concerning the function ψ, we recall that $\psi(V) = dV^{2/3}$ has been assumed in (Hahnfeldt et al. 1999).

It is easy to see that model (4)-(5) admits a unique positive equilibrium $V_e = K_e$, and it can be shown that this equilibrium is globally asymptotically stable in $\mathbb{R}^2_+$ (d'Onofrio and Gandolfi 2009).

As an example, Fig. 1 reports the tumour volume growth, predicted by the following model

$$V' = \alpha\big(1 - (\frac{V}{K})^{0.5}\big)V, \tag{9}$$

$$K' = bV - dV^{2/3}K, \tag{10}$$

for different initial values of K. Note that the equation for K is that proposed in (Hahnfeldt et al. 1999) with $\mu = 0$. The growth curve of mouse xenografts is taken as reference for this simulation. As the panels A and B show, different sets of parameters α and (b, d) can give very similar growth curves. When the dynamics of the vasculature is fast ((b, d) high, panel A), the system tends to reach quickly the K-nullcline, the evolution is thus scarcely influenced by the initial value of K, and the growth velocity is mainly under the control of the intrinsic proliferation rate α. Conversely, when the dynamics of the vasculature is rather low ((b, d) low, panel B), this dynamics starts to be limiting and thus to have a comparable growth velocity the proliferation rate of tumour cells must be higher, and the influence of the $K(0)$ value is increased. Panels C and D show the time course of the ratio $\rho(t) = K(t)/V(t)$. Since all the growths tend to an equilibrium state with $V_e = K_e$, $\rho(t)$ always will tend to 1. In the case of higher α and lower (b, d) (Panel D), ρ goes to the unity more rapidly and monotonically.

4 The chemotherapy model

To model the effect of a cytotoxic chemotherapeutic agent of concentration $c(t)$, we have to modify model (4)-(5) by adding a log-kill term in equation (4), as well as a term in Eq. (5) representing the possible cytotoxic actions of the chemotherapeutic drug on endothelial cells.

The killing efficacy of a blood-born agent on the tumour cells will depend on its actual concentration at the cell site, and thus it will be influenced by the geometry of the vascular network and by the extent of blood flow. The efficacy of a drug will be higher if vessels are close each other and sufficiently regular to permit a fast blood flow; it will be lower if vessels are distanced, or irregular and turtuous so to hamper the flow. To represent simply these phenomena, we assume that the log-kill term to be added in Eq. (4) be dependent on the vessel density, i.e. in our model on the ratio $\rho = K/V$, by writing it as $\gamma(\rho)c(t)V(t)$. We suppose $\gamma(0) = 0$, and γ increasing for ρ small. For larger values of the vessel density we make two hypotheses: either $\gamma(\rho)$ continues to increase tending to a saturation value, or it starts to decrease after having reached a unique absolute maximum. According to these assumptions we propose the

following model

$$V' = V\big(F(\frac{K}{V}) - \gamma(\frac{K}{V})c(t)\big) \tag{11}$$

$$K' = K\big(\beta(\frac{K}{V}) - \psi(V) - \mu - \chi c(t)\big) \tag{12}$$

where:

- $c(t) \geq 0$ is the blood concentration of the cytotoxic drug;
- $\chi \geq 0$. The case $\chi = 0$ corresponds to chemoterapic agents having no vessel disrupting effects;
- $\gamma(\rho)$, with $\gamma(0) = 0$, is continuous and either increasing with $\gamma(+\infty) < +\infty$, or it exists $\rho_M > 0$ such that at $\rho = \rho_M$ the function γ has an absolute maximum and $\gamma(+\infty) \geq 0$.

We shall call $\gamma(\rho)$ the efficacy curve of the drug.

A simple sufficient condition, although quite sharp, for the asymptotic eradication of the tumour is stated in the following:

Proposition 4.1 *Let us define*

$$C^o = \sup_{\rho\in[1,+\infty)} \frac{F(\rho)}{\gamma(\rho)}$$

If $C^o < +\infty$ *and*

$$\min_{t\in[t_0,+\infty)} c(t) > C^o \text{ for some } t_0 \geq 0, \tag{13}$$

then

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

for any positive initial condition, i.e. global eradication of the tumor is achieved.

It is interesting to note that, as far as the role of $\gamma(\rho)$ in the asymptotic behaviour is concerned, only the values of this function for $\rho > 1$ matter.

5 Constant infusion therapy: eradication and equilibrium points

5.1 Eradication

In the case of $c(t) = C$, i.e. in the case of constant continuous infusion therapy, the previous Proposition guarantees that if $C > C^o$ asymptotic eradication will occur. However, even if $C < C^o$, with $C^o \leq +\infty$, there may be tumour eradication. In such a case, the anti-angiogenic effect of the drug as well as of the spontaneous loss of vessels play a *necessary* role.

If $0 < C < C^o$, by setting $V' = 0$, equilibrium values for the ratio ρ are determined by solving the equation

$$F(\rho) = C\gamma(\rho), \tag{14}$$

which, since (13) does not hold, has $n \geq 1$ solutions:

$$1 < \rho_1(C) < \rho_2(C) < \cdots < \rho_n(C).$$

In the case of γ constant, obviously we would have $n = 1$. The corresponding equilibrium values $V_i(C)$ may be obtained by solving

$$\psi(V) = \beta\left(\rho_i(C)\right) - \mu - \chi C, \tag{15}$$

which, provided that

$$\beta\left(\rho_i(C)\right) - \mu - \chi C > 0,$$

has the unique positive solution:

$$V_i(C) = \psi^{-1}\left(\beta\left(\rho_i(C)\right) - \mu - \chi C\right)$$

Of course, this suggests that if there exists $C^* < C^o$ such that

$$\beta\left(\rho_1(C^*)\right) - \mu - \chi C^* = 0,$$

then there is no positive solution of (15) for $C = C^*$, since $\beta(\rho)$ is decreasing, and there is eradication. This fact is rigorously shown in the following proposition:

Proposition 5.1 *Let a positive $C^* < C^o$ exist such that*

$$\beta\left(\rho_1(C^*)\right) - \mu - \chi C^* = 0. \tag{16}$$

If

$$C \geq C^*, \tag{17}$$

then the tumour is globally eradicated.

We remark that an eradication threshold $C^* < C^o$ can exist only if $\chi > 0$ and/or $\mu > 0$, that is *only if* the chemotherapeutic drug exerts a vessel disrupting effect and/or a spontaneous loss of vessel is present. The converse is true if $\lim_{C \to C^o} \rho_1(C)) = +\infty$. In this case, in fact, if $\chi > 0$ and/or $\mu > 0$ a value $C^* < C^o$ always exists, because $\beta(\rho)$ tends to zero for ρ increasing.

In the case of delivering of a purely chemotherapeutic agent, i.e. $\chi = 0$, the eradication condition becomes

$$\beta\left(\rho_1(C)\right) - \mu \leq 0, \tag{18}$$

and then the possibility of eradication with doses smaller than C^o relies on the spontaneous loss of vessels. Since the rate of this loss is likely to be small (Hahnfeldt et al. 1999), it appears unlikely that an eradication threshold significantly smaller than C^o can exist in this case. Of course, if $\mu = 0$ and $C^o = +\infty$ the eradication would be impossible.

5.2 Non eradication: multiple equilibria and their local stability

In the case in which the eradication condition (17) cannot be fulfilled, indicating with $m \leq n$ the maximum index i such that

$$\beta\left(\rho_i(C)\right) - \mu - \chi C > 0$$

we have $m \geq 1$ coexisting equilibria:

$$E_i = (V_i(C), K_i(C)), \qquad i = 1, \ldots, m,$$

with $K_i = \rho_i V_i$.

Remark *Note that the integer m is a function of C. In fact, in the case of nonmonotone $\gamma(\rho)$, for small C there is typically a single equilibrium with ρ slightly greater than 1, for large C no or at most one equilibrium point is present (in this case the equation $F(\rho) = \gamma(\rho)C$ can have one solution but there may be no equilibria because the eradication condition may be satisfied), whereas for intermediate C values there may be multiple equilibria.*

From (15) we see that the tumour size at the equilibrium points under therapy, $V_i(C)$, $i = 1, \ldots, m$, will be smaller than the equilibrium tumour size in the absence of therapy also if $\mu = \chi = 0$. We have in fact

$$\beta(1) - \mu > \beta(\rho_i(C)) - \mu - \chi C,$$

and, since $\beta(\rho)$ is decreasing, the above inequality is true also if $\mu = \chi = 0$. Whereas the role of the chemoterapic action appears to be, in the framework of the present model, the determination of a steady-state vessel density greater than 1 through the equation

$$F(\rho) = \gamma(\rho)C,$$

the eventual value of the tumour volume is prescribed by the vessel dynamics. The role of the anti-angiogenic action of the drug is that of setting the tumour size to a value lower than the value which would be set in the absence of any anti-angiogenic action.

It is interesting to note that the local stability of the co-existing equilibria depends on the dynamics of V and on the shape of the function $\gamma(u)$ through a nice geometrical property, as shown in the following proposition:

Proposition 5.2 *Let $E_i = (V_i, K_i)$ be a critical point of (11)-(12). If*

$$\gamma'(\rho_i) < F'(\rho_i), \tag{19}$$

then E_i is locally asymptotically stable, whereas if

$$\gamma'(\rho_i) > F'(\rho_i), \tag{20}$$

then E_i is unstable.

In case of a unique equilibrium point ($m = 1$), it is easy matter to show that it must be locally asymptotically stable. As far as its global stability is concerned, it holds the following:

Proposition 5.3 *Let E be a unique equilibrium point for (11)- (12). Denoting by $\rho_M \leq +\infty$ the value such that $\gamma(\rho)$ is maximum, if*

$$C < \min_{\rho\in(0,\rho_M)} \frac{F'(\rho) - \beta'(\rho)}{\gamma'(\rho)} \tag{21}$$

then E is globally asymptotically stable.

Figure 2 illustrates the response to a constant continuous infusion therapy of model (11)-(12) with F, β and ψ as in (9)-(10), and $\mu = 0$. In the simulations we used the following simple form for $\gamma(\rho)$:

$$\gamma(\rho) = \bar{\gamma}\max\big[\frac{4}{9}\rho(3-\rho), 0\big], \tag{22}$$

so that γ attains its maximum, equal to $\bar{\gamma}$, for $\rho = 3/2$. With such a choice for the efficacy function of the drug, the treatment cannot be eradicative if the chemotherapic drug does not exert also an anti-angiogenic effect. However, for each value of C, a unique equilibrium exists, with a reduced tumour volume, and this equilibrium point can asymptotically be reached. In the absence of any vessel disrupting effect ($\chi = 0$, solid lines), the final volume is smaller in the case of model 1, as expected because this model is characterized by a smaller value of the proliferation rate α of tumour cells. The response pattern can be changed if the drug also exert a vessel disrupting action (dashed lines): the final volumes are smaller, and the smaller volume is predicted now by model 2, since the slow vessel dynamics which is characteristic of such a model is impaired at a larger extent by the anti-angiogenic action. This simulation show how the underlying vessel dynamics can affect the response to a chemotherapeutic treatment of different tumours that appear very similar on the basis of their growth curves. We note that this behaviour is not necessarily related to the non-monotonic shape of $\gamma(\rho)$, it can be achieved also for γ increasing or constant.

5.3 Bifurcations

In this section we shall deal with the analysis of bifurcations by taking as bifurcation parameter the drug concentration C. Preliminarly we note that, by applying the implicit function theorem, it follows that:

$$\frac{d\rho_i}{dC} = \frac{\gamma(\rho_i)}{F'(\rho_i) - \gamma'(\rho_i)C}.$$

As a consequence, it holds that if i is *odd* then $\rho_i'(C) > 0$, else if i is *even* then $\rho_i'(C) < 0$. This implies that if $m \geq 3$ then there is at least a hysteresis bifurcation (Hale and Kocak 1991). In fact, let us consider for the sake of simplicity a $\widehat{C}$ such that

$m(\widehat{C}) = 3$. Note that this is possible *both* in the case of non-monotone γ functions, and for γ increasing. We have:

$$\rho_1'(\widehat{C}) > 0 \ , \ \rho_2'(\widehat{C}) < 0 \ , \ \rho_3'(\widehat{C}) > 0,$$

(see middle panel of figure 3). It follows that by increasing C there is a threshold value $C_2 > \widehat{C}$ such that at $C = C_2$ the equilibria E_1 ad E_2 will collide and only E_3 will last (see lower panel of figure 3, and figure 4), which implies that the asymptotic tumour size – which is given by eq. (15) – may undergo a 'jump' towards a far lower value. On the contrary, by decreasing C one has that it exists a $C_1 < \widehat{C} < C_2$ such that at $C = C_1$ E_2 and E_3 will collide and only E_1 will last (see upper panel of figure 3, and figure 4), with a possible jump of tumour size towards a higher value.

We note that the latter transition could occur even if C were constant and the efficacy function $\gamma(\rho)$ were multiplied by a parameter continuously decreasing. A time-varying, decreasing γ might be the result of the development of drug resistance, and thus we might expect in such a case that the tumour control exerted by the chemotherapy can be abruptly lost.

Figure 5 illustrates this phenomenon. Let us consider the model (11)-(12) with F, β and ψ as in (9)-(10), $\mu = 0$, and

$$\gamma(\rho, t) = \frac{\bar{\gamma}}{1 + \left(\frac{\rho - \rho_m}{\sigma}\right)^2} \mathrm{e}^{-(t-t_0)/\tau} Heav(t - t_0), \qquad c(t) = C Heav(t - t_0),$$

where t_0 is the time at which the treatment starts, and $Heav(\cdot)$ is the Heaviside function. In the simulation, the tumour is treated from $t = 15$ day with a constant continuous infusion of drug. Its volume starts moving towards the equilibrium value characterized by the higher ρ value, and for a relatively long period tumour control is achieved with only a mild regrowth. As the value of γ decreases, however, an abrupt transition occurs towards high values of the tumour volume, when the root of $\gamma(\rho, t)C = F(\rho)$ jumps to a low ρ value. The tumour behaviour is qualitatively the same even in the presence of an anti-angiogenic effect of the drug.

6 Periodic therapy

In this section we shall assess the effects of a therapy periodically delivered by means of boluses. In the case of a chemotherapic agent with monoexponential pharmacokinetics, the drug concentration profile in blood is asymptotically given by

$$c(t) = D_c \frac{\mathrm{e}^{-qMod(t,T)}}{1 - \mathrm{e}^{-qT}}, \tag{23}$$

where T is the period of the delivering, q is the clearance rate of the drug, and D_c is the amount of drug delivered as bolus over the distribution volume. Such a profile of $c(t)$ is characterized by the following average value:

$$< c(t) >= \frac{D_c}{qT}. \tag{24}$$

For purely anti-angiogenic therapies, experimental studies suggested that intensifying the delivering frequency by maintaining the total administered dose per unit time constant, gives a better response (Drixler et al. 2000, Kisker et al. 2001). Such kind of drug scheduling has been called (with a slight abuse) metronomic, and the improving of the response with the increase of the delivering frequency is termed "metronomic effect". It has also been suggested that metronomic schedulings might also improve the results of chemotherapies (Kerbel and Kamen 2004, Orlando et al. 2006). However, Browder et al. (2000) showed that, at least in some cases, the metronomic effect is not the strict rule and that there is a somewhat optimal T for the reduction of the tumour volume. Thus, here, we aim at verifying whether our model is able to reproduce these effects.

In our simulations we used, as for Fig 2, model (11)-(12) with F, β and ψ as in (9)-(10), $\mu = 0$, and $\gamma(\rho)$ firstly given by (22). Moreover, since in metronomic therapy the dose delivered per time unit is constant, we impose

$$D_c = qTM_0, \tag{25}$$

so that:

$$< c(t) >= M_0.$$

After setting $\alpha = \ln 2/1.5$ day^{-1}, $b = 4.64$, $d = 0.01$, and $\bar{\gamma}M_0 = 0.2$, we simulated the model response changing T and the extent of the anti-angiogenic action, i.e the value of χ. The model, driven by a periodic $c(t)$, produces an asymptotically periodic $V(t)$ and we computed the average tumour volume, $V_m =< V(t) >$, when this regimen is in practice reached. Note that with the γ function given by (22), the continuous infusion therapy does not guarantee the eradication of the tumor whatever be the value of the drug concentration.

Figure 6 shows how the average asymptotic tumour volume depends on T for different values of χ, both in the case of constant γ, and in the case of drug efficacy changing with ρ. In the case of γ constant, V_m is an increasing function of T for all the values of χ tested, i.e. there is full metronomic effect (upper panel of figure 6). Quite different is the behaviour in case of non-constant $\gamma(\rho)$ (lower panel). For $\chi = 0$, namely in the case of delivering of an agent with pure chemotherapic action, the average volume V_m decreases as T decreases, i.e. there is full metronomic effect. By setting χ to small values, V_m is again increasing with T (not shown) and, of course, it sets to lower values because of the synergy between the chemotherapic and the anti-angiogenic action. However, for $\chi/\bar{\gamma} = 0.5$ or greater, V_m becomes non-monotone and it exists a T_{min} such that the average volume has a minimum. Thus our model was able to mimick the metronomic effect in the absence of anti-angiogenic action of drug, but also the presence of a *partial metronomic* effect characterized by an optimal delivering period, T_{min}, when a significant anti-angiogenic effect is present. However, the difference between the values of V_m for $T = 1$ and for $T = T_{min}$ results small. Thus, for $\chi/\bar{\gamma} = 0.5$ and for $\chi/\bar{\gamma} = 0.65$ it would be more correct to say that there is a more or less large (depending on χ) interval for T where V_m is approximately constant and delivering with the highest frequencies is not advantageous. The effect of higher values of χ is

reported in Fig. 7. If for $\chi/\bar{\gamma} = 1$ a (slight) anti-metronomic effect extends up to about $T = 9$ days, for higher χ values the therapy tends to be more and more characterized by a metronomic effect. It must be observed, however, that when $\chi/\bar{\gamma}$ attains the high values of 5 and 7 the drug is exherting mainly an anti-angiogenic, vessel disrupting action, and a better efficacy of time-dense schedulings of pure anti-angiogenic drugs is indeed expected (d'Onofrio et al. 2009, d'Onofrio and Gandolfi 2009).

The effect of varying the period of a periodic therapy (keeping the average concentration constant) can be subtly modulated by the shape of the function $\gamma(\rho)$. Assuming the form

$$\gamma(\rho) = \bar{\gamma}\mathrm{Min}(1, \rho/A),$$

which has a monotone profile, Figure 8 shows how different behaviours can be achieved by varying the value of A. The brisk change going from $A = 3$ to $A = 3.5$ corresponds, in the constant continuous therapy, to a bifurcation due to the occurrence of three equilibrium points.

Finally, we note that the metronomic effect appears to be related basically to the nonlinear nature of the tumour growth law. A slight metronomic effect (not shown) was indeed found using the model of Eq. (1). However, modelling the vessel dynamics and the dependence of the drug cytotoxicity on the vessel density makes it possible to expand the variety of the response patterns.

7 Combined chemotherapy and anti-angiogenic therapy. Role of vascular "pruning"

In this Section we will consider the case of a combined therapy in which a true anti-angiogenic agent is administered together with a chemotherapeutic drug. The most general setting is the following:

- a chemotherapeutic agent, with concentration $c(t)$;
- an anti-angiogenic agent exerting vessel disruption, with concentration $g(t)$;
- an anti-angiogenic agent, with concentration $h(t)$, exerting (directly or indirectly) a cytostatic action on the endothelial cells.

Of course, the same drug can exert both vessel disruption and inhibition of endothelial cell proliferation, and in this case it shall be $g(t) = h(t)$.

The model (11),(12) can be easily extended, obtaining

$$V' = VF(\frac{K}{V}) - \gamma(\frac{K}{V})c(t)V, \tag{26}$$

$$K' = K\big(\theta\left(h(t)\right)\beta(\frac{K}{V}) - \psi(V) - \mu - \chi c(t) - \eta g(t)\big), \tag{27}$$

where $\theta(u)$ is a decreasing function such that $0 \leq \theta_\infty < \theta(u) \leq 1$. $\theta(u) = 1$ corresponds to the case of absence of cytostatic action on the endothelial cells. The coefficient $\eta \geq 0$ represents the vessel disrupting efficacy of the anti-angiogenic agent.

As it easy to recognize, almost all the results of the previous sections can be translated for the model (26),(27). We state the main results in the case of constant continuous administration of all the drugs, i.e when $c(t) = C$, $g(t) = G$, and $h(t) = H$.

If $C^o < +\infty$ and $C > C^o$, asymptotic eradication of tumour is achieved thanks to Proposition 4.1. If $0 < C < C^o \leq +\infty$, the equation

$$\psi(V) = \beta\left(\rho_i(C)\right)\theta(H) - \mu - \chi C - \eta G, \tag{28}$$

where $\rho_i(C)$ is a generic solution of Eq. (14), has the unique positive solution

$$V_i(C, G, H) = \psi^{-1}\left(\beta\left(\rho_1(C)\right)\theta(H) - \mu - \chi C - \eta G\right), \tag{29}$$

provided that

$$\beta\left(\rho_i(C)\right)\theta(H) - \mu - \chi C - \eta G > 0.$$

(V_i, K_i), with $K_i = \rho_i V_i$, is therefore an equilibrium point. Of course, this suggests that if it exists a tratment (C, G, H) such that

$$\beta\left(\rho_1(C)\right)\theta(H) - \mu - \chi C - \eta G \leq 0,$$

then there is no positive equilibria and there is asymptotic eradication. This property is stated in the following proposition:

Proposition 7.1 *If $C < C^o \leq +\infty$ and*

$$\beta\left(\rho_1(C)\right)\theta(H) - \mu - \chi C - \eta G \leq 0, \tag{30}$$

then the tumour is globally eradicated.

In the case in which the eradication condition is not fulfilled, indicating with $m \leq n$ the maximum index i such that

$$\beta\left(\rho_i(C)\right)\theta(H) - \mu - \chi C - \eta G > 0,$$

we have m coexisting equilibria

$$E_1 = (V_1(C, G, H), \rho_1(C)), \ldots, E_m = (V_m(C, G, H), \rho_m(C)),$$

whose local stability properties are established by Proposition (5.2). Note that the number m itself will depend on C, G, H. As we have seen before, if a unique equilibrium point there exists, it will be locally asymptotically stable. Concerning its global stability, it holds the following:

Proposition 7.2 *Let E be a unique equilibrium point for (26)-(27). Denoting by $\rho_M \leq +\infty$ the value such that $\gamma(\rho)$ is maximum, if*

$$C < \min_{\rho \in (0, \rho_M)} \frac{F'(\rho) - \beta'(\rho)\theta(H)}{\gamma'(\rho)}, \tag{31}$$

then E is globally asymptotically stable.

We note that in the case of a cytostatic anti-angiogenic agent and in the absence of any vessel disrupting activity by both the drugs ($\theta(H) < 1$ and $\chi = \eta = 0$), the eradication condition for $C < C^o$ becomes

$$\theta(H)\beta\left(\rho_1(C)\right) - \mu \leq 0,$$

that is

$$H > \theta^{-1}\left(\frac{\mu}{\beta\left(\rho_1(C)\right)}\right). \tag{32}$$

Thus, the tumour eradication might be difficult if μ is small, unless $\theta(H)$ has a quite rapid decrease. Of course, if $\mu = 0$ the eradication would be impossible. In the case of an anti-angiogenic drug which is purely vessel disrupting ($\theta(H) = 1$ and $\eta > 0$), the eradication for $C < C^o$ is possible even if the chemotherapeutic drug does not impair the vasculature, provided that:

$$G > G^* = \frac{\beta\left(\rho_1(C)\right) - \mu}{\chi}.$$

As far as the asymptotic outcome of a constant infusion therapy is concerned, the administration of an anti-angiogenic drug, therefore, *always* produces a *synergistic* effect. If chemotherapy alone were not eradicative, the concomitant administration of an anti-angiogenic drug could induce tumour eradication, or could set the asymptotic tumour size to a value lower than the value reachable by using the chemotherapeutic drug alone.

Complex dynamics can arise, in the case in which multiple equilibria are present. In such a case, as the following simulations will show, the asymptotic outcome of a constant continuous infusion therapy can be dramatically altered if the administration of the chemoterapic drug follows a preliminary transient vessel disrupting treatment. This behaviour matches, at least qualitatively, experimental observations on the positive effect of preliminary anti-angiogenic treatments on chemotherapy (Jain 2001). For these observations, the causal role of the vascular "pruning", in order to obtain a "normalized" more efficient vascular network deprived of turtous and immature vessels, has been hypothesized (Jain and Munn 2007).

The simulations of Figures 9 and 10 are performed assuming

$$\gamma(\rho) = \frac{\bar{\gamma}}{1 + \left(\frac{\rho - \rho_m}{\sigma}\right)^2},$$

and

$$c(t) = Heav(t - t_2)C, \qquad g(t) = (Heav(t - t_1) - Heav(t - t_2))G,$$

where t_1 and t_2 are the times at which the anti-angiogenic treatment and, respectively, the chemotherapic treatment start. Figure 9 show how a mild chemotherapic treatment, capable if administered alone of only stopping the growth, become able, if proceeded by a transient (although rather vigorous) anti-angiogenic treatment, of achieving a remarkable tumour reduction. As the phase-portraits illustrate, the vessel

disruption, that means the reduction of ρ, allows to exit from the basin of attraction of the equilibium point with large tumour volume, and to enter the basin of the second locally stable equilibrium, characterized by a smaller tumour volume. It may be interesting to note that this shift can be achieved also by a very short anti-angiogenic treatment (pulse treatment), provided that its intensity be sufficiently high (see Figure 10).

Finally, we want to observe that:

- If in the case of three roots $\rho_1 < \rho_2 < \rho_3$, the root ρ_3 corresponds to tumour eradication, the preliminary anti-angiogenic treatment can allow to exit from the basin of attraction of the equilibium point with larger tumour volume, and to reach eradication.

- The shift of the state of the system to a more favourable basin of attraction can be achieved, in principle, also by a purely antiproliferative anti-angiogenic treatment ($H > 0$, $G = 0$).

8 Concluding remarks

Despite its inherent simplicity, our model appears able to explain a wide array of biomedically significant behaviours. Most traditional models of chemotherapy are characterized by monostability, namely they suggest that the effect of delivering a chemotherapic drug is either to eradicate the tumor or to drive the tumour into a unique equilibrium state, so that varying slightly the delivering of the chemotherapic agent would in any case have the effect of slightly moving the equilibrium value. Although allowing some nice biological inferences, this appears an excessively simple scenario, largely different from the clinical reality where more complex and puzzling phenomena are observed (see e.g. (Peckham et al. 1995)). Thanks to the possibility of being multistable, our model may represent an improvement in the understanding of relevant biological phenomena and also, at least conceptually, in suggesting therapeutic plans.

In particular, the multistability of our model may give a contribution in understanding some cases of failures of therapy, in which the tumour suddenly restarts growing. These occurrences might simply be explained as hysteresis bifurcations, driven by the gradual onset of drug resistance, that cause jumps in $V(t)$.

Moreover, we have shown how multistability and the beneficial effect of vascular "pruning" by anti-angiogenic agents can be strictly interlinked. In fact, if the tumour under a given (constant continuous) chemotherapy admits two stable equilibria, it appears in principle possible by the delivery of an anti-angiogenic treatment before the chemotherapy, to move the state in the basin of attraction of the equilibrium with the smaller (or null) tumour volume. In general (i.e also in the case of monostable behaviour), the model predicted synergy between constant continuous chemotherapy and anti-angiogenic treatment, and we were *not* able to find combinations in which the anti-angiogenic action was detrimental for the chemotherapy. However, we cannot

exclude *a priori* that particular cases may exist where competition can arise. In fact, although the synergy between antiangiogenic therapy and chemotherapy may appear as an intrinsic feature of our models, this property cannot plainly be considered as embedded in the dynamical system because it is not cooperative.

Furthermore, our model is able to describe how the effect of a periodic delivery of a cytotoxic drug can change by varying the time intervals between the administrations, keeping constant the average drug concentration. Our simulations show indeed that the vessel-disrupting action of some chemotherapic agents may imply the existence of an optimal delivery frequency that minimize the average tumour volume. This is a result of some interest also when the minimum is quite flat, since in such a case our simulations suggest that there is a quite large range of inter-boli time intervals where there is no sensible metronomic effect, i.e. an advantage in increasing the bolus frequency. In addition, the behaviour under a periodic therapy that keep constant the average drug concentration appears to be modulated, in a rather complex way, by the dependence of the drug efficacy on the vessel density.

We stress that we have considered here only tumour growth laws with bounded relative growth rate, so excluding the Gompertz law since its potentially unbounded relative growth rate is unphysical. An interesting property of models of anti-angiogenesis therapy incorporating a Gompertzian tumour growth law, namely the impossibility, in case of periodic therapy, of setting a sufficient condition for the tumour eradication only in terms of the average value of the drug concentration, is fully retained by models with generalised logistic growth law and $\nu < 1$, for which the function $F(K/V)$ is bounded (d'Onofrio et al. 2009, d'Onofrio and Gandolfi 2009). From a strict mathematical point of view, however, the results of this paper on the tumour eradication in the presence of vessel disrupting activity and those on the location, local stability and bifurcation of multiple equilibria continue to hold also in case of F unbounded.

The model assumes that the carrying capacity of the vasculature is simply proportional to the vessel amount. A more realistic view might consider the carrying capacity dependent on the vessel amount but also on the vessel density. The same volume of vessels can have, in fact, a different capability of supplying oxygen and nutrients to the tumour cells because of different vessel functionality. We intend to analyse the consequence of this hypothesis in a future investigation. Preliminary results suggest that if the carrying capacity of vessels, for a fixed tumour volume, is an increasing function of the vessel amount, the qualitative behaviour of the present model should be substantially conserved.

Of course our model is oversimplified in many other important aspects. We must mention the distinction between mature vessels and immature vessels, and the spatial heterogeneity of the vessels network, with the consequent heterogeneity of the distribution of cells between the proliferating and quiescent compartments. This last phenomenon could be significant in the case of drugs characterized by a marked cycle-specificity. Moreover, the onset of drug resistance is of paramount importance in all chemotherapies. To account for the above features, however, a far more complex modelling would be required.

As a final remark, we observe that in this initial work we focused on general prop-

erties and so we framed our study in a general setting, showing that non-trivial results - in particular the possible presence of multistability - are substantially independent of the choice of specific functions. A comprehensive analysis of the dependence of the tumour response on the specific functional form of the rates F, β, γ and Ψ, might reveal finer details of the problem under study.

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10 Appendix: Proofs

Proposition 4.1

Proof Rewriting (11) as follows

$$V' = V\gamma(\rho)\left(\frac{F(\rho)}{\gamma(\rho)} - c(t)\right),$$

and taking into account that $F(\rho) < 0$ for $\rho < 1$ and $F(\rho) > 0$ for $\rho > 1$, if (13) holds we have $V' < 0$ for any positive V. Thus it follows that

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

Thus the equation for K asymptotically becomes:

$$K' = -(\mu + \chi c(t))K$$

implying that also $K(t) \to 0^+$. $\diamondsuit$

Proposition 5.1

Proof If (17) is satisfied, we have

$$\beta\left(\rho_1(C)\right) - \mu - \chi C \leq 0.$$

This means that in the set

$$A = \{(V, K))|V > 0 \text{ and } K > \rho_1(C)V\}$$

it is $K' > 0$, implying that either $V(t) \to 0^+$ and $\text{minlim}_{t\to+\infty}\rho(t) > \rho_1$, or that the orbit enters in the set:

$$\Omega_o = \{(V,K))|K > 0 \text{ and } 0 < K < \rho_1 V\},$$

which is positively invariant and where $V' < 0$, so that $V(t) \to 0^+$. $\diamondsuit$

Proposition 5.2

Proof Preliminarly, for the sake of notation simplicity, let us define the following auxiliary function

$$H(\rho) = \frac{F(\rho)}{\gamma(\rho)}.$$

Since it is

$$\frac{\partial V'}{\partial V}\Big|_{E_i} = -\rho_i\gamma(\rho_i)H'(\rho_i), \qquad \frac{\partial V'}{\partial K}\Big|_{E_i} = \gamma(\rho_i)H'(\rho_i),$$
$$\frac{\partial K'}{\partial V}\Big|_{E_i} = -\left(\rho_i^2\beta'(\rho_i) + K_i\Psi'(V_i)\right), \qquad \frac{\partial K'}{\partial K}\Big|_{E_i} = \rho_i\beta'(\rho_i),$$

we get the following characteristic polynomial:

$$\lambda^2 + \rho_i\left(\gamma(\rho_i)H'(\rho_i) - \beta'(\rho_i)\right)\lambda + K_i\Psi'(V_i)\gamma(\rho_i)H'(\rho_i).$$

Thus if $H'(\rho_i) < 0$ then E_i is unstable, whereas if $H'(\rho_i) > 0$ then E_i is locally asymtptically stable (remember that $\beta' < 0$). Finally, since

$$H'(\rho_i) = \frac{\gamma(\rho_i)F'(\rho_i) - \gamma'(\rho_i)F(\rho_i)}{\gamma^2(\rho_i)} = \frac{F'(\rho_i) - \gamma'(\rho_i)}{\gamma(\rho_i)},$$

our claim immediately follows. $\diamondsuit$

Proposition 5.3

Proof From

$$\text{div}\left(\frac{1}{VK}(V',K')\right) = \frac{1}{V^2}\left(-F'(\rho) + \beta'(\rho) + \gamma'(\rho)C\right).$$

we have, from the Poincare's tricothomy, that condition (21) implies the global asymptotic stability of E. $\diamondsuit$

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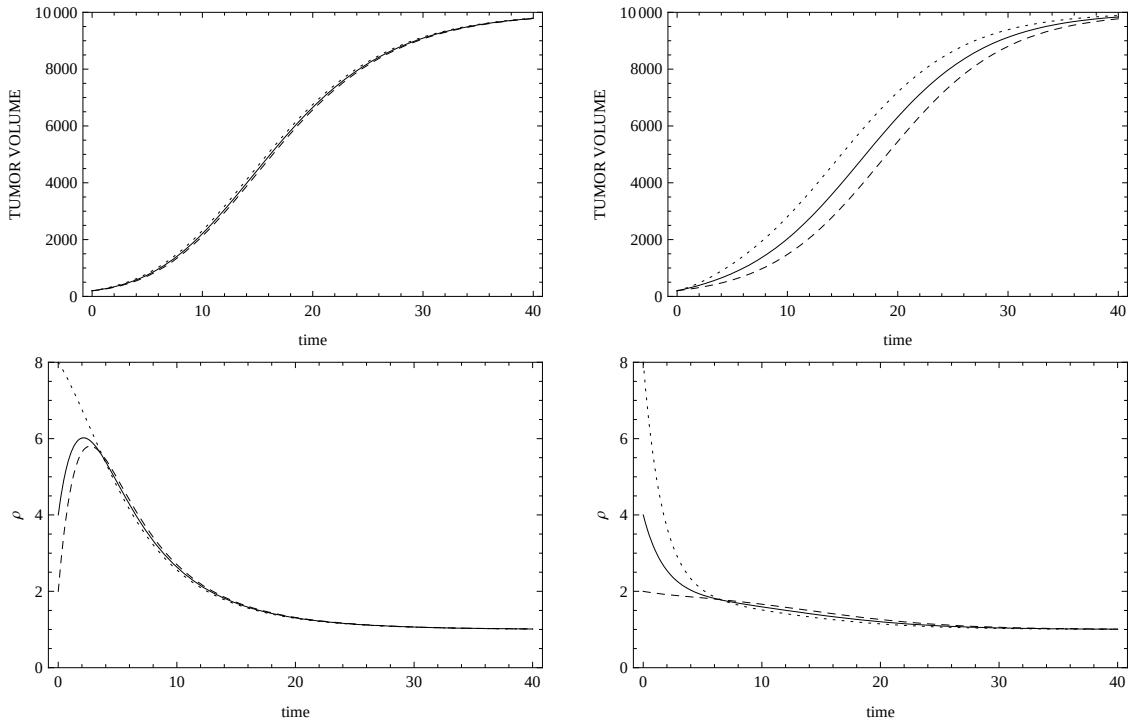

Figure 1: Tumour volume (panels A and B), and $\rho = K/V$ (panels C and D) vs. time, according to the model of Eqs. (9)-(10). Panels A and C: $\alpha = \ln 2/1.5$ day^{-1}, $b = 4.64$, $d = 0.01$. Panels B and D: $\alpha = \ln 2/0.9$ day^{-1}, $b = 0.464$, $d = 0.001$. In all panels: $V(0) = 200$ mm^3, $\rho(0) = 2$ (dashed), $\rho(0) = 4$ (solid), $\rho(0) = 8$ (dotted line).

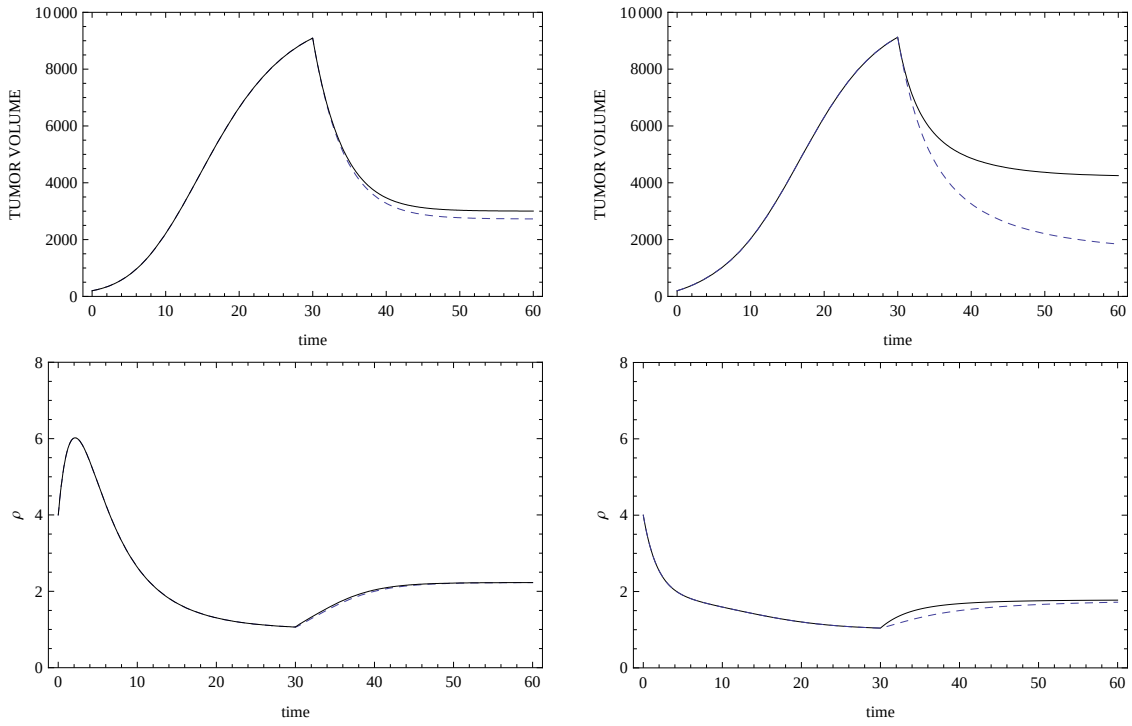

Figure 2: Tumour volume (panels A and B), and $\rho = K/V$ (panels C and D) vs. time, before and after a treatment with constant drug concentration starting at $t = 30$ days. Panels A and C: $\alpha = \ln 2/1.5$ day^{-1}, $b = 4.64$, $d = 0.01$, $\bar{\gamma}C = 0.2$ day^{-1}. Panels B and D: $\alpha = \ln 2/0.9$ day^{-1}, $b = 0.464$, $d = 0.001$, $\bar{\gamma}C = 0.2$ day^{-1}. In all panels: $V(0) = 200$ mm^3, $\rho(0) = 4$, $\chi = 0$ (solid lines), $\chi/\bar{\gamma} = 0.65$ (dashed lines).

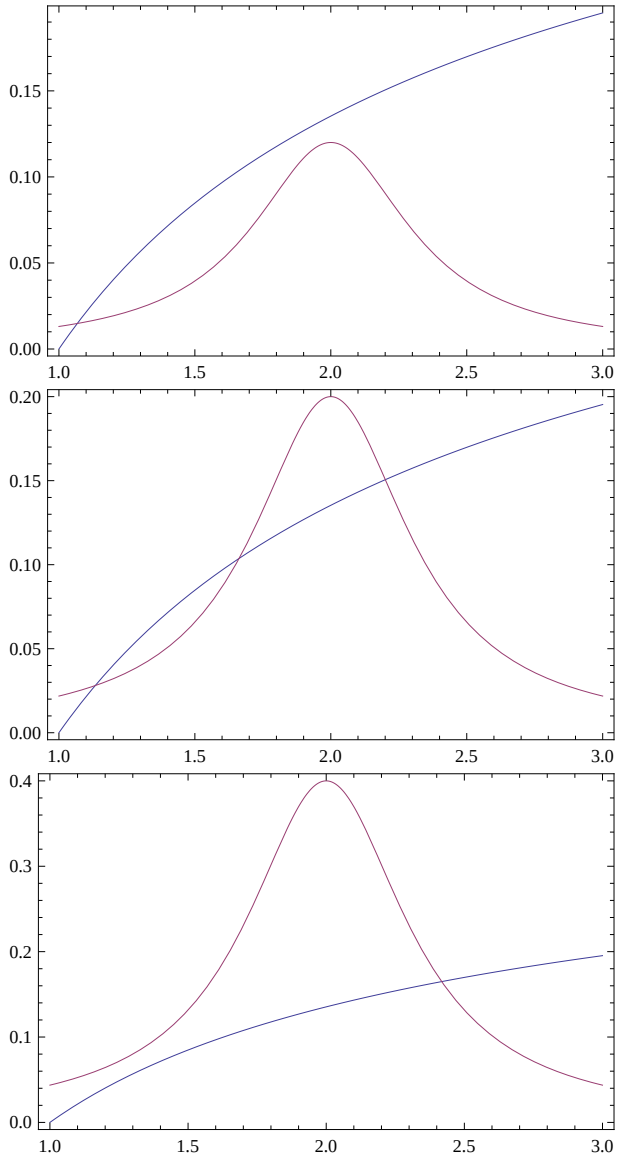

Figure 3: Upper panel, small C: a single equilibrium is present. Middle panel, intermediate C: 3 equilibria are present. Lower panel, high C: only the equilibrium with greater ρ remains. $F(\rho)$ as in Eq. (9), with $\alpha = Ln(2)/1.5$; $\gamma(\rho) = \bar{\gamma}/(1+((\rho-\rho_m)/\sigma)^2)$ with $\rho_m = 2$, $\sigma = 0.35$. $\bar{\gamma}C = 0.12$ (upper panel), $\bar{\gamma}C = 0.2$ (middle panel), $\bar{\gamma}C = 0.4$ (lower panel).

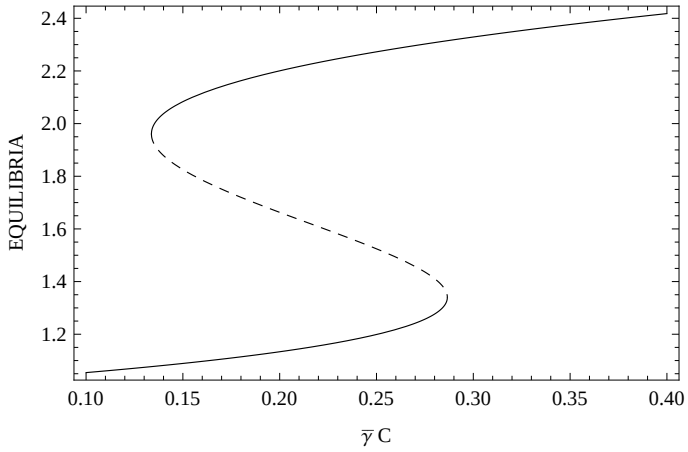

Figure 4: Bifurcation diagram. In red the central unstable equilibrium, in black the two locally asymptotically stable equilibria. Functions F and γ as in Fig. 3.

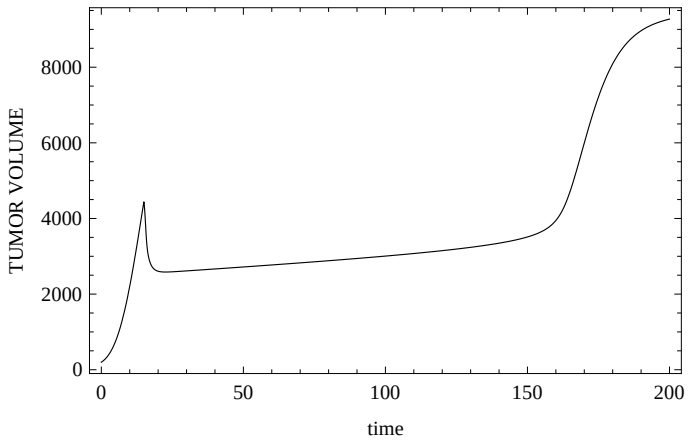

Figure 5: Tumour response to a constant continuous therapy, when Eq. (14) has 3 positive roots at the start of treatment, and drug efficacy decays with time (mimicking the rise of drug resistance). Model of Eqs. (9),(10), with $\gamma(\rho,t)c(t) = 0.5Heav\,(t-15)\,(1/(1+((\rho-2)/0.35)^2))\,Exp\,(-0.01(t-15))$. Other parameters as in the text.

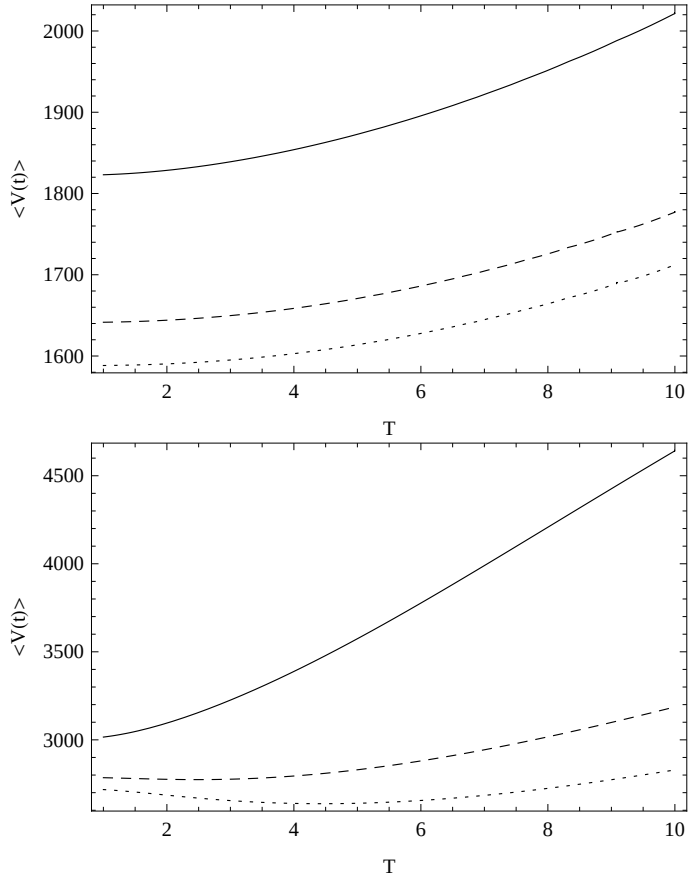

Figure 6: Periodic therapy. Average tumour volume V_m vs. the period T for different values of χ. Upper panel: $\gamma(\rho)$ constant; lower panel: $\gamma(\rho)$ as in formula (22). In both panels: $\chi = 0$ (solid line), $\chi/\bar{\gamma} = 0.5$ (dashed line), $\chi/\bar{\gamma} = 0.65$ (dotted line). Other parameters given in the text.

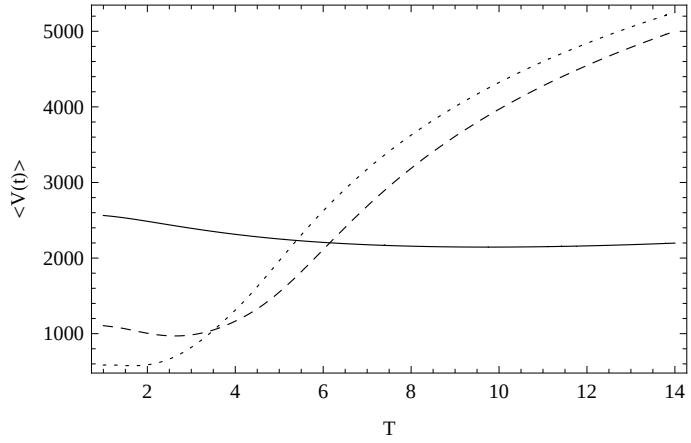

Figure 7: Periodic therapy. Average tumour volume V_m vs. the period T for different values of χ. $\gamma(\rho)$ as in formula (22). $\chi/\bar{\gamma} = 1$ (solid line), $\chi/\bar{\gamma} = 5$ (dashed line), $\chi/\bar{\gamma} = 7$ (dotted line). Other parameters as Fig. 6.

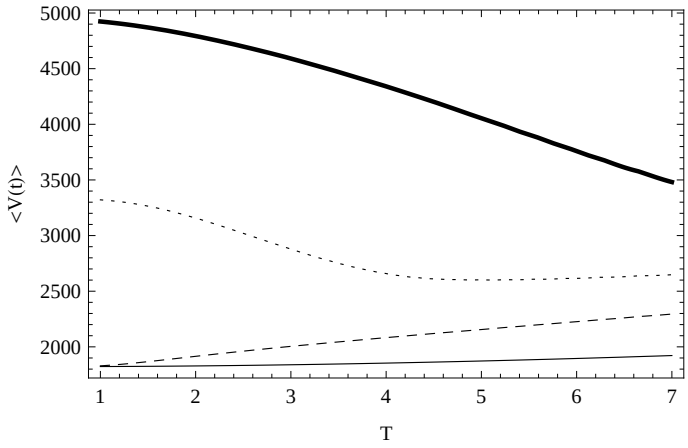

Figure 8: Periodic therapy. Average tumour volume V_m vs. the period T. Pure chemotherapy ($\chi = 0$) with $\gamma(\rho) = \bar{\gamma}\mathrm{Min}(1, \rho/A)$. $A = 1$ (solid line), $A = 3$ (dashed line), $A = 3.25$ (dotted line), $A = 3.5$ (thick line). Other parameters as in Fig. 6.

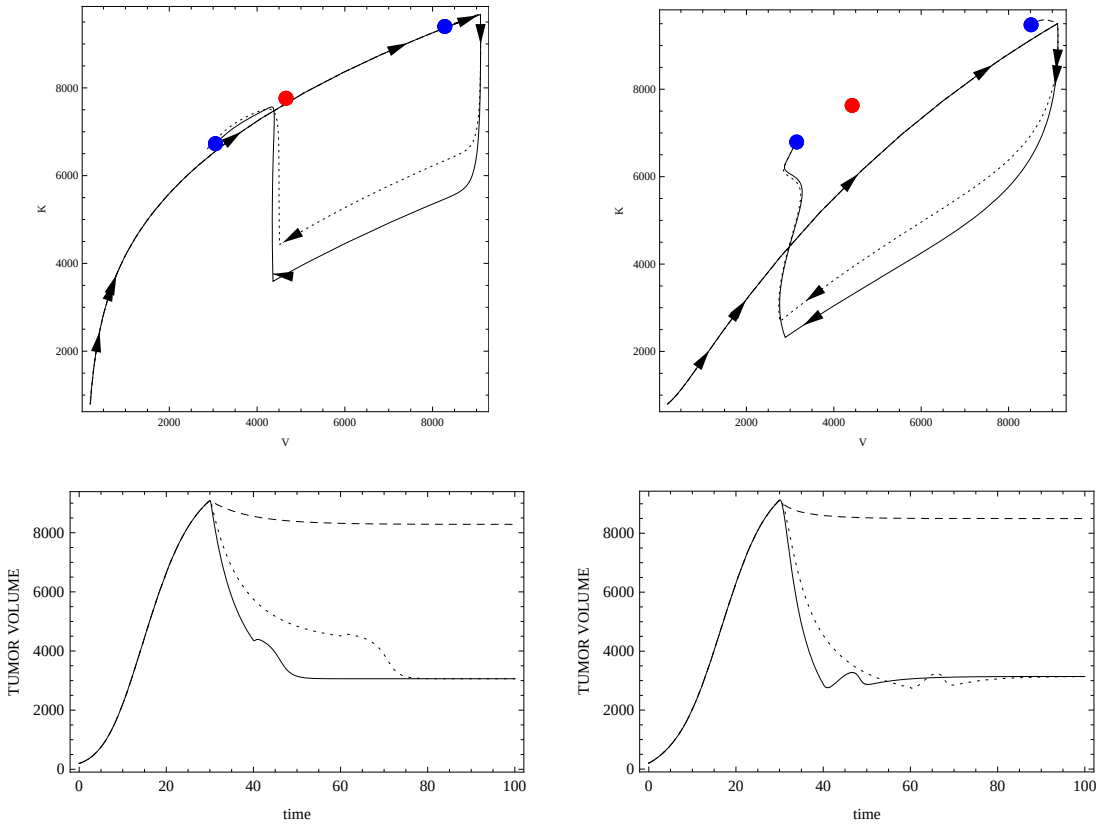

Figure 9: Combined treatment: tumour evolution in the (V, K) plane (panels A and B), and tumour volume vs. time (panels C and D). At $t = 30$ days, the tumour is treated with a vessel disrupting agent for 10 days (solid lines) or 30 days (small dashed lines). Thereafter, the anti-angiogenic treatment ceases and the tumour is treated with a pure chemoterapic drug. The effect of the chemotherapic drug alone, given at $t = 30$, is also shown (dashed line). In panels A and B the equilibrium points are reported as large dots (the unstable equilibrium is red). Model of Eqs. (9)-(10), with $\gamma(\rho) = \bar{\gamma}/(1 + ((\rho - \rho_m)/\sigma)^2)$ and $\rho_m = 2$, $\sigma = 0.35$. Panels A and C: $\alpha = \ln 2/1.5$ day^{-1}, $b = 4.64$, $d = 0.01$, $\bar{\gamma}C = 0.2$, $\chi = 0$, $\eta G = 3$ (solid), $\eta G = 2$ (small dashed lines). Panels B and D: $\alpha = \ln 2/0.9$ day^{-1}, $b = 0.464$, $d = 0.001$, $\bar{\gamma}C = 0.3$, $\chi = 0$, $\eta G = 0.45$ (solid), $\eta G = 0.29$ (small dashed lines).

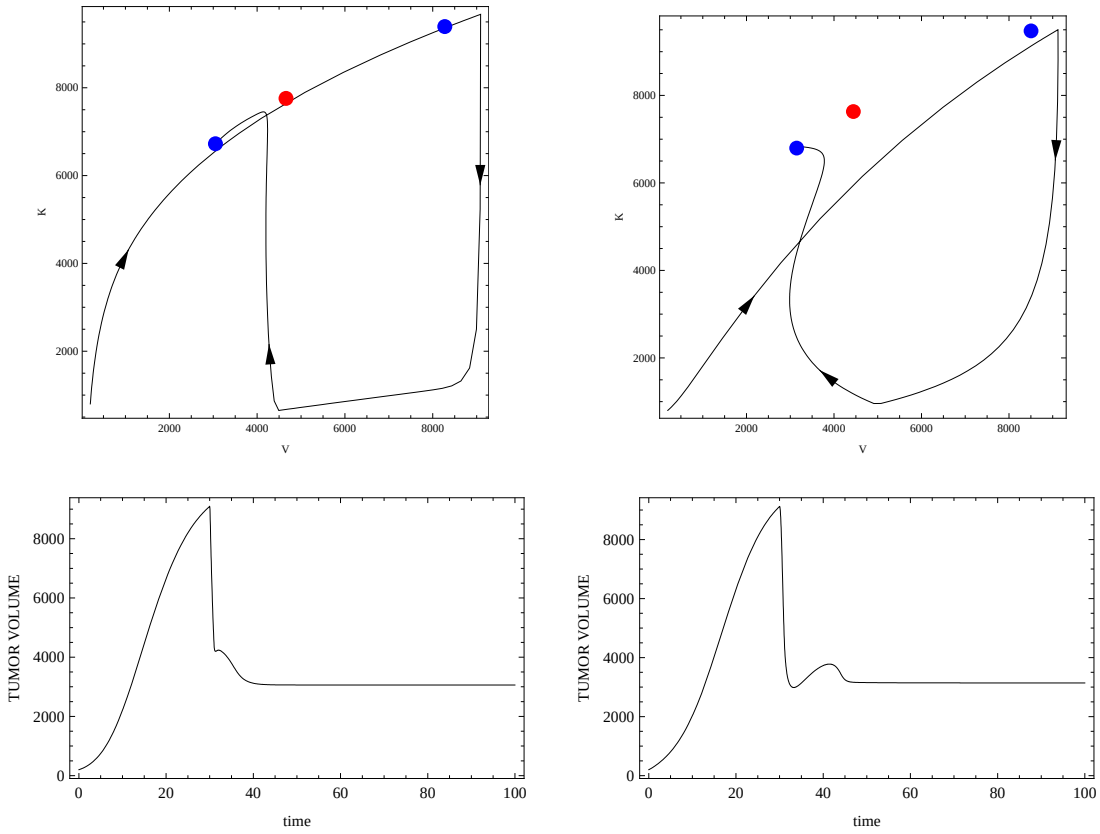

Figure 10: Combined treatment with pulsed anti-angiogenic treatment: tumour evolution in the (V, K) plane (panels A and B), and tumour volume vs. time (panels C and D). At $t = 30$ days, the tumour is treated with a high concentration of a vessel disrupting agent for 1 day. Thereafter, the tumour is treated with a pure chemoterapic drug. In panels A and B the equilibrium points are reported as large dots (the unstable equilibrium is red). Model of Eqs. (9)-(10), with $\gamma(\rho) = \bar{\gamma}/(1 + ((\rho - \rho_m)/\sigma)^2)$ and $\rho_m = 2$, $\sigma = 0.35$. Panels A and C: $\alpha = \ln 2/1.5$ day^{-1}, $b = 4.64$, $d = 0.01$, $\bar{\gamma}C = 0.2$, $\chi = 0$, $\eta G = 30$. Panels B and D: $\alpha = \ln 2/0.9$ day^{-1}, $b = 0.464$, $d = 0.001$, $\bar{\gamma}C = 0.3$, $\chi = 0$, $\eta G = 3.5$.