Combined therapies of antithrombotics and antioxidants delay \textit{in silico} brain tumour progression

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Glioblastoma multiforme (GBM), the most frequent type of primary brain tumour, is a rapidly evolving and spatially heterogeneous high-grade astrocytoma that presents areas of necrosis, hypercellularity and microvascular hyperplasia. The aberrant vasculature leads to hypoxic areas and results in an increase in oxidative stress, selecting for more invasive tumour cell phenotypes. In our study, we assay \textit{in silico} different therapeutic approaches which combine antithrombotics (ATs), antioxidants and standard radiotherapy (RT). To do so, we have developed a biocomputational model of GBM that incorporates the spatio-temporal interplay among two glioma cell phenotypes corresponding to oxygenated and hypoxic cells, a necrotic core and the local vasculature whose response evolves with tumour progression. Our numerical simulations predict that suitable combinations of ATs and antioxidants may diminish, in a synergistic way, oxidative stress and the subsequent hypoxic response. This novel therapeutical strategy, with potentially low or no toxicity, might reduce tumour invasion and further sensitize GBM to conventional RT or other cytotoxic agents, hopefully increasing median patient overall survival time.

\textbf{Keywords:} glioblastoma multiforme; antithrombotic; radiotherapy; antioxidants; combined therapies.
1. Introduction

Glioblastoma multiforme (GBM), a World Health Organization (WHO) grade IV astrocytic glioma, is the most aggressive and frequent primary brain tumour (Louis et al., 2007). GBM may develop (spanning from 1 year to more than 10 years) from lower-grade astrocytomas (WHO grade II) or anaplastic astrocytomas (WHO grade III), but more frequently, it manifests de novo. The median overall survival ranges from 12 to 15 months after diagnosis despite using the current standard care. This includes maximal safe resection followed by radiotherapy (RT) in combination with the chemotherapeutic (alkylating) agent temozolomide (Van Meir et al., 2010). After treatment, relapse occurs typically within a few months due to the GBM radio and chemoresistance and its remarkable infiltrative nature.

At the tissue level, the presence of necrosis and microvascular hyperplasia is characteristic of GBM, in contrast to lower-grade gliomas where peritumoural vascular damage is infrequent or absent. Histological samples of GBM frequently show thrombosed vessels within necrotic cores. Tumour cells actively migrate away from oxygen-deficient (hypoxic) regions, originated after vascular injury. They form hypercellular regions that surround the necrotic cores (Rong et al., 2006).

At the molecular level, adaptation of tumour cell subpopulations to strong spatio-temporal variations of oxygen availability is mediated by the hypoxia-inducible factor (HIF-1). This complex activates the transcription of hundreds of genes that play key roles, among others, in cell death avoidance, genetic instability, vascularization, glucose metabolism, pH regulation, immune evasion and invasion (Kelly et al., 2008; Semenza, 2012). Under acute hypoxia, tumour cells will tend to cease or reduce their proliferation rate as a means of decreasing oxygen consumption. This mechanism relies primarily on the subunit HIF-1α of the heterodimer HIF-1, which arrests DNA replication in the presence of oxygen stress (Hubbi et al., 2013). Moreover, the levels of free radicals increase during cycles of hypoxia and there is evidence that they are also molecular switches influencing the stabilization of HIF-1α (Wilson and Hay, 2011).

In contrast to normal cells, the survival of GBM cells is favoured by an increase in free radicals since the oxidative stress forces the cells to produce antioxidant enzymes such as catalase and superoxide dismutase to control the exacerbated level of free radicals (Kovacic & Osuna, 2001; Schumacker, 2006). The increase in these antioxidant enzymes strongly interferes with the action of radio/chemotherapy that kills tumour cells by inducing an increase in free radicals (Tennant et al., 2010). In addition, free radicals inactivate the tumour suppressor protein p53, enabling tumour cells to escape apoptosis (Cobbs et al., 2003). Thus, disrupting this signalling process in tumours may be expected to promote hypoxia-induced death (Joiner & Van der Kogel, 2009) and to restore redox homoeostasis, turning off the hypoxic response.

Many evidences indicate that the invasive ability of GBM and its resistance to chemo- and RT is mostly due to phenotypic changes that are intimately linked to the presence of hypoxia, initiated by oxygen deprivation after a vaso-occlusion. Under these conditions, the levels of HIF-1α increase, driving the expression of many pro-angiogenic factors such as the vascular endothelial growth factor (VEGF). The accumulation of VEGF promotes an aberrant neovascularization (Semenza, 2009) composed of a high density of non-functional microvessels which are leaky and devoid of pericytes. This structure allows the contact between blood and tumour cells and initiates the coagulation cascade and the formation of thrombi, a major cause of patient death (Jenkins et al., 2010; Young et al., 2012). Furthermore, the transmembrane protein tissue factor (TF) not only activates the extrinsic pro-coagulant pathway but also contributes to GBM cell invasion and neovascularization (Harter et al., 2013). In general, thromboembolism, i.e. vaso-occlusions of large vessels, is recognized as a major complication of cancer and a
common cause of death in cancer patients. There is strong evidence linking venous thromboembolism (VTE) and malignancy (Khorana & Francis, 2008; Green & Kwaan, 2009).

Thromboembolic complications include a broad spectrum of clinical problems, a fact that has led to the use of different ways of thromboprophylaxis (Khorana & Francis, 2008; Green & Kwaan, 2009) for cancer patients. Bastida et al. (1984) proved that glioma cell lines secrete pro-thrombotic factors, and in fact, glioma patients have a high incidence of VTE, with several studies suggesting that 25–30% of these patients suffer thromboembolic events (Streiff et al., 2004; Simanek et al., 2007).

It is also known that the more tumoural tissue is removed during surgery of high-grade gliomas, the less likely are the patients to die from VTE (Simanek et al., 2007; Brose & Lee, 2008). Thus, all this body of evidence has led to the consideration of thromboprophylaxis using anticoagulants, such as, for instance, low molecular weight heparin (LMWH), for glioma patients, especially in those with a high potential risk of developing VTE (Hamilton et al., 1994; Batchelor & Byrne, 2006; Khorana & Francis, 2008; Jenkins et al., 2010).

In addition to its potential use to diminish the risk of VTE, LMWH exerts a direct effect on tumour cells and tumour stroma, as it can directly kill cancer cells and also inhibit the neovascularization process, which down-regulates cell invasion (Green & Kwaan, 2009; Dos Santos et al., 2010; Svensson et al., 2011). In this context, there have been a limited number of small-scale clinical studies evaluating the safety of antithrombotic (AT) therapy in GBM patients with a VTE risk supporting the safety of the approach (Schneider et al., 2010). However, no extra benefits beyond the substantial reduction in the VTE risk have been proved yet.

In our previous study (Martínez-González et al., 2012), we developed a minimal mathematical model of GBM progression incorporating the evolution of different tumour cell subpopulations and chemicals in the tumour microenvironment. The implications of that work are that ATs would have a restraining effect on tumour progression, thus allowing a better local control of the tumour. However, the potential gain achieved by the use of ATs alone is modest. In this paper, we will discuss a way to potentially obtain a much more substantial tumour control by targeting simultaneously the vessel occlusion events and the accumulation of hypoxia inducible factors.

Thus, in this paper we study in silico a more elaborate system, including two different cell phenotypes, the oxygen concentration in the brain tissue and a dynamic vasculature to assess the potential effect of combined therapies targeting simultaneously the vessel-occlusion events and the ‘normalization’ therapy (antioxidation) together with standard RT as an example of cytotoxic therapy.

Our conceptual framework is summarized in Fig. 1. Vascular regularizing therapies and antioxidants follow different pathways aimed at reducing tumour hypoxia by means of directly impacting on HIF-1α levels. This effect would decrease oxidative stress and the subsequent hypoxic response. To achieve this goal, antioxidants act on the oxidation balance, whereas ATs reduce vaso-occlusions and hypoxic areas. The overall effect is an increment in oxygenation. Both therapies should ameliorate vessel functionality by reducing pro-thrombotic and angiogenic factors. In addition, the higher oxygen levels and decreased oxidative stress may provide the extra benefit of a sensitization to radio- and chemotherapy, which might result in a synergistic response to the treatment.

Our plan in this paper is as follows. First, in Section 2 we present the basic model equations describing tumour progression in detail and discuss the assumptions behind them. Next, in Section 3 we discuss how to incorporate the different therapies into the model. Parameter estimation is addressed in Section 4. Once the model is set up and realistic parameter ranges identified, we move on to studying the dynamics, the main results being outlined in Section 5. Finally, in Section 6 we discuss the implications of our findings and summarize our conclusions.
Fig. 1. Schematic description of how a combined treatment of ATs and antioxidants may slow down GBM invasion and sensitize the tumour to radio- and chemotherapy.

2. Mathematical model of tumour progression

In our work, we propose a mathematical model that incorporates the spatio-temporal interplay among two tumour cell phenotypes corresponding to well-oxygenated and hypoxic cells, a necrotic core, the oxygen distribution and a dynamic vasculature.

2.1 Cell phenotypes

The so-called migration/proliferation dichotomy in tumour cells refers to the commonly assumed fact that cancer cells do not move and proliferate simultaneously (Giese et al., 2003). The switch between a more proliferative and a more invasive phenotype cannot be only mutation driven (Hatzikirou et al., 2010; Onishi et al., 2011) and, in fact, it has been suggested that invasive glioma cells are able to revert to a proliferative cellular program and vice versa, depending on the environmental stimuli (Giese et al., 2003; Keunen et al., 2011). Specifically, several studies have linked hypoxia to the invasive behaviour of different types of tumours and their relationship with metastasis and negative prognosis (Elstner et al., 2007; Bristow & Hill, 2008; Kalliomaki et al., 2009).

Following these biological facts we will assume in our model that the heterogeneous oxygen distribution is the driving force that triggers phenotypic changes of tumour cells as shown schematically in Fig. 2. Thus, the local oxygen concentration will be considered to have a major influence on the cell phenotype expression. It will be assumed to induce spatio-temporal variations of phenotypic expressions via the HIF-1α activation/deactivation pathways. Therefore, we will focus only on two dominant tumour cell phenotypes. A first phenotype with a spatio-temporal density described by a non-negative function $C_n(x, t) : \mathbb{R}^2 \rightarrow \mathbb{R}_0^+$ corresponds to more proliferative tumour cells. A second phenotype will be described by (a non-negative) function $C_h(x, t) : \mathbb{R}^2 \rightarrow \mathbb{R}_0^+$ accounting for tumour cells which are...
Fig. 2. Oxygen pressure influences the phenotype of a tumour cell. Depending on the oxygen pressures various switching mechanisms arise coupling the populations: Normoxic to hypoxic $S_{nh}$ for oxygen concentrations $O_2^{(S)}$ with characteristic time $\tau_{nh}$; hypoxic to normoxic $S_{hn}$ above $O_2^{(S)}$ with characteristic time $\tau_{hn}$ and hypoxic to necrotic $S_{hd}$ for pressures below $O_2^{(D)}$ with characteristic time $\tau_{hd}$. High oxygen pressures favour the existence of less mobile phenotypes with shorter doubling times $\tau_n$. In contrast, cells respond to low oxygen pressures by expressing more motile phenotypes with larger doubling times $\tau_h$ (Brat et al., 2004).

more mobile and resistant to therapies. Both phenotypes have described to have a major role in GBM progression (Giese et al., 2003; DeBerardinis et al., 2007; Keunen et al., 2011; Onishi et al., 2011). For convenience, we will refer to them somewhat imprecisely as ‘normoxic’ and ‘hypoxic’ phenotypes since they correspond to the limit behaviour of the tumour cell density after either very long oxygenation periods ($C_n(x,t)$) or persistent oxygen deprivation periods ($C_h(x,t)$).

The equations governing the interplay between the dominant phenotypes are

$$\frac{\partial C_n}{\partial t} = D_n \nabla^2 C_n + \frac{C_n}{\tau_n} \left( 1 - \frac{C_n + C_h + C_d}{C^{(M)}} \right) + \frac{S_{hn}}{\tau_{hn}} C_h - \frac{S_{nh}}{\tau_{nh}} C_n, \quad (2.1a)$$

$$\frac{\partial C_h}{\partial t} = D_h \nabla^2 C_h + \frac{C_h}{\tau_h} \left( 1 - \frac{C_n + C_h + C_d}{C^{(M)}} \right) - \frac{S_{hn}}{\tau_{hn}} C_h + \frac{S_{nh}}{\tau_{nh}} C_n - \frac{S_{hd}}{\tau_{hd}} C_h. \quad (2.1b)$$

The first right-hand side terms in Equations (2.1a) and (2.1b) account for cellular motility. Since the hypoxic phenotype is more migratory than the normoxic one (Berens & Giese, 1999; Giese et al., 2003; Gorin et al., 2004; Bristow & Hill, 2008), the hypoxic cell diffusion coefficient $D_h$ should be chosen to be larger than the normoxic one $D_n$. This is a key assumption of our model whose implications will be elucidated in detail.

The second terms in Equations (2.1a) and (2.1b) are classical logistic growth terms for the tumour cell populations with proliferation times $\tau_n$ and $\tau_h$, respectively, and a maximum cell density $C^{(M)}$ that the brain tissue can accommodate. The migration–proliferation dichotomy suggests that $\tau_n < \tau_h$; this is also supported by the observation that hypoxic cells reduce their proliferation rate by arresting DNA replication (Hubbi et al., 2013). Since growth is assumed to be space-limited, we incorporate also the necrotic tissue density $C_d(x,t)$ into the saturation terms (see below).

The third and fourth terms in Equations (2.1a) and (2.1b) represent the phenotypic switch under different oxygenation levels. The switch functions $S_{nh}, S_{hn}$ depend on the oxygen pressure. Under low oxygen conditions ($O_2^{(S)} < 7$ mmHg), normoxic cells change their phenotype to the hypoxic one, whereas above $O_2^{(S)}$ hypoxic cells recover their oxic phenotype. By $\tau_{nh}$ and $\tau_{hn}$ we denote the characteristic
switching times of these processes. Usually $\tau_{nh}$ is much shorter than $\tau_{hn}$ since cells incorporate mechanisms to respond almost instantaneously to the absence of oxygen. However, the reverse process is not so fast, especially in cells that have already suffered several oxygen deprivation episodes (Hsieh, 2010).

To close the model, we have to incorporate an equation for the necrotic tissue density $C_d(x, t)$ as follows:

$$\frac{\partial C_d}{\partial t} = \frac{S_{hd}}{\tau_{hd}} C_h.$$ (2.1c)

Hypoxic cells (2.1b) feed the necrotic tissue in Equation (2.1c) under persistent anoxic ($O_2^{(D)} < 0.7 \text{mmHg}$) conditions. This is accounted for by the switch function $S_{hd}$ and a rate $1/\tau_{hd}$. The explicit forms of the switch functions will be taken to be (Martínez-González et al., 2012) as follows:

$$S_{nh}(O_2) = \frac{1}{2} \left[ 1 - \tanh \left( \frac{O_2 - O_2^{(S)}}{\Delta O_2} \right) \right],$$ (2.2a)

$$S_{hn}(O_2) = \frac{1}{2} \left[ 1 + \tanh \left( \frac{O_2 - O_2^{(S)}}{\Delta O_2} \right) \right],$$ (2.2b)

$$S_{hd}(O_2) = \frac{1}{2} \left[ 1 - \tanh \left( \frac{O_2 - O_2^{(D)}}{\Delta O_2} \right) \right],$$ (2.2c)

where $S_{nh}$, $S_{hn}$ and $S_{hd} \in [0, 1]$ are the three step-like switch functions and the parameter $\Delta O_2$ describes the characteristic range of oxygen variations where the transitions occur.

### 2.2 Modelling oxygen distribution through the tissue

To simplify the analysis, yet retaining the basic interplay of the key underlying biological processes in GBM, we will stick to a 1D geometry with the oxygen flow coming from a network (1D lattice) of vessels. In the brain, spatio-temporal inhomogeneities arise in the parameter values (e.g. different propagation speeds in white and grey matter, which also vary in time as the tumour progresses) and anisotropies (e.g. on the diffusion tensor with preferential propagation directions along white matter tracts (Painter & Hillen, 2013)). However, those complexities would add on top of the phenomena to be described in this paper. Thus, in what follows, we will focus our study on a representative spatial section which will provide key spatial and time metrics enabling us to incorporate, at a later stage, the action of various therapeutic modalities.

The spatio-temporal evolution of oxygen $O_2(x, t)$ is similar to the one chosen in Martínez-González et al. (2012), although now oxygen sources are located on the blood vessels, and is governed by

$$\frac{\partial O_2}{\partial t} = D_{O_2} \nabla^2 O_2 - \frac{\alpha_n C_n + \alpha_h C_h}{O_2^{(T)} + O_2} O_2 + J(x) C(\chi F O_2^v - O_2).$$ (2.3)

The first term in the right-hand side of Equation (2.3) accounts for oxygen diffusion in the brain tissue and assumes a homogeneous and isotropic diffusion coefficient $D_{O_2}$. The second term models the oxygen consumption by both normoxic and hypoxic cells at rates $\alpha_n$ and $\alpha_h$, respectively. The saturation Michaelis–Menten constant $O_2^{(T)}$ corresponds to the oxygen pressure level at which the reaction rate is halved. The third term describes the oxygen flow from the vessels to the tissue. The oxygen pressure in the $i$th blood vessel will be denoted as $O_2^v_i(t)$. The multiplicative function $J(x)$ in Equation (2.3) accounts...
for the spatial distribution of the oxygen supply and depends on the positions $p_i$ of blood vessels and their sizes $v_i$. Here, $J$ will be taken to be a combination of Gaussian functions of the form

$$J(x) = J_{O_2} \sum_{i=1}^{N} e^{-(x-p_i)^2/v_i^2}, \quad (2.4)$$

with $J_{O_2} = 10^{-6}$ s$^{-1}$ as the estimation for the oxygen exchange coefficient which is considered to be constant in time and the same for all of the vessels. The positions $p_i$ of the vessels will be considered to be equidistant, with separations of 300 µm. The vessel widths $v_i$ (being capillaries) will be taken to be 30 µm in diameter. Since oxygen diffuses along its partial pressure gradient and through the vasculature, it is reasonable to think that vessels of similar size will have similar nutrient concentrations. Therefore, $O_{2i}$ is considered to be constant over time for all $i$. Furthermore, $\chi^C$ and $\chi^F$ in Equation (2.3) are factors accounting for the chronic and fluctuating hypoxia induced by the tumour and will be explained in depth in Section 2.3.

Oxygen diffuses successively through the intracellular fluid, cell membranes and cytoplasm, which have abrupt spatial variations. However, previous works (Pogue et al., 2001; Daçu et al., 2003; Powathil et al., 2012) have proved that using an average diffusion coefficient for oxygen as in Equation (2.3) provides a good approximation to the diffusion process.

Given that the mean oxygen pressure in arterial blood is around 95 mmHg (Kimura et al., 1996) and venous values are around 30–40 mmHg, we will employ for the oxygen pressure within the capillaries a constant value $O_{2i} = 80$ mmHg.

### 2.3 Vasculature and coagulation

The sources of nutrients and oxygen in the brain are the blood vessels (capillaries here). We will also assume that oxygen concentration in the capillary network does not follow the variations induced by the cardiac pumping in the major blood vessels (Daçu et al., 2003), because of the fast time scale of those variations.

Thrombi formation leading to the developing of pseudopalisading structures (Brat et al., 2004; Rong et al., 2006) may be in part connected with the vascular remodelling induced by tumour cells. The pro-angiogenic stimuli give rise to hyperplasia in the endothelial cells which lose cell to cell unions causing vascular permeability and a malfunctioning of the blood–brain barrier. The generated orifices allow the blood to be in contact with the tumour cells. It results in plasma coagulation factors in direct connection with the tumour tissue. As a consequence, fibrin coagulates and platelets aggregate. The tumour secretes TFs that activate the so-called extrinsic coagulation pathway that provides an additional source for the strong pro-thrombotic activity of GBM (Ruf et al., 2010; Rong et al., 2005).

We will consider that vessel functionality decreases as tumour cell density increases. Once cells accumulate around the vessels, the constitutive expression of TF by cancer cells will trigger local and systemic activation of the coagulation cascade as described above.

In our approach, blood vessels will be first assumed to become semifunctional when the tumour cell density is above a certain threshold $C_i^{(F)}$, implying the onset of fluctuating hypoxia. The step-like switch function $\chi^F$ reproduces this phenomena depending on a cellular density $C_i^{(F)}$ and a random
dynamic noise \( z (0 < z < 1) \) given by the equation

\[
\chi^F(C_n + C_h + C_d, t^*) = 1 - \frac{1}{2} \left[ 1 + \tanh \left( \frac{(C_n + C_h + C_d) - C^{(F)}}{\Delta c} \right) \right].
\]

(2.5a)

We will also assume that when the tumour cell density around the vessel grows beyond a higher threshold value \( C^{(C)} \), the coagulation process is irreversible and chronic hypoxia sets in due to the complete lack of oxygen flow. This process is modelled by a step-like switch function \( \chi^C \) given by

\[
\chi^C(C_n + C_h + C_d, t^*) = \min \left\{ \frac{1}{2} \left[ 1 - \tanh \left( \frac{(C_n + C_h + C_d) - C^{(C)}}{\Delta c} \right) \right] : t \leq t^* \right\}.
\]

(2.5b)

Finally, the vasculature sensitivity threshold \( \Delta c \) is estimated to be around 0.02 \( C^{(M)} \) cells.

3. Modelling therapies

3.1 AT therapy

We will assume that low molecular weigh heparin (LMWH) decreases the expression of TF and increases the vascular functionality. This therapy will be modelled by increasing the thresholds at which the vessels become unstable from \( C^{(F)} = 0.5 C^{(M)} \) to \( C^{(F)} = 0.6–0.8 C^{(M)} \) and from \( C^{(C)} = 0.7 C^{(M)} \) to \( C^{(C)} = 0.8–0.99 C^{(M)} \). Although the precise values are not known and probably depend on a number of additional features, they will allow us to simulate the proof-of-principle of the AT therapy.

In addition to the prevention of VTE there are other several direct mechanisms of action of LMWH on tumour cells: direct cell killing (Dos Santos et al., 2010), antiangiogenic effects (Svensson et al., 2011) and many others (see e.g. Green & Kwaan, 2009, Chapter 15). Since those effects are difficult to quantify, we will assume in this paper that the net effect of the AT therapy is to increase the vessel functionality thresholds \( C^{(C)} \) and \( C^{(F)} \).

In this paper, we will assume that drug administration leads to a blood concentration level that is sufficient to induce the required effect and as such we will not include the details of the pharmacokinetics of the drugs. The same fact applied to the treatment described in Section 3.2.

3.2 HIF-\( \alpha \) stabilization treatment

When oxygen is present, HIF-\( \alpha \) is continuously synthesized by the cell, but it is unstable and degraded with a half-life of about 2–3 min. However, as a consequence of the cycles of hypoxia due to the anomalous vasculature, free radicals are originated, regulating and stabilizing the expression of HIF-\( \alpha \) even when vascular functionality is restored. In fact, the U87 glioma cell line shows higher HIF-\( \alpha \) expression under cyclic than under chronic hypoxia (Hsieh, 2010). It is believed that the sequence of oxygen deprivation episodes may drive the accumulation of HIF-\( \alpha \) in the cell nucleus. Therefore, cells need longer times to return to the normoxic phenotype state (Semenza, 2009). This HIF-\( \alpha \) stabilization significantly increases invasiveness and secretion of angiogenic factors and drives the intrinsic and extrinsic coagulation routes.

Free radicals are usually detoxified by a complex system of proteins and antioxidant macromolecules which maintain the cell redox homoeostasis. Antioxidative (AO)-based treatments, such as those with caulerpine or tempol, result in a HIF-\( \alpha \) inhibition and consequently in an antiproliferative effect in GBM murine xenograft models (Hsieh, 2010). Thus, AO-based adjuvant therapies would provide faster
ways for cells to revert to their normoxic state underoxic conditions. They decrease the HIF-1α accumulation and enable the recovery of the redox homoeostasis. In our model, we will include the effect of the AO therapy by decreasing the time of recovery of the normoxic phenotype $\tau_{hn}$ from 96 h to a smaller value in the range 8–48 h.

It is worth mentioning that based on the go or grow hypothesis, the diffusion coefficient of hypoxic cells is larger than for the normoxic cells; however, it is not known whether targeting HIF-1α via AO therapy would induce a significant change in them. Consequently, these parameters have a 10% variability in each simulation and are constant in time along the treatments.

3.3 Radiotherapy

The standard RT protocol for high-grade gliomas consists of a total of 60 Gy in fractions of 2 Gy given in 30 sessions from Monday to Friday, leading to a treatment duration of 6 weeks. Treatment is usually started several (2–4) weeks after surgery.

To describe the effect of the therapy in our model, cell death induced by radiation is included in the simulations instantaneously once per day (Monday to Friday) during 6 weeks. Since the simulations start 2 weeks after surgery, the RT starts at Week 2.

In the absence of oxygen, the unstable free radicals generated by radiation have a longer half life. Then, they can react with $H^+$ restoring its chemically original form without the need for biological and enzymatic intervention. The overall result is that better oxygenated cells are more radiosensitive (Joiner & Van der Kogel, 2009). Thus, we will assume the effect of RT to depend on the local oxygen concentration $O_2(x, t)$ at the time of the therapy. Denoting by $RT_{ox}$ the cell surviving fraction underoxic conditions and $RT_{hyp}$ the surviving fraction under hypoxic conditions, we will employ for the total surviving fraction under a local oxygen pressure $O_2(x, t)$ the equation

$$S(x, t) = RT_{ox} \frac{O_2(x, t)}{O_{2v}} + RT_{hyp} \left(1 - \frac{O_2(x, t)}{O_{2v}}\right).$$

(3.1)

4. Parameter estimation and computational details

4.1 Parameter estimation

We resort to available experimental values from human glioma models to obtain order-of-magnitude estimates of the intervening parameter in our equations. Typical values of the used biological parameters are shown in Table 1 (see also further parameters details in Martínez-González et al. (2012)).

First, the maximum cell density $C^{(M)}$ has been estimated in previous works (see e.g. Rockne et al., 2010) to be about $10^6$ cell/cm$^2$. The oxygen pressure threshold for hypoxic metabolism $O_2^{(S)}$ is cell line dependent but experimental evidence supports for glioma the choice of 7 mmHg (Vaupel, 2004). The Michaelis–Menten constant $O_2^{(T)}$ has to be smaller than this parameter yet larger than the anoxia threshold $O_2^{(D)}$, about 0.7 mmHg (Brown & Wilson, 2004). We have chosen it to be 2.5 mmHg (Daçu et al., 2003). The oxygen diffusion coefficient $D_O$ is classically known to be around $10^{-5}$ cm$^2$/s (Daçu et al., 2003), while the cell diffusion coefficients are not so readily accessible in vivo.

The hypoxic diffusion coefficient has been considered to be around the mean data reported in Rockne et al. (2010) and ten times larger than the normoxic one. In addition, for high-grade glioma, the largest radial velocity $v (\approx 3$ cm/y) can be related to the hypoxic cell propagation, whereas the smallest radial velocity ($\approx 1$ cm/y) can be related to the normoxic one. For instance, considering the Fisher–Kolmogorov approximation to the radial cell propagation ($v \approx 2\sqrt{D/\tau}$), the normoxic and hypoxic
Table 1  Typical values of the biological parameters taken for our model equations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value and units</th>
<th>Meaning</th>
<th>Reference</th>
<th>Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C^{(M)}$</td>
<td>$10^6$ cell/cm²</td>
<td>Maximum tumour cell density</td>
<td>Rockne et al. (2010)</td>
<td>No</td>
</tr>
<tr>
<td>$O_2^{(S)}$</td>
<td>7 mmHg</td>
<td>Oxygen concentration level switch to hypoxia</td>
<td>Vaupel (2004)</td>
<td>No</td>
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<tr>
<td>$O_2^{(T)}$</td>
<td>2.5 mmHg</td>
<td>Michaelis–Menten threshold</td>
<td>Daçu et al. (2003)</td>
<td>No</td>
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<tr>
<td>$O_2^{(D)}$</td>
<td>0.7 mmHg</td>
<td>Critical anoxia level</td>
<td>Brown &amp; Wilson (2004)</td>
<td>No</td>
</tr>
<tr>
<td>$O_2$</td>
<td>80 mmHg</td>
<td>Typical oxygen pressure within vessels</td>
<td>Kimura et al. (1996)</td>
<td>No</td>
</tr>
<tr>
<td>$\alpha_n$</td>
<td>$7.5 \times 10^{-4}$ mmHg c/s</td>
<td>Typical normoxic cell oxygen consumption</td>
<td>Daçu et al. (2003)</td>
<td>No</td>
</tr>
<tr>
<td>$\alpha_h$</td>
<td>$\alpha_n/5$ mmHg c/s</td>
<td>Typical hypoxic cell oxygen consumption</td>
<td>Griguer et al. (2008)</td>
<td>No</td>
</tr>
<tr>
<td>$D_{O_2}$</td>
<td>$10^{-5}$ cm²/s</td>
<td>Oxygen diffusion coefficient</td>
<td>Daçu et al. (2003)</td>
<td>No</td>
</tr>
<tr>
<td>$\tau_{nh}$</td>
<td>0.15 h</td>
<td>Normoxic to hypoxic phenotype switch time</td>
<td>Jewel et al. (2001)</td>
<td>No</td>
</tr>
<tr>
<td>$\tau_{hd}$</td>
<td>7 days</td>
<td>Anoxic death time</td>
<td>Martínez-González et al. (2012)</td>
<td>No</td>
</tr>
<tr>
<td>$J_{O_2}$</td>
<td>$10^{-6}$ s⁻¹</td>
<td>Oxygen flow coefficient</td>
<td>Estimated</td>
<td>No</td>
</tr>
<tr>
<td>$\Delta C$</td>
<td>0.02 $C^{(M)}$ cells</td>
<td>Sensitivity vascular threshold</td>
<td>Estimated</td>
<td>No</td>
</tr>
<tr>
<td>$D_n$</td>
<td>$5 \times 10^{-10}$ cm²/s</td>
<td>Normoxic and hypoxic diffusion coefficients</td>
<td>Rockne et al. (2010)</td>
<td>10%</td>
</tr>
<tr>
<td>$D_h$</td>
<td>$5 \times 10^{-9}$ cm²/s</td>
<td>Normoxic and hypoxic doubling times</td>
<td>Estimated by</td>
<td>10%</td>
</tr>
<tr>
<td>$\tau_n$</td>
<td>14 days</td>
<td></td>
<td>Fisher–Kolmogorov approximation</td>
<td>10%</td>
</tr>
<tr>
<td>$\tau_h$</td>
<td>24 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\tau_{hn}$</td>
<td>96 h</td>
<td>Hypoxic to normoxic phenotype switch time</td>
<td>Semenza (2009), Hsieh (2010)</td>
<td>10%</td>
</tr>
<tr>
<td>$C^{(F)}$</td>
<td>0.5 $C^{(M)}$</td>
<td>Critical cell number for fluctuating hypoxia</td>
<td>Ruf et al. (2010)</td>
<td>10%</td>
</tr>
<tr>
<td>$C^{(C)}$</td>
<td>0.7 $C^{(M)}$</td>
<td>Critical cell number for chronic hypoxia</td>
<td>Ruf et al. (2010)</td>
<td>5%</td>
</tr>
<tr>
<td>$\tau_{hn}$</td>
<td>8–48 h</td>
<td>Hypoxic to normoxic phenotype switch time under AO therapy</td>
<td>Semenza (2009), Hsieh (2010)</td>
<td>5%</td>
</tr>
<tr>
<td>$C^{(F)}$</td>
<td>0.6–0.8 $C^{(M)}$</td>
<td>Cell number thresholds for fluctuating and chronic hypoxia under AT</td>
<td>Ruf et al. (2010)</td>
<td>5%</td>
</tr>
<tr>
<td>$C^{(C)}$</td>
<td>0.8–0.99 $C^{(M)}$</td>
<td></td>
<td>Ruf et al. (2010)</td>
<td>1%</td>
</tr>
<tr>
<td>$R_{Tox}$</td>
<td>0.8</td>
<td>RT surviving fractions in normoxia and hypoxia</td>
<td>Joiner &amp; Van der Kogel (2009)</td>
<td>5%</td>
</tr>
<tr>
<td>$R_{Thyp}$</td>
<td>0.95</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Doubling times $\tau$ (14 and 24 days, respectively) can be extrapolated, which are also similar to those used in Rockne et al. (2010). The normoxic oxygen uptake was obtained from a healthy tissue of $0.2C^{(M)}$ as the cell density with oxygen consumption of 15 mmHg/s (Daçu et al., 2003). Hypoxic oxygen uptake is several times smaller than the normoxic one as it was observed in U251 glioma cells (Griguer et al., 2008).

4.2 Computational details

Henceforth, we consider two possible scenarios: one corresponding to a biopsied but not totally/partially resected tumour, and a second one where the tumour was either partially or totally resected. In both scenarios we will assume that, prior to the application of RT, or AT and AO therapies, there is a mixture of normoxic cells plus smaller fractions of hypoxic cells and a necrotic core.

In the case of resected tumours initially there will be a small infiltrative tumour remnant located around the surgical border. In that case simulations start 2 weeks after surgery and all blood vessels are assumed to work correctly; thus $\chi^C = \chi^F = 1$. Since the initial cell density is low, there is a good oxygen supply at $t = 0$ and its redistribution is very fast, we can take $O_2(x, 0)$ to be roughly uniform in space.

Non-resected tumours will be considered of size $\approx 1.6$ cm of diameter at diagnosis, with a necrotic core at its centre occupying $\approx 0.8$ cm of diameter. Around the necrotic tissue a high tumour cell density of about 0.6 (in units of $C^{(M)}$) exists and is formed by normoxic and hypoxic cells. This hypercellular region is related to the high-contrast ring which is frequently observed in T1 MRIs (with Gadolinium) of GBMs. In our simulations, the width of this ring is about 4 mm. A very low density of hypoxic cells infiltrating into the healthy tissue (brain parenchyma) is also present. Only blood vessels located within the necrotic compartment are considered to be non-functional. We will also study smaller tumours for partially resected tumours, of diameter $\approx 1$ cm after surgery, where the necrotic core is in the centre occupying $\approx 0.2$ cm of diameter and radial tumour infiltration of size $\approx 0.4$ cm formed by normoxic and hypoxic cells. In that situation we will assume that there are no functional vessels in the centre of the tumour.

To solve Equations (2.1) and (2.3) numerically, we have used a standard finite difference method of second order in time and space with zero boundary conditions along the sides of the computational domain. We have employed large integration domains and cross-checked our results for different domain sizes to avoid spurious edge effects. All the results provided in the following sections are the outcome of sets of ten simulations. Each simulation allows a random static variability (in the range of 1–10%) in the parameters according to the last column in Table 1. All variations are constant over time except $z$ in Equation (2.5a), which is related to the stochastic temporal variability of oxygen perfusion from the vessels. The error bars shown in the figures correspond to the standard deviations of the results after the full set of simulations corresponding to small variations of the parameters is computed.

5. Results

5.1 Tumour evolution

We have performed extensive simulations of the model Equations (2.1–2.5) with different initial data and for broad parameter ranges of biological significance. A typical result of the tumour density evolution is shown in Fig. 3 for two times (Days 72 and 168). In spite of the extensive cytoreduction assumed for the surgery, tumour cells proliferate, giving rise to a relapse. The first stages (not shown in
Fig. 3. Typical evolution of the cell densities and oxygen on a tissue of size 2 cm. Intermediate distribution (Day 72) on the left column and final distribution (Day 168) on the right. (a) and (e) depict tissue oxygenation levels, (b) and (f) the normoxic cell densities, (c) and (g) the hypoxic cell densities and, finally, (d) and (h) show the areas of necrosis. Red vertical lines at (a) and (e) indicate the positions of blood vessels along the tissue.

As the tumour progresses, the area occupied by damaged vessels and necrosis increases with time, and oxygenation decreases due to the destruction of the functional vasculature (Fig. 3e). After 168
Fig. 4. Volumes of tumours treated with ATs or antioxidants related to non-treated tumours. (a) Relative tumour volumes for different AT regimens (T3: \( C_C = 0.8C_M \), \( C_F = 0.6C_M \), T2: \( C_C = 0.9C_M \), \( C_F = 0.7C_M \), T1: \( C_C = 0.99C_M \), \( C_F = 0.8C_M \)). (b) Relative tumour volumes for different AO regimens (O3: \( \tau_{hn} = 48 \text{ h} \) O2: \( \tau_{hn} = 24 \text{ h} \) O1: \( \tau_{hn} = 8 \text{ h} \)). Dotted lines in (a) and (b) are the respective relative volumes for non-treated tumours. Error bars display the standard deviation for the 10 simulations developed for each treatment, varying the parameters as indicated in Table 1.

days the necrotic core already has a size of 8 mm (Fig. 3h). Necrotic areas are typically surrounded by hypercellular regions generated by the migration of cells from low oxygen areas, resembling the pseudopalisades observed in high-grade gliomas (Brat et al., 2004; Rong et al., 2006).

5.2 Targeting vaso-occlusions leads to a small reduction in tumour volume

We have first studied the effect of AT therapy improving vessel functionality as described in Section 3.1. This therapy reduces invasion speed by keeping cells in their oxic and less motile phenotype, but the effect on tumour progression is small. An example is shown in Fig. 4a. Each point represents the average of 10 simulations with mean values and static random variations (Table 1). The error bars indicate the standard deviation of the results of those sets of simulations.

We have used different AT doses T1, T2 and T3 to delay vessel degradation by the tumour. We suppose that there is a dose-response effect. The threshold parameters for chronic and fluctuating hypoxia for T3 are \( C_C = 0.8C_M \) and \( C_F = 0.6C_M \); for T2: \( C_C = 0.9C_M \) and \( C_F = 0.7C_M \); and for T1: \( C_C = 0.99C_M \) and \( C_F = 0.8C_M \). It is clear from Fig. 4 that there are no significant differences with the non-treated tumour volumes until the sixth week; this is the time when the tumour density becomes large enough to impair the vessels. This therapy is able to delay
the damage of the vessels, but once the new (higher) thresholds are reached, no further gains are observed. We have verified this conclusion with a large range of parameters obtaining very similar results. Thus, only minor gains are to be expected from AT therapy unless it is combined with other agents.

5.3 **AO therapy has the potential to improve survival substantially**

The heterodimer HIF-1 has complex effects on tumour cell biology. On the one hand, the suppression of the HIF-1α subunit activity severely compromises the ability of tumour cells to undergo anaerobic glycolysis. This reduces the proliferation rate of hypoxic cells and promotes the apoptotic programme in cells that are deprived of both oxygen and glucose (Joiner & Van der Kogel, 2009). This is why many therapies have tried to target HIF-1α, although their efficacy in treating brain tumours has not been proved yet. In our case, we propose AO therapies as a way to normalize the cell response under oxic conditions.

We have used different antioxidant doses O3, O2 and O1, corresponding to a reduction in the normalization times to $\tau_{hn} = 48, 24$ and $8$ h, respectively. Our simulations encompass a time window of 6 months for the tumour volume evolution with and without AO therapies. The results are depicted in Fig. 4b. Since the AO therapy promotes the proliferative phenotype, vaso-occlusion takes place earlier than in the control case. This leads to the formation of cells actively migrating away from collapsed vessels, and the AO therapy does not decrease the tumour volume during the first 4 weeks. However, maintenance of the therapy for longer times produces a significant benefit, coming from the essential lessening in the invasion speed. Even assuming a minor effect of the AO therapy (as would be the case with O3), there is a significant tumour volume reduction (12%) after 6 months. Higher doses/effects of antioxidants (O1 or O2), associated to smaller recovery times, result in substantial tumour volume reductions (up to 40%; Fig. 4b). We have used a large range of parameters, obtaining similar results that would suggest that a prolonged AO therapy may be beneficial for a large number of patients.

5.4 **ATs and antioxidants may have a synergistic effect leading to a substantial tumour volume reduction**

Having AT and AO together in our mathematical model has the biological meaning that we are simultaneously targeting the coagulation cascade to delay the invasiveness process and the high levels of free radicals that also have an effect on invasiveness.

Figure 5 shows the typical outcome of our simulations. We have combined the therapies T1, T2, T3, O1, O2 and O3, as described in Sections 5.2 and 5.3, to get *in silico* several treatment modalities: $T_3 + O_3$, $T_2 + O_2$ and $T_1 + O_1$, besides the control C, which is the non-treated tumour group. The error bars in Fig. 5 display the standard deviation for ten simulations, varying the parameters in Table 1, as described previously.

First, Fig. 5a exhibits the tumour volume evolution related to the control for the combined treatments. All treatments show a visible volume reduction from the sixth week. After 24 weeks, the relative tumour volume reduction, when compared with the control, is 0.83, 0.56 and 0.45 for $T_3 + O_3$, $T_2 + O_2$ and $T_1 + O_1$, respectively.

Secondly, Fig. 5(b–d) shows the tumour volumes after 6 months for each different therapeutic modality. All of them result in a reduction in the final tumour volume after 6 months of treatment, but in all cases the combination of AT and AO gives the best outcome. In some instances, such as with
**Fig. 5.** Evolution in time of the relative tumour volume under combination therapies with ATs and antioxidants. (a) Tumour volume as a function of time for the three combined treatments relative to the control. The dotted constant line represents the volumes for non-treated tumours. AT treatments are T3: $C_{C} = 0.8 C_{M}$, $C_{F} = 0.6 C_{M}$; T2: $C_{C} = 0.9 C_{M}$, $C_{F} = 0.7 C_{M}$; and T1: $C_{C} = 0.99 C_{M}$, $C_{F} = 0.8 C_{M}$). AO treatments are O3: $\tau_{thn} = 48$ h, O2: $\tau_{thn} = 24$ h and O1: $\tau_{thn} = 8$ h. (b–d) Final relative tumour volumes for the different therapeutic modalities considering C as the control (non-treated tumour group). Error bars display the standard deviation for the 10 simulations developed for each treatment, varying the parameters as indicated in Table 1.

the most effective therapies T1 + O1, a synergistic effect is observed when combining both therapies (see Fig. 5d).

### 5.5 ATs and antioxidants sensitize the tumour for cytotoxic therapies

An interesting result of our simulations is that the combination of ATs and antioxidants results in a reduction of hypoxic areas and the normalization of cell phenotypes that results in a higher sensitivity to cytotoxic therapies. In what follows, we will discuss the action of RT together with AT and AO therapies. As discussed in Section 3.3, well-oxygenated cells suffer more damage when exposed to radiation. In addition, more aggressive phenotypes, such as those induced by hypoxia, have higher levels of repair enzymes that makes them less sensitive to cytotoxic therapies.

We have run a set of simulations combining RT with AT and AO for tumours of different initial sizes at diagnosis. We display examples of their evolution, comparing different combination therapies and initial conditions: totally resected tumours after surgery (Fig. 6), biopsied tumours without an extensive tumour volume reduction (Fig. 7) and partially resected tumours (Fig. 8).
Fig. 6. Tumour volume and tumour radial velocity for different therapeutic modalities. (a and b) The tumour volume (cm$^3$) and the tumour radial velocity (cm/y) during 58 weeks, respectively. Each line provides the results for a therapeutic modality: C is the control (non-treated), T1 (antithrombotics): $C^{(C)} = 0.99C^{(M)}$, $C^{(F)} = 0.8C^{(M)}$, O1 (antioxidants): $\tau_{\text{adm}} = 8\text{h}$, T1 + O1 (antithrombotics + antioxidants), R (standard radiotherapy with 30 sessions, 2 Gy/session, Monday–Friday), R + T1 (radiotherapy + antithrombotics), R + O1 (radiotherapy + antioxidants), R + T1 + O1 (radiotherapy + antithrombotics + antioxidants). Red curves correspond to non-radiated tumours and blue curves to radiated ones. (c) The relative final volume for all treatments after 58 weeks. Error bars display the standard deviation for the three simulations developed for each treatment, varying the parameters as indicated in Table 1.

A first example of our results is plotted in Fig. 6 where we show the in silico predictions for the tumour volume evolution and radial tumour velocity (Fig. 6b), for different therapeutic modalities during a period of 58 weeks, together with the final volumes comparing all therapies (Fig. 6c). Following the standard practice for GBM, we will assume that RT starts 2 weeks after surgery and runs for 6
Fig. 7. Tumour volume evolution for different therapeutic modalities without prior surgery. (a) The initial cell density distribution at diagnosis for hypoxic, normoxic and necrotic cells. Initial tumour diameter $\approx 1.6$ cm, necrotic core $\approx 0.8$ cm and radial tumour infiltration $\approx 0.5$ cm. (b) The tumour volume evolution (cm$^3$) during 24 weeks. Each line provides the results for a therapeutic modality: C is the control (non-treated), T1 (antithrombotics): $C_C = 0.99 C_M$, $C_T = 0.8 C_M$, O1 (antioxidants): $\tau_{th} = 8$ h, T1 + O1 (antithrombotics + antioxidants), R (standard radiotherapy with 30 sessions, 2 Gy/session, Monday–Friday), R + T1 (radiotherapy + antithrombotics), R + O1 (radiotherapy + antioxidants), R + T1 + O1 (radiotherapy + antithrombotics + antioxidants). Red curves correspond to non-radiated tumours and blue curves to radiated ones. (c) The relative final volume for all treatments after 24 weeks. Error bars display the standard deviation for the three simulations developed for each treatment, varying the parameters as indicated in Table 1.

weeks from Monday to Friday in fractions of 2 Gy. It is clear from Fig. 6a and b that there are no significant volume or growth speed differences between the irradiated tumours until Week 12. The effect of RT results in a substantial reduction in tumour volume and tumour growth speed that is still apparent several months after the therapy has finished. It is also obvious that combination therapies of RT with the different agents are always more effective than RT alone. It is remarkable that the effect of AT + AO is equivalent, after 1 year, to the effect of RT, though with a much lower toxicity (and long-term effects). At the end of the studied period of 58 weeks, there is a 12% reduction for AT alone and a remarkable 42% decrease with AO alone. The mean final tumour volume for the combination of T1 + O1 is similar to the one obtained for tumours receiving only RT; both displaying a reduction of more than 50% when compared with the control group. RT + AT gives rise to about 55% reduction, RT + AO to around 65%
and the combination RT + AT + AO produces a reduction of tumour volume of over 70% at the 58th week compared with the non-treated tumours.

Our results suggest that a substantial increase of several months in the median overall survival for GBM patients may be obtained by combining RT + AT + AO.

5.6 ATs and antioxidants might be more effective for patients having only a biopsy or subtotal resections

Figure 7 displays the tumour volume evolution for the different therapeutic modalities during 24 weeks after diagnosis. In this case, the tumour was not resected and its diameter at diagnosis was higher than 1.6 cm. Only vessels located between 20 and 30 mm were considered non-functional since almost this space was occupied by dead cells as illustrated in the initial cell density subplot Fig. 7a. Tumour volume evolution is shown in Fig. 7b, which gives evidence regarding how all therapies delay tumour progression almost from the first day, a fact that did not occur for totally resected tumours.

At the end of the 24 weeks, we find a benefit in the reduction of tumour volume similar to the one obtained from totally resected tumours after 58 weeks of treatment modalities (Fig. 7c). Thus, the group of patients with biopsy or subtotal resection might benefit even more from a combination of AT and AO therapies. In addition, when both AT and AO are used together with RT, a significant advantage is obtained when compared with RT alone. This gain is more evident for patients having only a biopsy or subtotal resections, than in tumours with small remnants that are expected to be initially well oxygenated.

5.7 Combination of ATs and antioxidants might be more effective than RT alone

The last example shown in Fig. 8 displays the tumour volume evolution for the different therapeutic modalities during the first 48 weeks after subtotal resection. In this case, the tumour diameter following surgery was around 1 cm. Initially, the vessels in the centre of the tumour were taken to be non-functional leading to the formation of a small necrotic core. Hypoxic and normoxic cells, surrounding the necrotic areas, infiltrate into healthy tissue (Fig. 8a). The initial distribution of the partial pressure of oxygen is displayed in Fig. 8b.

After 48 weeks all treated tumours significantly reduced their volume compared to the non-treated ones. It is remarkable that RT implies a 35% reduction versus the 55% reduction observed in Fig. 6c for totally resected tumours. The cause of this fact could be the better initial oxygenation and the absence of necrosis and hypoxic cells in the second case. However, antioxidants and ATs are still as effective as in Fig. 6. In addition, after 48 weeks, the benefit obtained from the combination of AT and AO with RT is more apparent for these cases (see Fig. 8c).

Although the best result is obtained with a combination of the three proposed therapies, it is important to emphasize the fact that AO alone or AO with AT (without radiation) produced a reduction in tumour volume larger than the one observed under RT alone (see Fig. 8d).

6. Discussion and conclusions

As a consequence of cyclic hypoxia during GBM progression, free radicals are mainly originated from the mitochondrial respiratory chain which regulates the transcription hypoxia-inducible factor HIF-1α expression. Indeed, hypoxia (particularly via HIF-1α) is responsible for metabolic and phenotypic
COMBINED THERAPIES DELAY BRAIN TUMOUR PROGRESSION

Fig. 8. Tumour volume evolution for different therapeutic modalities after subtotal resection. (a) The initial cell density distribution for hypoxic, normoxic and necrotic cells. Initial tumour diameter \( \approx 1 \) cm, necrotic core \( \approx 0.2 \) cm and radial tumour infiltration \( \approx 0.4 \) cm. (b) The initial distribution of the partial pressure of oxygen. Vertical red lines represent the functional vessels and blue lines the oxygenation along the space. There are no functional vessels at the centre of the tumour. (c) The relative final volume for all treatments after 48 weeks. (d) The tumour volume evolution (cm\(^3\)) during 48 weeks. Each line provides the results for a therapeutic modality: C is the control (non-treated), T1 (antithrombotics): \( C^{(C)} = 0.99C^{(M)} \), \( C^{(F)} = 0.8C^{(M)} \), O1 (antioxidants): \( \tau_{in} = 8 \) h, T1 + O1 (antithrombotics + antioxidants), R (standard radiotherapy with 30 sessions, 2 Gy/session, Monday–Friday), R + T1 (radiotherapy + antithrombotics), R + O1 (radiotherapy + antioxidants), R + T1 + O1 (radiotherapy + antithrombotics + antioxidants). Red curves correspond to non-radiated tumours and blue curves to radiated ones. Error bars display the standard deviation for the three simulations computed for each treatment, varying the parameters as indicated in Table 1.

changes which induce the invasiveness of GBM cells and initiate coagulation mechanisms that promote tumour growth acceleration.

The AO treatment may result in the inhibition of HIF-1\( \alpha \), leading to a reduction of tumour invasion, delaying progression of GBM in humans. In addition, the increase of free radicals could be stabilized by means of an AO therapy, controlling or delaying the vascular degeneration due to the malformations of endothelial cells induced by HIF-1\( \alpha \) pathway activation. In this way, targeting HIF-1\( \alpha \) can be seen as an attractive approach to complement RT and chemotherapy, which kill well-oxygenated cells (Joiner & Van der Kogel, 2009; Chen et al., 2011), and also to inactivate extrinsic HIF-1\( \alpha \) pathways, forcing the tumour to display a lower-grade behaviour by reducing invasion and angiogenesis.
Other potential interesting agents for GBM treatment are ATs, such as LMWH, a low molecular weight heparin that promotes the release of TF pathway inhibitors for preventing VTE and with very low toxicities (Planès, 2003). Thus LMWH might be incorporated as a part of GBM treatment to avoid vaso-occlusion phenomena and to delay the neovascularization process associated with this pathology. The results from our model seem to imply that an additional indirect antitumoural effect from thromboprophylaxis might be expected related to the delay of tumour invasion, different from the direct antitumoural effect or the increase in survival and quality of life to be expected from the prevention of the formation of big coagulates. Furthermore, from our simulations shown in Fig. 4a, it follows that an optimum AT treatment regimen exists (in our case, T2: \( C^C(0.9) C^M(0.7) \) in comparison with T3 or T1) as evidenced by a U-effect on the relative tumour volume.

In addition, AT therapy could prevent early tumour-induced vaso-occlusions delaying the so-called malignant transformation of low-grade gliomas, were they to transition to a high-grade glioma. Consequently, AT therapy administered to WHO grade II astrocytic and oligodendroglial patients may help to preclude the malignant transformation into secondary GBM associated with the onset of hypoxia once local vascular damage is produced.

Finally, Targeting HIF-1\( \alpha \) and vasculature normalization simultaneously in GBM may have a synergistic effect, decreasing tumour invasion and increasing patient overall survival time by changing the tumour microenvironmental behaviour from a high-grade to low-grade glioma. In addition, tumour cells have high levels of AO enzymes which contribute to eliminate more efficiently the free radicals produced by alkylating agents such as carmustine (BCNU) or by radiation. Besides the RT sensitization obtained with better tumour oxygenation, the treatment would also reduce the need to activate AO enzymes, rendering the tumour more susceptible to the effect of these agents. Figure 1 summarizes the possible synergistic effect predicted by our \textit{in silico} model combining antioxidants, ATs and RT in the GBM treatment.

It is worth mentioning the use of antiangiogenics in the context of the go or grow hypothesis. On the one hand, anti-angiogenic treatments impair tissue oxygenation and promote motile phenotypes to dominate the tumour population and invade faster the adjacent tissue. On the other hand, a pro-vascular therapy may oxygenate the tumour with the risk of producing leaky vessels through which tumour cells can intravasate and form distant foci in the brain parenchyma. This has led to a long discussion on the potential existence of a window where antiangiogenic therapies may be useful mainly as concomitant ones. It would be very interesting to develop mathematical models of these phenomena in order to guide the use of these therapies, especially after the recent results of clinical trials investigating the combination of antiangiogenic therapies with the standard of care that have shown no impact on overall survival (Herriksson et al., 2013; Wick et al., 2013), though the progression free survival seems to benefit from the use of these drugs.

It is important to remark that stochastic fluctuations in the therapy might affect the tumour dynamics. These changes can be due to stochastic variations of the drug pharmacokinetics as shown by d’Onofrio & Gandolfi (2010). However, in this paper we have assumed that drug administration is such that the drugs achieve their therapeutical regimes that is preserved during treatment and constant in each simulation.

Other results from our simulations are that the different treatment modalities proposed suggest a more substantial effect on larger initial tumours that on smaller ones. Thus, the combination of ATs and antioxidants might be more effective for patients having only a biopsy or subtotal resections than for those in which the tumour could be macroscopically resected. In fact, for non-resected tumours, a combination of ATs and antioxidants could be more effective than RT alone, probably due to the poor oxygenation and the presence of necrotic and hypoxic areas within the tumour. In addition,
this combined therapy could even be more favourable for those patients with a high risk of vessel coagulation.

In conclusion, we have developed a biocomputational model of GBM that incorporates the spatio-temporal interplay among two glioma cell phenotypes corresponding to oxygenated and hypoxic cells, a necrotic core and the local vasculature whose response evolves with the tumour progression. Our *in silico* approach reveals that the different therapeutic modalities which combine ATs and antioxidants would improve vessel functionality, avoiding vaso-occlusions and reducing oxidative stress and the subsequent hypoxic response. This combined treatment would reduce glioma cell invasion and sensitize glioblastoma to conventional RT, hopefully increasing patients’ survival.

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