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# Slow-fast model and therapy optimization for oncolytic treatment of tumors

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## Abstract

The present work studies models of oncolytic virotherapy without space variable in which virus replication occurs at a faster time scale than tumor growth. We derive the asymptotic of a three-species slow-fast model and obtain a two-species dynamical system, where the variables are tumor cells and infected tumor cells. We fully characterize the behavior of this system depending on the various biological parameters. In a second part we address the modelling of virus injection and its expression in the two-species system, where the amount of virus is not explicitly a variable. We prove that the injection can be described by an instantaneous jump in the phase plane, where a certain amount of tumors cells is transformed instantly into infected tumor cells. This description allows to discuss qualitatively the timing of different injections in the frame of successive treatment strategies. This work is illustrated by numerical simulations. The timing and amount of injected virus may have counterintuitive optimal values, nevertheless the understanding is clear from the phase space analysis.

**Keywords:** oncolytic therapy, slow-fast system, boundary layer, asymptotic system

# 1 Introduction

Oncolytic virotherapy is an emerging cancer treatment. An oncolytic virus (OV) is a virus that has potential to kill selectively tumor cells. OVs can either be natural viruses, or be genetically engineered for that purpose. The key point is that OV replicates in a tumor cell before lysis, and therefore a larger number of virions are released to infect other cells. In the most favorable case, the tumor is cured before the virus is eliminated by the immune system. The action of OVs was reported as early as mid-twentieth century [1], and the first OV for treatment of head-and-neck cancer was approved in 2005 in China and then in 2015 by FDA and EMA for Herpes Simplex Virus (HSV)-based virotherapy for melanoma. To date, more than 1500 patients with cancer have been treated by virotherapy, with few clinical success and no severe adverse secondary events. However many questions remain open regarding the optimal parameters for an OV to achieve its objective, and the design and experimentation of OVs are an active field of research. More than 80 clinical trials were registered in the review [2].

Different treatment protocols and options are described in the review [3], where it is advocated that no consensus on optimal protocols has emerged due to the lack of comparative studies.

We believe that a deeper mathematical understanding of the behavior of the system would help research scientists and clinicians towards informed therapeutic decisions. The present work is a contribution in this direction.

Over the last two decades, many mathematical models have been proposed and a complete review is not possible here. The pioneering works in the domain are [4, 5] where population dynamics methods were adapted to the specific field of oncolytic therapy. The first reference does not consider a space variable—in other words the tumor and the viruses are assumed to be well-mixed—while the second reference assumes spherical symmetry, which reduces the domain to a one-dimensional space. The determination of fixed points, together with their stability analysis provides a first insight. A general framework was proposed in [6], and in most models an endemic fixed point with reduced tumor burden is present. The bifurcation study of the endemic fixed point was performed in numerous studies with different variants of the models, see e.g. [7, 8], or [9] in the case of macrophage-tumor interaction. A detailed study of the early infection and the comparison with experimental data was proposed in [10].

The use of space variables leads to systems of partial differential equations that can be investigated numerically, but theoretical insights are more difficult to obtain. In [11] a stochastic model is simulated on a 2D lattice, and extensive numerical simulations guide the discussion towards the most desirable virus parameters. The key parameters describe the spatial spreading properties and the oncolytic activity. A counterintuitive conclusion is that if the virus is too aggressive (i.e., tumor cell lysis is fast) the treatment may fail, and an optimal choice of parameters would combine fast spreading and slow lysis. The fact that an optimal value for the viral cytotoxicity allows reaching the smallest tumor burden was already a conclusion in the spaceless model studied in [4].

Modeling biological processes requires to perform some simplifications, since no model can account for every detail of a highly complex phenomenon. One of the most frequent simplifications is to consider Ordinary Differential Equations (ODE) models, where the space variable is not considered. This eases the mathematical analysis, while introducing a simplifying assumption. Nevertheless, the ODE study is a preliminary step towards the mathematical understanding of spatial models. In [12] the infection is modeled by syncytia e.g., cell-cell fusion following infection, and a three species ODE model is analyzed and calibrated with data. It is proved that the system admits an equilibrium with reduced tumor burden, and this equilibrium can undergo a Hopf bifurcation leading to oscillating solutions. The work [13] considers a 4-species ODE model which includes the infection of tumor cells both by free virus and syncytia. This model allows reproducing total tumor volume observed in animal experiments.

A number of contributions study the effect of treatment schedule, and evaluate different therapeutic options. In [14] the administration of successive doses of viruses is modeled, and a target is defined that consists in minimizing a combination of tumor eradication and maximum tumor burden over a time window  $[0, T]$ . The choice of an optimal strategy of administration's timing is determined numerically. In [15] the timing of a second administration of viruses was studied experimentally and numerically. The work [16] uses optimal control methods to show that a combination of TNF- $\alpha$  inhibitors with oncolytic therapy can be optimized using bang-bang control for the virus administration, which means that the virus has to be injected at high doses during short time intervals (in contrast with continuous administrations). In [17] the model is calibrated with experimental data where mice have been administered virus doses at regular time intervals.

All existing models account for the three following phenomena that are at the root of oncolytic treatment: 1) the tumor cells follow a growth that may be limited by a maximal load constrained by physical limits, which is termed the carrying capacity 2) the tumor cells become infected when they encounter some free virus or following cell-to-cell virus transmission (a cluster) 3) infected cells release virus continuously during infection and massively when they are lysed. These basic behaviors can be expressed by various mathematical equations, and more complex phenomena can be accounted for, e.g., spatial configuration, complex biological processes or complementary therapies.

In the present work, we aim at unveiling the mechanisms that govern the optimal timing of successive administrations. For this purpose, the three basic phenomena discussed above, are modeled as simply as possible, which leads to a simple 3 species model similar to what was already proposed in [18]. The variables are the number of tumor cells, the number of infected cells and the quantity of free virus. We make the hypothesis that the infection by viruses is faster than the tumor proliferation, more precisely we consider the ratio of these two rates as a small parameter  $\epsilon$ . Note that the validity of this hypothesis largely depends on the viral strain, and our analysis does not apply to

a case where virus cycle is slower than tumor cell proliferation, a “high-risk” scenario that may be of interest to favor the production of high viral load within tumors. The system becomes then a slow-fast system that can asymptotically be described with only 2 variables, following classical boundary layer techniques [19]. The 2-species limit system obtained using singular perturbation techniques is still realistic when  $\epsilon=0.2$  as we can see using numerical experiments.

As a second contribution, we study the effect of virus administration in the 2-species system. We show that this amounts to performing a singular perturbation of the slow-fast system. The trajectory of the 2D limit system in the phase plane is affected in an explicit way by each administration, and the optimal timing of the administrations can be specified depending on the therapeutic option: the aim can be either to eradicate the tumor (or to drive the tumor burden under a small threshold), or to converge rapidly towards the stable endemic equilibrium or attracting cycle. The choice between these different options may be governed by the parameters of the particular system that is considered, and by complementary therapies that may be available. The main message is that the understanding of the effect of an administration on the trajectory in the phase plane should be accounted for when deciding the timing of successive administrations, and this understanding is backed by an analysis of the 2 species limit system.

The present article is organized as follows. In Section 2 we present the models that we use: the 3-species model and the 2-species model that is obtained when the infection rate is faster than tumor proliferation. Section 3 contains the analysis of the 2-species model. In Section 4 we model the treatment that consists of virus administration, and describe it in terms of the 2-species model where no variable describes the quantity of free virus. Numerical simulations are presented in Section 5, and Section 6 contains a discussion that summarizes the results obtained. Appendices A.1, A.2 and A.3 contain technical material: the detailed stability analysis of the equilibria, the bifurcation analysis and the proof of the treatment modeling in the two species model (theorem 5).

## 2 The different models

We present here the different models that will be used. Our aim is to design models that can reproduce the qualitative behavior observed in the experiments and *in-vivo*, while depending on a few parameters, in order to allow for qualitative discussion and possibly parameter identification. As noted in [20], the domain of validity of such a model is necessary limited, and further events like immune system response are not taken into account.

A model that is both rich enough and relatively lean was proposed in [12, 14], describing the interplay between tumor cells, infected tumor cells and free virus. We use a very close variant of this model as our first three species model, and derive the reduction to a two species model under the assumption

that infection is faster than tumor growth. This two species approximation allows us to recover other well studied systems (see for e.g. [18]).

Let us emphasize that the model considered here does not take into account any spatial dimension contrary to [5], but it allows to derive theoretical results and approximations when the dynamics of viruses is faster than the dynamics of cancer cells.

## 2.1 A three species model

We consider three populations that depend on the time variable  $t$ . We denote by  $z(t)$  the quantity of free virus. We differentiate the cancer cells depending on whether they are infected by viruses or not and denote by  $x(t)$  the quantity of cancer cells not infected by the virus and  $y(t)$  the quantity of cancer cells infected by the virus.

We assume that non infected cancer cells grow according to a logistic law, with a growth rate of  $\mu$ , and carrying capacity of 1. Thus the unit for the tumor cells corresponds to the carrying capacity. These cells are then turned to infected tumor cells, at a rate proportional to the quantity of free virus, and we denote by  $\lambda$  the infection rate.

We assume that infected tumor cells  $y(t)$  are lysed at a rate  $\gamma$ . Finally, free virus is consumed for the infection of cancer cells at rate proportional to the quantity of cells. The unit to measure free virus was chosen so that the quantity of virus required to infect one cell is 1, therefore a proportion  $\lambda x(t)$  of viruses disappear due to new infections. Free viruses are released into the system when an infected cell is lysed and the parameter  $N$  scales the quantity released by each lysed infected cell. We also assume that the viruses are cleared by the immune system and diffusion at a rate  $\delta$ .

The population dynamic  $(x(t), y(t), z(t))_{t \geq 0}$  is then solution of the following system of ordinary differential equations

$$\begin{cases} \frac{d}{dt}x(t) = -\lambda x(t)z(t) + \mu x(t)(1 - x(t)), \\ \frac{d}{dt}y(t) = \lambda x(t)z(t) - \gamma y(t), \\ \frac{d}{dt}z(t) = -\lambda x(t)z(t) + N\gamma y(t) - \delta z(t). \end{cases} \quad (1)$$

This system is very close to the system proposed in [12, 14], with a slightly different term describing the logistic growth.

## 2.2 Slow-fast system and two species models

### *Slow-fast model*

As mentioned before, the speed of infection is largely dependent on the viral strain. Some "lazy" viruses such as H-1PV take days ( $\sim 2$ -3) to actively infect and propagate in cultures, while others, like vesicular stomatitis virus, have an explosive behavior.

6 *Slow-fast model for oncolytic therapy*

In the sequel we will assume that the viruses' dynamics is fast compared with the tumor cells' dynamic. We introduce a small parameter  $\epsilon$  and consider the modified system:

$$\begin{cases} \frac{d}{dt}x_\epsilon(t) = -\lambda x_\epsilon(t)z_\epsilon(t) + \mu x_\epsilon(t)(1 - x_\epsilon(t)), \\ \frac{d}{dt}y_\epsilon(t) = \lambda x_\epsilon(t)z_\epsilon(t) - \gamma y_\epsilon(t), \\ \epsilon \frac{d}{dt}z_\epsilon(t) = -\lambda x_\epsilon(t)z_\epsilon(t) + N\gamma y_\epsilon(t) - \delta z_\epsilon(t). \end{cases} \quad (2)$$

We expect that as  $\epsilon \rightarrow 0$ , the virus' dynamics would be infinitely faster and the system can be described by a two species system where the virus population is always at equilibrium.

***Reduced two species model***

The reduced system is obtained by setting  $\epsilon = 0$  in (2). This implies

$$\begin{aligned} 0 &= -\lambda x_0(t)z_0(t) + N\gamma y_0(t) - \delta z_0(t) \\ \Leftrightarrow \quad z_0(t) &= \frac{N\gamma y_0(t)}{\lambda x_0(t) + \delta}. \end{aligned} \quad (3)$$

By replacing in the other equations one obtains the following two species model:

$$\begin{cases} \frac{d}{dt}x_0(t) = -\frac{\lambda N\gamma x_0(t)y_0(t)}{\lambda x_0(t) + \delta} + \mu x_0(t)(1 - x_0(t)), \\ \frac{d}{dt}y_0(t) = \frac{\lambda N\gamma x_0(t)y_0(t)}{\lambda x_0(t) + \delta} - \gamma y_0(t). \end{cases} \quad (4)$$

In the sequel we will prove that this reduced two species model describes adequately the complete three species model when  $\epsilon \rightarrow 0$  in the following sense: in Proposition 1 we prove that the trajectories of the reduced system converge towards the trajectories of the complete system when  $\epsilon \rightarrow 0$ , uniformly on any compact time interval. Moreover Theorem 5 states that the perturbation of the complete system that describes the administration of a certain amount of virus is approximated by a singular perturbation of the reduced system.

***Approximation of the slow-fast system by the two species system***

We use Theorem 1.2 from [19] on the solutions of singularly perturbed differential equations in order to derive a rigorous link between the slow-fast system (2) and its reduced form (4).

**Proposition 1** *For any initial condition  $(x^0, y^0, z^0) \in (0, \infty)^3$  then uniformly on any time interval  $[t_0, t_1]$  with  $0 < t_0 \leq t_1$ , the solutions  $(x_\epsilon, y_\epsilon, z_\epsilon)$  of (2) and  $(x_0, y_0, z_0)$  solution of (4)-(3) satisfy*

$$|x_\epsilon - x_0| + |y_\epsilon - y_0| + |z_\epsilon - z_0| \rightarrow_{\epsilon \rightarrow 0} 0.$$

*Proof* Let us briefly verify that the assumptions of Theorem 1.2 from [19] are satisfied. The algebraic part of the perturbed system consists of one equation, namely

$$\epsilon \frac{d}{dt} z_\epsilon(t) = g(x_\epsilon(t), y_\epsilon(t), z_\epsilon(t)),$$

where

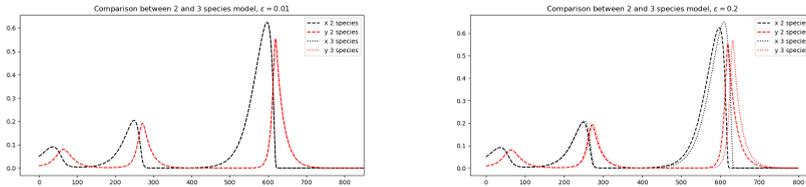
$$g(x, y, z) = -\lambda xz + N\gamma y - \delta z.$$

Since

$$\frac{\partial g}{\partial z} = -\lambda x - \delta \leq -\delta < 0,$$

the hypothesis H3 of [19] is satisfied. It follows that if  $\epsilon$  is sufficiently small the solution of the perturbed problem (2) are approximated by the solution of the singular problem (4) uniformly on any interval that does not contain the initial time.  $\square$

We present in Figure 1 the comparison between the 2 and 3 species models for different values of  $\epsilon$ . Note that even for the value  $\epsilon = 0.2$  which is not so small, the slow-fast model approximates reasonably the 3 species model for a few pseudo-periods.



**Fig. 1** The comparison between the 2 and 3 species models. The parameter  $\epsilon$  is  $10^{-2}$  (left) and 0.2 (right).

### 3 Dynamical analysis of two species system

This section is devoted to the analysis of the solution of the 2 species dynamical system. We focus on the stability of equilibria and the convergence of the solutions.

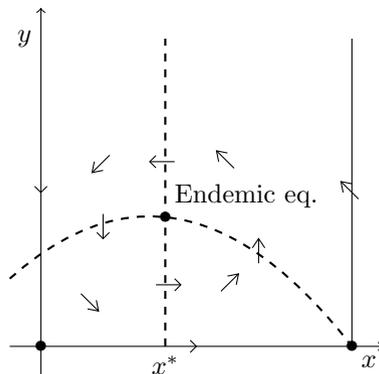
#### 3.1 Reducing the number of parameters

We first remark that the number of parameters of the two species model (4) can be reduced. We set

$$\beta = \frac{\delta}{\lambda},$$

then the system becomes

$$\begin{cases} \frac{d}{dt}x(t) = -\frac{N\gamma x(t)y(t)}{x(t) + \beta} + \mu x(t)(1 - x(t)), \\ \frac{d}{dt}y(t) = \frac{N\gamma x(t)y(t)}{x(t) + \beta} - \gamma y(t), \end{cases} \quad (5)$$



**Fig. 2** Phase portrait of the 2 species system. The equilibria are indicated by dots  $\bullet$ . The stability of the endemic equilibrium depends on the bifurcation parameter  $\beta$ , as discussed in the text.

where we recall that the variables are:

- $x$ : number of cancer cells,
- $y$ : number of infected cancer cells.

In this form, the number of parameters is reduced to 4:

- $\mu$ : growth rate of cancer cells,
- $\gamma$ : death rate of infected cancer cells,
- $N > 1$ : number of viruses released at the lysis of an infected cell,
- $\beta$ : ratio between the clearing rate of the virus and the infection rate of tumor cells by the virus.

### 3.2 Equilibria and stability analysis

**Proposition 2** *Assume that  $N > 1$ . The system (5) admits three equilibria that are*

- $(0, 0)$ : *therapeutic success. This equilibrium is always unstable.*
- $(1, 0)$ : *therapeutic failure. This equilibrium is stable if and only if  $N - \alpha \leq \beta$ .*
- $(x^*, y^*) = \left( \frac{\beta}{N-1}, \frac{\mu}{\gamma} x^* (1 - x^*) \right)$  *called endemic equilibrium. This equilibrium is stable if and only if  $\beta > \beta_c = \frac{N-1}{N+1}$ .*

This proposition is proved in Appendix A.1. We illustrate in Figure 2 the phase portrait of the system in the  $(x, y)$  plane. The region of interest is  $\{0 \leq x \leq 1\}$ . The derivative of  $y$  cancels for  $x = x^*$ , and the derivative of  $x$  cancels for  $y = \frac{\mu}{N\gamma}(x + \beta)(1 - x)$ . These loci where the derivatives are along the axes of coordinates are indicated by dotted lines.

The endemic equilibrium  $(x^*, y^*)$  admits a Hopf bifurcation with respect to the parameter  $\beta$ . From the previous computation we have

$$\beta_c = \frac{N-1}{N+1}.$$

The following result can be proved using a standard bifurcation analysis [21], see Appendix A.2 for the detailed calculations.

**Proposition 3** *The dynamical system (5) admits a supercritical Hopf bifurcation associated with the parameter  $\beta$ . Namely if  $\beta < \beta_c$  the the solution of the system converges to a stable limiting cycle. Otherwise the endemic equilibrium  $(x^*, y^*)$  is globally attractive for every positive initial condition.*

Note that high values of  $\beta$  and therefore cycling behaviour of the system correspond to viruses with low infection rate or which are easily eliminated by the immune system.

## 4 Treatment modeling and optimization

In this section we focus on modeling treatment options associated with the administration of free viruses in the tumor. First we justify that a sudden administration of viruses at a time  $t_0$  can be modeled as a jump in the position  $(x, y)$  of the reduced two species system.

Then we explore numerically different treatment options and discuss the optimal timing of viruses' administration.

### 4.1 Modeling the introduction of viruses in the slow-fast model

The treatment amounts to add to the system a quantity  $V$  of viruses at the time  $t_0$ . A protocol composed of successive administrations of virus is described by the administration of different quantities  $V_0, V_1, \dots, V_k$  at the instants  $t_0, t_1, \dots, t_k$ .

For the slow-fast system (2), the treatment is modeled by adding a source term in the equation for  $z_\epsilon$  that is a Dirac in time at  $t = t_0$ . The system reads

$$\begin{cases} \frac{d}{dt}x_\epsilon(t) = -\lambda x_\epsilon(t)z_\epsilon(t) + \mu x_\epsilon(t)(1 - x_\epsilon(t)), \\ \frac{d}{dt}y_\epsilon(t) = \lambda x_\epsilon(t)z_\epsilon(t) - \gamma y_\epsilon(t), \\ \epsilon \frac{d}{dt}z_\epsilon(t) = -\lambda x_\epsilon(t)z_\epsilon(t) + N\gamma y_\epsilon(t) - \delta z_\epsilon(t) + D_{t=t_0}V, \end{cases}$$

where  $D_{t=t_0}$  denotes the Dirac distribution in time.

An equivalent possibility is to consider that before the treatment the state of the system tends to

$$(x_{\epsilon,0}, y_{\epsilon,0}, z_{\epsilon,0}) = (x_{\epsilon}(t_0^-), y_{\epsilon}(t_0^-), z_{\epsilon}(t_0^-)),$$

and after the treatment, when  $t \rightarrow t_0^+$  the state of the system tends to

$$(x_{\epsilon,0}, y_{\epsilon,0}, z_{\epsilon,0} + V/\epsilon).$$

It remains to study the evolution of the system starting from such an initial condition, and its behavior when  $\epsilon \rightarrow 0$ . This is the object of the next section.

## 4.2 Treatment modeling in the 2 species system

The main issue in this part is that the amount of virus at initial time explodes as  $\epsilon \rightarrow 0$  such that standard results do not apply. We have to deal with a singular perturbation (i.e. of order  $1/\epsilon$ ) of a singular system. The strategy here consists in proving that this burst induces in the limit system an instantaneous change in the amount of cancer cells, while the quantity of viruses rapidly decreases towards its equilibrium value. This phenomenon is called a boundary layer.

To simplify the notation we denote by  $(\bar{x}_{\epsilon}, \bar{y}_{\epsilon}, \bar{z}_{\epsilon})_{t \geq t_0}$  the solutions of (2) whose values at  $t_0$  are  $(x_{\epsilon,0}, y_{\epsilon,0}, z_{\epsilon,0} + V/\epsilon)$ .

We aim at comparing these solutions with the solutions  $(x_{\epsilon}, y_{\epsilon}, z_{\epsilon})$  of the same system without treatment, i.e. with initial condition at  $t = t_0$  equal to  $(x_{\epsilon,0}, y_{\epsilon,0}, z_{\epsilon,0})$ . One writes

$$\bar{x}_{\epsilon} = x_{\epsilon} + \zeta, \quad \bar{y}_{\epsilon} = y_{\epsilon} + \eta, \quad \bar{z}_{\epsilon} = z_{\epsilon} + \xi.$$

By difference it comes

$$\begin{cases} \zeta'(t) = -\lambda\zeta(t)z_{\epsilon}(t) - \lambda\xi(t)x_{\epsilon}(t) - \lambda\zeta(t)\xi(t) + \mu\zeta(t)(1 - 2x_{\epsilon}(t) - \zeta) \\ \eta'(t) = \lambda\zeta(t)z_{\epsilon}(t) + \lambda\xi(t)x_{\epsilon}(t) + \lambda\zeta(t)\xi(t) - \gamma\eta(t) \\ \epsilon\xi'(t) = -\lambda\zeta(t)z_{\epsilon}(t) - \lambda\xi(t)x_{\epsilon}(t) - \lambda\zeta(t)\xi(t) + N\gamma\eta(t) - \delta\xi(t). \end{cases} \quad (6)$$

together with the initial condition  $\zeta(t_0) = \eta(t_0) = 0$  and  $\xi(t_0) = V/\epsilon$ . This is a singular source term since the amplitude tends to  $+\infty$  as  $\epsilon \rightarrow 0$ . We want to determine the behavior of the boundary layer, that is the transitory solutions. For any  $t \geq t_0$ , we write

$$t = t_0 + \epsilon u$$

with  $u \geq 0$  and consider the following change of variable:

$$\zeta(t) = X\left(\frac{t-t_0}{\epsilon}\right), \quad \eta(t) = Y\left(\frac{t-t_0}{\epsilon}\right), \quad \xi(t) = \frac{1}{\epsilon}Z\left(\frac{t-t_0}{\epsilon}\right).$$

We then deduce that the new variables  $(X, Y, Z)$  satisfy:

$$\begin{cases} X'(u) = -\lambda x_\epsilon(t_0 + \epsilon u)Z(u) - \lambda X(u)Z(u) \\ \quad + \epsilon [-\lambda X(u)z_\epsilon(t_0 + \epsilon u) + \mu X(u)(1 - 2x_\epsilon(t_0 + \epsilon u) - X(u))] \\ Y'(u) = \lambda x_\epsilon(t_0 + \epsilon u)Z(u) + \lambda X(u)Z(u) + \epsilon [\lambda X(u)z_\epsilon(t_0 + \epsilon u) - \gamma Y(u)] \\ Z'(u) = (-\lambda x_\epsilon(t_0 + \epsilon u) - \delta)Z(u) - \lambda X(u)Z(u) + \epsilon [-\lambda X(u)z_\epsilon(t_0 + \epsilon u) + N\gamma Y(u)] \end{cases} \quad (7)$$

and  $X(0) = Y(0) = 0, Z(0) = V$ . We will sometimes use the notation  $(X_\epsilon, Y_\epsilon, Z_\epsilon)$  in order to emphasize the dependence on  $\epsilon$ . Let us remark that  $x_\epsilon(t_0 + \epsilon u) \rightarrow x_{\epsilon,0}$  for small  $\epsilon$  since  $x$  is continuous. The first order approximation reads

$$\begin{pmatrix} X'_0(u) \\ Y'_0(u) \\ Z'_0(u) \end{pmatrix} = \begin{pmatrix} -\lambda x_0(t_0)Z_0(u) - \lambda X_0(u)Z_0(u) \\ \lambda x_0(t_0)Z_0(u) + \lambda X_0(u)Z_0(u) \\ (-\lambda x_0(t_0) - \delta)Z_0(u) - \lambda X_0(u)Z_0(u) \end{pmatrix} \quad (8)$$

with initial conditions  $X_0(0) = 0 = Y_0(0)$  and  $Z_0(0) = V > 0$ .

**Proposition 4** *As  $t \rightarrow \infty$  the solutions of (8) converge to  $(-Q, Q, 0)$  where the positive constant  $Q$  is defined as*

$$Q = V - \delta \int_0^\infty Z_0(s) ds. \quad (9)$$

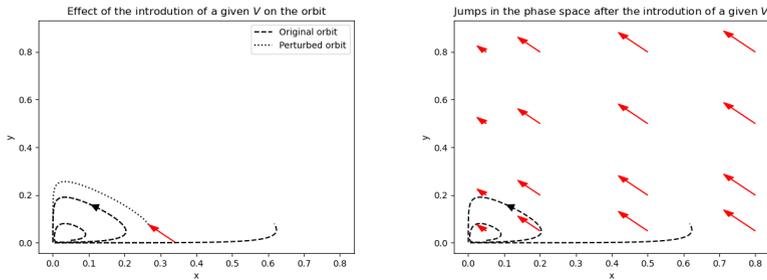
The proof is provided in Appendix A.3. This proposition gives the limiting behaviour of the boundary layer. It allows to prove the following approximation result which states that after a large input of virus, the system behaves as if the amount of infected, resp. non infected, cancer cells are shifted from the quantity plus, resp. minus,  $Q$ . In other words an amount  $Q$  of cancer cells have been instantaneously infected.

**Theorem 5** *Let  $(\bar{x}_\epsilon, \bar{y}_\epsilon \bar{z}_\epsilon)$  be the solution of the slow-fast system (2) starting from  $(x(t_0), y(t_0), z(t_0) + \frac{V}{\epsilon})$  and  $(x_0^Q, y_0^Q, z_0^Q)$  the solutions of (5)-(3)  $(x(t_0) - Q, y(t_0) + Q)$ . Then the difference*

$$|(\bar{x}_\epsilon, \bar{y}_\epsilon \bar{z}_\epsilon) - (x_0^Q, y_0^Q, z_0^Q)|$$

*vanishes as  $\epsilon \rightarrow 0$  uniformly on any time interval  $[t, T]$  with  $T > t > t_0$ .*

See Appendix A.3 for the proof. The amount  $Q$  is related to the quantity  $V$  of viruses injected, but no simple algebraic relation can be provided. We present in Figure 3 the equivalent amount  $Q$  computed at different starting points  $(x_0, y_0)$  in the  $(x, y)$ -phase plane. Note that for a given  $V$ , the value of  $Q$  depends on the initial position in the  $(x, y)$  plane.



**Fig. 3** Left: representation of one orbit in the phase space, together with the perturbed orbit after the introduction of virus. Right: illustration of the different values of  $Q$  for a given value of  $V = 0.1$  in the phase space, together with an orbit. When the oncolytic virus is introduced, the position in the phase space jumps from  $(x, y)$  to  $(x - Q, y + Q)$  and the segments between these two points are represented in red at different locations in the phase space.

### 4.3 Modeling multiple treatments

Without treatment, the evolution of cancer grows to its maximal size. In our framework this is expressed by the fact that  $t \mapsto x(t)$  is increasing and converges towards 1. Here we aim at modeling a treatment which should avoid this fatal outcome and in the perfect scenario cure the cancer, that is obtain  $x = 0$ .

When cancer is treated through a viral administration, the 2 species system state jumps from  $(x_0, 0)$  to  $(x_0 - Q, Q)$  (see Fig 3, right). Afterwards, the amount of cancer cells evolves according to (4) along a new trajectory. This allows to decrease the total amount of cancer cells.

We propose to explore how a second administration can contain the cancer proliferation. More precisely we study the impact of the time interval between the two administrations in the treatment of the patient. When changing the timing of the second administration, the jump in the phase space will lead to different orbits that are more or less desirable, and we discuss below the options that are at hand.

Here we will consider that the first administration occurs at  $T_{1st} = 0$  and that after that instant the state of the patient is described by  $(x_0, y_0)$  and evolves according to the 2 species model (4). We denote by  $T_{2nd} > 0$  the time of the second administration.

#### *Strategy 1*

The first possible objective is to cure completely the cancer, which amounts to reach the equilibrium  $(0, 0)$ . This is possible following a single precise orbit of our model since this equilibrium is a saddle and that would require attaining the attracting manifold for  $(0, 0)$  which is the line  $x = 0$ . In other words all the cancer cells  $x$  should have been turned into infected cancer cells  $y$  at once, which is highly unrealistic.

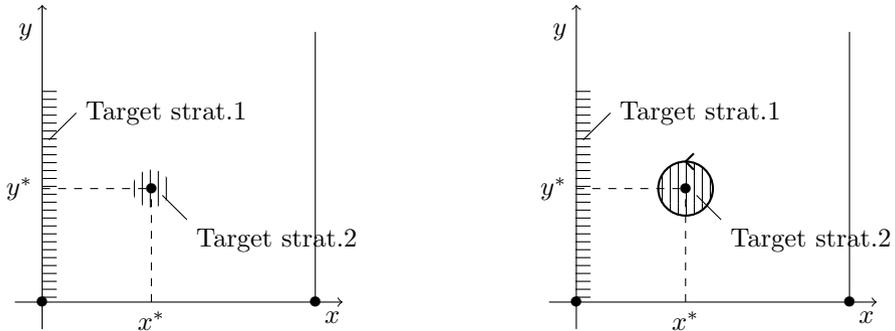
A more realistic objective [14] would be to reach the region  $\{x \leq \eta\}$  where  $\eta$  is a small constant (say  $\eta = 10^{-8}$ ) so that  $x \leq \eta$  in our continuous modeling amounts to saying that the number of tumor cells is below 1, or the number of tumor cells is so small that an alternative therapy such as tumor resection should have a great probability of success. This therapeutic option is desirable.

In order to measure the efficiency of the second administration, we compute the minimum value of  $x(t)$  for  $t \geq t_0$ . In practice we do not study the whole time interval but we restrict ourselves to first pseudo-period of the system. More precisely we call the pseudo-period the time to the second local maximum for  $x$  after  $T_{1st}$ .

### Strategy 2

The second possible objective is to reach the endemic equilibrium (when it is stable), or the limit cycle that loops around the endemic equilibrium (when it is unstable). This amounts to control the volume of the tumor, so that it stays at a reasonable load. This strategy is adapted when the tumor load at the endemic equilibrium  $(x^*, y^*)$  is bearable, which means that  $x^*$  is small.

We illustrate in Figure 4 the target zones for these different strategies.



**Fig. 4** Target zones for the different strategies. Left: case of a stable endemic equilibrium  $(x^*, y^*)$ . Right: case of a stable cycle close to the unstable equilibrium  $(x^*, y^*)$ .

## 5 Numerical simulations

We provide here test cases that document the strategies that were presented in Section 4.3. We used two sets of parameters for the model, that are given in Table 1. These parameters, excepted  $\delta$ , were obtained by fitting *in-vivo* experimental data for two different oncolytic virus strains (unpublished work), and therefore may not be realistic in a clinical therapy. However we believe that they exhibit diverse behaviors that reflect various situations.

The parameter  $\delta$  was given different values, that allowed to study cases where  $\beta > \beta_c$  (stable endemic equilibrium) and  $\beta < \beta_c$  (unstable endemic

equilibrium, stable cycle). Instead of giving the value of  $\delta$ , we provide the value of  $\beta$  as a fraction of  $\beta_c$ .

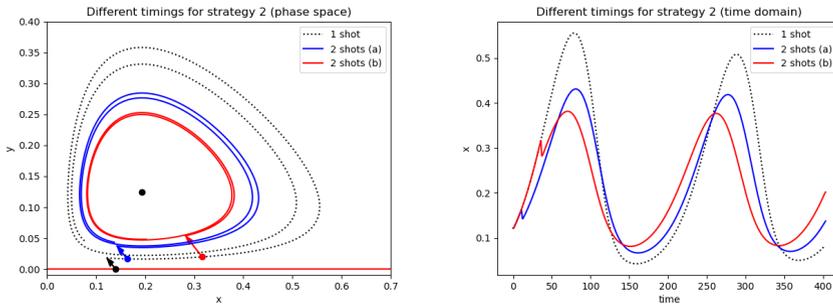
	$\lambda$	$\mu$	$\gamma$	$N$	$\delta$
set 1	$8.8 \cdot 10^{-6}$	0.04	0.05	4	variable
set 2	$1.53 \cdot 10^{-7}$	0.1	10.7	1.76	variable

**Table 1** The two sets of parameters that were used.

### *Effect on the timing for strategy 2, parameter set 1*

We use parameter set 1, with  $\beta = 0.967\beta_c$  (attracting cycle close to the endemic equilibrium). The initial condition is  $x_0 = 0.14$  at time  $T_{1st} = 0$ , and we apply an administration of a quantity  $V = 0.1$  of viruses. The evolution of the system is then termed "1 shot".

The same quantity  $V$  is again injected at a later time  $T_{2nd}$ , when  $T_{2nd} = 10$  we term the evolution "2 shots (a)", and when  $T_{2nd} = 35$  we term it "2 shots (b)". All these cases are illustrated in Figure 5 both in the phase plane and in the time domain. It is clear that the second option "2 shots (b)" is preferable, since the maximum reached by the tumor burden  $x$  is smaller than in the option "2 shots (a)". The fact that the second administration is performed later allows the jump in phase space provoked by this administration to be more efficient in the sense that it allows to reach orbits that are closer to the limit cycle, hence the maximal value of  $x$  that is attained is diminished.

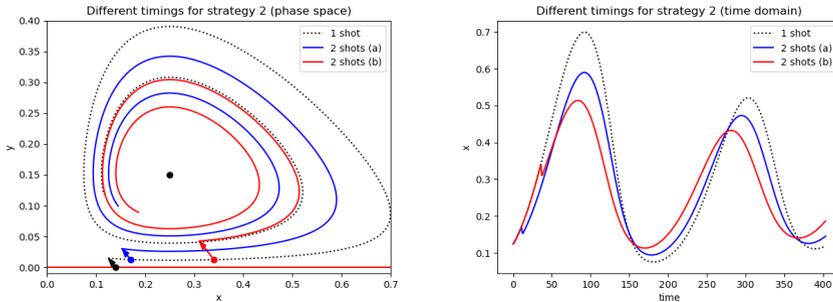


**Fig. 5** Different timings for strategy 2, for parameter set 1 and  $\beta = 0.967\beta_c$ . Evolution in the  $(x, y)$  plane (left) and  $x$  in the time domain (right).

In Figure 6 we present the same simulation excepted that  $\beta = 1.25\beta_c$ , in other words the endemic equilibrium is stable. The orbits revolve around the equilibrium, and the two-shots strategy aims at minimizing the maximal value reached by  $x$ . We have used the same parameters  $T_{2nd} = 10$  and  $T_{2nd} = 35$ .

Note that for these values of the parameters, the maximal value of  $x$  reached for  $\beta = 0.967\beta_c$  is significantly smaller than the maximal value reached for

$\beta = 1.25\beta_c$  ( $x \cong 0.38$  vs  $x \cong 0.5$ ). It is therefore not necessary easier to control the tumor when the endemic equilibrium is stable than when it is unstable.

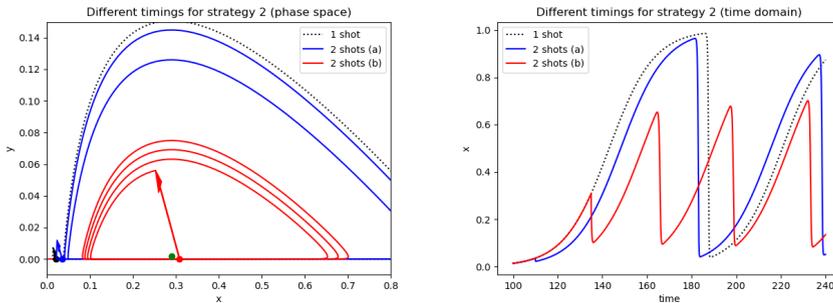


**Fig. 6** Different timings for strategy 2, for parameter set 1 and  $\beta = 1.25\beta_c$ . Evolution in the phase space (left) and  $x$  in the time domain (right).

### *Effect on the timing for strategy 2, parameter set 2*

We now discuss analogous strategies for the parameter set 2, with  $\beta = 0.8\beta_c$ . The equilibrium point  $(x^*, y^*) \cong (0.29, 0.002)$  is located close to the horizontal axis, and the attracting cycle reaches high values of  $x$  that are non desirable.

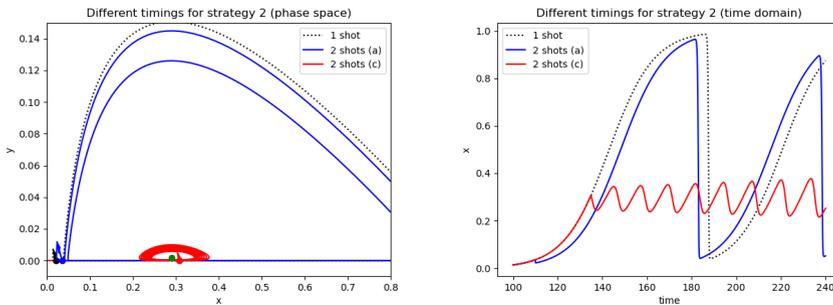
The strategy "2 shots (a)", with second shot at  $T_{2\text{nd}} = 10$  escapes rapidly close to the attracting cycle, and reaches large values of  $x$ . The strategy "2 shots (b)", with a second shot at  $T_{2\text{nd}} = 35$  overshoots the equilibrium point, and also escapes to high values of  $x$  although slower, see Figure 7.



**Fig. 7** Different timings for strategy 2, for parameter set 2. Evolution in the phase space (left) and  $x$  in the time domain (right).

In this case, we propose to inject a smaller quantity of virus  $0.1V = 0.01$  at the time  $T_{2\text{nd}} = 35$ . This modified strategy termed "2 shots (c)" allows to hit a point closer to the equilibrium and therefore loop around the equilibrium

for a longer time (however the limit cycle will be approached, but in a longer time horizon). The results are presented in Figure 8.



**Fig. 8** Different timings for strategy 2 (parameter set 2), with reduced virus quantity 0.1V for strategy 2b. Evolution in the phase space (left) and  $x$  in the time domain (right).

### *Effect on the timing for strategy 1*

Strategy 1 consists in reaching a position as close as possible from the vertical axis  $x = 0$ , using jumps between orbits at instants of the virus administrations. However it is not desirable that during the evolution the variable  $x$  takes a high value.

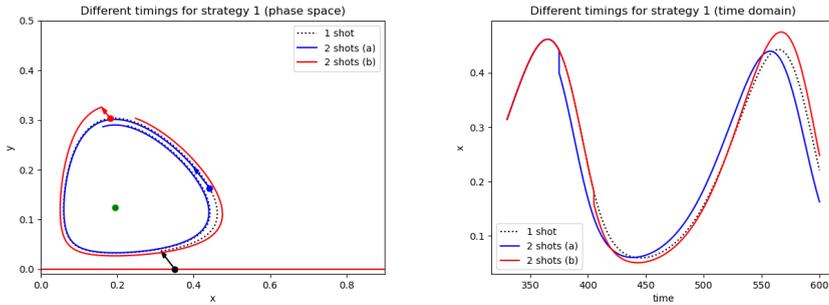
We present illustrations with parameters set 1, and  $\beta = 0.967\beta_c$ , as in the first test case. In order to reach small values of  $x$ , it is desirable to jump to outer trajectories w.r.t. the equilibrium point. Therefore the timing should be modified accordingly.

We present in Figure 9 a case where the first administration  $T_{1st}$  is later than compared to Figure 5. The timing for the second administration is  $T_{2nd} = T_{1st} + 45$  for strategy "2 shots (a)", and  $T_{2nd} = T_{1st} + 75$  for strategy "2 shots (b)". It is clear that the strategy "2 shots (a)" has no effect since the trajectory jumps to almost the same orbit. The strategy "2 shots (b)" has a better effect since the minimal value of  $x$  obtained later is smaller. However note that it is difficult to reach a really small value of  $x$  and for the parameters set at hand it may not be desirable to follow strategy 1.

For parameter set 2, the orbits reach very high value of  $x$  and strategy 1 appears to be undesirable.

## Discussion

This work studied different options to determine the optimal timing of the second administration. The main findings are the following: the second administration should be determined differently according to the therapeutic goal, that can be either to eliminate the tumor, or to reach a reasonable size in the case the endemic equilibrium occurs at a bearable tumor load. Of course the



**Fig. 9** Different timings for strategy 1, for parameter set 1. Evolution in the phase space (left) and  $x$  in the time domain (right). The strategy "2 shots" does not modify the trajectory but merely shifts the trajectory in time, this is due to an inadequate timing.

ideal goal is to eliminate the tumor, but a reduction in size can also be a first step before alternative therapies like curative surgery.

We have shown that for the second administration, a larger dose does not necessarily entail a more efficient treatment, see Figures 7 and 8. Another experiment showed that inadequate timing of the second administration has no effect on the trajectory of the system (but for a shift in time), see Figure 9.

These observations and numerical experiments were performed on a 2 species model that was derived from a 3 species slow-fast model, under the assumption that the viral infection is faster than the tumor growth. The ODE model that was used did not consider the spatial aspect of the question, and therefore a more complete and realistic study should account for space variables and ultimately tumor heterogeneity in composition (cell, matrix...). However we believe that the illustration in the phase plane provides an original understanding of the different outcomes of a multiple administrations strategy.

## Appendix A Proofs

### A.1 Proof of Proposition 2 (stability of equilibria)

*Proof* The fact that the system admits only the three equilibria  $(0, 0)$ ,  $(1, 0)$ ,  $(x^*, y^*)$  follows from a simple computation. We now study the stability of these equilibria.

**Equilibrium**  $(0, 0)$  The Jacobian matrix is given by

$$\begin{pmatrix} \mu & 0 \\ 0 & -\lambda \end{pmatrix},$$

it is a saddle equilibrium. The only possibility to converge towards this equilibrium is to follow its stable manifold, which is the line  $x = 0$ . In other words all cancer cells are infected.

**Equilibrium**  $(1, 0)$  The Jacobian matrix is given by

$$\begin{pmatrix} -\mu & \frac{-N\gamma}{\alpha + \beta} \\ 0 & \frac{N\gamma}{\alpha + \beta} - \gamma \end{pmatrix}.$$

This equilibrium is a saddle if and only if  $N - \alpha > \beta$ , otherwise it is stable.

**Endemic equilibrium** The Jacobian matrix is given by

$$\begin{pmatrix} -\mu\lambda x^*(1 + \frac{1}{N}) + \lambda\mu\frac{1}{N} & -\gamma \\ (1 - x^*)(1 - \frac{1}{N})\mu & 0 \end{pmatrix}.$$

The determinant is positive for  $N > 1$  since  $x^* < 1$ . Moreover the trace is negative if and only if

$$\frac{1}{N} < x^*(1 + \frac{1}{N}) \Leftrightarrow \beta > \frac{N-1}{N+1}.$$

Therefore the endemic equilibrium is stable iff  $\beta > \beta_c = \frac{N-1}{N+1}$ .  $\square$

## A.2 Proof of Proposition 3 (Hopf bifurcation analysis)

*Proof* We study the following orbitally equivalent system obtained by changing the time parametrization:

$$\begin{cases} x'_0(t) = -x_0y_0N\gamma + \mu x_0(1 - x_0)(\beta + x_0), \\ y'_0(t) = x_0y_0\gamma(N - 1) - \gamma\eta y_0. \end{cases}$$

The equilibrium of interest is

$$x^*(\beta) = \frac{\beta}{N-1}, \quad y^*(\beta) = \frac{\mu}{N\gamma} x^*(1 - x^*).$$

The Jacobian matrix of the system in  $(x^*, y^*)$  for  $\beta = \beta_c$  is

$$A(\beta_c) = \begin{pmatrix} 0 & -N\gamma x_c^* \\ (N-1)\gamma y_c^* & 0 \end{pmatrix}.$$

Its eigenvalues are  $i\omega$  and  $-i\omega$  with

$$\omega^2 = N\gamma^2(N-1)x_c^*y_c^*.$$

The following two eigenvectors  $p$  and  $q$  are such that  $Aq = i\omega q$ ,  $A^T p = -i\omega p$  and  $\langle p, q \rangle = 1$ :

$$q = \begin{pmatrix} N\gamma x_c^* \\ -i\omega \end{pmatrix}, \quad p = \frac{1}{2\omega N\gamma x_c^*} \begin{pmatrix} \omega \\ -iN\gamma x_c^* \end{pmatrix}.$$

The system in the appropriate basis is approximated by

$$\dot{\xi} = A\xi + \frac{1}{2}B(\xi, \xi) + \frac{1}{6}C(\xi, \xi, \xi) + \dots$$

where

$$B(\xi, \eta) = 2 \begin{pmatrix} -\frac{N\gamma}{2}(\xi_1\eta_2 + \xi_2\eta_1) + \xi_1\eta_1(\mu(1-\beta) - 3\mu x_c^*) \\ \frac{1}{2}\gamma(N-1)(\xi_1\eta_2 + \xi_2\eta_1) \end{pmatrix}$$

and

$$C(\xi, \eta, \zeta) = 6 \begin{pmatrix} -\xi_1\eta_1\zeta_1 \\ 0 \end{pmatrix}.$$

Finally it remains to compute

$$g_{20} = \langle p, B(q, q) \rangle, \quad g_{11} = \langle p, B(q, \bar{q}) \rangle, \quad g_{2,1} = \langle p, C(q, q, \bar{q}) \rangle.$$

The computations lead to

$$g_{20} = N\gamma x_c^* (\gamma(N-1) + \mu(1-\beta) - 3\mu x_c^*) + i\omega N\gamma,$$

$$g_{11} = N\gamma x_c^* (\mu(1 - \beta) - 3\mu x_c^*), \quad g_{21} = -3\mu N^2 \gamma^2 (x_c^*)^2.$$

The first Lyapunov exponent is then given by

$$l_1 = \frac{1}{2\omega^2} \Re(ig_{20}g_{11} + \omega g_{12}) = \frac{-N^2 \gamma^2 \mu (x_c^*)^2}{\omega(N+1)}.$$

The first Lyapunov exponent is thus negative, therefore the unique and stable limit cycle bifurcates from the equilibrium via a supercritical Hopf bifurcation for  $\beta < \beta_c$ .  $\square$

### A.3 Treatment approximation

*Proof of Proposition 4* We consider the solutions  $(X_0, Y_0, Z_0)$  of the first order system (8):

$$\begin{pmatrix} X_0'(u) \\ Y_0'(u) \\ Z_0'(u) \end{pmatrix} = \begin{pmatrix} -\lambda x_0(t_0)Z_0(u) - \lambda X_0(u)Z_0(u) \\ \lambda x_0(t_0)Z_0(u) + \lambda X_0(u)Z_0(u) \\ (-\lambda x_0(t_0) - \delta)Z_0(u) - \lambda X_0(u)Z_0(u) \end{pmatrix}$$

with initial conditions  $X_0(0) = 0 = Y_0(0)$  and  $Z_0(0) = V > 0$ .

Since  $Y_0'(u) = -X_0'(u)$  it suffices to study the couple  $(X_0, Z_0)$ . From the last equation, we deduce that  $Z_0(u) > 0$  for all  $u \geq 0$ . Similarly we obtain that  $X_0(u) + x_0(t_0) > 0$  for all  $u \geq 0$ . Thus we deduce that

$$Z_0'(u) < -\delta Z_0(u).$$

This leads to

$$Z_0(u) \leq V \exp(-\delta u). \quad (\text{A1})$$

As a consequence  $Z_0(u) \rightarrow 0$  as  $u \rightarrow \infty$ . Let us now remark that

$$X_0'(u) = Z_0'(u) + \delta Z_0(u)$$

and therefore by integration

$$X_0(u) = -V + \int_0^u Z_0(s) ds.$$

From (A1) we deduce that  $X_0$  converges as  $u \rightarrow \infty$  towards a negative limit

$$-Q = \lim_{u \rightarrow \infty} X_0(u) = -V + \delta \int_0^\infty Z_0(s) ds < 0.$$

$\square$

*Proof of Theorem 5*

Step 1: Prove that the solutions of (8) are close approximations of  $(X, Y, Z)$ .

To do so we first study the regularity of the solutions of (7) with respect to the parameter  $\epsilon$  as a parameter. Let us denote by  $G$  the function  $\mathbf{R} \times \mathbf{R}^3 \times \mathbf{R} \rightarrow \mathbf{R}^3$  such that the system (7) reads

$$\begin{pmatrix} X'(u) \\ Y'(u) \\ Z'(u) \end{pmatrix} = G(u, (X(u), Y(u), Z(u)), \epsilon).$$

To study the dependency in  $\epsilon$ , we consider the extended system for a fixed value  $\epsilon_0 > 0$

$$\begin{cases} (X'(u), Y'(u), Z'(u), \epsilon'(u)) = (G(u, (X(u), Y(u), Z(u), \epsilon(u)), 0), \\ (X(0), Y(0), Z(0)) = (0, 0, V), \quad \epsilon(0) = \epsilon_0. \end{cases}$$

Let us denote by  $u \mapsto \psi(u, (0, 0, V, \epsilon_0))$  the flow associated with this differential equation, then our aim is to control

$$\psi(u, (0, 0, V, \epsilon_0)) - \psi(u, (0, 0, V, 0)) = \int_0^u \tilde{G}(s, \psi(s, (0, 0, V, \epsilon_0))) - \tilde{G}(s, \psi(s, (0, 0, V, 0))) ds,$$

where  $\tilde{G}(s, (X, Y, Z, \epsilon)) = (G(s, X, Y, Z, \epsilon), 0)$ . If  $\tilde{G}$  has bounded derivatives with respect to its second variable, then there exist a positive constant  $L$  such that

$$|\psi(u, (0, 0, V, \epsilon_0)) - \psi(u, (0, 0, V, 0))| \leq L \int_0^u |\psi(s, (0, 0, V, \epsilon_0)) - \psi(s, (0, 0, V, 0))| ds,$$

which leads using Grönwall lemma to

$$\psi(u, (0, 0, V, \epsilon_0)) - \psi(u, (0, 0, V, 0)) \leq \epsilon_0 e^{Lu}. \quad (\text{A2})$$

Step 2: Regularity of  $\tilde{G}$ .

We recall that:

$$\tilde{G}(u, X, Y, Z, \epsilon) = \begin{pmatrix} -\lambda x_\epsilon(t_0 + \epsilon u)Z - \lambda XZ + \epsilon[-\lambda Xz_\epsilon(t_0 + \epsilon u) + \mu X(1 - 2x_\epsilon(t_0 + \epsilon u) - X)] \\ \lambda x_\epsilon(t_0 + \epsilon u)Z + \lambda XZ + \epsilon[\lambda Xz_\epsilon(t_0 + \epsilon u) - \gamma Y] \\ (-\lambda x_\epsilon(t_0 + \epsilon u) - \delta)Z(u) - \lambda XZ + \epsilon[-\lambda Xz_\epsilon(t_0 + \epsilon u) + N\gamma Y] \\ 0 \end{pmatrix}$$

Clearly,  $\tilde{G}$  is smooth, but its derivative are not uniformly bounded. We now prove that the trajectories remain in a compact set, where  $\tilde{G}$  has thus bounded derivatives.

Let us first prove that the solutions  $(x_\epsilon, y_\epsilon, z_\epsilon)$  of (2) are bounded uniformly in time, by a constant that depends only on the initial condition (and not on  $\epsilon$ ). Clearly, all coordinates remain positive. The upper bound can be obtain since  $x_\epsilon$  has a logistic behavior, and  $x_\epsilon + y_\epsilon$  has negative derivative if  $y_\epsilon \geq \frac{\mu}{\lambda} x_\epsilon(1 - x_\epsilon)$  and  $z_\epsilon$  has negative derivative as soon as  $z_\epsilon \geq \frac{N\gamma y_\epsilon}{\delta + \lambda x_\epsilon}$ . A similar argument shows that the solutions  $(X, Y, Z)$  of (8) are uniformly bounded through times and for  $\epsilon$  in some compact  $[0, \epsilon_{max}]$  This allows to obtain (A2).

Step 3: Conclusion

Finally combining (A2) and (9), if  $(X_\epsilon, Y_\epsilon, Z_\epsilon)$  denote the solution of (7) for a given value of  $\epsilon$ , we obtain:

$$\begin{aligned} |X_\epsilon(u) + Q| &\leq |X_\epsilon(u) - X_0(u)| + |X_0(u) + Q| \\ &\leq \epsilon e^{Lu} + \int_u^\infty Z_0(s) ds \\ &\leq \epsilon e^{Lu} + \frac{V}{\delta} e^{-\delta u} \end{aligned}$$

Let us fix  $A > 0$ , then for a time  $u_\epsilon = -\frac{1}{\delta} \ln(\epsilon^A \delta / V)$  we have that

$$|X_\epsilon(u_\epsilon) + Q| \leq \epsilon e^{Lu_\epsilon} + \epsilon^A$$

In particular if  $A$  satisfies that  $1 - AL/\delta > 0$  then it comes

$$\lim_{\epsilon \rightarrow 0} |X_\epsilon(u_\epsilon) + Q| = 0$$

which leads as  $\epsilon u_\epsilon \rightarrow 0$  to

$$\lim_{\epsilon \rightarrow 0} \bar{x}_\epsilon(t_0 + \epsilon u_\epsilon) = x_0 - Q.$$

Similarly we have that

$$\lim_{\epsilon \rightarrow 0} \bar{y}_\epsilon(t_0 + \epsilon u_\epsilon) = y_0 + Q.$$

To conclude let us prove that  $\lim_{\epsilon \rightarrow 0} \bar{z}_\epsilon(t_0 + \epsilon u_\epsilon)$  is finite. The naive bound given by (A2) is not sufficient to conclude since  $u_\epsilon \rightarrow \infty$  and

$$\frac{1}{\epsilon} Z_\epsilon(u_\epsilon) \leq e^{Lu_\epsilon} \rightarrow \infty$$

Therefore, let us come back to the exact ODE satisfied by  $Z_\epsilon$ :

$$Z'_\epsilon(u) = (-\lambda(x_\epsilon(t_0 + \epsilon u) + X_\epsilon(u)) - \delta)Z_\epsilon(u) + \epsilon[-\lambda X_{\epsilon z_\epsilon}(t_0 + \epsilon u) + N\gamma Y_\epsilon]$$

From the previous computations we have that on the time interval  $[0, u_\epsilon]$ , there exists a positive constant  $M$  independent of  $\epsilon$  such that

$$Z'_\epsilon(u) \leq -\delta Z_\epsilon(u) + \epsilon M.$$

As a consequence for all  $u \in [0, u_\epsilon]$

$$Z_\epsilon(u) \leq (V - \frac{\epsilon M}{\delta})e^{-\frac{\delta}{\epsilon M}u} + \frac{\epsilon M}{\delta}$$

Using this upper-bound, and the fact that  $\bar{z}_\epsilon$  remains positive for all times, we deduce that

$$\bar{z}_\epsilon(t_0 + \epsilon u_\epsilon) = z_\epsilon(t_0 + \epsilon u_\epsilon) + \frac{1}{\epsilon} Z_\epsilon(u_\epsilon)$$

remains bounded as  $\epsilon \rightarrow 0$ . We finally use Theorem 1.2 from [19] on the solutions of singularly perturbed differential equations to conclude that for any  $T > t > t_0$ , the solution  $(\bar{x}_\epsilon, \bar{y}_\epsilon)$  are asymptotically equal to the solution of the system (5) starting from  $(x_0 - Q, y_0 + Q)$  on the interval  $[t, T]$ .  $\square$

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