Differential Equation Techniques for Modeling a Cycle-Specific Oncolytic Virotherapeutic

Joanna R. Wares, Joseph J. Crivelli, and Peter S. Kim

Abstract The development of a mathematical model of oncolytic virotherapeutic vesticular stomatitis virus (VSV) is presented in stages. Standard mathematical tools are discussed along with the development and analysis of the model. A defining property of VSV is that it only affects tumor cells when they are in the active phases of the cell cycle. To model this characteristic, we first model tumor growth and separate cells into active and resting, which takes the form of a linear system of differential equations. We then take into account the minimum time needed for cells to travel through the active phases of the cell cycle, first using delay-differential equations and then later age-structured partial differential equations. Our basic tumor growth model allows us to investigate linear systems analysis (eigenvalue analysis). We then study similar techniques for delay differential equations, after adding the minimum time necessary to travel through the active phases of the cell cycle to the model. After tumor growth alone has been modeled, we include viral dynamics, which takes the form of a nonlinear system of ordinary differential equations. We investigate how linearization helps us understand how to properly develop the model. Finally we add the minimum biological time to the viral model. With the model fully developed, we arrive at a system of differential equations, one of which is an age-structured partial differential equation, which provides a nice example for discussing the method of characteristics. Finally, we show how our model can be used to investigate the dynamics of the tumor-virus system. As we travel through the development of our model, we discuss various techniques to analyze ordinary, delay, and partial differential equations.

J.R. Wares (✉)
University of Richmond, Richmond, VA 23173, USA
e-mail: jwares@richmond.edu

J.J. Crivelli
Weill Cornell Medical College, New York, NY 10065, USA
e-mail: jjc2004@med.cornell.edu

P.S. Kim
University of Sydney, Camperdown, NSW 2006, Australia
e-mail: pkim@maths.usyd.edu.au

A. Eladdadi et al. (eds.), *Mathematical Models of Tumor-Immune System Dynamics*, Springer Proceedings in Mathematics & Statistics 107,
DOI 10.1007/978-1-4939-1793-8_10

1 Introduction

In this chapter, we provide a review of the mathematical techniques used to develop and analyze a model of oncolytic virotherapeutic vesicular stomatitis virus, VSV [9]. The model provides a platform for understanding the dynamics of systems of ordinary differential equations (ODE), delay differential equations (DDE), as well as a starting point for understanding partial differential equations (PDE).

Oncolytic virotherapeutics (OV), specially engineered cancer-killing viruses, differ based on the mechanisms of the underlying virus used. Examples of oncolytic viruses that have demonstrated anti-tumor efficacy include adenoviruses [12], Coxsackieviruses [1], herpes simplex viruses [21], measles viruses [10], Newcastle disease virus [18], reoviruses [8], Seneca Valley virus [20], vaccinia viruses [17], and vesicular stomatitis virus [5]. Various models have been proposed as representations for treatment of cancer with oncolytic virotherapeutics, and frequently the models are novel specifically because of the differences in the underlying virus.

Early OV modeling efforts by Wodarz et al. [23] explored the different oncolytic mechanisms at play, death from replication of the virus, from an immune response mounted against the virus, or from an immune response due to molecules secreted by the tumor cells in response to the virus invasion. Wu et al. [25] looked at the race between the tumor, the OV, and the immune system (which attacks both the tumor and the OV) in a partial differential equations model which included spatial dynamics. More specific models followed, Friedman et al. looked at Glioma virotherapy in combination with an immunosuppressant, cyclophosphamide [11]. Bajzer et al. and Biesecker et al. [2, 6] look at optimal dosing and timing of doses using recombinant measles virus. Wodarz and Komarova followed up in 2009 with a more general study of virus therapy, looking at which models were consistent with various experimentally validated tumor dynamics [13, 24].

In a previous work, we developed a model of the oncolytic virotherapeutic, VSV [9]. VSV is an RNA virus that has demonstrated anti-tumor efficacy in a large range of human tumor cell lines, including prostate, breast, cervical, and hematologic cancers [4]. VSV also has the distinguishing characteristic that it is only transmissible when the tumor cells are in the active phases of the cell cycle [16]. We therefore developed the model to differentiate between tumor cells in active phases and the quiescent phase of the cell cycle. To do so, the tumor population was separated into two compartments, one compartment for the cells in the active phases and one compartment for cells in the quiescent phase. In the first part of this chapter, we describe the movement of cells between these two compartments, including cell division and natural cell death. The differential equations system that was developed is simple being linear with constant coefficients. To begin our discussion, we describe how linear systems analysis was used to analyze the dynamics of the tumor growth system alone.

Next, we include the idea that there is a minimum time necessary for cells to travel through the active phases of the cell cycle. To force cells to remain in the active phases for a minimum time, the model is converted into a system of

differential equations, one of which includes a delay. Basic theory of analyzing delay differential equations is then presented, along with some results particular to the VSV model, as an example of how to utilize the analysis methods.

Next we incorporate virotherapy into the base model, while at first excluding the delay. To build the model properly, an investigation of the transmission term was necessary. We explain the basics of local nonlinear systems analysis and reveal how it was helpful in developing our model. Upon completion of this stage, the model became a four-dimensional model, with cycling cells separated into infected and susceptible, with an additional compartment for the virions [2].

Finally, we bring all of the components together, transmission and delay, and arrive at a system of five equations, one of which is an age-structured partial differential equation, which captures the minimum time necessary to travel through the active phases of the cell cycle. We review the method of characteristics and show how this method was utilized twice in the paper, in one case to solve an equation and later in a proof that shows that solutions of the PDE system remain nonnegative.

The development of the model and underlying mathematical theory are interesting alone, but mathematical biology is at its best when we can say something about the underlying biological system using the mathematical model. Therefore, at the end of this chapter, we review the biological results in the original paper, obtained through numerical simulations and stability analysis, which elucidate the factors that promote complete remission, controlled tumor growth, or uncontrolled tumor growth.

2 Linear System Techniques

First, tumor growth alone is modeled. The model comprises two compartments, $Q(t)$ and $S(t)$, representing the volume of tumor cells in the quiescent phases and the active phases of the cell cycle at time t, respectively. Later in the chapter, the minimum biological time needed to travel through the active phases of the cell cycle and the viral dynamics will be added to the model. But for now, the model will simply track tumor growth, accounting for the transition to resting and back to the active phases of the cell cycle. The equations of the model are

$$Q'(t) = 2a_2 S - a_1 Q - d_1 Q, \tag{1}$$

$$S'(t) = a_1 Q - a_2 S - d_2 S. \tag{2}$$

The parameters a_1 and a_2 are the rates that cells move from Q to S and S to Q, respectively, with cells dividing into two when they leave the active phase, hence the $2a_2$ in the first term of the $Q'(t)$ equation. Cells die naturally at rates d_1 and d_2 for Q and S, respectively.

The system is linear with constant coefficients and provides a nice example of how linear analysis is used to qualitatively understand a system of differential

equations. The system is also solvable but the solution is in terms of parameters, and understanding how the parameters affect the dynamics of the system is easier if we analyze the stability of the equilibria, rather than looking at the analytical forms of the solutions. To fully appreciate this, we will look at both methods.

The solutions can be found using the eigenvalue method. If the eigenvalues of the coefficient matrix are real, the general solution of the system has the form

$$\begin{bmatrix} Q(t) \\ S(t) \end{bmatrix} = c_1 e^{\lambda_1 t} \begin{bmatrix} v_1 \\ v_2 \end{bmatrix} + c_2 e^{\lambda_2 t} \begin{bmatrix} v_3 \\ v_4 \end{bmatrix}, \tag{3}$$

where, λ_1 and λ_2 are the eigenvalues of the coefficient matrix. The corresponding eigenvectors are $[v_1\ v_2]^T$ and $[v_3\ v_4]^T$, respectively, and c_1 and c_2 are constants that can be found once initial values, $Q(0)$ and $S(0)$ are given.

The coefficient matrix of our model system is

$$A = \begin{bmatrix} -(a_1 + d_1) & 2a_2 \\ a_1 & -(a_2 + d_2) \end{bmatrix}, \tag{4}$$

so the eigenvalues of A are

$$\lambda_1 = \frac{-(a_1 + a_2 + d_1 + d_2) - \sqrt{\Delta}}{2}, \tag{5}$$

$$\lambda_2 = \frac{-(a_1 + a_2 + d_1 + d_2) + \sqrt{\Delta}}{2}, \tag{6}$$

where

$$\Delta = (a_1 + a_2 + d_1 + d_2)^2 - 4\left(a_1(d_2 - a_2) + d_1(a_2 + d_2)\right),$$

with corresponding eigenvectors

$$\begin{bmatrix} v_1 \\ v_2 \end{bmatrix} = \begin{bmatrix} 3a_2 + d_2 - \sqrt{\Delta} \\ 2a_1 \end{bmatrix}, \tag{7}$$

$$\begin{bmatrix} v_3 \\ v_4 \end{bmatrix} = \begin{bmatrix} 3a_2 + d_2 + \sqrt{\Delta} \\ 2a_1 \end{bmatrix}. \tag{8}$$

As you can imagine, trying to divine anything from (3) with these eigenvalues and eigenvectors inserted would be quite difficult. Instead, qualitative analysis is employed to study the long term behavior of the tumor. With qualitative analysis, we can ask, based solely on the growth and death parameters, will the tumor prosper or decline? Unless A is singular, which is highly improbable, the only equilibrium of the system is the tumor free equilibrium ($Q(t)$, $S(t)$) = (0,0), so another way to ask our question is, will nonzero solutions of the system approach (0,0) or move away from it?

To determine our answer, we look at the eigenvalues of the matrix A. Linear systems analysis allows us to determine the stability of the equilibrium (0,0) solely from the sign of the real parts of the eigenvalues of the coefficient matrix. If the real parts of the eigenvalues of A are less than zero, then (0,0) is asymptotically stable, and solutions move toward (0,0) as $t \to \infty$. Therefore, the tumor will be extinguished naturally. On the other hand, if the eigenvalues of A have positive real parts, then (0,0) is unstable and the tumor will grow indefinitely. If one eigenvalue has positive real part and the other has negative real part, then (0,0) is a saddle and is unstable (with only two trajectories moving toward the equilibrium). If the eigenvalues have real part equal to zero, then the situation is more complicated. Also note that the eigenvectors are not used in understanding the stability of (0,0). Using qualitative analysis, we can more easily discuss the long term behavior of the solutions of the system than if we only had the analytical form of the solution alone.

Using the information in the preceding paragraph and the equations for the eigenvalues, we can come up with conditions, based on the parameters of the model, that determine when (0,0) will be stable. We only need to determine when the real parts of the eigenvalues are both negative [9].

From (5) and (6), we know both eigenvalues are always real when all parameters are nonnegative, since

$$\begin{aligned}
&(a_1 + a_2 + d_1 + d_2)^2 - 4(a_1(d_2 - a_2) + d_1(a_2 + d_2)) \\
&= d_1^2 + (a_1 + a_2 + d_2)^2 - 2d_1(a_1 + a_2 + d_2) + 4a_1a_2 \\
&= (d_1 - (a_1 + a_2 + d_2))^2 + 4a_1a_2 \geq 0.
\end{aligned}$$

Hence, if $a_1(d_2 - a_2) + d_1(a_2 + d_2) > 0$, both eigenvalues are negative and the cancer-free equilibrium is asymptotically stable, implying that the tumor would disappear naturally. On the contrary, if $a_1(d_2 - a_2) + d_1(a_2 + d_2) < 0$, then one eigenvalue is positive, the cancer-free equilibrium is unstable, implying that the tumor will grow without bounds. Notice that if either $d_1 > a_1$ or $d_2 > a_2$ (i.e., either compartment has a death rate which dominates the corresponding rate of transfer within the system), then the cancer-free equilibrium is stable [9]. We note that these results are analogous to those of Crivelli et al. [9] and Villasana and Radunskaya [22].

3 Delay Differential Equations

If we model cell transitions as above, there is a possibility that the cell will move into the active phases of the cell cycle and immediately split and transition to quiescence. In reality, cells take some amount of time to transit through the active phases of the cell cycle, due to various biological process in mitosis. The amount of time it takes to travel through the cell cycle is not pre-determined, but is stochastic in nature.

We think of the total amount of time needed to travel through the cell cycle as some minimum time τ plus some additional time that is Poisson in nature. The minimum time is deterministic and is modeled with a delay. The additional time is modeled through the exponential rate that cells transition back to resting.

To model the minimum time necessary to complete the active phases of the cell cycle, we add a delay to the model. Cells transition from quiescence to the active phases of the cell cycle at a rate of a_1 and remain there for a minimum time τ, representing the duration of mitosis. The way we model this mathematically, is to transition cells through a holding compartment, $\bar{S}$, representing mitosis, for τ days. Cells move from S to $\bar{S}$ at a rate of a_2, but cannot move back to Q until the minimum time is over. After the requisite time τ, cells move from $\bar{S}$ into Q. If this were the whole story, we could account for the transition through $\bar{S}$ by moving cells out of $\bar{S}$ at rate $a_2 S(t-\tau)$, so that the rate of cells leaving at time t would be precisely equal to the rate that cells entered τ days ago at time $t-\tau$. However, cells still die at a rate of d_3 while traveling through $\bar{S}$. Therefore, the model equations are

$$Q'(t) = 2a_2 e^{-d_3\tau} S(t-\tau) - a_1 Q - d_1 Q, \tag{9}$$

$$S'(t) = a_1 Q - a_2 S - d_2 S, \tag{10}$$

$$\bar{S}'(t) = a_2 S - d_3 \bar{S} - a_2 e^{-d_3\tau} S(t-\tau), \tag{11}$$

where the term $e^{-d_3\tau}$ accounts for the proportion of cells that have died over the τ days in the holding compartment, $\bar{S}$. To have a well-defined model, we also include history functions given by $Q(t) = \phi_q(t)$, $S(t) = \phi_s(t)$ and $\bar{S}(t) = \phi_{\bar{s}}(t)$, for $-\tau \le t \le 0$.

Notice that the first two equations in the system are not coupled with the holding compartment, so we can analyze the behavior of the system by only considering the first two equations and solving the equation for $\bar{S}$ in terms of $S(t)$.

Even though our system is still linear, the delay makes the system much more difficult to analyze. In general, systems of DDEs lead to characteristic quasipolynomials that include terms of the form $e^{-\lambda\tau}$, where τ is a time delay in the system.

To obtain the characteristic equation for our DDE system, we guess a solution of the form $e^{\lambda t}\mathbf{v}$ for some constant vector $\mathbf{v}$. Substituting this solution into (9) and (10) and simplifying, we obtain

$$\left(\begin{bmatrix} -(a_1+d_1) & 2a_2 e^{-d_3\tau} e^{-\lambda\tau} \\ a_1 & -(a_2+d_2) \end{bmatrix} - \lambda I_2 \right) \mathbf{v} = 0$$

where I_2 is the 2×2 identity matrix. Hence, it follows that

$$\det \begin{bmatrix} -(a_1+d_1)-\lambda & 2a_2 e^{-d_3\tau} e^{-\lambda\tau} \\ a_1 & -(a_2+d_2)-\lambda \end{bmatrix} = 0.$$

Calculating this determinant, we obtain the characteristic equation

$$P(\lambda) = 2a_1a_2e^{-d_3\tau}e^{-\lambda\tau} - (a_1 + d_1 + \lambda)(a_2 + d_2 + \lambda) = 0. \tag{12}$$

As is the case with transcendental equations of this form, in general, there are infinitely many roots. As in the ODE case, we use the eigenvalues to prove something about the tumor-free-equilibrium instead of finding the actual solutions to the delay equations.

Given below, a result from the original work [9] which is proved using the eigenvalues from the characteristic equation, describes a condition on τ, which, if achieved, results in a stable cancer-free equilibrium.

Theorem 1. *For any a_1, a_2, d_1, d_2, $d_3 > 0$, $(Q, S) = (0, 0)$ is stable when*

$$\tau > \frac{1}{d_3}\log\left(\frac{2a_1a_2}{(a_1 + d_1)(a_2 + d_2)}\right) > 0$$

and unstable when

$$0 < \tau < \frac{1}{d_3}\log\left(\frac{2a_1a_2}{(a_1 + d_1)(a_2 + d_2)}\right).$$

Theorem 1 shows that for any growth and death rates, there is a τ, given by the condition in the theorem, for which the tumor would be naturally eliminated. To prove this theorem, we first proved the following lemma [9]. See the original work for the proof.

Lemma 1. *For any a_1, a_2, d_1, d_2, $d_3 > 0$, the rightmost eigenvalue derived from the characteristic equation (12) is real.*

Having proven the previous lemma, we proved the following proposition [9]. The theorem directly follows.

Proposition 1. *For any parameters a_1, a_2, d_1, d_2, $d_3 > 0$, the cancer-free equilibrium $(Q, S) = (0, 0)$ of the system (9)–(10) is globally asymptotically stable if*

$$2a_1a_2e^{-d_3\tau} - (a_1 + d_1)(a_2 + d_2) < 0,$$

and unstable if

$$2a_1a_2e^{-d_3\tau} - (a_1 + d_1)(a_2 + d_2) > 0.$$

Using our result, it is also possible to determine a threshold delay value of τ, about which stability switches occur. We see that lengthening the time spent in the active phases of the cell cycle can cause the doubling time of the tumor population to

increase and thereby cause the tumor to be eliminated. Many therapeutics work in this way to lengthen the time that cells stay in the cell cycle so that they reproduce more slowly.

4 Virus System

So far, we have been investigating a system that describes tumor growth, including the minimum time necessary for the cell to travel through the active phases of the cell cycle. But the main goal of the work was to understand the dynamics of the oncolytic virus, VSV. At this point, the virus is introduced into the system. To add the virus, we must add compartments for the virus, V, and for cells that are infected by the virus, I.

Mathematically, the most interesting question here is how to model transmission of the virus to cells that are in the active phases of the cell cycle. As not to confound the situation, we first look at transmission, ignoring the minimum time spent in the active phases of the cell cycle.

As previously considered in (1) and (2), the model without virus is

$$Q'(t) = 2a_2S - a_1Q - d_1Q, \tag{13}$$

$$S'(t) = a_1Q - a_2S - d_2S. \tag{14}$$

After adding the virus and infected cell populations, we arrive at

$$Q'(t) = 2a_2S - a_1Q - d_1Q, \tag{15}$$

$$S'(t) = a_1Q - a_2S - d_2S - \kappa\frac{VS}{N}, \tag{16}$$

$$I'(t) = \kappa\frac{VS}{N} - \delta I, \tag{17}$$

$$V'(t) = \alpha I - \kappa\frac{VS}{N} - \omega V. \tag{18}$$

Let us examine how the system changes when the virus and infected cells are added. The top equation is the same because the virus cannot act when cells are in the Q state. The next equation, which describes how the size of the population of active cells changes, has a new term, $-\kappa\frac{VS}{N}$, which describes the rate that cells become infected. This term then appears again in the next equation, as cells move from S to I when they become infected. Here, N, is the total volume of tumor cells and virions in the system ($N = S + I + V$). Modelers frequently use a mass-action term for transmission (here that would be $-\kappa VS$). On the other hand, the term we used is called a ratio- or frequency-dependent term.

If we use a mass-action term to describe the dynamics of the virus, then the virus has no effect on the local stability of the tumor-free equilibrium ($(Q, S, I, V) = (0, 0, 0, 0)$). To understand this, we extend our linear analysis from Sect. 3 to nonlinear systems. To do so, we must call on the Hartman–Grobman Theorem.

Formally, the Hartman–Grobman Theorem is a topological result. Informally, it is likely the most used tool for understanding the long term behavior of nonlinear differential equations systems. A formal statement is given in Perko [19]:

Theorem 2 (Hartman–Grobman Theorem). *Let E be an open subset of $\mathbb{R}^n$ containing the origin, let $\mathbf{f} \in C^1(E)$, and let ϕ_t be the flow of the nonlinear system $\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x})$. Suppose that $\mathbf{f}(\mathbf{0}) = \mathbf{0}$ and that the matrix $A = D\mathbf{f}(\mathbf{0})$ has no eigenvalue with zero real part. Then there exists a homeomorphism H of an open set U containing the origin onto an open set V containing the origin such that for each $\mathbf{x_0} \in U$, there is an open interval $I_0 \subset \mathbb{R}$ containing zero such that for all $\mathbf{x_0} \in U$ and $t \in I_0$*

$$H \circ \phi_t(\mathbf{x_0}) = e^{At} H(\mathbf{x_0}). \tag{19}$$

A friendlier (but less technical) version of this theorem can be found in Cain and Reynolds [7] and is helpful in this discussion:

Theorem 3 (Hartman–Grobman Theorem, Friendly Version). *Suppose $\mathbf{x_0}$ is an isolated equilibrium of a nonlinear system $\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x})$. Then in the vicinity of $\mathbf{x_0}$, the linearization $\mathbf{x_0} = J\mathbf{f}(\mathbf{x_0})(\mathbf{x} - \mathbf{x_0})$ about that equilibrium has the same qualitative behavior as the original nonlinear system.*

In the virus system, the vectors $\mathbf{x} = (Q, S, I, V)^T$ and $\mathbf{f}$ is the vector formulated right hand side of our system of differential equations.

The Hartman–Grobman Theorem tells us that solutions of a nonlinear system act like solutions of their corresponding linearized system near hyperbolic equilibria. Assuming that the tumor-free equilibrium is hyperbolic (no eigenvalues with zero real part), we can linearize the system and see how the virus affects the stability of the tumor-free equilibrium.

But what is this term $J\mathbf{f}(\mathbf{x_0})(\mathbf{x} - \mathbf{x_0})$, and what does it have to do with linearization? Linearizing a nonlinear system means that we take the multivariate functions on the right hand side of each differential equation in the system and Taylor expand each one around each equilibrium ($\mathbf{x_0}$), so that

$$\frac{d\mathbf{x}}{dt} = \mathbf{f}(\mathbf{x_0}) + J\mathbf{f}(\mathbf{x_0})(\mathbf{x} - \mathbf{x_0}) + \text{higher order terms}, \tag{20}$$

where J is the Jacobian, the matrix of all first derivatives of the vector valued function, $\mathbf{f}$. We then drop the higher order terms, since we want to know about the local stability of the equilibria. We can drop the higher order terms because we are interested in the local behavior of the system and the higher order terms are small when we are near the equilibrium.

Since $\mathbf{x_0}$ is an equilibrium of the system, we know that $\mathbf{f}(\mathbf{x_0}) = 0$ so that

$$\frac{d\mathbf{x}}{dt} = J\mathbf{f}(\mathbf{x_0})(\mathbf{x} - \mathbf{x_0}). \tag{21}$$

Next, we want to change the main vector of variables to be the distance from the equilibrium instead of the total distance. We do so by substituting $\bar{x} = (\mathbf{x} - \mathbf{x_0})$ and noting that $\frac{d\mathbf{x}}{dt} = \frac{d\bar{\mathbf{x}}}{dt}$ since $\frac{d\mathbf{x_0}}{dt} = 0$. We then have

$$\frac{d\bar{\mathbf{x}}}{dt} = J\mathbf{f}(\mathbf{x_0})(\bar{\mathbf{x}}). \tag{22}$$

This is the linearized version of the system around the equilibrium $\mathbf{x_0}$. From the Hartman–Grobman Theorem, we know we can determine the stability of any hyperbolic equilibrium by eigenvalue analysis of the Jacobian evaluated at the equilibrium of interest.

Now going back to virus system, we want to know how the stability of the tumor-free equilibrium ($(Q, S, I, V) = (0, 0, 0, 0)$) is affected by the introduction of the virus. The real question is, by introducing the virus, can we eliminate the tumor over time, or in mathematical terms, can introduction of the virus change the sign of the real part of the eigenvalues of the Jacobian so that they go from at least one being positive to all negative?

In the original paper, we make the argument that the virus cannot affect local stability of the tumor-free equilibrium if transmission is modeled using a mass-action term κVS instead of the ratio-dependent term $\frac{\kappa VS}{N}$, where $N = Q + S + I + V$. Now that we understand what it means to linearize a system and talk about stability of the tumor-free equilibrium under linearization, let us look at what happens when we linearize the system which includes mass-action transmission:

$$Q'(t) = 2a_2 S - a_1 Q - d_1 Q, \tag{23}$$

$$S'(t) = a_1 Q - a_2 S - d_2 S - \kappa VS, \tag{24}$$

$$I'(t) = \kappa VS - \delta I, \tag{25}$$

$$V'(t) = \alpha I - \kappa VS - \omega V. \tag{26}$$

The Jacobian, J, of the right-hand side of this system is

$$J(Q, S, I, V) = \begin{bmatrix} -a_1 - d_1 & 2a_2 & 0 & 0 \\ a_1 & -a_2 - d_2 - \kappa V & 0 & -\kappa S \\ 0 & \kappa V & -\delta & \kappa S \\ 0 & -\kappa V & \alpha & -\omega \end{bmatrix}.$$

So far, it looks like the parameters of the virus are coming into play repeatedly in the Jacobian matrix and will have an important role to play in determining the sign

of the eigenvalues. But we must remember that before we find the eigenvalues, we evaluate the Jacobian at the tumor-free equilibrium $(Q, S, I, V) = (0, 0, 0, 0)$), so anywhere we see a V or an S, that term will be zero. These are all of the transmission terms. At the tumor-free equilibrium, the Jacobian is

$$J(0,0,0,0) = \begin{bmatrix} -a_1 - d_1 & 2a_2 & 0 & 0 \\ a_1 & -a_2 - d_2 & 0 & 0 \\ 0 & 0 & -\delta & 0 \\ 0 & 0 & \alpha & -\omega \end{bmatrix}.$$

This is a block matrix and the eigenvalues from the top-left block only depend on the parameters a_1, a_2, d_1, and d_2, which is the coefficient matrix of the linear submodel describing tumor growth alone without virus (see (13) and (14)). The two eigenvalues of the lower-right block are $-\delta$ and $-\omega$, which are always negative, because δ and ω are always positive. So we see that if we use mass-action transmission, the virus is not able to affect the stability of the tumor-free equilibrium because the terms related to the virus do not alter the signs of the eigenvalues associated with tumor growth. The eigenvalues due to the virus-associated parameters are always negative and the others are unaffected by the virus.

However, if we use ratio-dependent transmission, as is in (15) and (18), it has been shown that the virus can affect the stability of the tumor-free equilibrium [15]. Ratio dependence also makes sense biologically because it allows for the spatial size of the tumor to change, whereas mass action makes the assumption that the spatial dimension is staying constant, while the density changes, which is not usually the case for tumors.

It is noted that the biological relevance of these results is not certain. In the mass-action type model, oscillations are frequently seen that drive the tumor size to near zero [9, 13, 24]. In the true biological system, when the tumor is near zero, it can be removed completely due to the stochasticity of the underlying dynamics. Additionally, the formulation of the ratio-dependent term is sensitive to perturbations, causing the model to not be entirely robust.

5 PDE Virus System

First, a model of tumor growth was developed. We then investigated how incorporating the time needed to travel through the cell cycle affected the stability of the tumor-free equilibrium of the model by developing a system of equations that included a delay. Next, we created a model of tumor growth and viral dynamics without the delay. In this section, we finally put all of the components together into one model.

We can no longer use delay differential equations, because the transmission term is nonlinear and we can no longer solve directly for the loss of cells in the holding

state $\bar{S}$, as we did in the simpler growth-only model. After developing the model, we want to make sure that the solutions match those of the simpler model, if the viral parameters are set to zero. To do so, we must first complete our analysis of (11) by solving for $\bar{S}$ in terms of S, which we think of as a known function of t.

Begin with the differential equation

$$\bar{S}'(t) = a_2 S - d_3 \bar{S} - a_2 e^{-d_3 \tau} S(t-\tau), \tag{27}$$

which is *linear* in $\bar{S}$.

The integrating factor is $e^{d_3 t}$. After multiplying by the integrating factor on both sides and integrating between 0 and t, we find that

$$\bar{S}(t) = \bar{S}(0) e^{-d_3 t} + a_2 e^{-d_3 t} \int_{t-\tau}^{t} e^{d_3 u} S(u) du - a_2 e^{-d_3 t} \int_{-\tau}^{0} e^{d_3 u} \phi_s(u) du. \tag{28}$$

We also assume that cells that are in the holding state before $t = -\tau$, leave before $t = 0$. Mathematically we can do this by setting $\bar{S}(0) = a_2 \int_{-\tau}^{0} e^{d_3 u} \phi_s(u) du$. Therefore, the solution is

$$\bar{S}(t) = a_2 e^{-d_3 t} \int_{t-\tau}^{t} e^{d_3 u} S(u) du. \tag{29}$$

After developing the full model, we will check that it is consistent with this solution.

Now to the full model. Our full PDE model, including the minimum biological time needed to complete the active phases of the cell cycle, as well as the viral transmission, is

$$\frac{dQ}{dt} = 2\hat{S}(\tau, t) - a_1 Q - d_1 Q, \tag{30}$$

$$\frac{dS}{dt} = a_1 Q - a_2 S - d_2 S - \kappa \frac{VS}{N}, \tag{31}$$

$$\frac{\partial \hat{S}}{\partial t} + \frac{\partial \hat{S}}{\partial x} = -d_3 \hat{S} - \kappa \frac{V\hat{S}}{N}, \tag{32}$$

$$\frac{dI}{dt} = -\delta I + \kappa \frac{VS + V\bar{S}}{N}, \tag{33}$$

$$\frac{dV}{dt} = \alpha I - \omega V - \kappa \frac{VS + V\bar{S}}{N}. \tag{34}$$

where $\bar{S}(t) = \int_0^\tau \hat{S}(x,t) dx$, $N(t) = Q(t) + S(t) + \bar{S}(t) + I(t) + V(t)$, and the boundary condition is given by

$$\hat{S}(0,t) = a_2 S(t).$$

$\hat{S}$ is a function of two variables: t–time, and x–the length of time already spent in the cell cycle. A diagram of the full PDE model is given in Fig. 1.

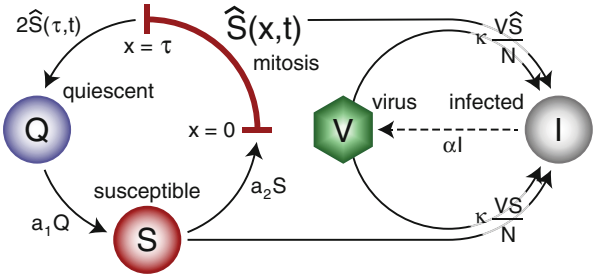

Fig. 1 Compartmental diagram for the full model of virotherapy, given by (30)–(34). Transfer occurs from the quiescent to the non-quiescent, or susceptible, cell population at rate a_1, and susceptible cells begin mitosis at rate a_2. Cells undergoing mitosis remain in a holding state for τ units of time. After completing mitosis, two daughter cells enter the quiescent population. Susceptible cells are infected through contact with the free virus population at rate $\kappa V/N$ and enter the infected state. Viral reproduction in infected cells, combined with lysis, leads to production of free virions at a rate α. Although not shown in the diagram, all cell and virus populations, Q, S, $\hat{S}$, I, and V die or decay at rates d_1, d_2, d_3, δ, and ω, respectively

Note that the PDE now accounts for the loss of susceptible cells in the delay period. For biological relevance, the initial conditions, $Q(0)$, $S(0)$, $\hat{S}(x,0)$, $I(0)$, and $V(0)$, are all assumed to be nonnegative. To extend the model to the origin, when $N = 0$, we let the right-hand sides of (30)–(34) equal zero. Note that the system (30)–(34) also reduces to (15)–(18) when $\tau = 0$.

Remember, we want to show that if we remove virotherapy, the solution we obtain is the same as (29). To do so, we will use the method of characteristics, a technique that can be used to solve certain partial differential equations (PDE). We use it here to find the solution of the full system when virotherapy is turned off, and then afterward, to prove that solutions of the full system with virotherapy do not become negative.

The main idea behind the method of characteristics is that you divide the domain into characteristic curves. Along these characteristic curves, the PDE becomes an ODE that you can solve, given suitable initial value data. After you find solutions on the characteristic curves, you convert the solutions into one concise surface solution for the PDE.

A standard example involves the advection equation

$$a\frac{\partial u}{\partial t} + b\frac{\partial u}{\partial x} = 0, \tag{35}$$

where a and b are not zero. We are looking for solutions $u(t,x)$ that satisfy this PDE.

The characteristic curves are found by realizing that the normal to the surface $(t, x, u(t, x))$ is given by $(u_t, u_x, -1)$. Our PDE tell us that $(a, b, 0)\cdot(u_t, u_x, -1) = 0$, so we look for $(a, b, 0)$ which lies in the tangent plane to $(t, x, u(t, x))$. To do so, we let

$$\frac{dt}{ds} = a \tag{36}$$

$$\frac{dx}{ds} = b \tag{37}$$

$$\frac{du}{ds} = 0, \tag{38}$$

where we are parameterizing the characteristic curve in the tangent plane by s. Solving the system of ordinary differential equations in terms of s we find

$$\begin{aligned} t(s) &= as + c_1 \\ x(s) &= bs + c_2 \\ u(s) &= c_3. \end{aligned}$$

We can get rid of the parameter s, noting that the characteristic curves are $ax - bt$ and that the solution u is constant along these characteristic curves. Therefore, the solution is an arbitrary differentiable function $u(t, x) = f(ax - bt)$. The particular function needed when modeling is determined from auxiliary conditions. To see why the solution makes sense, notice from the chain rule that

$$a\frac{\partial u}{\partial t} + b\frac{\partial u}{\partial x} = abf'(ax - bt) - baf'(ax - bt) = 0.$$

Now, let us examine how we used the method of characteristics in a couple different ways in the paper. Going back to the model, if we remove virotherapy, and assume that, for $0 \leq x \leq \tau$, $\hat{S}(x, 0) = a_2\phi_s(-x)e^{-d_3 x}$, then this system acts like (9)–(11). We want to show that is the PDE model really is equivalent to (9)–(11) by showing that $\bar{S}$ is the same as (29).

Similar to the preceding example, if we use the method of characteristics, we let

$$\begin{aligned} \frac{dt}{ds} &= 1 \\ \frac{dx}{ds} &= 1 \\ \frac{d\hat{S}}{ds} &= -d_3\hat{S}, \end{aligned}$$

Solving the system of ODEs, we find

$$\begin{aligned} t(s) &= s + c_1 \\ x(s) &= s + c_2 \\ \hat{S}(s) &= \hat{S}(s=0)e^{-d_3 s}, \end{aligned}$$

which implies

$$\hat{S}(s + c_2, s + c_1) = \hat{S}(c_2, c_1)e^{-d_3 s}.$$

Letting $s = \tilde{x}$, $c_1 = \tilde{t} - \tilde{x}$, and $c_2 = 0$ for $\tilde{t} \geq \tilde{x}$ and removing the tildes, we obtain

$$\hat{S}(x, t) = \hat{S}(0, t - x)e^{-d_3 x}.$$

We can then find $\bar{S}(t)$ by noting that $\hat{S}(0, t - x) = a_2 S(t - x)$:

$$\bar{S}(t) = \int_0^\tau \hat{S}(x, t)dx = \int_0^\tau \hat{S}(0, t - x)e^{-d_3 x}dx \tag{39}$$

$$= \int_0^\tau a_2 S(t - x)e^{-d_3 x}dx \tag{40}$$

$$= \int_{t-\tau}^t a_2 S(u)e^{-d_3(t-u)}du \tag{41}$$

$$= a_2 e^{-d_3 t} \int_{t-\tau}^t e^{d_3(u)} S(u)du. \tag{42}$$

And so we have achieved our goal, showing that $\bar{S}$ is the same as for the virus free system, see (29).

We also used the method of characteristics and integrating factor techniques to prove that solutions that begin nonnegative remain nonnegative for all time. We include the theorem and proof here as a more complicated example of using the method of characteristics [9].

Theorem 4. *Assume that $Q(0)$, $S(0)$, $\hat{S}(x, 0)$, $I(0)$, and $V(0)$ are nonnegative. Then, solutions of the system (30)–(34) are nonnegative for $t \geq 0$.*

Proof. If $Q(0) = S(0) = \hat{S}(x, 0) = \hat{S}(0, t) = I(0) = V(0) = 0$, then $Q(t) = S(t) = \hat{S}(x, t) = I(t) = V(t) = 0$ for all t, and we are at equilibrium.

Otherwise by assumption, at $t = 0$, all compartments are greater than or equal to zero and the total population $N(0) > 0$. In this case, we assume

$$t_0 = \inf_{t>0}\{t \mid Q(t) < 0, S(t) < 0, \bar{S}(t) < 0, I(t) < 0 \text{ or } V(t) < 0\},$$

with $t_0 < \infty$ and proceed to arrive at a contradiction.

Let $W = I + V$. We first assume that $\omega \geq \delta$, so

$$W' = -\omega W + (\alpha - \delta + \omega)I.$$

If $f(t) = (\alpha - \delta + \omega)I$, then $f(t) \geq 0$ for $t \in [0, t_0]$. Second, we assume that $\omega < \delta$, so

$$W' = -\delta W + \alpha I + (\delta - \omega)V.$$

If $f(t) = \alpha I + (\delta - \omega)V$, then $f(t) \geq 0$ for $t \in [0, t_0]$, so in general,

$$W' = -c_1 W + f(t), \tag{43}$$

for some $c_1 > 0 \in \mathbb{R}$ and $f(t) \geq 0$ for $t \in [0, t_0]$.

The solution of (43) is

$$W(t) = W(0)e^{-c_1 t} + e^{-c_1 t} \int_0^t e^{c_1 \xi} f(\xi) d\xi.$$

If $W(0) = 0$, the system reduces to the model with no treatment. Otherwise, because $W(0) > 0$ and $f(t) \geq 0$, it follows that $W(t) > 0$ for $t \in [0, t_0]$. Then, since $W(t_0) > 0$, the total population $N(t_0) > 0$. Now, with $N(t_0) > 0$, we can show that all compartments will stay nonnegative past t_0.

We begin with the age-structured PDE (32) and show that $\hat{S}(\tau, t) \geq 0$ for $t \in [0, t_0 + \eta)$ where $\eta = \min\{\epsilon, \tau\}$ for some $\epsilon > 0$. For each $\zeta \in \mathbb{R}$, we define

$$S_\zeta^*(T) = \hat{S}(\zeta + T, T).$$

and find solutions along the characteristic lines $x = \zeta + T$ with $t = T$. Then,

$$\frac{dS_\zeta^*}{dT} = \frac{\partial \hat{S}}{\partial x} + \frac{\partial \hat{S}}{\partial t}$$

and

$$(S_\zeta^*)' = -d_3 S_\zeta^* - \kappa \frac{V S_\zeta^*}{N}.$$

Hence, for each ζ, we have converted (32) into an ODE.

Since $t = T$,

$$S_\zeta^{*'}(t) = -d_3 S_\zeta^*(t) - \kappa \frac{V(t) S_\zeta^*(t)}{N(t)}.$$

Letting $g(t) = d_3 + \frac{\kappa V(t)}{N(t)}$, we rewrite the equation above as

$$S_\zeta^{*'}(t) = -g(t)S_\zeta^*(t). \tag{44}$$

Replacing Eq. (32) with (44), we obtain a system of ODEs for each characteristic line. From the form of the system of equations and the nonnegativity of initial conditions, it follows that $t_0 > 0$.

Since $N(t_0) > 0$, the ODE system is well-posed and a solution exists on an interval $(t_0 - \epsilon, t_0 + \epsilon)$. Moreover, by continuity, we may assume $N(t) > 0$ for $t \in (t_0 - \epsilon, t_0 + \epsilon)$.

Then solutions of Eq. (44) along the characteristic lines $x = \zeta + t$ are

$$S_\zeta^*(t) = S_\zeta^*(0)e^{-\int_0^t g(u)du}.$$

Each characteristic line in the (x, t) plane intersects either the nonnegative x-axis or the positive t-axis. If $\zeta \geq 0$, then the characteristic line intersects the nonnegative x-axis and $S_\zeta^*(0) = \hat{S}(\zeta, 0)$, which is nonnegative by assumption. Otherwise, if $\zeta < 0$, the characteristic line intersects the positive t-axis at $-\zeta$, and $S_\zeta^*(0) = \hat{S}(0, -\zeta) = a_2 S(-\zeta)$. By definition of t_0, we know that $S(-\zeta)$ is nonnegative when $-\zeta \in [0, t_0]$. Thus, $S_\zeta^*(0) \geq 0$ for each characteristic line that intersects the positive t-axis at or below t_0, $(\zeta \geq -t_0)$.

Since $g(t)$ is bounded $(0 \leq g(t) \leq d_3 + \kappa)$ and $S_\zeta^*(0) \geq 0$ for $\zeta \geq -t_0$, on the corresponding characteristic lines, $S_\zeta^*(t)$ will remain nonnegative for as long as the solution exists. Therefore, $\hat{S}(\tau, t)$ will also remain nonnegative for $t \in (t_0 - \eta, t_0 + \eta)$ where $\eta = \min\{\epsilon, \tau\}$. The constant η is defined in this way to ensure that solutions lie on the proper characteristic lines and that solutions exist.

Next, we evaluate (30)–(34), excluding the PDE. Each of the four equations is of the form $B'(t) = A(t) - r(t)B(t)$, and each equation has a solution of the form

$$B(t) = B(0)e^{-\int_0^t r(s)ds} + \int_0^t e^{-\int_\xi^t r(s)ds} A(\xi)d\xi,$$

where B is Q, S, I or V. For (30), the variable $A(t) = 2\hat{S}(\tau, t)$, and we know that $\hat{S}(\tau, t)$ is greater than or equal to zero for $t \in [0, t_0 + \eta)$ from the earlier argument using the method of characteristics. By assumption, the initial condition $Q(0) \geq 0$, so we obtain $Q(t) \geq 0$ for $t \in (t_0 - \eta, t_0 + \eta)$. For (31), the variable $A(t) = a_1 Q(t)$, so by similar reasoning, it follows that $S(t) \geq 0$ for $t \in (t_0 - \eta, t_0 + \eta)$. Since $S(t) \geq 0$ for $t \in (t_0 - \eta, t_0 + \eta)$, it follows that $S_\zeta^*(0) \geq 0$ for characteristic lines intersecting the t-axis up to $t_0 + \eta$. So, $\hat{S}(x, t) \geq 0$ for $t \in (t_0 - \eta, t_0 + \eta)$, and therefore the same holds for $\bar{S}(t)$. Finally, since $Q, S, \hat{S} \geq 0$, it follows from (33) and (34) that solutions cannot leave the positive quadrant of the $I - V$ plane and I and V will remain nonnegative for $t \in (t_0 - \eta, t_0 + \eta)$.

We have shown that all compartments remain nonnegative for $t \in (t_0 - \eta, t_0 + \eta)$. This contradicts the definition of t_0. We conclude that solutions remain nonnegative for all time.

6 Numerical Simulations

Models of biological systems are interesting from a mathematical point of view, but more importantly, for what they say about the biology. We used the models we developed above to understand the dynamics of the tumor-virus system in terms of biological parameters. In our paper [9], we numerically studied the dynamics from two perspectives: trajectories over time and stability regions using parameters from the literature (Table 1). By investigating trajectories over time, we showed that increasing the delay or adding virus can change the stability of the tumor free equilibrium. We also showed, using stability regions, that the specific parameters of the tumor or the virus affect the stability of the tumor-free equilibrium.

6.1 Non-Delay Case

We numerically simulated the various models using solvers, such as dde23, in MATLAB (Mathworks, MA), first without delay, then with the delay. Figure 2 plots solutions under three different conditions. The leftmost plot displays exponential tumor growth resulting from the system (13) and (14). The middle plot shows solutions of the system when a mass-action transmission term is used, whereas the plot on the right shows solutions for ratio-dependent transmission, using system (15)–(18) for the case of ratio-dependent transmission and system (23)–(26) for the case of mass-action transmission. Note that the untreated tumor grows exponentially (Fig. 2, left), whereas the treated tumor is eliminated (Fig. 2, right). Mass-action solutions are presented to show how solutions oscillate under such dynamics (Fig. 2, middle).

Two parameters, α and κ, are strongly correlated with the effectiveness of VSV treatment in system (15)–(18). Increase in viral replication is modeled by increasing α. Increasing viral replication increases virus-cell contact and results in a better treatment. As κ increases, likelihood of infection increases, also increasing the efficacy of the treatment. Figure 3 shows the effects of changing α and κ on the stability or instability of the cancer-free equilibrium.

6.2 Delay Case

Next, we looked at the models including the minimum time biologically necessary to complete division and compared to the non-delay system. In the left panel of Fig. 4, we plot solutions of the system, including the delay, but without VSV treatment.

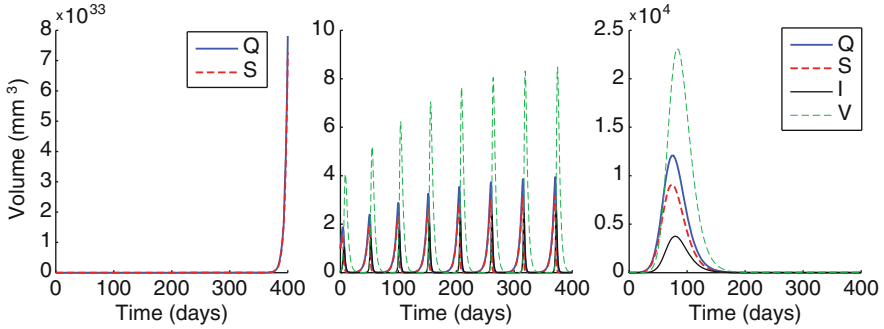

Fig. 2 Numerical solutions of VSV, excluding delay. *Left*: exponential tumor growth in the absence of therapy, (13) and (14). *Middle*: growing oscillatory behavior of solutions when virus-cell contact is modeled using mass action, (23)–(26). *Right*: complete tumor elimination; virus-cell contact is modeled using ratio dependence, (15)–(18). Parameter values: $a_1 = 0.9$, $a_2 = 0.6$, $d_1 = 0.00001$, $\delta = 1.119$, $\omega = 0.3$, $\alpha = 3$, $\kappa = 1$ in the case of mass-action transmission (*middle*), and $\kappa = 1$, in the case of ratio-dependent transmission (*right*)

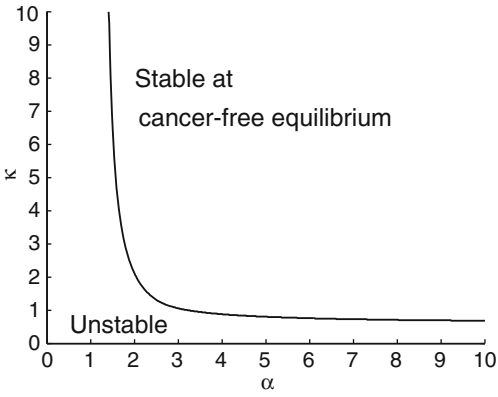

Fig. 3 Stability diagram for viral reproduction (α) and contact (κ). Other parameter values are as in Fig. 2, i.e. $a_1 = 0.9$, $a_2 = 0.6$, $d_1 = 0.00001$, $\delta = 1.119$, $\omega = 0.3$, and $\alpha = 3$

Parameters were chosen so that the solutions grow exponentially. In the middle panel, using the same parameters for tumor growth, we look at the VSV model that excludes the delay. We once again see exponential growth of the tumor (middle panel). The right most panel shows how interaction of the treatment and the delay causes successful elimination of the tumor. When the time delay is included, VSV successfully eliminates the tumor (right panel), demonstrating how the delay and the treatment interact, leading to successful eradication of the tumor.

Finally, we study stability diagrams of the VSV treatment parameters, α, κ, and τ. Figure 5 shows how the parameters interact, two at a time, to change the stability of the tumor free equilibrium.

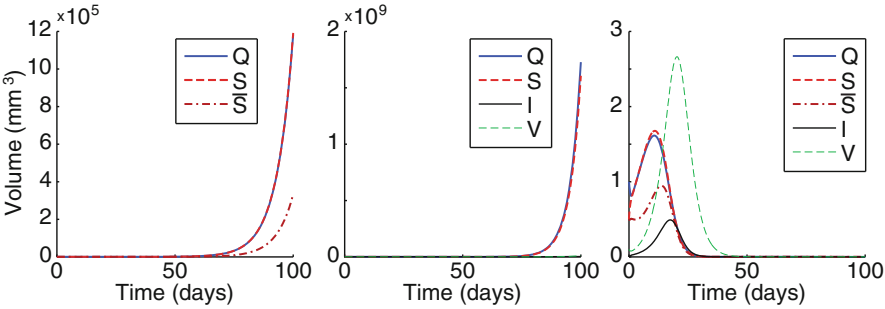

Fig. 4 *Left*: uncontrolled tumor growth (in the absence of virotherapy) under prolonged cell cycle progression ($\tau = 0.5$). *Middle*: when $\kappa = 0.8$, virotherapy treatment fails; minimum cell cycle time is not accounted ($\tau = 0$). *Right*: when $\kappa = 0.8$, virotherapy with a minimum cycling time ($\tau = 0.5$) results in a stable cancer-free state. All other parameter values are the same as in Fig. 2, i.e. $a_1 = 0.9$, $a_2 = 0.6$, $d_1 = 0.00001$, $\delta = 1.119$, $\omega = 0.3$, and $\alpha = 3$

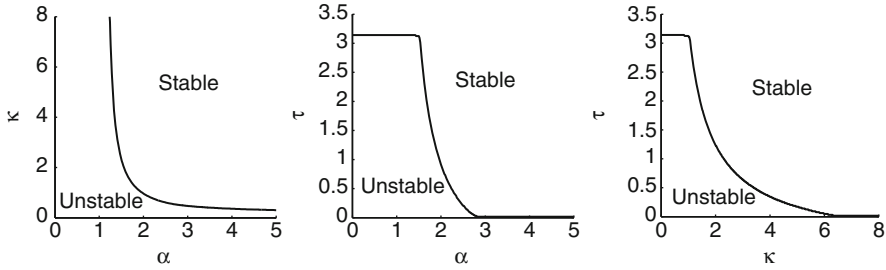

Fig. 5 Stability maps when α, κ, and τ are varied, two at a time. For small α and κ, a delay value (τ) beyond a certain threshold will ensure stability of the origin. Parameter values, if not varied, are $a_1 = 0.9$, $a_2 = 0.6$, $d_1 = 0.00001$, $\delta = 1.119$, $\omega = 0.3$,$\alpha = 1.5$, $\kappa = 1$, and $\tau = 1$

7 Discussion

In this chapter, we have developed a model of vesicular stomatitus virus (VSV), a candidate oncolytic virus, which has the defining feature that it can only infect tumor cells when they are in the active phases of the cell cycle.

We began with a simple tumor growth model containing compartments for resting and proliferating cells. This model took the form of a linear system of differential equations. We used the model to discuss basic techniques for linear systems analysis, the eigenvalue method. We presented results from the original work [9], giving conditions, in terms of parameters, for which the tumor would grow indefinitely or decay based solely on parameters related to the tumor.

Next, we extended the model to account for the minimum biological time course of the active phases of the cell cycle. In doing so, we arrived at a three-dimensional system of linear delay differential equations. Eigenvalue analysis for delay differential equations was discussed and a basic example given. We also

Table 1 Table of parameters given by (30)–(34). Parameters were obtained from the references cited in the fourth column. For all simulations, it was assumed that $d_3 = d_2$

Parameter	Description	Estimate	Reference
a_1	Quiescent cell entrance into active phases (day^{-1})	0.9	[14]
a_2	Active cell entrance into quiescence (day^{-1})	0.6	[14]
d_1	Quiescent cell death (day^{-1})	1×10^{-5}	[14]
d_2	Active cell death (day^{-1})	0.15	[14]
α	Virion production (day^{-1})	3	Variable
δ	Infected cell elimination (day^{-1})	1.119	[3]
ω	Free virion decay (day^{-1})	0.3	[3]
τ	Minimum duration of active phases (day)	[0, 3]	Variable
κ	Kinetic coefficient (day^{-1})	[0, 5]	Variable

reviewed the main result for that model: for a given set of model parameters, there exists a minimum value of the delay τ that will drive the system towards a globally stable cancer-free state, which can be calculated in terms of the growth and death rates of tumor populations.

Our next extension involved introducing virotherapy treatment by including two additional compartments: infected cells and free virions, creating a nonlinear system of differential equations. We discussed linearization techniques and methods of analysis. These methods helped us understand why transmission kinetics should be modeled through ratio-dependent contact between free virions and tumor cells. Our model complements experimental results that suggest that initiation of virotherapy treatment can drive the system towards the cancer-free equilibrium.

Finally, we developed our full model using an age-structured PDE model. We introduced the method of characteristics, a method commonly used to solve basic hyperbolic PDEs. We then showed how we used this method to obtain results in our original work. We first showed that the PDE without virotherapy is identical to the DDE model. The method of characteristics was used in a more complicated example to show that the solutions of our full model remain nonnegative. As a last note, we showed how numerical simulations could further the discussion by allowing us to examine time trajectories and stability regions.

This work reveals how techniques and tools from differential equations can be used to develop and analyze models of oncolytic viruses. Each virus is unique and therefore different models will be needed to study the characteristics of each one. Here, we progressively developed a model of VSV using ordinary, delay, and partial differential equations and presented the necessary tools to build and analyze that particular model.

References

1. Au, G.G., Lindberg, A.M., Barry, R.D., Shafren, D.R.: Oncolysis of vascular malignant human melanoma tumors by Coxsackievirus A21. Int. J. Oncol. **26**(6), 1471–1476 (2005)
2. Bajzer, Z., Carr, T., Dingli, D., Josic, K.: Optimization of tumor virotherapy with recombinant measles virus. In: Lim, G.J., Lee, E.K. (eds.) Optimization in Medicine and Biology. Auerbach Publications, New York (2008)
3. Bajzer, Z., Carr, T., Josic, K., Russell, S.J., Dingli, D.: Modeling of cancer virotherapy with recombinant measles viruses. J. Theor. Biol. **252**(1), 109–122 (2008)
4. Balachandran, S., Barber, G.N.: Vesicular stomatitis virus (VSV) therapy of tumors. IUBMB Life **50**(2), 135–138 (2000)
5. Bell, J., Parato, K., Atkins, H.: Vesicular stomatitis virus. In: Harrington, K.J., Vile, R.G., Pandha, H.S. (eds.) Viral Therapy of Cancer. Wiley, Hoboken (2008)
6. Biesecker, M., Kimn, J.H., Lu, H., Dingli, D., Bajzer, Z.: Optimization of virotherapy for cancer. Bull. Math. Biol. **72**(2), 469–489 (2010)
7. Cain, J.W., Reynolds, A.M.: Ordinary and Partial Differential Equations. An Introduction to Dynamical Systems. Creative Commons. Virginia Commonwealth University, Richmond (2010)
8. Comins, C., Spicer, J., Protheroe, A., Roulstone, V., Twigger, K., White, C.M., Vile, R., Melcher, A., Coffey, M.C., Mettinger, K.L., Nuovo, G., Cohn, D.E., Phelps, M., Harrington, K.J., Pandha, H.S.: REO-10: a phase I study of intravenous reovirus and docetaxel in patients with advanced cancer. Clin. Cancer Res. **16**(22), 5564–5572 (2010)
9. Crivelli, J.J., Foldes, J., Kim, P.S., Wares, J.R.: A mathematical model for cell cycle-specific cancer virotherapy. J. Biol. Dyn. **6**(Suppl 1), 104–120 (2012)
10. Dingli, D., Peng, K.W., Harvey, M.E., Greipp, P.R., O'Connor, M.K., Cattaneo, R., Morris, J.C., Russell, S.J.: Image-guided radiovirotherapy for multiple myeloma using a recombinant measles virus expressing the thyroidal sodium iodide symporter. Blood **103**(5), 1641–1646 (2004)
11. Friedman, A., Tian, J.P., Fulci, G., Chiocca, E.A., Wang, J.: Glioma virotherapy: effects of innate immune suppression and increased viral replication capacity. Cancer Res. **66**(4), 2314–2319 (2006)
12. Heise, C., Sampson-Johannes, A., Williams, A., McCormick, F., Von Hoff, D.D., Kirn, D.H.: ONYX-015, an E1B gene-attenuated adenovirus, causes tumor-specific cytolysis and antitumoral efficacy that can be augmented by standard chemotherapeutic agents. Nat. Med. **3**(6), 639–645 (1997)
13. Komarova, N.L., Wodarz, D.: ODE models for oncolytic virus dynamics. J. Theor. Biol. **263**(4), 530–543 (2010)
14. Liu, W., Hillen, T., Freedman, H.I.: A mathematical model for M-phase specific chemotherapy including the G0-phase and immunoresponse. Math. Biosci. Eng. **4**(2), 239–259 (2007)
15. Novozhilov, A.S., Berezovskaya, F.S., Koonin, E.V., Karev, G.P.: Mathematical modeling of tumor therapy with oncolytic viruses: regimes with complete tumor elimination within the framework of deterministic models. Biol. Direct **1**, 6 (2006)
16. Oliere, S., Arguello, M., Mesplede, T., Tumilasci, V., Nakhaei, P., Stojdl, D., Sonenberg, N., Bell, J., Hiscott, J.: Vesicular stomatitis virus oncolysis of T lymphocytes requires cell cycle entry and translation initiation. J. Virol. **82**(12), 5735–5749 (2008)
17. Park, B.H., Hwang, T., Liu, T.C., Sze, D.Y., Kim, J.S., Kwon, H.C., Oh, S.Y., Han, S.Y., Yoon, J.H., Hong, S.H., Moon, A., Speth, K., Park, C., Ahn, Y.J., Daneshmand, M., Rhee, B.G., Pinedo, H.M., Bell, J.C., Kirn, D.H.: Use of a targeted oncolytic poxvirus, JX-594, in patients with refractory primary or metastatic liver cancer: a phase I trial. Lancet Oncol. **9**(6), 533–542 (2008)
18. Pecora, A.L., Rizvi, N., Cohen, G.I., Meropol, N.J., Sterman, D., Marshall, J.L., Goldberg, S., Gross, P., O'Neil, J.D., Groene, W.S., Roberts, M.S., Rabin, H., Bamat, M.K., Lorence, R.M.: Phase I trial of intravenous administration of PV701, an oncolytic virus, in patients with advanced solid cancers. J. Clin. Oncol. **20**(9), 2251–2266 (2002)

19. Perko, L.: Differential Equations and Dynamical Systems, 3rd edn. Springer, New York (2001)
20. Reddy, P.S., Burroughs, K.D., Hales, L.M., Ganesh, S., Jones, B.H., Idamakanti, N., Hay, C., Li, S.S., Skele, K.L., Vasko, A.J., Yang, J., Watkins, D.N., Rudin, C.M., Hallenbeck, P.L.: Seneca Valley virus, a systemically deliverable oncolytic picornavirus, and the treatment of neuroendocrine cancers. J. Natl. Cancer Inst. **99**(21), 1623–1633 (2007)
21. Todo, T., Martuza, R.L., Rabkin, S.D., Johnson, P.A.: Oncolytic herpes simplex virus vector with enhanced MHC class I presentation and tumor cell killing. Proc. Natl. Acad. Sci. USA **98**(11), 6396–6401 (2001)
22. Villasana, M., Radunskaya, A.: A delay differential equation model for tumor growth. J. Math. Biol. **47**(3), 270–294 (2003)
23. Wodarz, D.: Computational approaches to study oncolytic virus therapy: insights and challenges. Gene Ther. Mol. Biol. **8**, 137–146 (2004)
24. Wodarz, D., Komarova, N.: Towards predictive computational models of oncolytic virus therapy: basis for experimental validation and model selection. PLoS ONE **4**(1), e4271 (2009)
25. Wu, J.T., Byrne, H.M., Kirn, D.H., Wein, L.M.: Modeling and analysis of a virus that replicates selectively in tumor cells. Bull. Math. Biol. **63**(4), 731–768 (2001)