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Mathematical Analysis for Oncolytic Virotherapy, Considering the Role of the Lytic Cycle in the Presence of Immune System Response

Hajimohammad Mohammadinejad[1],
Saeed Jani[2] and Omid RabieiMotlagh[3]
[1,2,3] Department of Mathematics, University of Birjand, Iran

Abstract. The immune system of the cancer patient's body and the viral lytic cycle play important roles in cancer virotherapy. Most mathematical models for virotherapy do not include these two agents simultaneously. In this paper, based on clinical observations we propose a mathematical model including the time of the viral lytic cycle, the viral burst size, and the immune system response. The proposed model is a nonlinear system of delay differential equations in which the period of the viral lytic cycle is modeled as a delay parameter and is used as the bifurcation parameter. We analyze the stability of equilibrium points and the existence of Hopf bifurcation and obtain some conditions for the stability of equilibrium points in terms of the burst size and delay parameter. Finally, we confirm the results with a numerical example and describe them from a biological point of view.

Keywords: Delay differential equation; Hopf bifurcation; Stability.

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[1]Corresponding author: hmohammadin@birjand.ac.ir
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1. Introduction

Cancer is one of the main causes of death in the world today and the second most fatal disease after cardiovascular disease. The World Health Organization (WHO) estimates that the annual cancer-induced mortality number exceeds six million people hence the fight against cancer is one of the most important public health interests [**?**]. The usual therapies of cancer are surgery, radiotherapy, chemotherapy, and immunotherapy (For a history of cancer therapy see [**?**, **?**]). Although cancer treatment has progressed in many ways, some specific forms of cancer still have very limited treatment options. Many tumors are completely untreatable and so require a wider set of treatments. Oncolytic virotherapy is a novel therapeutic idea to treat cancer. The overall strategy in oncolytic virus therapy is to infect the tumor with specific viruses that kill the tumor cells but ignore the normal cells. For more than a hundred years, viruses had been pursued as experimental agents of cancer destruction. Interest in the field has fluctuated during this time. Some results were reached in the early 20th century and then due to technological limitations, followed by near-abandonment in the 1970s and 1980s. Now that genetic engineering and virology have advanced rapidly over the past two decades, the interest in oncolytic virus therapy has been revisited[**?**]. When oncolytic viruses are inoculated into a cancer patient or directly injected into a tumor, they spread throughout the tumor, and infect tumor cells. The viruses that are in the infected tumor cells replicate themselves. Upon lysis of infected tumor cells, new virion particles burst out and proceed to infect additional tumor cells. Oncolytic viruses have two types: oncolytic wild viruses and gene-modified viruses. Oncolytic wild viruses that naturally occur with preferential in human cancer cells include the parvoviruses H-1, Vesicular Stomatitis Virus (VSV), Newcastle Disease Virus (NDV), Coxsackievirus A21, etc. While gene-modified oncolytic viruses include Adenovirus, Herpes simplex, Vaccinia virus, etc.(Some of the other oncolytic viruses introduced in [**?**]). Moreover, a large number of viruses is being tested for potential as oncolytic viruses [**?**, **?**].

Although virotherapy is low-cost and elite in comparison to other classical treatments of cancer, it has not yet lived up to its expectations. The reason maybe is that different factors influence virotherapy. One of the major problems in virotherapy is the replicative ability of oncolytic viruses within tumor cells [**?**]. However genetic engineering has made it possible to modify the viral genome to improve the replicative ability of oncolytic viruses. Another fundamental problem in cancer research is to understand the complex dynamics of the interaction between the tumor and the anti-tumor elements of the body's immune response. Immune

responses are largely of two types: the innate immune response and the adaptive immune response. Broadly they are of two types, the antibodies, and the killer cells. While the antibodies fight foreign elements like viruses, bacteria, etc. By recognizing foreign proteins outside the cells, the killer cells recognize the mutated proteins of the cancer cells on display on cell walls. These killer cells are also known as cytotoxic T-lymphocytes (CTL). After the recognition, the CTLs undergo a process of cell division. Then they release certain molecules, perforin for instance, which kills cancer affected cells. The process is called reactivity. It is also possible that the CTLs fail to recognize the mutated proteins as different from normal proteins of the body. In this case, the phenomena of tolerance are said to take place. It is believed that the progress of cancer is in the period when the adaptive immune system exhibits tolerance [**?**]. Another challenge is that when an oncolytic virus is injected into a tumor for the first time, the immune system makes a memory of it and in the next injections the immune system distinguishes the virus and starts an immunity mechanism by CTLs to suppress the infection. Indeed, the influence of the immune system at the second injection of the virus is much better in comparison with that of the first injection [**?**]. Thus, it is a real problem in the second injection that the immune system annihilates the viral infection before the virus can annihilate the tumor.

On the other hand from [**?**] we know that at the molecular level, the lytic cycle of a virus has six stages. To infect a cell, a virus must enter the cell through the plasma membrane. The virus attaches to a receptor on the cell membrane and then releases its genetic materials into the cell. These are the first two stages, called adsorption and penetration. The third stage is the integration that the host cell gene expression is arrested, and viral materials are embedded into the host cell nucleus. The fourth stage is biosynthesis that the virus uses the cell machinery to make a large number of viral components, and in the meantime, destroys the host's DNA. Then, it enters the last two stages, maturation and lysis. When many copies of viral components are made, they are assembled into complete viruses. The number of newly formed viruses is called the burst size of the virus. These phages direct the production of enzymes that break down the host cell membrane. The cell eventually bursts, and new viruses come out. During the lytic cycle, each stage is mediated by a diverse group of proteins, and each stage needs some time to complete. Overall, the burst size and the time of the intracellular viral life cycle are important factors in viral therapy. So different agents can influence the outcomes of virotherapy.

Although a clear picture of the dynamic of virotherapy seems hard to

obtain, mathematical modeling can help us to understand such a complicated process. To model the virotherapy process, researchers use ordinary differential equations, delayed differential equations, and partial differential equations. Each presented mathematical model studied the effects of some special factors influence in virotherapy. One of the first of these models was presented by Wodarz [**?**, **?**]. He formulated a simple model with three ordinary differential equations including three hypothetical situations: viral cytotoxicity alone kills tumor cells, a virus-specific lytic CTL response contributes to killing of infected tumor cells, and the virus elicits immunostimulatory signals within the tumor, which promote the development of tumor-specific CTL. Bajzer et al. proposed a mathematical model for recombinant measles viruses. Their model includes the free virus population besides the tumor cell population and infected tumor cell populations [**?**]. Based on the Bajzers model, in [**?**] Tian proposed the following model for virotherapy

$$\begin{cases} \frac{dx}{dt} = \lambda x(1 - \frac{x+y}{K}) - \beta xv \\ \frac{dy}{dt} = \beta xv - \delta y \\ \frac{dv}{dt} = b\delta y - \beta xv - \gamma v \end{cases} \tag{1.1}$$

where x, y and v represent uninfected tumor cell ,infected tumor cell, and virus population respectively. He found that the viral burst size plays an important role in the dynamics of oncolytic treatments. Choudhury and Nasipuri [**?**] considered a simple model of three ordinary differential equations for the dynamics of oncolytic virotherapy in the presence of immune response. However, this model did not include the free virus population, and it may not give a complete picture of the dynamics of viral therapy with innate immune response.

In this paper we consider the role of lytic cycle of oncolytic viruses and the immune system in virotherapy. In our study based on the common basic model given [**?**] we propose a mathematical model for the dynamics of cancer virotherapy. As we mention, the population of viruses and the immune system are the factors that affect the outcome of virotherapy, so it is necessary and realistic to incorporate the lytic cycle and immune system responding, in the original model. In our model, we describe the competition between tumor cells, immune system, and oncolytic viruses. We consider the effect of the immune system in the role of virus-specific CTLs. The model we propose is given by

$$\begin{cases} \frac{dx}{dt} = rx(1 - \frac{x}{C}) - \beta xv \\ \frac{dv}{dt} = b\delta x(t - \tau) - \beta xv - dvz - ev \\ \frac{dz}{dt} = pvz - qz. \end{cases} \tag{1.2}$$

The first equation represents the change of the tumor population. The tumor cells growth is modeled by common logistic growth, $rx(1-\frac{x}{C})$ where C is maximal tumor size. The term βxv represents the infection of tumor cells by viruses. The second equation of (**??**) gives the change in the population of the new viruses which are coming out from the lysis of infected tumor cells. In fact, we simulated the population of newborn viruses by this idea: a part of tumor cells(which we denote it by coefficient δ) is infected by viruses and after time τ the lytic cycle of viruses is completed and new viruses are born. So we take the discrete time delay for the lytic cycle of the virus in the second equation. b is burst size of the virus. The term dvz represents the killing of viruses by virus-specific CTLs. e is the clearance rate of the virus. The third equation describes the population of virus-specific CTLs. We assumed that the production of CTLs depends on both the population of viruses and CTL cells. So we modeled activation of CTLs by pvz. q is death rate of CTLs. However for the simplicity of mathematical analysis, we neglect the infected tumor cells from the original model, but the term $\delta x(t-\tau)$ implicitly simulates the infected tumor cells population. The advantage of this model over other presented models is that we consider the role of the immune system and the lytic virus cycle in the dynamics of viral therapy simultaneously. So this model is more realistic than others. By using the following notations

$$\bar{x}=\frac{x}{C},\quad \bar{v}=\frac{v}{C},\quad \bar{z}=\frac{z}{C},\quad \bar{\beta}=\beta C,\quad \bar{d}=dC,\quad \bar{p}=pC,\quad \bar{b}=\delta b$$

and then dropping the over bar, system (**??**) can be written as

$$\begin{cases} \frac{dx}{dt}=rx(1-x)-\beta xv \\ \frac{dv}{dt}=bx(t-\tau)-\beta xv-dvz-ev \\ \frac{dz}{dt}=pvz-qz. \end{cases} \tag{1.3}$$

This paper is organized as follows. We will provide some mathematical analysis for the model (**??**) in the absence of delay in the next section. This analysis includes positivity of solutions, existence periodic solutions, calculating equilibria and determining conditions for their stability in terms of burst size b . In section (**??**) we will study the delayed model. We determine the stability of equilibrium solutions and conditions that the system undergoes Hopf bifurcation in terms of delay value. To validate our analytical results, we present a numerical simulation and biological arguments in section (**??**).

2. Preliminary results

In this section, we present some common analysis of the model in the absence of delay which will be used in the next section for the original

model. When $\tau = 0$ we have the following system.

$$\begin{cases} x' = rx(1-x) - \beta xv \\ v' = bx - \beta xv - dvz - ev \\ z' = pvz - qz. \end{cases} \tag{2.1}$$

First, we state the following lemma for the solutions of the system (**??**), which shows that the solutions are non-negative. Boundedness of the solutions has proved in the next section for the general case. In fact the set

$$\Omega^+ = \{(x, v, z) | x \geq 0, v \geq 0, z \geq 0\} \subset R^3$$

is positively invariant for the system.

Lemma 2.1. *Suppose that* $(x(t), v(t), z(t))$ *be a solution of system (**??**) and* $x(0) > 0, v(0) > 0, z(0) > 0$. *Then* $x(t) \geqslant 0, v(t) \geqslant 0$ *and* $z(t) \geqslant 0$ *for all* $t \geq 0$.

Proof. If the conclusion $x(t) \geqslant 0, v(t) \geqslant 0$ and $z(t) \geqslant 0$ for all $t \geq 0$ is not true, there must be a time t_1, such that there is at least one component that will be zero first. If $x(t_1) = 0$ first, then $x'(t_1) = 0$. From the first equation, by the uniqueness of the solution we know $x(t) = 0$ for all $t \geqslant t_1$. Then the second equation becomes $v'(t) = -dvz - ev$. Its solution is $v(t) = v(t_1)e^{\int_{t_1}^{t}(-dz-e)ds}$. So for all $t \geq t_1$, $v(t) > 0$ since $v(t_1) > 0$. Similarly from the third equation we have $z'(t) = (pv - q)z$ which has the solution $z(t) = z(t_1)e^{\int_{t_1}^{t}((pv-q)ds}$. Since $z(t_1) > 0$, $z(t) > 0$ for all $t \geqslant t_1$.
If $v(t_1) = 0$ first, from the second equation $v'(t_1) = bx(t_1) > 0$. So $v(t) \geqslant 0$ after t_1. In this case it is clear that $x(t) \geqslant 0$ and $z(t) \geqslant 0$.
Finally if $z(t_1) = 0$ first, then from the third equation and uniqueness of the solution, $z(t) = 0$ when $t \geqslant t_1$. If $z(t) = 0$ the second equation becomes $v'(t) = bx - \beta xv - ev$ which is first order linear differential equation and has the solution $v(t) = e^{-\int_{t_1}^{t}(\beta x+e)ds}(v(t_1) + \int_{t_1}^{t} bxe^{\int(\beta x+e)d\tau}ds)$. Since $v(t_1) > 0$ and $x(t) \geqslant 0$ then $v(t) \geqslant 0$ when $t \geqslant t_1$. □

In the continuation of this section, we calculate the fixed points of the system. Then we present the conditions for their stability and describe their stability from a biological point of view.

2.1. **Stability of the fixed points.** In this study we assume that growth rate of tumor is bigger than infection rate and activation rate of CTLs is bigger than the death rate of CTLs. So $\beta < r$ and $q < p$. These assumptions are biologically reasonable. It is clear that $E_0 = (0, 0, 0)$ is a fixed point of this system. To obtain other fixed points, from the third equation we have $pvz - qz = 0$. So $z = 0$ or $v = \frac{q}{p}$. If $v = \frac{q}{p}$ and $x \neq 0$,

from the first equation we have $x = 1 - \frac{\beta v}{r}$ and by the second equation $z = \frac{bx-\beta xv-ev}{dv}$. Then we get

$$x^* := 1 - \frac{\beta q}{pr}, \qquad v^* := \frac{q}{p}, \qquad z^* := \frac{p}{qd}((b - \frac{\beta q}{p})(1 - \frac{\beta q}{pr}) - \frac{eq}{p})$$

On the other hand if we assume that $x \neq 0$ and $z = 0$, then by the first equation $v = \frac{r}{\beta}(1 - x)$. By replacing in the second equation we get the the equation

$$-rx^2 + (r - b - \frac{er}{\beta})x + \frac{er}{\beta} = 0.$$

Since the parameters are positive this equation has one positive solution for x as:

$$\overline{x} = \frac{r - b - \frac{er}{\beta} + \sqrt{(r - b - \frac{er}{\beta})^2 + 4r^2\frac{e}{\beta}}}{2r}.$$

As we assumed that $\beta < r$ and $q < p$, so x^* and v^* are positive. If we assumed $b > \frac{\beta q}{p} + \frac{eqr}{pr-\beta q}$, then z^* is positive. In the other word when the burst size is bigger than the certain value $b^* := \frac{\beta q}{p} + \frac{eqr}{pr-\beta q}$, then we have a new positive fixed point. Furthermore we see that $\overline{x}$ is positive and

$$\frac{r-b-\frac{er}{\beta}+\sqrt{(r-b-\frac{er}{\beta})^2+4r^2\frac{e}{\beta}}}{2r} < \frac{r-b-\frac{er}{\beta}+\sqrt{(r+b+\frac{er}{\beta})^2}}{2r} = \frac{2r}{2r} = 1.$$

This implies that $\overline{x} < 1$ and then $\overline{v} = \frac{r}{\beta}(1-\overline{x}) > 0$. So this fixed points have positive components and are biologically valid. We summarize this argument in the next lemma.

Lemma 2.2. *Suppose $\beta < r$, $q < p$ and $b > b^*$. Then system has three non-negative fixed points: $E_0 = (0, 0, 0), \overline{E} = (\overline{x}, \overline{v}, 0)$ and $E^* = (x^*, v^*, z^*)$, where $\overline{x}, \overline{v}, x^*, v^*$ and z^* are given as below:*

$$\overline{x} = \frac{r - b - \frac{er}{\beta} + \sqrt{(r - b - \frac{er}{\beta})^2 + 4r^2\frac{e}{\beta}}}{2r}, \qquad \overline{v} = \frac{r(1 - x)}{\beta},$$

$$x^* = 1 - \frac{\beta q}{pr}, \quad v^* = \frac{q}{p}, \quad z^* = \frac{p}{qd}((b - \frac{\beta q}{p})(1 - \frac{\beta q}{pr}) - \frac{eq}{p}).$$

To determine the stability of the fixed points we use the variational matrix of the system which is given by

$$\begin{pmatrix} r - 2rx - \beta v & -\beta x & 0 \\ b - \beta v & -\beta x - dz - e & -dv \\ 0 & pz & pv - q \end{pmatrix}.$$

So the variational matrix of system in E_0 is

$$\begin{pmatrix} r & 0 & 0 \\ b & -e & 0 \\ 0 & 0 & -q \end{pmatrix}.$$

Since the eigenvalue of matrix are $r, -e, -q$ and $r > 0$, the fixed point E_0 is unstable.

Lemma 2.3. *Suppose that* $K_1 = \frac{2\beta q}{p} - \frac{er}{\beta} - r$, $K_2 = (\frac{q}{\beta q - pr})(\frac{\beta^2 q}{p} - r\beta - er)$ *and* $K_3 = max\{K_1, K_2\}$. *If* $b > K_3$, *then* $\overline{E}$ *is asymptotically stable. Otherwise* $\overline{E}$ *is unstable when* $K_1 < b < K_2$.

Proof. The variational matrix in the fixed point $\overline{E}$ is given by

$$\begin{pmatrix} r - 2r\overline{x} - \beta\overline{v} & -\beta\overline{x} & 0 \\ b - \beta\overline{v} & -\beta\overline{x} - e & -d\overline{v} \\ 0 & 0 & p\overline{v} - q \end{pmatrix}.$$

On the other hand,

$$r - 2r\overline{x} - \beta\overline{v} = -r\overline{x} \quad \beta\overline{x} + e = \frac{b\overline{x}}{\overline{v}} \quad \beta\overline{v} - b = -\frac{e\overline{v}}{\overline{x}}.$$

So the characteristic polynomial is given by

$$f(\lambda) = (\lambda + q - p\overline{v})(\lambda^2 + (b\frac{\overline{x}}{\overline{v}} + r\overline{x})\lambda + (\frac{b\overline{x}}{\overline{v}})(r\overline{x}) + \beta e\overline{v}).$$

We take $a_0 = b\frac{\overline{x}}{\overline{v}} + r\overline{x}$ and $a_1 = (\frac{b\overline{x}}{\overline{v}})(r\overline{x}) + \beta e\overline{v}$. Because the parameters, $\overline{x}$ and $\overline{v}$ are positive, we conclude that $a_0 > 0$ and $a_1 > 0$. Therefore by the Routh-Hurwitz Criterion, all roots of the polynomial $\lambda^2 + a_0\lambda + a_1$ have negative real part. So stability of $\overline{E}$ is determined by the sign of $p\overline{v} - q$. We know that if $p\overline{v} - q < 0$ then $\overline{E}$ asymptotically stable. $p\overline{v} - q < 0$ is equivalent to:

$$\sqrt{(r - b - \frac{er}{\beta})^2 + \frac{4er^2}{\beta}} < r + b + \frac{er}{\beta} - \frac{2\beta q}{p}.$$

Solving this inequality (note that $\beta q < pr$ and we suppose that $b > \frac{2\beta q}{p} - \frac{er}{\beta} - r$) in term of b, we get $b > (\frac{q}{\beta q - pr})(\frac{\beta^2 q}{p} - r\beta - er)$. Otherwise if

$$b < (\frac{q}{\beta q - pr})(\frac{\beta^2 q}{p} - r\beta - er),$$

then $p\overline{v} - q > 0$ and so $f(\lambda)$ has a positive root which means that $\overline{E}$ is unstable. □

Stability of fixed point E^*

When the burst size of virus (b)is bigger than value b^* then system has

fixed point E^* with non-negative components. The variational matrix of system in E^* is given by

$$\begin{pmatrix} -rx^* & -\beta x^* & 0 \\ b-\beta v^* & \frac{bx^*}{v^*} & -dv^* \\ 0 & pz^* & 0 \end{pmatrix}.$$

The characteristic equation in E^* is $f(\lambda)=\lambda^3+a_1\lambda^2+a_2\lambda+a_3$ where:

$$a_1=-(A+D), \qquad a_2=AD-EF-BC, \qquad a_3=AEF,$$

$$A=\frac{\beta q-pr}{p}, \qquad B=\frac{\beta}{pr}(\beta q-pr) \qquad C=\frac{bp-\beta q}{p},$$

$$D=\frac{b}{qr}(\beta q-pr), \qquad E=\frac{-qd}{p} \qquad F=\frac{p^2}{qd}((b-\frac{\beta q}{p})(1-\frac{\beta q}{pr})-\frac{eq}{p}).$$

By the Routh-Hurwitz ceriterion, all roots of $f(\lambda)$ have negative real parts if and only if:

$$\mathbf{H_1}=\left|a_1\right|>0, \quad \mathbf{H_2}=\begin{vmatrix} a_1 & a_3 \\ 1 & a_2 \end{vmatrix}>0, \quad \mathbf{H_3}=\begin{vmatrix} a_1 & a_3 & 0 \\ 1 & a_2 & 0 \\ 0 & a_1 & a_3 \end{vmatrix}=a_3H_2>0.$$

Because $F=pz^*>0$ and we assumed that $b>b^*$, $r>\beta$ and $p>q$ so

$$a_1=-(A+D)=-(\beta q-pr)(\frac{1}{p}+\frac{b}{qr})>0,$$

$$a_3=AEF=(\frac{\beta q-pr}{p})(-\frac{dq}{p})(\frac{p^2}{qd}((b-\frac{\beta q}{p})(1-\frac{\beta q}{pr})-\frac{eq}{p})>0.$$

So all roots of $f(\lambda)$ have negative real parts if and only if $a_1a_2-a_3>0$. If we take $\Phi(b)=a_1a_2-a_3$, then

$$\Phi(b)=\frac{pr-\beta q}{p^3q^2r^2}(\xi_1b^2+\xi_2b+\xi_3), \tag{2.2}$$

where

$$\xi_1=p^4qr+p^4r^2-p^3q^2\beta-p^3qr\beta, \quad \xi_3=q^4\beta^3r-pq^3r^2\beta^2,$$

$$\xi_2=-pq(p^2qre-(pr-\beta q)(p(r^2-\beta q)-\beta^2q)). \tag{2.3}$$

So all roots of $f(\lambda)$ have negative real parts if and only if $\Phi(b)>0$. Now we state the following theorem for the stability of E^*.

Theorem 2.4. *Suppose that $b>b^*$ and Φ,ξ_1,ξ_2 and ξ_3, are defined by* **??** *and* **??**. *Then the following statements are valid.*

I). If $\xi_1>0$, then there exist a value $\overline{b}>0$, such that E^* is asymptotically stable for $b>\overline{b}$.
II). If $\xi_1<0$ and $\xi_2>0$, there exist an interval $I\subset R$, which E^* is

asymptotically when $b \in I$.
III). If $\xi_1 < 0$ *and* $\xi_2 < 0$, E^* *is unstable for any* $b > 0$.

Proof. Since we assumed that $r > \beta$ and $p > q$ so $\xi_3 < 0$ and $\frac{pr-\beta q}{p^3q^2r^2} > 0$. Now

$$\xi_2^2 - 4\xi_1\xi_3 = p^2q^2[4pqr\beta^2(q+r)(pr-\beta q) + (p^2qre - (pr-\beta q)(p(r^2 - \beta q) - \beta^2 q))^2] > 0,$$

which implies $\Phi(b) = 0$ has two real roots. If $\xi_1 > 0$, then $\Phi(b) = 0$ has a unique positive root $\bar{b}$. So when $b > max\{\bar{b}, b^*\}$, then $\Phi(b) > 0$ therefore all roots of $f(\lambda)$ have negative real parts which implies that E^* is asymptotically stable. If $\xi_1 < 0$ and $\xi_2 > 0$ then $\Phi(b)$ has two positive roots $0 < b_1 < b_2$. If $b^* < b_1$ then for $b \in (b_1, b_2)$, $\Phi(b) > 0$ and therefore E^* is asymptotically stable. If $b_1 < b^* < b_2$, then for $b \in (b^*, b_2)$, $\Phi(b) > 0$ and so E^* is asymptotically stable. Otherwise if $\xi_1 < 0$ and $\xi_2 < 0$, then for any $b > 0$, $\Phi(b) < 0$. The above discussion concludes that E^* is unstable for any $b > 0$. □

2.2. **Existence periodic solutions.** We employ the Bendixon-Dulac criterion for systems as follow. Define

$$f_1 = rx - rx^2 - \beta xv, \quad f_2 = bx - \beta xv - dvz - ev, \quad f_3 = pvz - qz.$$

So the system can be written as following form

$$\begin{cases} \frac{dx}{dt} = f_1(x, v, z) \\ \frac{dv}{dt} = f_2(x, v, z) \\ \frac{dz}{dt} = f_3(x, v, z). \end{cases}$$

Firs we take $N_1 = \frac{1}{xv}$. So we have

$$L_1 = \frac{\partial}{\partial x}(N_1 f_1) + \frac{\partial}{\partial v}(N_1 f_2) = -(\frac{r}{v} + \frac{b}{v^2}) < 0.$$

Similarly by $N_2 = \frac{1}{xz}$, we get

$$L_2 = \frac{\partial}{\partial x}(N_2 f_1) = \frac{\partial}{\partial z}(N_2 f_3) = -\frac{r}{z} < 0.$$

And by $N_3 = \frac{1}{vz}$,

$$L_3 = \frac{\partial}{\partial v}(N_3 f_2) + \frac{\partial}{\partial z}(N_3 f_3) = -\frac{bx}{zv^2} < 0.$$

Now the Bendixon-Dulac criterion satisfies, so there is no limit cycle or homoclinic connections for the system under consideration.

3. Analysis of delayed model

In this section we study behavior of the solutions of the presented model. Fixed points and their stability will be considered. Because from the biological view E^* has more importance we study Hopf bifurcation occurrence in E^*. In fact, the asymptotical stability of E^* means that we can control the size of the tumor and it is an important part of cancer therapy. Also, the Hopf bifurcation means biologically that the tumor will relapse exactly when it seems that the therapy is successful.
As our model is a system of delay differential equations first we show that this model can be considered as functional differential equation. Consider the delay differential equation

$$\dot{x} = g(x(t), x(t-r)), \tag{3.1}$$

where $r \geq 0$ is a real number. Suppose that $C = C([-r, 0], R^n)$ is the Banach space of continuous functions defined on the interval $[-r, 0]$ with value in R^n with the usual supremum norm. Let $\sigma \in R$, $A > 0$ and let $x \in C([\sigma - r, \sigma + A], R^n)$. For any $t \in [\sigma, \sigma + A]$, let x_t denote element of C defined by $x_t(\theta) = x(t + \theta)$ for $\theta \in [-r, 0]$. Now let D be an open subset of C and $f : D \longrightarrow R^n$ be a given function. the equation

$$\dot{x} = f(x_t) \tag{3.2}$$

(where the dot denotes the right-hand derivative) is called a functional differential equation. Obviously if we take

$$f(\phi) = g(\phi(0), \phi(-r)), \qquad \phi \in C([-r, 0], R^n,$$

then Eq. **??** is special case of Eq. **??**. So any delay differential equation can be considered as a functional differential equation [**?**].
Based on the [**?**] the existence and uniqueness of the solutions of the Eq.**??** is guaranteed according to the uniqueness and existence theorems. So we focus on the positivity and boundedness of the solutions of Eq. **??** . The next lemma guarantees that the solutions of (**??**) remain in the feasible region.

Lemma 3.1. *Suppose that $(x(t), v(t), z(t))$ is a solution of system (**??**). If $x(0) \geqslant 0, v(0) \geqslant 0, z(0) \geqslant 0$, then $x(t) \geqslant 0, v(t) \geqslant 0, z(t) \geqslant 0$ for all $t \geqslant 0$. Furthermore if $0 < x(0) < 1$,$0 < v(0) < 1$ and $0 < z(0) < 1$ then $0 < x(t) < 1$, $\limsup_{t\to\infty} v(t) < \frac{b}{e}$ and $z(t)$ remains bounded.*

Proof. From the first equation of (**??**) we get $x(t) = x(0)e^{\int_0^t (r - rx(s) - \beta v(s))ds}$. Since $x(0) \geqslant 0$, it implies that $x(t) \geqslant 0$ for $t \geqslant 0$.
Similarly from third equation we have $z(t) = z(0)e^{\int_0^t (pv(s) - q)ds}$. Since we take $z(0) \geqslant 0$, so $z(t) \geqslant 0$ for $t \geqslant 0$. The second equation implies

that

$$v(t) = e^{-\int_0^t(\beta x(s-\tau)+dz(s)+e)ds}[v(0)+b\int_0^t x(s-\tau)e^{\int_0^s(\beta x(u-\tau)+dz(u)+e)du}ds].$$

Because $v(0) \geqslant 0$ and $x(t) \geqslant 0$, so $v(t) \geqslant 0$ for $t \in [0, \tau]$. By induction, we have $v(t) \geq 0$ for $t \geq 0$.

Furthermore suppose that $0 < x(0) < 1$, $0 < v(0) < 1$ and $0 < z(0) < 1$. Because the initial values of each component of solutions is non-negative, so the solutions remain non-negative. We have $x'(t) = rx(1-x)-\beta xv \leqslant rx(1-x)$ and $x(0) \leqslant 1$. So by the comparison theorem for ODEs we get $x(t) \leqslant 1$. Similarly because $x(t) \leqslant 1$, $v'(t) = bx(t-\tau)-\beta xv-dvz-ev \leqslant bx - ev \leqslant b - ev$. So by the comparison theorem , we get $v(t) \leqslant \frac{b}{e} + v(0)exp(-et)$. Taking lim sup both sides yield $\limsup_{t\to\infty} v(t) \leqslant \frac{b}{e}$. Moreover z is bounded. Becuse if $z(t) \longrightarrow \infty$ as $t \longrightarrow \infty$, then as $x < 1$, so from second equation we get $v'(t) < b - dvz$. since $z \longrightarrow \infty$, so $v'(t) \longrightarrow -\infty$. Therefore there exists $t_1 > 0$ such that $pv - q < 0$ for $t > t_1$. Now from the third equation

$$z(t) = z(0)e^{\int_0^t(pv-q)ds} = z(0)[e^{\int_0^{t_1}(pv-q)ds+\int_{t_1}^t(pv-q)ds}].$$

Becuse for $t > t_1$, $pv - q < 0$, so $e^{\int_{t_1}^t(pv-q)ds} \leq 1$. Thus we get $z(t) \leq z(0)e^{\int_0^{t_1}(pv-q)ds} \leq z(0)M$, where $M = e^{\int_0^{t_1}(pv-q)ds}$. This is a contradiction since we assumed that $z \longrightarrow \infty$, so $z(t)$ remains in the bounded region. □

3.1. **Stability of E^* and Hopf bifurcation.** By the transformation $u_1 = x - x^*, u_2 = v - v^*, u_3 = z - z^*$, (**??**) is changed to

$$\frac{dU}{dt} = M_1U(t) + M_2U(t-\tau) + f(u_1, u_2, u_3)$$

where $U = (u_1, u_2, u_3)^T$,

$$\mathbf{f(u_1, u_2, u_3)} = \begin{pmatrix} -\beta u_1u_2 - ru_1^2 \\ -\beta u_1u_2 - du_2u_3 \\ pu_2u_3 \end{pmatrix}, \mathbf{M_1} = \begin{pmatrix} A & B & 0 \\ C-b & D & E \\ 0 & F & 0 \end{pmatrix},$$

$$\mathbf{M_2} = \begin{pmatrix} 0 & 0 & 0 \\ b & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix}$$

and A, B, C, D, E, F are same as the previous section. The characteristic equation corresponding to linearized system is $\xi(\lambda) = det(\lambda I - M_1 - M_2e^{-\lambda\tau}) = 0$. So the characteristic equation can be written as

$$\xi(\lambda) = \lambda^3 + m_1\lambda^2 + m_2\lambda + m_3 + n_1\lambda e^{-\lambda\tau} = 0, \tag{3.3}$$

$m_1 = -(A+D) \quad m_2 = AD+bB-BC-EF, \quad m_3 = AEF, \quad n_1 = -bB.$

Clearly, $i\omega(\omega > 0)$ is a root of $\xi(\lambda) = 0$ if and only if

$$-i\omega^3 - m_1\omega^2 + im_2\omega + m_3 + in_1\omega(\cos w\tau - i\sin\omega\tau) = 0.$$

Separating the real and imaginary part, we have

$$\begin{cases} -m_1\omega^2 + m_3 = -n_1\omega\sin\omega\tau \\ -\omega^3 + m_2\omega = -n_1\omega\cos\omega\tau. \end{cases}$$

Adding the squares of both equations together gives

$$\omega^6 + (m_1^2 - 2m_2)\omega^4 + (m_2^2 - 2m_1m_3 - n_1^2)\omega^2 + m_3^2 = 0. \tag{3.4}$$

We take

$$z = \omega^2, p = m_1^2 - 2m_2, q = m_2^2 - 2m_1m_3 - n_1^2, r = m_3^2.$$

So the equation (**??**) becomes

$$h(z) = z^3 + pz^2 + qz + r = 0. \tag{3.5}$$

Because $h(0) = m_3^2 > 0$ and $lim_{z\to-\infty}h(z) = -\infty$ so $h(z) = 0$ has at least one negative root. Suppose the equation (**??**) has positive roots. Without loss of generality, we assume that it has two positive roots, denoted by z_1 and z_2 respectively. So the equation (**??**) has two positive roots, say $\omega_1 = \sqrt{z_1}$ and $\omega_2 = \sqrt{z_2}$. Take

$$\tau_k^j = \frac{1}{\omega_k}\arccos[\frac{n_1\omega_k^4 - m_2n_1\omega_k^2}{n_1^2\omega_k^2}] + \frac{2\pi j}{\omega_k}, \quad k = 1, 2, \quad j = 1, 2, ... \tag{3.6}$$

then $\pm i\omega_k$ is a pair of purely imaginary roots of (**??**) with $\tau = \tau_k^j, k = 1, 2, j = 1, 2, ...$. Obviously $\lim_{j\to\infty}\tau_k^j = \infty$. Now we can define

$$\tau_0 = \tau_{k_0}^{j_0} = \min_j^k \tau_k^j, \quad \omega_0 = \omega_{k_0}. \tag{3.7}$$

For $\tau = 0$ the equation (**??**) becomes

$$\lambda^3 + m_1\lambda^2 + (m_2 + n_1)\lambda + m_3 = 0. \tag{3.8}$$

As we saw in the previous section because $m_1 > 0, m_3 > 0$, by the Routh-Hurwits criterion, all roots of (**??**) have negative real parts, if and only if $\Phi(b) > 0$. We take tree conditions:
(Q_1):$\xi_1 > 0$ and $b > max\{\bar{b}, b^*\}$.
(Q_2):$\xi_1 < 0$, $\xi_2 > 0$, $b^* < b_1$ and $b \in (b_1, b_2)$.
(Q_3): $\xi_1 < 0$, $\xi_2 > 0$, $b_1 < b^* < b_2$ and $b \in (b^*, b_2)$.
$b^*, \bar{b}, b_1, b_2$ are defined in the previous section. Because $r = m_3^2 \geqslant 0$, so from [**?**] we have the following lemma.

Lemma 3.2. *(see* [**?**]*). Suppose that one of conditions Q_1, Q_2 or Q_3 is true. And take $\bar{z}_1 = \frac{-p+\sqrt{\Delta}}{3}$. Then*
*(i) if $\Delta = p^2 - 3q < 0$, then all roots of the equation (**??**) have negative*

real part for all $\tau \geqslant 0$
(ii) if $\bar{z}_1 > 0$ *and* $h(\bar{z}_1) \leqslant 0$*, than all roots of equation (**??**) have negative real part when* $\tau \in [0, \tau_0)$*.*

Let $\lambda(\tau) = \alpha(\tau) + i\omega(\tau)$ be the root of the equation (**??**) satisfying $\alpha(\tau_0) = 0$ and $\omega(\tau_0) = \omega_0$, $z_0 = \omega_0^2$. To arise Hopf bifurcation we need $h'(z_0) \neq 0$. Suppose that $\bar{z}_1 = \frac{-p+\sqrt{p^2-3q}}{3}$, $\bar{z}_2 = \frac{-p-\sqrt{p^2-3q}}{3}$ be the roots of $h'(z) = 3z^2 + 2pz + q = 0$. If $\bar{z}_1 > 0$ and $h(\bar{z}_1) < 0$, then because $h(0) = m_3^2 > 0$ and $lim_{z\to-\infty} h(z) = -\infty$ so $\bar{z}_1$ and $\bar{z}_2$ are local minimum and maximum of $h(z)$ respectively. In this case it is clear that $h(z)$ has two distinct positive roots z_1, z_2 and moreover $h'(z_1) \neq 0, h'(z_2) \neq 0$.
Now we differentiate both sides of the equation (**??**) with respect to τ, and obtain

$$\{3\lambda^2 + 2m_1\lambda + m_2 + [n_1 - \tau n_1\lambda]e^{-\lambda\tau}\}\frac{d\lambda}{d\tau} = n_1\lambda^2 e^{-\lambda\tau}.$$

Solving for the derivative

$$(\frac{d\lambda}{d\tau})^{-1} = \frac{(3\lambda^2 + 2m_1\lambda + m_2)e^{\lambda\tau}}{n_1\lambda^2} + \frac{n_1 - \tau n_1\lambda}{n_1\lambda^2}.$$

Then

$$Sign\frac{dRe\lambda(\tau_0)}{d\lambda} = Sign\{Re(\frac{d\lambda}{d\tau})^{-1}|_{\tau=\tau_0}\} = Sign\{\frac{1}{n_1^2\omega_0^2}h'(z_0)\} \neq 0.$$

So the transversality condition holds and the system undergoes Hopf bifurcation at $\tau = \tau_0$. We state the results above as the next theorem.

Theorem 3.3. *Suppose that one of the conditions* Q_1, Q_2, Q_3 *holds.*
(i) If $\Delta = p^2 - 3q < 0$*, then all roots of equation (**??**) have negative real parts. So* E^**is asymptotically stable.*
(ii) If $\Delta = p^2 - 3q > 0, \bar{z}_1 > 0$ *and* $h(\bar{z}_1) < 0$*, then there exist a positive value* τ_0*, such that all roots of the equation (**??**) have negative real parts when* $\tau \in [0, \tau_0)$*. At* $\tau = \tau_0$*, the equation (**??**) has a pair of simple purely imaginary roots,* $\pm i\omega_0$*, and all other roots have negative real parts. Furthermore,*
$Sign\{\frac{d\alpha(\tau_0)}{d\tau}\} = Sign\{h'(z_0)\} \neq 0.$

4. Numerical simulation and conclusion

In this paper, we studied our proposed mathematical model and obtained some conditions for stability of E^* in terms of parameters. When E^* is asymptotical stable it means that the tumor size remains in controlled size and virotherapy may be successful. The biological importance of theorem (**??**) is that determines conditions such that E^* is asymptotically stable. If we use an oncolytic virus with a certain burst

FIGURE 1. solution of system (**??**) for initial values $x(0) = 0.16, v(0) = 0.85, z(0) = 13.328$.(a): E^* is asymptoticaly stable for $\tau = 25.2 < \tau_0$. (b):E^* is unstable when $\tau = 36.65 > \tau_0$

size such that provides part (i) of theorem, then we will have a good controlling on the size of the tumor; however, sometimes it is impossible. In this case, we may have a Hopf bifurcation around E^* which means that after a time, the tumor will relapse and therapy fails. As a result, it is better to avoid happening the first Hopf bifurcation at τ_0 . So we should use oncolytic viruses in which the time to complete the lytic cycle is in the interval $\tau \in [0, \tau_0)$. Thus the burst size and delay parameter must be controlled carefully. This may be possible by genetic engineering.

Now to demonstrate the model behavior, we choose a set of parameters based on the [**?**], and simulate the stability and Hopf bifurcation in E^*. We take $p = 0.04, q = 0.036, r = 0.1, e = 0.01, \beta = 0.09, b = 10, d = 0.16$. So the equilibrium point is $E^* = (x^*, v^*, z*) = (0.19, 0.9, 13.02)$. We obtain $h(z) = r + qz + pz^2 + z^3 = 2.03195 \times 10^{-6} - 0.022z + 4.30z^2 + z^3$, $\Delta = p^2 - 3q \approx 18.64 > 0, \bar{z}_1 \approx 0.0025 > 0$ and $h(\bar{z}_1) \approx -0.00002699 < 0$. $h(z)$ has two positive roots: $z_1 \approx 0.000092, z_2 \approx 0.005$. By (**??**) and (**??**), we get $\tau_0 \approx 31.659, \omega_0 = 0.0713, z_0 = 0.005$ and $h'(z_0) \approx 0.02 > 0$. So $Sign\frac{d\lambda(\tau_0)}{d\tau} = Signh'(z_0) > 0$. On the other hand $\xi_1 \approx 6.61504 \times 10^{-9} > 0$. So condition Q_1 holds and therefore by theorem (**??**), the positive equilibrium E^* is asymptotically stable when $\tau \in [0, 31.659)$. When

$\tau \approx 31.659$, the system undergoes Hopf bifurcation and E^* becomes unstable.

From the biological view, these results mean that, if the time to complete the lytic cycle of the virus be less than 31.659, the tumor remains in controlled size, while therapy can fail when $\tau > \tau_0$. We simulate a solution of system (**??**) in the figure (**??**) for initial values $x(0) = 0.16, v(0) = 0.85$ and $z(0) = 13.328$.

Note that we studied the immune system effect in the virus-specific CTLs role, however, one can study the role of tumor-specific CTLs in the outcome of virotherapy.

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