

Glioma Virotherapy: The Effects of Innate Immune
Suppression and Increased Viral Replication
Capacity

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Abstract

Oncolytic viruses are genetically altered replication-competent viruses
which infect, and reproduce in, cancer cells but do not harm normal

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cells. Upon lysis of the infected cells, the newly formed viruses burst out and infect other tumor cells. Experiments with injecting mutant Herpes Simplex Virus 1 (hrR3) into glioma implanted in brains of rats show lack of efficacy in eradicating the cancer. This failure is attributed to interference by the immune system. Initial pretreatment with immunosuppressive agent Cyclophosphamide (CPA) brings only marginal improvement. We introduce a mathematical model and use it to determine how different protocols of CPA treatment and how increased burst size of the mutated virus will affect the growth of the cancer. One of our conclusions is that the diameter of the cancer will decrease from 4 mm to eventually 1 mm if the burst size of the virus is triple that which is currently available, even without CPA. The effect of repeated CPA treatment is to maintain a low density of uninfected cells in the tumor, thus reducing the probability of migration of tumor cells to other locations in the brain.

1 Introduction

Oncolytic viruses (OV) are genetically altered replication-competent viruses which can infect, and reproduce in, cancer cells but leave healthy normal cells unharmed. Upon lysis of an infected cell, a swarm of new viruses burst out of the dead cell and infect neighboring tumor cells. Tumor therapy by OV has been and continues to be actively tested in clinical trials for variety of malignancies ([1],[2],[3],[4],[5],[6],[7],[8]). Agents includes ONYX-015 ([9],[10]), the herpes virus G207 [11], the prostate-specific adenovirus CN706 and CN708 [12], and the reovirus that attacks tumors with activated Ras pathways [13]. Additional OV species are in development.

At present, despite producing many thousands of infectious virions per infected tumor cell, most virus species are unable to eradicate the majority

of tumor modules. There is increasing evidence that the host response to an active viral infection plays a critical role in determining the overall efficacy of viral therapy. Indeed, it has been manifested that the innate immune system destroys infected cells as well as free virus particles, thus enabling the tumor to grow [14]. Until very recently the course of the immune response has been defined in the wild-type viral infection of normal organs in humans and animals, but not in recombinant, oncolytic viral infections of tumors [15].

A recent experimental paper by Fulci et al [16] reports for the first time that CD163+ macrophage population rapidly infiltrate experimental gliomas in rats and inhibits OV therapy. This is likely done by phagocytosis of infected tumor cells through generation of antiviral molecules such as iNOS, and by setting up an innate inflammatory reaction coordinated by initial expression of $\text{IFN}\gamma$, iNOS and Zn-APN (CD13). A drug that appears effective in suppressing the innate immune response and thereby enhancing oncolysis is cyclophosphamide (CPA) [17] and [18]. Fulci et al [16] experimented with CPA and found that inhibition of the CD163+ macrophages response by CPA, in glioma, leads to enhanced virotherapy. This then suggests a clinical application to improve efficacy of OV trials.

In this paper we formulate and analyze a mathematical model of spherical glioma that has been injected at its center with oncolytic virus hrR3, which is a mutant of Herpes Simplex Virus 1 (HSV). The model includes uninfected and infected tumor cells, necrotic cells, free virus particles, innate immune cells and CPA. Our model is similar to a model introduced by Wu et al [19], [20]; however there are several important differences. First, in our model we have included the effects of immunosuppression drug CPA. Indeed one of the main aims of our paper is to determine, by analysis of

the mathematical model, the effect of administering CPA under different protocols, on the progress of glioma.

A second difference between our model and the model of [19] is that we include the presence of innate immune cells in the tumor tissue, whereas [19] includes only the immune response (tumor necrotic factor TNF) which consists of molecules with negligible volume. This difference is important, since the immune cells make up to 50% of the total number of cells at some stages of the tumor progression [16].

Since the free virus particles are very small, they disperse in the fluid tissue like Brownian particles. We have therefore incorporated into our model a diffusion term for the free viruses. We have also added a term which accounts for the destruction of virus particles by the immune cells [21]. Finally, in the mathematical model of [19], the parameters are estimated by using data from head and neck cancer [9], [22] and [23]. In our model, the parameters are estimated so as to conform with experimental results for glioma [16]. There is a substantial difference in the values of some of the parameters due to the fact that glioma is much more aggressive cancer. In fact, the course of OV therapy in neck and head cancer is considered over a period of weeks or months, while in gliomas it is determined in a matter of days.

2 materials and methods

The mathematical model describes a spherical tumor undergoing OV therapy. The model parameters are based on experimental results of [16]. In these experiments D74/HveC Rat Glioma cell lines were implanted into the

brain of rats. After 7 days, the tumor reached the size of 4mm in diameter, and then the oncolytic virus hrR3 (which is a mutant of Herpes Simplex virus 1 (HSV)) was injected into the center of the tumor. This mutant attacks tumor cells, but does not attack healthy normal cells [6]. Six hours after injection, some rats were sacrificed, the tumor was stained and the JPEG pictures were taken, and then the infected area of the tumor was measured. This procedure was repeated after 72-76 hours from the time of virus injection, and then once more, one week after virus injection. Five different stains were used: one for each of four innate immune cells, and one for the infected cells. The immune cells are CD11b (dendritic cells), CD68+ (main monocytes, also called ED1 in rats), CD163+ (also called ED2 in rats) and CD169 (also called ED3 in rats). In order to study the effects of the immunosuppression drug cyclophosphamide (CPA), some rats were pre-administered by CPA on the fifth day after tumor cell implantation, that is, 2 days before virus injection.

The mathematical model includes infected tumor cells (y), uninfected tumor cells (x), necrotic cells (n), immune cells (z) and free virus particles (v). The quantity y represents the number density of infected cells, that is, the number of infected cells in mm^3 ; the same meaning is associated with the quantities x , n , z and v . The experiments in [16] revealed that OV therapy induced a rapid up-regulation of CD163+ and CD68+ markers in the brain tumor, while CPA pretreatment inhibited this up-regulation. Although there was also increase in other populations of immune cells within the OV-treated brain tumors, the CPA treatment did not appear to inhibit this increase significantly. In our model we shall take only the average response of all the immune cells, rather than the response of each population of immune cells.

The partial differential equations of our model are listed in the Appendix. The parameters are derived as follows.

The uninfected cells proliferate at a rate λ , where λ is computed from the experimental results of [16] for untreated gliomas. The infection rate of tumor cells by virus, β , is based on the observation [16] that approximately 70% of the virus particles successfully invade uninfected cells. According to [16] the mean life time of infected tumor cells is about 18 hours. Hence the infected tumor cell lysis rate, δ , is $\frac{1}{18}h^{-1}$. Necrotic cells are removed at the average rate of 2-3 days [24]; we take the removal rate μ to be $\frac{1}{48}h^{-1}$. With regard to the diffusion coefficient of virus particles, D , according to Olmsted [25] for HSV particles, $\frac{D}{D_{pbs}} = 0.0089 \pm 0.0052$, and from [26] $D_{pbs} = 1.1 \times 10^{-5} cm^2/sec$. We take $D = 3.6 \times 10^{-2} mm^2/h$. This is nearly twice the diffusion coefficient in Chaplain [27] as computed by the Einstein formula $D = \frac{kT}{6\pi R\eta}$ where η is the viscosity. But, this difference is to be expected since virus particles are not spherical and thus their "effective" radius is smaller than the radius R used in Einstein's formula.

The burst size, b , of new viruses per one infected cell, upon its lysis, is an important parameter. In wild type of Herpes Simplex Virus it is in the thousands. However for the oncolytic virus hrR3 it is much smaller, ranging from 10 to 100. Our default number is taken to be 50, but we shall also try to see how OV therapy is effected if this number can be made larger.

The way immune cells are cleared is typically by membrane protein Fas and FasL which, when combined on the cell surface, signal to caspase protein to execute the cell. The brain is an immune privilege organ, so that the percentage of immune cells is small, typically 1-2%. When stimulated by infected cells in glioma, this percentage arises sharply. As the number of infected cells drops, the need for immune cells diminishes, so they undergo

apoptosis, either by killing themselves (using their own Fas and FasL to activate caspase) or by killing each other (Fas from one cell ligands to FasL from another cell)[21] [28]. The first process occurs when z is small, and yields a linear clearance; the second process occurs when z is large, and it yields a quadratic clearance. Hence,

$$c(z)z = \begin{cases} cz & \text{if } z < z_0, \\ \frac{c}{z_0}z^2 & \text{if } z \geq z_0. \end{cases}$$

From [28] one can infer the value of z_0 , and combining this with the linear clearance rate in [27] and [29], we arrive at the number $\omega = \frac{c}{z_0} \approx 20 \times 10^{-8} \text{mm}^3/\text{hour} \cdot \text{cell}$. For simplicity we shall assume only quadratic clearance with a rate ω as above.

There remain four more parameters to be determined: a first order clearance rate of virus, γ , the killing rate of infected cells by the innate immune system, k , the immune take-up rate of virus, k_0 , and the stimulation rate of innate immune cells by infected tumor cells, s . The experimental results of [16] with and without CPA treatments suggest that $k = 2 \times 10^{-8} \text{mm}^3/\text{hour} \cdot (\text{immune cell})$. We assume that the immune cells take notice of, and are attracted to, the infected cells more than they are to the free virus. We therefore take k_0 , to be smaller than k : $k_0 = 10^{-8} \text{mm}^3/\text{hour} \cdot (\text{immune cell})$.

Infected cells stimulate the innate immune system and cause a second order increase $s \cdot y \cdot z$ of the number density of the immune cells. It is difficult to measure the stimulation rate s , but here again we derive an estimate from the experimental results of [16] which show that the percentage of immune cells increased from 1% before OV injection to 30-50% after 6 hours.

Accordingly, we take $s = 5.6 \times 10^{-7} \text{mm}^3/\text{hour} \cdot (\text{immune cell})$. Finally, we take the first order clearance rate of virus to be $\gamma = 2.5 \times 10^{-2}/\text{hour}$. Table 1 summarizes the model parameters and their numerical values

Table.1 **Parameters**

Parameters	Description	Numerical values	dimensions
λ	Proliferation rate of tumor cells	2×10^{-2}	$1/h$
δ	Infected cell lysis rate	$\frac{1}{18}$	$1/h$
μ	Removal rate of necrotic cells	$\frac{1}{48}$	$1/h$
b	Burst size of infected cells	50	$\text{viruses}/\text{cell}$
θ	Density of tumor cells	10^6	cells/mm^3
D	Diffusion coefficient of viruses	3.6×10^{-2}	mm^2/h
β	Infection rate	$\frac{7}{10} \times 10^{-9}$	$\text{mm}^3/h \cdot \text{virus}$
k	Immune killing rate	2×10^{-8}	$\text{mm}^3/h \cdot \text{imcell}$
k_0	Take-up rate of viruses	10^{-8}	$\text{mm}^3/h \cdot \text{imcell}$
s	Stimulation rate by infected cells	5.6×10^{-7}	$\text{mm}^3/h \cdot \text{incell}$
ω	Clearance rate of immune cells	20×10^{-8}	$\text{mm}^3/h \cdot \text{cell}$
γ	Clearance rate of viruses	2.5×10^{-2}	$1/h$

The parameters k , k_0 , s have been estimated rather crudely from the experiments of [16], and γ was determined by trying to fit the percentages of infected and immune cells derived by the mathematical model to the experimental percentages reported in [16] 6 hours and 72 hours after injection of OV.

3 Results

3.1 Fitting of data

In order to compare our numerical simulation with the experimental results of [16] we take, as in [16], the initial radius of the tumor to be 2 mm, and the number of particle forming units (pfu) of virus injected at the center to be 10^8 - 10^9 . The initial time is the seventh day after tumor implantation in the rat's brain.

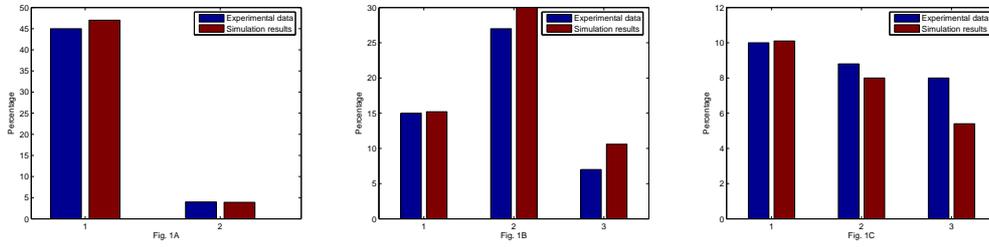


Figure 1: Experimental data vs simulation results. Fig.1A is for infected tumor cells without CPA; Fig.1B is for immune cells without CPA; Fig.1C is for innate immune cells with CPA. In each picture, data group 1 is after 6 hours, data group 2 is after 72 hours and data group 3 is before the rat dies.

Figure 1A compares the experimental measurements with our numerical simulation of the percentage of infected tumor cells (relative to the total number of all cells) without pretreatment of CPA. Figure 1B compares the experimental measurement data with the numerical simulation of the percentage of the innate immune cells without pretreatment of CPA.

After CPA is administered to the rats, the level of CPA arises and reaches a plateau after 2 days. This level is maintained for approximately 3 days

and then begins to drop off to zero in the next two days. We simulate the CPA level in the tumor by a piecewise linear function

$$P(t) = \begin{cases} 8.5 \times 10^{-2} & \text{if } 0 \leq t \leq 72, \\ \frac{8.5 \times 10^{-2}}{48}(120 - t) & \text{if } 72 \leq t \leq 120, \\ 0 & \text{if } t \geq 120, \end{cases} \quad (1)$$

where the unit of time is hour; the unit of $P(t)$ is 1/hour.

Figure 1C compares the experimental measurements of immune cells, when CPA was administered, with our numerical simulation. The discrepancy between measurements and simulation develops only after a relatively long time both in Figures 1B and 1C. This discrepancy may be caused by the fact that in our model we have lumped together all types of the innate immune cells, whereas in [16] measurements were done only for the innate immune cells CD68+ and CD163+.

Figure 1 shows that our model fits quite well with experiments. We next proceed to compare the cancer progression with and without CPA.

3.2 comparison results

Figure 2A shows the profiles of the averages over space, of immune cell densities with and without CPA pre-treatments. Without CPA, the immune cells reach the maximum 52% at 26th hour after virus injection; with CPA, the immune cells reach the maximum 34% at 24th hour after virus injection. Thus, CPA suppresses the maximum level of innate immune cells and shortens the time that the immune system reaches its peak. Since the effect of CPA disappears after 120 hours, the percentage of the immune cells climbs up after 120 hours, thus forming a bimodal profile.

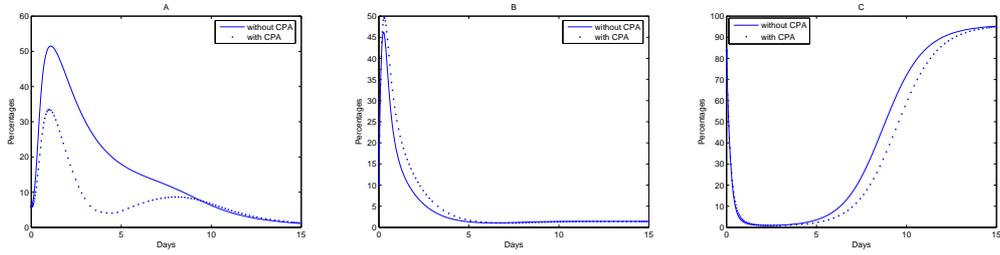


Figure 2: Profiles of the percentages of cell populations within the tumor with and without CPA. Figure A is for innate immune cells, B is for infected tumor cells, and C is for uninfected tumor cells.

The Figure 2B shows the profiles of the percentage of infected tumor cell with and without CPA. Clearly with CPA we expect to have more infected cells. As the simulation shows, without CPA, the infected cells reach the maximum 46% at 5th hour; with CPA, the infected cells reach the maximum 50% at 7th hour approximately.

The Figure 2C shows the profiles of the percentage of uninfected tumor cells with and without CPA. The first thing to notice is that there is a time delay in the effect of CPA; the immune suppression does not begin right away; in fact, there is a 3 days delay. The effect of CPA becomes negligible after approximately 17 days. However, during the intermediate period, it is significant. For example, in day 10, the percentages of uninfected cells with and without CPA is, respectively, 42% and 62%.

4 Discussion

We will use our model to make certain predictions on oncolytic therapy. We first examine what will happen within the glioma if we change the burst size without administering CPA, and then examine the effect of administering

CPA using different protocols.

4.1 Burst sizes

Suppose we inject into the tumor OV which replicates at a faster rate than hrR3. A large burst size b of virus will increase the stimulation of the immune system, which will then attack the infected cells and the free viruses. As a result, the population of viruses will decrease and this will be followed by a decrease in the population of immune cells. the population of virus and infected cells will then be able to increase, and it will follow by a new phase of stimulation of the immune system, etc. Thus, we may expect a "feedback mechanism" which will cause an oscillatory behavior of the percentages of infected cells, immune cells, and uninfected cells, with slight time-shift of the corresponding maxima. This is indeed demonstrated in Figures 3, for burst size $b = 400$ and 1000 . For smaller values of b such as $b = 200$, oscillations occur only in the first 20-30 days. For $b = 50$ (not shown here), we do not see any oscillation.

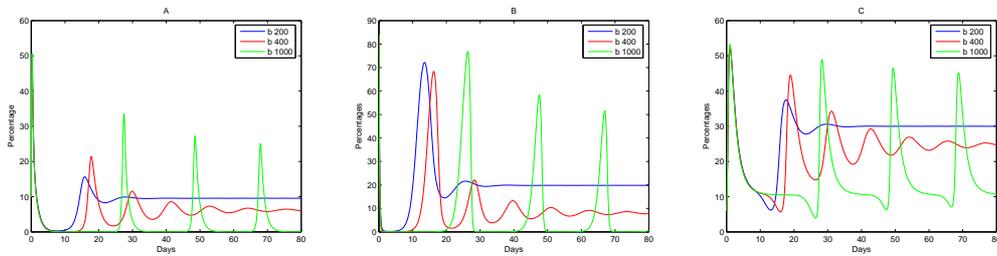


Figure 3: Profiles of cell populations within the tumor with different burst sizes. Figure A is for infected tumor cells, B is for uninfected tumor cells, and C is for innate immune cells.

4.2 CPA treatments

We shall compare two different protocols for administering CPA. The first protocol is to administer a "normal" amount of CPA weekly (as in (1)), and the second protocol is to administer twice the normal amount every two weeks. The simulation results of the percentage of uninfected tumor cells for burst sizes $b = 100, 200$ and 400 is shown in Figure 4.

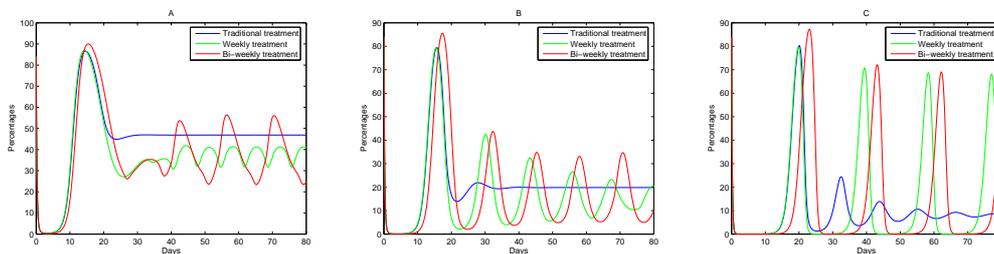


Figure 4: Profiles of uninfected tumor cell populations with different treatments and different burst sizes. Figure A is for burst size 100, B is for burst size 200, and C is for burst size 400.

These figures show that there is little difference between the weekly and bi-weekly treatments. If we take weekly averages of the uninfected cells we find a slight advantage to the weekly treatment if $b = 100, b = 200$, and a slight advantage to bi-weekly treatment if $b = 400$.

It is instructive to compare these treatments with the "traditional" treatment, where we pre-administer normal amount CPA just once. The weekly or bi-weekly treatment reduce the average (over time) of the uninfected cells, but due to oscillations (as was already noted in section 4.1) there are periods of time of when this traditional treatment yields a small percentage of uninfected cells.

4.3 the tumor radius

All the preceding numerical results are based on solving the partial differential equations (PDEs) of the model given in the Appendix and then taking averages over the tumor region. We have obtained approximately the same numerical results using the much simpler ordinary differential equations (ODEs) which one can obtain from the PDEs by neglecting spatial variations.

There is however one important quantity that we have not yet taken into account, namely, the radius of the tumor, and in order to compute it we need to work directly with the PDE system.

As the simulations presented above show, uninfected tumor cells will persist even with large burst sizes and with repeated CPA treatments. However, if the radius of the tumor can be kept small enough then long term survival of the animals can be assured, unless cell invasion and metastasis will occur as a result of cell shedding and migration.

Figure 5(a) simulates the growth of the tumor radius for $b = 50$ without CPA and with one pre-treatment of CPA.

According to Fulci et al [16], without CPA the rats die after 8-10 days after the injection of viruses, and with one CPA pretreatment the rats die after 11-13 days after the injection of viruses; at death, the radius of the tumor is approximately 6 mm. These experimental results roughly fit with the simulations in Figure 5(a).

Figure 5(b) simulates the growth of the tumor's radius without CPA and with weekly CPA treatment for different burst sizes.

We see that with weekly CPA treatment, the radius of the tumor will decrease as long as the burst size is bigger than 100; without any CPA

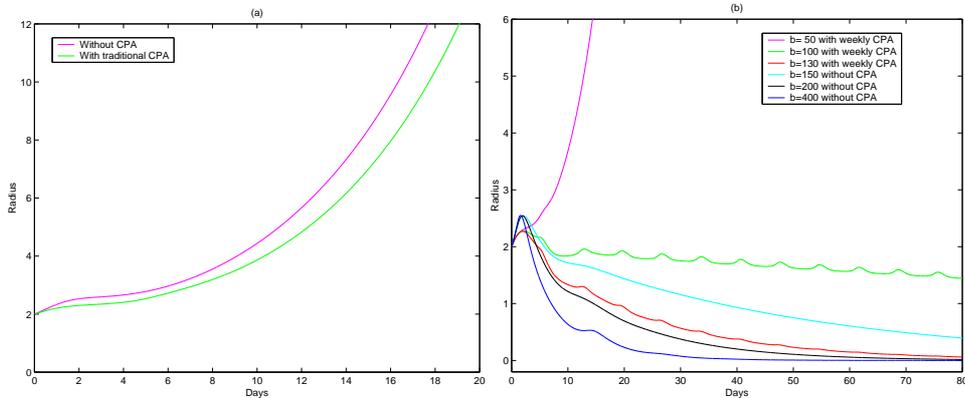


Figure 5: Tumor radius with different burst sizes and CPA treatments.

treatment and with burst size b already as high as 150, the radius of the tumor will decrease to 1 mm. If the burst size could be increased to 400, the tumor will shrink to an extremely small size. Finally, the weekly treatment by CPA helps in decreasing the radius of the tumor, as can be seen by comparing the profiles in Figure 5(b) for burst size $b = 130$ and $b = 150$: For $b = 130$ with CPA we achieve a smaller radius than for $b = 150$ without CPA.

4.4 Summary

We have shown that with the current burst size of oncolytic virus hrR3 the tumor cannot be eradicated with any reasonable CPA treatment; in fact, its radius will grow, and the rats will die within several weeks. If, however, the virus can be further altered to yield a burst size $b \geq 150$, then the tumor will shrink to a very small size, even with no CPA treatment. It is well known that tumor cells in glioma may shed and migrate into other areas in the brain. Thus even when the tumor size can be kept very small,

there is still a chance of developing a secondary tumor. In this respect, a repeated treatment of the tumor by CPA is important, for it decreases (on the average) the percentage of uninfected tumor cells, and thus reduces the risk of secondary tumors.

As was shown, by our model, that there is little difference between the weekly and bi-weekly CPA treatments. The protocol of choice should therefore depend on the side effects to this chemotherapy.

5 Appendix

We shall describe our mathematical model in the appendix. We consider the tumor to be radially symmetric and denote by r the distance from a point to the center of the tumor. We denote the boundary of the tumor by $r = R(t)$. We set

$$\begin{aligned}
 x(r, t) &= \text{number density of uninfected tumor cells,} \\
 y(r, t) &= \text{number density of infected tumor cells,} \\
 n(r, t) &= \text{number density of dead tumor cells,} \\
 z(r, t) &= \text{number density of immune cells (the different} \\
 &\quad \text{types of immune cells are lumped together),} \\
 v(r, t) &= \text{number density of free virus particles, that is virus} \\
 &\quad \text{particles which are not contained in the cells,} \\
 P(t) &= \text{concentration of CPA.}
 \end{aligned}$$

The proliferation and removal of cells cause a movement of cells within the tumor. If we denote the resulting velocity field by \vec{U} , then all cells undergo convection with velocity \vec{U} . In the radially symmetric case, we can

express this convection term, for the uninfected tumor cells x , in the form

$$\frac{1}{r^2} \frac{\partial}{\partial r} (r^2 u(r, t) x(r, t))$$

where $u(r, t)$ is the radial velocity, so that, in particular, $u(0, t) = 0$. The other cells undergo the same convection. The HSV particles have a small diameter, typically $0.13\mu m$, as compared to the typical diameter of $10\mu m$ of a cell. Hence, free virus particles, undergo diffusion rather than convection. Using mass conservation laws and taking convection of cells and dispersion of virus particles into account, we deduce the following equations in the tumor $r < R(t)$:

$$\frac{\partial x(r, t)}{\partial t} + \frac{1}{r^2} \frac{\partial}{\partial r} (r^2 u(r, t) x(r, t)) = \lambda x(r, t) - \beta x(r, t) v(r, t), \quad (2)$$

$$\frac{\partial y(r, t)}{\partial t} + \frac{1}{r^2} \frac{\partial}{\partial r} (r^2 u(r, t) y(r, t)) = \beta x(r, t) v(r, t) - ky(r, t) z(r, t) - \delta y(r, t), \quad (3)$$

$$\frac{\partial n(r, t)}{\partial t} + \frac{1}{r^2} \frac{\partial}{\partial r} (r^2 n(r, t) u(r, t)) = ky(r, t) z(r, t) + \delta y(r, t) - \mu n(r, t), \quad (4)$$

$$\frac{\partial z(r, t)}{\partial t} + \frac{1}{r^2} \frac{\partial}{\partial r} (r^2 z(r, t) u(r, t)) = sy(r, t) z(r, t) - c(z(r, t)) z(r, t) - P(r, t) z(r, t) \quad (5)$$

$$\frac{\partial v(r, t)}{\partial t} - D \frac{1}{r^2} \frac{\partial}{\partial r} (r^2 \frac{\partial v}{\partial r}) = b\delta y(r, t) - k_0 v(r, t) z(r, t) - \gamma v(r, t), \quad (6)$$

We assume that all the cells have the same size and that they are uniformly distributed in the tumor. Then the combined densities of the x , y ,

n and z cells is a constant, which is approximately $1gm/cm^3$; the density of the free virus particles is negligible. We can thus write

$$x(r, t) + y(r, t) + n(r, t) + z(r, t) = \text{const.} = \theta \quad (7)$$

and, by [31],

$$\theta \approx 10^6 \text{ cells}/mm^3.$$

Combining the equations (2)-(5) and using (7), we obtain an equation for the velocity $u(r, t)$:

$$\begin{aligned} & \frac{\theta}{r^2} \frac{\partial}{\partial r} (r^2 u(r, t)) \\ & = \lambda x(r, t) - \mu n(r, t) + sy(r, t)z(r, t) - c(z(r, t))z(r, t) - P(r, t)z(r, t). \end{aligned} \quad (8)$$

We also have

$$u(0, t) = 0, \quad \text{for } t > 0, \quad (9)$$

and

$$\frac{\partial v}{\partial r} (0, t) = 0, \quad \text{for } t > 0. \quad (10)$$

Since the free viruses remain in tumor,

$$\frac{\partial v}{\partial r} (R(t), t) = 0, \quad \text{for } t > 0. \quad (11)$$

Finally, the free boundary is subject to the kinematic condition

$$\frac{dR(t)}{dt} = u(R(t), t). \quad (12)$$

In the simulation study of this paper, we have taken

$$R(0) = 2mm$$

and

$$\begin{aligned}x(r, 0) &= 0.84 \times 10^6, y(r, 0) = 0.1 \times 10^6, \\z(r, 0) &= 0.06 \times 10^6, v(r, 0) = Ae^{-\frac{r^2}{22}},\end{aligned}$$

where, $0 \leq r \leq 2$,

$$5.2\pi \times 10^8 \leq 4\pi \int_0^2 Ae^{-\frac{r^2}{22}} r dr \leq 11.2\pi \times 10^8.$$

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