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ORIGINAL ARTICLE

Optimal Control in a Model of Dendritic Cell Transfection Cancer Immunotherapy

Filippo Castiglione*, Benedetto Piccoli

Istituto Applicazioni del Calcolo (IAC) "M. Picone," Consiglio Nazionale delle Ricerche (CNR), Viale del Policlinico, 137–00161 Rome, Italy

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Abstract We construct a population dynamics model of the competition among immune system cells and generic tumor cells. Then, we apply the theory of optimal control to find the optimal schedule of injection of autologous dendritic cells used as immunotherapeutic agent.

The optimization method works for a general ODE system and can be applied to find the optimal schedule in a variety of medical treatments that have been described by a mathematical model.

Keywords Optimal control · Necessary conditions · Cancer · Immunotherapy · Autologous dendritic cells transfection

1. Background

Mathematical models in biology are being used since Lotka (1925) and, separately, Volterra (1926) formulated the predator–prey model of biological species. Today, by means of powerful computers, to solve numerically complex mathematical formulations of biologically motivated problems is a routine task.

Computational and mathematical models are helping biologists to understand various aspects of the complex realm of living matter, from the beating of the heart to the molecular machinery underlying the cell-division cycle and cell movement.

A specific area of study is that of the immune system dynamics. Mammalian immune system can be considered as one of the most complex systems nature has ever created. It is in charge to fight against all kinds of potentially dangerous agents that break the anatomic barriers of the host organism (Goldsby et al., 2000). It is composed of a variety of organs, cells, and molecules acting in concert to achieve few basic functions, that is, recognition, response and memory. A malfunction of the immune system usually results in a disease for the host. In the case of a tumor,

*Corresponding author.
E-mail addresses: f.castiglione@iac.cnr.it (F. Castiglione), b.piccoli@iac.cnr.it (B. Piccoli).

for example, the immune system fails to detect and kill the anomalous cells. Such failure allows for an uncontrolled growth of the tumor mass.

Immunotherapy is the "art" of stimulating the immune system to react against something specific or, in contrast, to suppress its dangerous response as in the case of allergic reactions or autoimmune diseases. For example, for people allergic to bee sting the immunotherapy consists of repeatedly injecting small doses of venom until the immune system "changes" its way of reacting to the venom and becomes, so-to-say, more tolerant to it. Another example which is relevant to us is the immunotherapy applied to cancer. In that case the goal of the immunotherapy is just the opposite, to teach the immune system to react against something which is otherwise considered not foreign like a cancer cell.

In general, there are different ways of stimulating the immune system. One of these is to instruct it to react against cells bearing the so-called *tumor-associated antigens* (TAA) by actually inducing a TAA-specific immune response. The hope is that, once instructed, the immune system cells will guard against any appearance of cells bearing the same bits of tumor.

The immune system is composed of a variety of cell types. Among these, the *dendritic cells* are perhaps the best of the so-called *antigen presenting cells* (APC) in that their work consists in capturing the *antigens* and show them to other cells called *effector cells*. If the presented molecules are "labeled" as dangerous, then the immune system mounts a specific response to eliminate the danger. The immune response is two faceted. There is the *humoral* immune response made by antibodies that is more successful in eliminating soluble antigens like bacteria and toxins, and the *cellular* immune response that is more efficacious in deleting malignant cells or cells infected by viruses.

Dendritic cell transplantation is the practice of cultivating autologous dendritic cells (i.e., previously extracted from the same patient), together with some characteristic molecules of the cancer cells (the tumor associated antigen) and then inject them back into the patient. The resulting vaccine made by autologous TAA-loaded dendritic cells is called *dendritic cell vaccine* (DCV). The idea is that the immune system, confronted with such amount of tumor-antigen, starts to mount a response against it, in place of an otherwise weak or completely absent response due to the fact that the TAA belongs to the self. In fact, as a side effect of the immune response against the vaccine, the immune system will eventually recognize the same TAA molecule on tumor cells and kill them (Dietz et al., 2001; Boczkowski et al., 2000).

Our first goal is to build a mathematical model to investigate the effect of tumor immunotherapy for a generic solid a-vascular tumor (e.g., malignant melanoma). The model obtained is made of five variables representing respectively the population of cancer cells and those of four key immune cells involved in the immune response.

Secondly, by applying the theory of optimal control we want to find the optimal protocol for the administration of DCVs loaded with TAA, that is, fixing the number of vaccine injections, the optimal timing within the treatment period. Therefore, the injected vaccine represents the *control* part of the model and affects directly only the population of the dendritic cells while the tumor mass at the end of the treatment period (that is, our optimization horizon) is the cost function.

Since the typical treatment consists of a finite number of DCV injections, the problem reduces itself to an optimization over a finite dimensional space, assuming that the vaccine administration follows always the same procedure. Moreover, given that the typical time scale of the model evolution is much bigger than that of the vaccine administration, we can use an approximation of the optimal control tools to compute the gradient of the cost function with respect to the DCVs time schedule. More precisely, we consider a generalized setting for defining weakly differentiable control variations of a reference trajectory. Changes in the DCVs time schedule give rise to control variations that are weakly differentiable, hence we use the variational equation, along the reference trajectory, to compute the gradient of the cost function. The obtained approximation consists in considering each DCV injection as an impulse in the system evolution.

From the computational point of view, our approach amounts to calculate the reference trajectory corresponding to the actual schedule and solve a linear system with a time varying matrix that is computed along the reference trajectory. Therefore, any optimization algorithm relying only on the gradient of the cost function can be used to search for the minima of our problem.

The results are quite satisfactory showing a decrease of the cost function (i.e., the final value of the tumor mass) with the use of a simple steepest descent method.

This paper is organized as follows. In the following sections we first describe the mathematical model of cancer-immune competition, then we give a background of the theory of optimal control and, finally, we describe how we implemented the optimization schema and the results obtained.

2. The mathematical model

The most popular mathematical models in theoretical immunology involve ordinary differential equations (ODE) to represent reaction kinetics. In general, the equations are used to represent concentration or population of cells and/or molecules and the parameters represent kinetic or affinity constants. Partial differential equations (PDE) are generally used to have a spatial representation of the diffusion of reagents or, as in the case of cancer, when one wants to study the tumor formation and the phenomena of vascularization (Preziosi, 2003).

There are many mathematical models of the immune system known in the literature (Perelson and Weisbuch, 1997) and a relatively small subset considers the interactions between immune system and cancer (Preziosi, 2003). A quite known model of tumor-immune interaction is those of Kirschner and Penetta (1998). However its dynamics is quite complex as it includes oscillatory behavior of the tumor. Since this characteristic makes the treatment from the control point of view more difficult, we decided to start with a model showing a simpler dynamics and we constructed the one described in the following paragraph. Simple enough for our purpose of applying methods from control theory, nevertheless the model shows a quite interesting and rich dynamics that fits reasonably to that of the tumor-immune interaction observed at the level of the cells population.

2.1. ODE modelling of tumor growth and dendritic cell presentation of tumor-associated-antigen

The following ODE model is quite simple but it is probably the only one specialized for autologous dendritic cell transfection therapy. We decided from the beginning to start with a simple representation of TAA-specific cells. We take the following assumptions:

1. the time resolution is of 1 h;
2. it is a monoclonal model, that is, we consider only the dynamics of those clones of cells which actually recognize the TAA, neglecting the effect of cross-reactivity of other clones (this is a common approximation in this kind of models of the immune system);
3. no special reference to a specific tumor is necessary at a first stage of the mathematical model development; the only requirement is that of working in the range of the tumor mass where the effects of immune escape, immune down-regulation or vascularization, is still negligible (Preziosi, 2003);
4. no geometry is considered at this time.

The model consists of few key immune cell populations. The lymphocytes CD4 T-helper cells and CD8 cytotoxic T cells are modeled together with the population of cancer cells. Dendritic cells (the major antigen representing cells in vertebrate immune systems) are the source of TAA presentation and are introduced externally. The system is:

$$\frac{\mathrm{d}H}{\mathrm{d}t} = a_0 - b_0 H + c_0 D\left[d_0 H\left(1 - \frac{H}{f_0}\right)\right] \tag{1}$$

$$\frac{\mathrm{d}C}{\mathrm{d}t} = a_1 - b_1 C + c_1 I(M + D)\left[d_1 C\left(1 - \frac{C}{f_1}\right)\right] \tag{2}$$

$$\frac{\mathrm{d}M}{\mathrm{d}t} = \left[d_2 M\left(1 - \frac{M}{f_2}\right)\right] - e_2 MC \tag{3}$$

$$\frac{\mathrm{d}D}{\mathrm{d}t} = -e_3 DC \tag{4}$$

$$\frac{\mathrm{d}I}{\mathrm{d}t} = a_4 HD - c_4 CI - e_4 I \tag{5}$$

where **H** are the tumor-specific CD4 T helper cells, **C** are the tumor-specific CD8 T cells or CTLs cytotoxic cell, **M** are the cancer cells that expose the TAA, **D** are the **mature** dendritic cells loaded with the TAA (that is, that expose the tumor peptides on the HLA molecule[1]) and **I** is the IL-2 secreted by H and responsible for T cell growth.

[1]HLA: Human Leukocyte Antigen, proteins located on the surface of white blood cells and other tissues in the body that play a key role in regulating the self/nonself discrimination (Goldsby et al., 2000).

Note that we decide to use the logistic growth factor proposed by Verhulst in 1836. $R(x) = r(1 - \frac{x}{f})$ with r and f positive constants, where f is the carrying capacity of the environment that is usually determined by the available sustaining resources. $R(x)$ represents the per capita birth rate, that is, it is dependent on x.

The term $a_0 - b_0 H$ in Eq. (1) represents the production by the bone marrow of a very small number of tumor specific cells. Although tumor antigens are very poor immunogenic, it is reasonable to think that *very few* cells able to recognize them circulates in the host body. An equivalent term in Eq. (2) is given for tumor specific cytotoxic cells.

The term $c_0 D[d_0 H(1 - \frac{H}{f_0})]$ in Eq. (1) represents the clone expansion of tumor specific helper cells upon presentation of tumor associated antigen by dendritic cells. Dendritic cells are injected into the host already loaded, hence presenting the tumor peptides bind both to HLA class I or II. A saturation term for the growth of helper cells is considered.

The term $c_1 I(M + D)[d_1 C(1 - \frac{C}{f_1})]$ in Eq. (2) represents the clone expansion of tumor specific cytotoxic cells either by interaction with tumor cells or with dendritic cells. Since such clone expansion is possible only in presence of IL-2 which is secreted by T-helper cells upon recognition of dendritic loaded cells, no tumor specific response is possible without tumor antigen presentation by dendritic cells.

Cancer (e.g., myeloid) cells grow (limited grow) $[d_2 M(1 - \frac{M}{f_2})]$ in Eq. (3) and are killed by tumor specific cytotoxic cells; $-e_2 MC$.

Dendritic cells in Eq. (4) are killed by cytotoxic cells; $-e_3 DC$.

Interleukin IL-2 is produced by helper cells upon recognition of tumor loaded dendritic cells $a_4 HD$ and is consumed by cytotoxic cells during clonal growth $-c_4 CI$ (Eq. (5)), while the term $-e_4 I$ represents degradation of free interleukin.

The parameters of Table 1 have been chosen by tuning the system to a qualitatively reasonable dynamics starting from the set of values used for another mathematical model of the tumor-immune interaction (Kirschner and Panetta, 1998). The model in Eqs. (1)–(5) shows the following dynamics:

- In case of no treatment there is no immune response and the tumor grows up to the saturation limit;
- If we administer a vaccine, an immune response is obtained, that is a tumor specific response consisting of a growth of cytotoxic cells and helper cells recognizing the tumor. In Fig. 1 we show the dynamics of the system subjected to a given schedule of injections of DCs CTL cells kill dendritic cells because they show the tumor peptides. As a side effect, they also kill tumor cells. IL-2 is produced by helper cells upon contact with dendritic cells presenting tumor peptide together with MHC class II molecules. On the other hand, dendritic cells presenting tumor peptides on the class I MHC molecule are able to bind cytotoxic cell receptors and stimulate growth.

In the following section we give a short introduction of the theory of optimal control used herein and then the way we apply it. Finally, the results are shown together with some consideration for the work we plan for the future.

Table 1 Parameters of the model in Eqs. (1)–(5).

Name	Description	Value	Units (c = cells, h = hours)
a_0	birth of CD4 T	10^{-4}	$\mathrm{c\,h^{-1}\,mm^{-3}}$
b_0	death of CD4 T	0.005	$\mathrm{h^{-1}}$
c_0	max prolif of CD4 T	10	
d_0	1/2 satur const of CD4 T	10^{-2}	$\mathrm{c^{-1}\,h^{-1}\,mm^{-3}}$
f_0	carrying capacity of CD4 T	1	$\mathrm{c\,mm^{-3}}$
a_1	birth of CD8 T	10^{-4}	$\mathrm{c\,h^{-1}\,mm^{-3}}$
b_1	death of CD8 T	0.005	$\mathrm{h^{-1}}$
c_1	max prolif of CD8 T	10	
d_1	1/2 satur const of CD8 T	10^{-2}	$\mathrm{c^{-1}\,h^{-1}\,mm^{3}}$
f_1	carrying capacity of CD8 T	1	$\mathrm{c\,mm^{-3}}$
d_2	1/2 satur const of tumor	0.02	$\mathrm{h^{-1}}$
e_2	killing by CD8 of tumor	0.1	$\mathrm{c^{-1}\,h^{-1}\,mm^{3}}$
f_2	carrying capacity of tumor	1	$\mathrm{c\,mm^{-3}}$
e_3	CD8 T killing of DC	0.1	$\mathrm{c^{-1}\,h^{-1}\,mm^{3}}$
a_4	production by CD4 T	10^{-2}	$\mathrm{c^{-1}\,h^{-1}\,mm^{3}}$
c_4	IL-2 uptake by CD8 T	10^{-7}	$\mathrm{c^{-1}\,h^{-1}\,mm^{3}}$
e_4	degradation rate	10^{-2}	$\mathrm{h^{-1}\,mm^{-3}}$

Note: $e_2 = e_3$ since both dendritic and cancer cells express the same TAA by assumption.

3. Optimal control

Consider a general control system:

$$\frac{\mathrm{d}x}{\mathrm{d}t} = F(x, u), \tag{6}$$

where $x \in R^n$ and $u \in U$ compact subset of R^m. We fix a time horizon $T > 0$ and assume that F is regular so that for every measurable control function $u : [0, T] \to U$ and $\bar{x} \in R^n$ there exists a unique solution $x(\cdot, u)$ satisfying $\dot{x}(t, u) = F(x(t), u(t))$ a.e. on $[0, T]$ and $x(0, u) = \bar{x}$. An optimal control problem in Mayer form is given by:

$$\min_{u(\cdot)\in\mathcal{U}} \psi(x(T, u)), \qquad x(0) = \bar{x}, \tag{7}$$

where $\mathcal{U}$ is the class of admissible controls, ψ the final cost and $\bar{x}$ the initial condition. For example, under our assumptions, $\mathcal{U}$ can be defined as the set of all measurable controls. The aim is then to find a control $u(\cdot) \in \mathcal{U}$ so that $\psi(x(T, u))$ is minimized.

The well known Pontryagin Maximum Principle (PMP) (Pontryagin et al., 1961) provides, under suitable assumptions, a necessary condition for optimality. For problems in Mayer form with fixed final time and without final constraint as (7), PMP can be stated as follows:

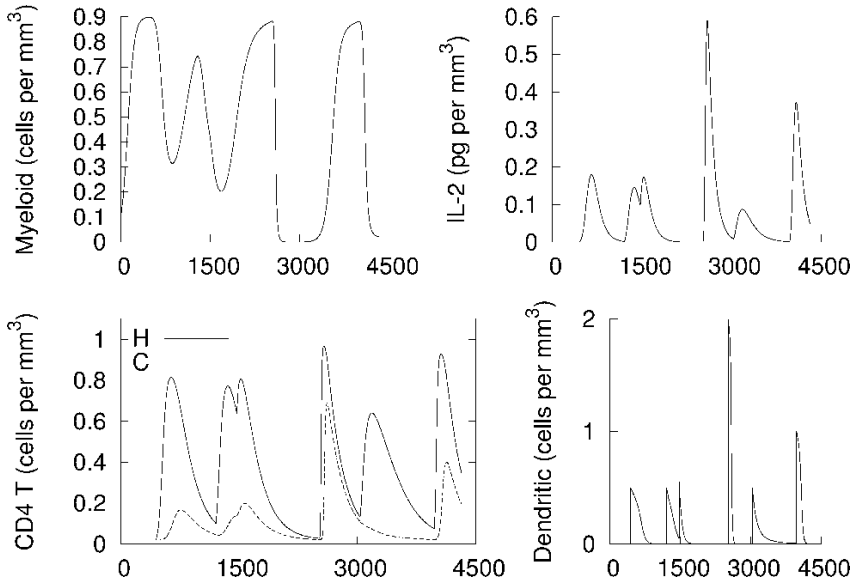

Fig. 1 The dynamics of the system subjected to the optimized schedule of injections of DCs (cfr. Fig. 4). The four plots show peaks of immune activity after each injection and, at the same time, reduction of the tumor ma ss.(x-axis is time in hours).

Theorem 1. *let $u^*(\cdot)$ be a (bounded) admissible control whose corresponding trajectory $x^*(\cdot) = x(\cdot, u^*)$ is optimal. Call $p : [0, T] \mapsto R^n$ the solution of the adjoint linear equation*

$$\frac{\mathrm{d}p}{\mathrm{d}t}(t) = -p(t) \cdot D_x F(x^*(t), u^*(t)), \qquad p(T) = \nabla\psi(x^*(T)). \tag{8}$$

Then the maximality condition

$$p(t) \cdot F(x^*(t), u^*(t)) = \max_{\omega \in U} p(t) \cdot F(x^*(t), \omega), \tag{9}$$

holds for almost every time $t \in [0, T]$.

The proof of the PMP relies on a special type of variations, called *needle variations*, of a reference trajectory. Given a candidate optimal control u^* and corresponding trajectory x^*, a time τ[2] and $\omega \in U$, a needle variation is a family of controls u_ε obtained replacing u^* with ω on the interval $[\tau - \varepsilon, \tau]$, see Fig. 2. A needle variation gives rise to a variation v of the trajectory satisfying, for $t \geq \tau$, the variational equation

$$\frac{\mathrm{d}v}{\mathrm{d}t}(t) = D_x F(x^*(t), u^*(t)) \cdot v(t) \tag{10}$$

[2]of approximate continuity for $F(x^*(\cdot), u^*(\cdot))$.

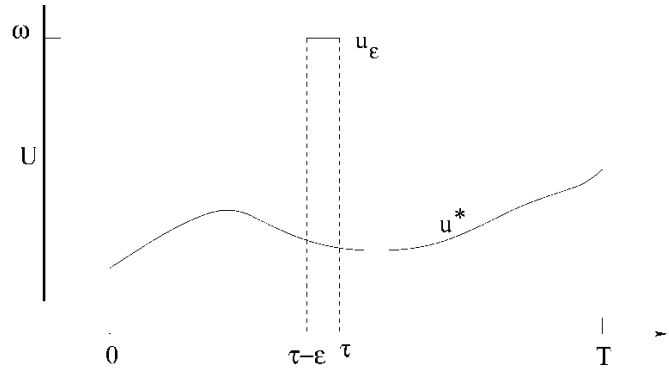

Fig. 2 Representation of a needle variation.

where $D_x F$ is the Jacobian of F w.r.t. x, with initial condition

$$F(x^*(\tau), \omega) - F(x^*(\tau), u^*(\tau)). \tag{11}$$

Notice that v satisfies Eq. (10) in classical sense only after time τ. Recently, see (Piccoli and Sussmann, 2000,G), it was introduced a setting in which needle and other variations happen to be differentiable. Since we use this more general (and mathematically natural) approach for the proof of a proposition in next section, we report some basic facts in the Appendix.

4. Optimal control theory applied to cancer immunotherapy

We now consider the DCV as a possible control on (1)–(5), hence we obtain the control system

$$\frac{\mathrm{d}x}{\mathrm{d}t} = F(x, u) = f(x) + ug(x) \tag{12}$$

where $x = (H, C, M, D, I)$ represents the cells population, the field f is given by (1)–(5) and, since DCV acts only on dendritic cells, we have $g(x) = \mathbf{e_4}$ the fourth coordinate vector. Our cost is the final value of the tumor mass M. Thus we consider the optimal control problem:

$$\min_{u(\cdot)\in\mathcal{U}} M(x(T, u(\cdot))), \qquad x(0) = x_0, \tag{13}$$

where T is the final time of the treatment period, x_0 is some fixed initial value of cells population and the set $\mathcal{U}$ is still to be defined.

We consider a vaccine administration procedure described by a control function

$$\bar{u} : [0, \eta] \mapsto [0, \bar{V}]$$

where $\bar{V}$ is the maximal vaccine quantity. The function $\bar{u}$ represents the value of injected dendritic cells population as a function of time.

The most important time scale, in the evolution of the system (1)–(5), is given by the cellular duplication time, which is estimated about $\frac{1}{3}$ of a day. On the other side, the time duration of the vaccine administration is often of the order of one hour or less, hence very small compared to the natural time scale of (1). Therefore we assume that η is very small.

Consider now a family of controls u_ε corresponding to a single vaccine administration procedure that happens at time $t_\varepsilon = \bar{t} + \varepsilon$. Then the family u_ε gives rise to a trajectory variation characterized by next:

Proposition 1. *Let u_ε be a family of controls corresponding to a single vaccine administration procedure at time $t_\varepsilon = \bar{t} + \varepsilon$. If $\bar{u}$ is constant, then, recalling that η is the time duration of vaccine administration, the corresponding variation for $t \geq \bar{t}$ is given by:*

$$\begin{cases} \frac{\mathrm{d}v(t)}{\mathrm{d}t}(t) = D_x f(x_0(t)) \cdot v(t) \\ v(\bar{t}) = f(x_0(\bar{t})) - f(x_0(\bar{t}) + V) + o(\eta), \end{cases} \tag{14}$$

where $V = \eta u(0)\mathbf{e_4}$.

The proof of Proposition 1, under slightly more general assumptions, is postponed to the Appendix.

The clinical treatment of a patient via immunotherapy consists in a series of DCVs that are scheduled over a time range of some months. We then consider a control procedure that consists in N vaccinations inoculated according to a *schedule* $S = \{t_i : i = 0, \ldots, N-1, 0 \leq t_0 \leq t_1 - \eta < t_1 \leq \cdots \leq t_{N-1} \leq T - \eta\}$. Let $\mathcal{S}$ be the space of schedules, then for every $S \in \mathcal{S}$ we define $u(S)$ to be the corresponding control

$$u_S(t) = \sum_{i=0}^{N-1} \bar{u}(t - t_i)\chi_{[t_i, t_i+\eta]}.$$

The control u_S corresponds to N vaccine administration procedures that occur at time t_i. Finally we set:

$$\mathcal{U} = \{u_S : S \in \mathcal{S}\}$$

and the optimal control problem (13) is now well defined:

(P) Given the initial condition x_0 determine a schedule $S \in \mathcal{S}$ so that the trajectory x_S of $\frac{\mathrm{dx}}{\mathrm{d}t} = f(x(t)) + u_S(t)g(x)$ attains the minimum of $M(x(T, u))$.

It is easy to notice that such optimal control problem is indeed a (finite dimensional) optimization problem. In fact the space S can be clearly parameterized by a subset of R^N.

Remark 1. Thanks to Proposition 1, we can approximate this optimization problem considering the set of controls given by finite sums of delta functions centered

at vaccination times of the schedule, thus considering formally $\eta = 0$. This can be checked by computing the difference obtained by shifting a delta function.

The set $\mathcal{S}$ is obviously compact and the function $S \mapsto M(x_S(T))$ continuous, hence there exists a solution to (P). Analogously to Proposition 1, one can easily prove:

Proposition 2. *For the problem (P) we have:*

$$\frac{\partial M(x_S(T))}{\partial t_i} = \nabla\psi(x_S(T)) \cdot v_i(T) = \mathbf{e_4} \cdot v_i(T) + o(\eta)$$

where $v_i(\cdot)$ is the solution to (14) for $\bar{t} = t_i$ and $x_0 = x_S$.

To solve numerically problem (P) we can use Proposition 2 and steepest descent or other optimization methods.

5. Optimization

The optimization algorithm is coded in C and consists of the following iterative procedure:

Step 0 Fix the time T horizon, the number N of vaccine administrations, the value V of vaccine quantity, an initial value x_0 of cells population and an initial schedule S_0 (the vector of vaccine administration time);
Step 1 Solve the system (1)–(5) with initial value x_0 via the fourth-order Runge-Kutta integrator generating an approximation of the trajectory x_S. At the same time solve the variational equation $\frac{\mathrm{d}v_i}{\mathrm{d}t} = D_x f(x_S) \cdot v_i$ with initial condition $v_i(t_i) = f(x_S(t_i)) - f(x_S(t_i) + V)$ for $i = 1, \ldots, N$;
Step 2 Compute $\partial M(x_S(T))/\partial t_i$ via Proposition 2.
Step 3 Update the schedule according to steepest descent, i.e., add to t_i the value $h \cdot \partial M(x_S(T))/\partial t_i$ for some small parameter h. Goto Step 1.

Remark 1. Notice that in Step 1 we compute at the same time the solution to (1)–(5) and the variational Eq. (14), this allows to spare memory also in view of more complex models. One has to notice, however, that in order to use a Runge–Kutta method of step Δt for the variation equations for v, the trajectory x_S must be computed with a step $\Delta t/2$. In fact the computations for v require the knowledge of the values of x_S. Simulations based on more simple integrator, such as first order Euler, for the variation equations give erroneous behavior of the optimization procedure.

In particular a time horizon T of six months and $N = 10$ DCVs injections are chosen. Moreover, we take the initial value of the tumor $M(0) = 0.1$, the H and C initial levels are set to equilibrium that is $H(0) = a_0/b_0$ and $C(0) = a_1/b_1$, while $I(0)$ and $D(0)$ are set to zero, meaning that there is no specific response at the

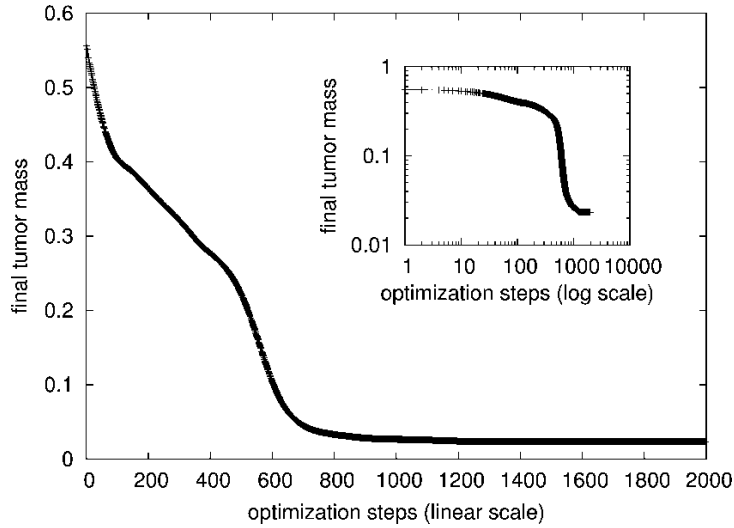

Fig. 3 This figure shows the final value of the tumor (linear and logarithmic scale) for each optimization step.

initial time. The vaccine quantity injected at each administration cycle is $V(0) = 0.5$.

We run the simulations with the initial schedule S_0 chosen randomly and 2000 optimization steps. In Fig. 1 we represent the dynamics of the model variables correspondent to the administration of the final schedule $\tilde{S}$, that is the output of the optimization procedure.

In Fig. 3 we represent the outcome of final cancer cells population during the same optimization run. Notice that the final value of the tumor decreased consistently from about 0.55 of the first optimization step to an almost zero level, which means essentially clearance of the tumor. Unfortunately, given the fast growth rate of the tumor cells (M), these population tends to increase immediately after the effect of a vaccination cycle and corresponding immune response. Moreover, given the fairly rapid decrease of immune cells coding for the immune memory, the final value of the tumor mass is quite sensitive with respect to the time of the last vaccination. While this is due to the relative simplicity of the model (something we are going to improve as described in the conclusions) it does not affect the optimization scheme presented in this work.

In Fig. 4 it is shown the evolution of the schedule during the iterations of the program. The number of iterations is plotted on the first axis, while in the second axis the vaccine administration times are reported. The time value is represented in hours (4320 time steps equal to six months). Also here we notice that, as expected, the cost function is more sensitive with respect to late vaccinations. Again, this is consequential to the fact that the effect of the vaccine injection expires in time and is consistent with mice experiments that only a continued therapy (chronic therapy) is able to keep lifelong immunity and correspondent control of the tumor.

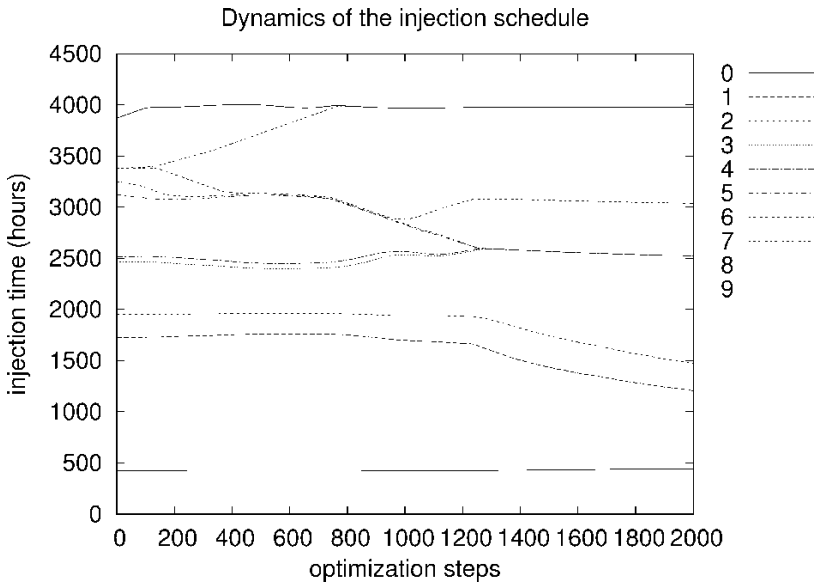

Fig. 4 We plot here the injection schedule versus the optimization steps. The initial schedule (or protocol) is chosen at random. The dynamics shows a non trivial aggregation of injections at the beginning and at about half of the period.

In this precise example the first vaccination time remains basically the same during all the optimization phase (injection 0 in Fig. 4). The last five (i.e., injections 5–9) vary most rapidly. Injections 5, 6, and 7 essentially collapse in a unique triple vaccination from the very beginning. Other joins take place during the optimization and are described in more detail in Fig. 5.

The inset plot (a) of Fig. 5 reports the evolution of the injection vector S as a function of the optimization step while inset (f) shows the cost function of the same optimization run. Inset (b) zooms on the dynamics of injections 8 and 9 (the upper box of (a)); it shows that the two injection times get very close in the first place, then run together for few hundred steps and finally start to fluctuate as shown in the zoomed area of inset plot (c). Also note in the latter box the three spikes due to the discretization of the optimization procedure. Going back to (a), note that the second box zooms to inset (d). This plot together with plot (e) zooming from (f) reveals drastic changes in the search path that correspond to different regimes in the minimization of the cost function.

Summarizing, Fig. 5 discloses the complexity of the optimization problem and in particular that the variation of the injection times is not far from being trivial.

In Fig. 1 we have shown the evolution of all variables at the end of the optimization algorithm. For the chosen values, the tumor is highly affected by the presence of the H (tumor specific T helper) cells and the C (tumor specific cytotoxic) cells. The decay of these cells is quite rapid and even more is that of dendritic (D) and interleukin (I). Let us remark that the value of the tumor is not under control for the whole time horizon but only the final value is taken as the cost of the optimization procedure. This often results in concentrating some injection in the last part

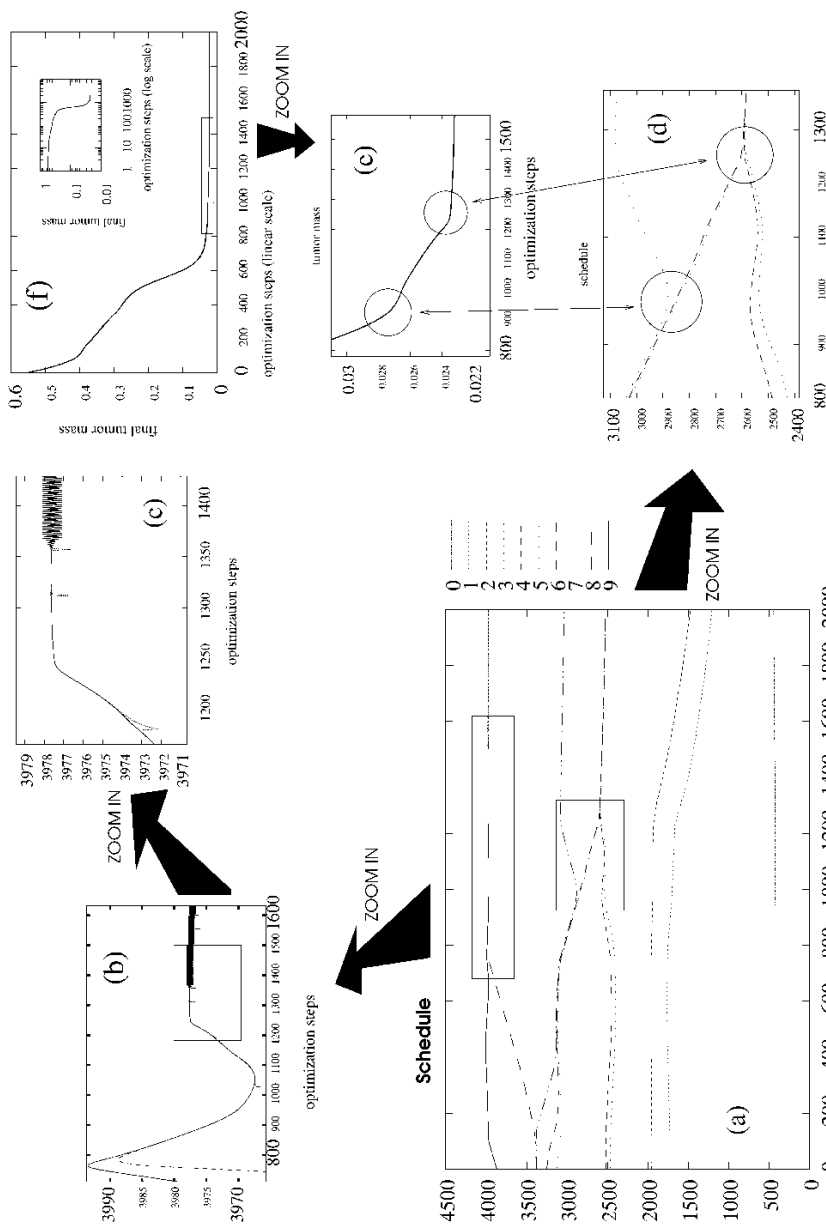

Fig. 5 Figures 3 and 4 are reported here together with some zooming on a couple of interesting features revealing the richness of the behavior of the optimization dynamics (see text for details).

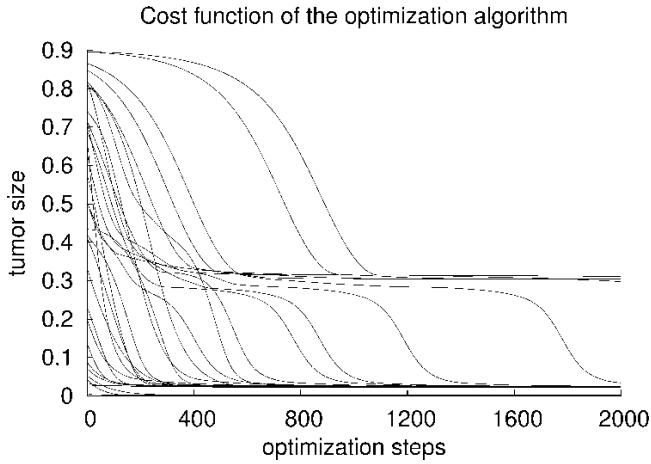

Fig. 6 This figure shows the value of the cost function during the optimization for a number of runs starting from a different random schedule.

of the therapeutic horizon. Unfortunately, inoculating the vaccine at times which are too close, may result in toxic effects like allergic reactions or other undesired alterations of the physiologic state of the patient.

In order to have a better allocation of the injections in the therapeutic period, one could, for instance, add a constraint or cost on the maximal value of the tumor during the whole time horizon. While this is natural for the model on one side, on the other side the optimal control problem obtained is much more difficult to be treated. This question constitutes a topic of future investigation and is further addressed in the conclusions.

5.1. Statistics

In Fig. 6 we show the value of the tumor mass at the end of each optimization step, versus the optimization time for different runs of the algorithm starting from a random initial schedule of vaccine injections. The figure shows that there are some saddle points corresponding to the value of the cost function equal to about 0.3 and another at 0.025. From Figs. 4 and 6 we conclude that the optimization procedure reaches some critical points (saddles) where the decrease of the cost function is strongly reduced.

To get an idea of how the optimization algorithm affects a random initial schedule, we computed the distribution of the optimal schedule and compared with the initial one. In other words, representing by $S_0 = (t_0, \ldots, t_9)$ the schedule of injections, the optimization can be seen as a transformation into another vector $\tilde{S} = (\tilde{t}_0, \ldots, \tilde{t}_9)$ of injection times. Then, by running a large number of optimizations from a randomly chosen initial schedule $t_k = \text{rand}(0, T) : t_k < t_j, \forall i,\ j \in \{0, 9\}$, we compared the distribution of each t_j with that of the corresponding $\tilde{t}_j$. This is depicted in Fig. 7. To show the differences better we plotted the initial distribution

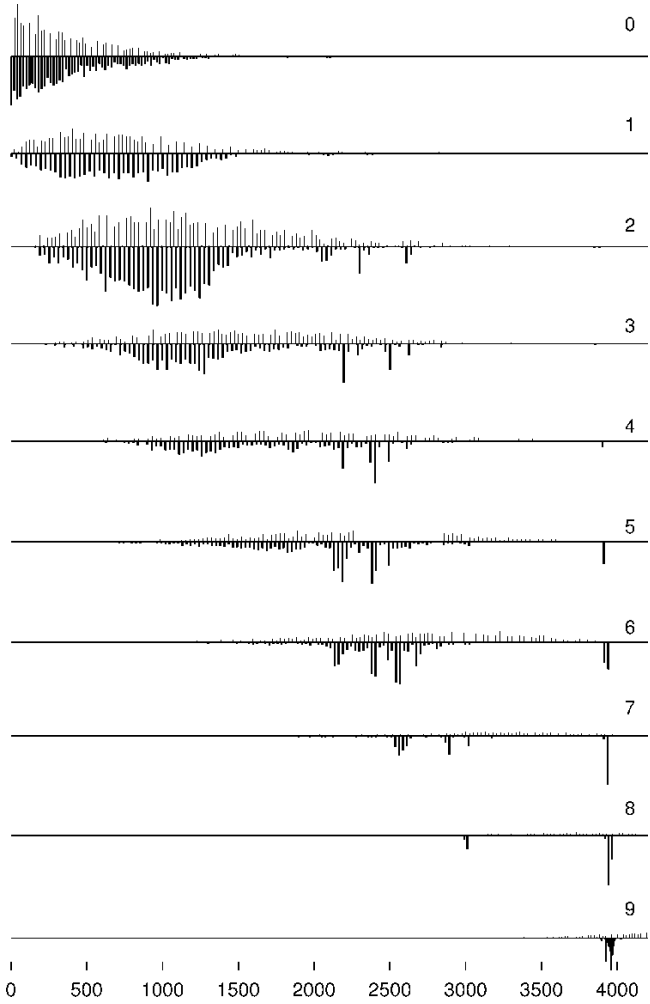

Fig. 7 On the x-label we have the therapeutic time horizon in hours (equivalent to about six months). On the y-label we have the normalized frequency (distribution). For visual comparison negative values are assigned to the distribution of the final optimized schedule. The number on the right of each plot is the index of the injection vector starting from 0.

of t_j in gray on the y-positive semiplane and the distribution of the schedule at the end of the optimization phase (i.e., the $\tilde{t}_j$s) on the y-negative semiplane in black.

Again, what is immediately evident is that those injections that are mostly changed by the optimization algorithm are those at the end of the optimization horizon while the first show little or no difference with respect to their initial random values, meaning that they are less important for the final reduction of the tumor mass. Moreover, a clear indication for the optimal values of $\tilde{t}_j$ exist for the last injections (i.e., $j = 8$ and $j = 9$) while injection 7 shows to have at

least two possible optima. This is somehow interpretable as the need to keep low the final value of the tumor that, indeed, is the cost of the optimization function. Clearly, by choosing a different cost function (as for example the integral of the tumor on the time horizon), we would get quite diverse distributions of $\tilde{S} = (\tilde{t}_0, \ldots, \tilde{t}_9)$.

5.2. Robustness of the optimization algorithm

For what concerns the dependency of the solution of the optimization problem on the parameters' values one can divide the space of possible changes of the parameters, with respect to the choice in Table 1, in two parts: the first subspace is that of parameters' changes that favor the tumor growth and the other is the one that makes a more effective immune system response (note that this is the "dual" of the first). Examples of parameters' changes in the first subset are: the increase of tumor growth d_2; decrease of cytolytic efficacy e_2; of the immune responsiveness to the vaccine (called immunogenicity) c_0 and c_1; increase of degradation rate of IL-2 e_4; just to mention few. The impact of a parameters' changes that favor the tumor growth consists in a slower convergence of the optimization algorithm (smaller reduction of the tumor at the end of the therapeutic period for a fixed number of optimization steps). In the opposite case of an increase of the immune system efficiency (as for example having a slower degradation of IL-2 that keeps a sustained immune response) the optimization algorithm converges faster but vary less the vaccination schedule.

In both cases the optimization procedure shows to be robust with respect to parameters' changes since a tumor reduction is always attained.

The number of injections required to keep the tumor mass small is very much dependent on the choice of the parameters that define the "balance" of the tumor aggressiveness with the efficiency of the immune response. In other words N is a function of all system parameters and of the initial condition.

Clearly a small N is better from the clinical point of view (less burden for the patient, smaller risk of toxicity, cost, etc.) but it is quite difficult to find its lower bound just by numerical analysis. Finally it is worth to say that for a fixed setting of the carrying capacities in Eqs. (1)–(5), there is a threshold above which one does not have any improvements in increasing the number of injections N, simply because the immune system efficacy is limited.

6. Conclusions

We have constructed a mathematical model of the immune-cancer interaction to study the effect of immunotherapy via dendritic cell vaccines (DCVs). The model though quite simple reproduces the expected dynamics of tumor-immune interaction both in the case of presence and absence of treatment. The question is then how to allocate the injections within the time horizon so to have an optimal control of the tumor.

Therefore we consider an optimal control problem with final value of tumor mass as cost function and, as optimization horizon, the treatment period of six months. Since the possible schedules take values in a finite dimensional set, the

optimal control problem reduces to an optimization one. However, we used typical tools of optimal control to approximate the effect of DCVs and compute the gradient of the cost function with respect to the schedule. The latter is obtained via the solution of a generalized variational equation, while the optimization algorithm is based on the steepest decent method.

The results are very satisfactory since the optimized schedule is always able to reduce the tumor consistently within the therapeutic horizon. However, given the fast grow of the tumor and the chosen cost function of the optimization schema, there is a strong dependencies with respect to the last vaccine injections.

Further research should go in the direction of improving the model by choosing a more realistic law for cancer growth and by adjusting the effects of DCVs on tumor levels and other cells. Another possible direction is to consider more complicate optimal control problems, e.g., including the maximal level of the tumor during the treatment period as cost (and/or constraint) or asking for a minimal separation of vaccination times. Whereas on one hand this leads to a more realistic model and avoids the undesired side-effects of clustered injections, on the other hand it leaves us with an optimal control and optimization problem that is much more difficult to be treated.

Appendix

First let $\mathcal{C} = \mathcal{C}([0, T]; R^n)$ be the space of R^n–valued continuous functions defined on $[0, T]$ and $\mathcal{M}$ its dual, the space of bounded R^n–valued Radon measures on $[0, T]$, see (Folland, 1999) for the basic theory.

Definition 1. A parameterized family of controls $\{u_\varepsilon : [0, T] \to U;\ \varepsilon \in [0, \bar{\varepsilon}]\}$ with corresponding trajectories x_ε is weakly differentiable at $\varepsilon = 0$ if u_ε converges strongly to u_0 in L^1 and

$$w_\varepsilon(\cdot) = \frac{F(x_0(\cdot), u_\varepsilon(\cdot)) - F(x_0(\cdot), u_0(\cdot))}{\varepsilon}$$

converges for the weak*-topology of $\mathcal{M}$ to some measure $\mu \in \mathcal{M}$. In other words, for every continuous function $\phi \in \mathcal{C}$ the map $\varepsilon \mapsto \int \phi_i(t)(w_\varepsilon)_i(t)\,\mathrm{d}t$ converges to $\int \phi_i(t)\,\mathrm{d}t\mu_i(t)$ for every $i = 1, \ldots, n$.

Given a weakly differentiable family u_ε the corresponding trajectory variation v satisfies:

$$\mathrm{d}v = D_x F(x_0, u_0) \cdot v\,\mathrm{d}t + \mathrm{d}\mu,$$

where now the equation is understood in integral sense. Otherwise stated, denoting by $M(\cdot, \cdot)$ the fundamental matrix solution to (10) with x^*, u^* replaced by x_0, u_0, we get:

$$v(t) = M(t, t')v(t') + \int_{t'}^{t} M(t, s)\mathrm{d}\mu(s).$$

(Notice that now in the integral $\int_{t'}^{t}$ it matters to include endpoints, otherwise, since μ may fail to be absolutely continuous w.r.t. Lebesgue measure, the value of the second integral may change.) Thus for a needle variation we obtain:

$$\mathrm{d}v = D_x F(x^*, u^*) \cdot v \,\mathrm{d}t + \big(F(x^*(\tau), \omega) - F(x^*(\tau), u^*(\tau))\big)\mathrm{d}\delta_\tau,$$

where δ_τ indicates a Dirac delta centered at τ. Restating in integral form:

$$\begin{aligned} v(t) &= M(t,0)v(0) + \int_0^t M(t,s)(F(x^*(\tau),\omega) - F(x^*(\tau),u^*(\tau)))\,\mathrm{d}\delta_\tau(s) \\ &= \chi_{[\tau,T]}(t)M(t,\tau)(F(x^*(\tau),\omega) - F(x^*(\tau),u^*(\tau))), \end{aligned} \tag{A.1}$$

where χ_A is the indicator function of the set A. Then one can prove PMP in the usual way.

Consider now a family of controls of type:

$$u_\varepsilon = \sum_{i=0}^{N} u^i(t)\chi_{[t_i^\varepsilon, t_{i+1}^\varepsilon[} \tag{A.2}$$

where $u^i : [0,T] \to U$ is continuous and $0 = t_0^\varepsilon < t_1^\varepsilon < \cdots < t_{N+1}^\varepsilon = T$. If $t_i^\varepsilon = t_i^0 + \varepsilon + o(\varepsilon)$, $i = 1, \ldots, N$, then such a family is weakly differentiable and the variation v of the corresponding trajectories x_ε satisfies:

$$\mathrm{d}v = D_x F(x_0, u_0) \cdot v \,\mathrm{d}t + \sum_{i=1}^{N} \mathrm{d}\delta_{t_i} \cdot (F(x_0(t_i), u^{i-1}(t_i)) - F(x_0(t_i), u^i(t_i))).$$

Proof of Proposition 1.
We prove Proposition 1 under the more general assumptions: $t_\varepsilon = \bar{t} + \varepsilon + o(\varepsilon)$, $\bar{u}$ is $\mathcal{C}^1$ and satisfies $\frac{\mathrm{d}}{\mathrm{d}t}\bar{u}(t) = o(\eta)$.
Notice that the family u_ε is formed by control functions of type (A.2), thus it is weakly differentiable. The corresponding expression for w_ε, defined as in Definition 1, is given for $\varepsilon < \eta$ by:

$$w_\varepsilon(t) = \begin{cases} \frac{1}{\varepsilon}(F(x_0(t),0) - F(x_0(t),\bar{u}(t-\bar{t}))) & \text{if } \bar{t} \le t < \bar{t} + \varepsilon \\ \frac{1}{\varepsilon}(F(x_0(t),\bar{u}(t-\bar{t}-\varepsilon)) - F(x_0(t),\bar{u}(t-\bar{t}))) & \text{if } \bar{t}+\varepsilon \le t \le \bar{t} + \eta \\ \frac{1}{\varepsilon}(F(x_0(t),\bar{u}(t-\bar{t}-\varepsilon)) - F(x_0(t),0)) & \text{if } \bar{t}+\eta < t < \bar{t} + \eta + \varepsilon. \end{cases}$$

Thus, since $\bar{u}$ is continuous, passing to the weak* limit in $\mathcal{M}$ as ε tends to 0 we get:

$$\begin{aligned} w_\varepsilon(t) \to^* \mu = {} & \delta_{\bar{t}}(t)(F(x_0(\bar{t}),0) - F(x_0(\bar{t}),\bar{u}(0))) \\ & + \delta_{\bar{t}+\eta}(t)\big(F(x_0(\bar{t}+\eta),\bar{u}(\eta)) - F(x_0(\bar{t}+\eta),0)\big) \\ & + \chi_{[\bar{t},\bar{t}+\eta]}(t) D_u F(x_0(t),\bar{u}(t-\bar{t})) \cdot \frac{\mathrm{d}}{\mathrm{d}t}\bar{u}(t-\bar{t})\,\mathrm{d}t, \end{aligned}$$

thus using (12) and $\frac{\mathrm{d}}{\mathrm{d}t}\bar{u}(t) = o(\eta)$:

$$w_\varepsilon(t) \to^* \mu = -\delta_{\bar{t}}(t)(\bar{u}(0)g(x_0(\bar{t}))) + \delta_{\bar{t}+\eta}(t)(\bar{u}(\eta)g(x_0(\bar{t}+\eta))) + o(\eta).$$

Therefore, denoting by $M(\cdot,\cdot)$ the fundamental matrix solution to

$$\frac{\mathrm{d}v}{\mathrm{d}t} = D_x F(x_0(t), u_0(t)) \cdot v(t), \tag{A.3}$$

the variation v satisfies:

$$\begin{aligned} v(\bar{t}+\eta) &= \int_{\bar{t}}^{\bar{t}+\eta} M(\bar{t}+\eta, s)\,\mathrm{d}\mu(s) \\ &= -\bar{u}(0)M(\bar{t}+\eta,\bar{t})g(x_0(\bar{t})) + \bar{u}(\eta)M(\bar{t}+\eta,\bar{t}+\eta)g(x_0(\bar{t}+\eta)) \\ &= -\bar{u}(0)\left(M(\bar{t}+\eta,\bar{t})\mathbf{e_4} - \mathbf{e_4}\right) + o(\eta). \end{aligned}$$

Denoting by ζ the solution to (A.3) with $\zeta(\bar{t}) = \bar{u}(0)\mathbf{e_4}$, we write:

$$\begin{aligned} v(\bar{t}+\eta) &= \zeta(\bar{t}) - \zeta(\bar{t}+\eta) + o(\eta) \\ &= -\int_{\bar{t}}^{\bar{t}+\eta} D_x F(x_0(s), u_0(s)) \cdot \zeta(s)\,\mathrm{d}s + o(\eta) \\ &= -\int_{\bar{t}}^{\bar{t}+\eta} D_x f(x_0(s)) \cdot \bar{u}(0)\mathbf{e_4}\,\mathrm{d}s + o(\eta) \\ &= -\int_{\bar{t}}^{\bar{t}+\eta} \left[\frac{\mathrm{d}}{\mathrm{d}s} f(x_0(\bar{t}) + (s-\bar{t})\bar{u}(0)\mathbf{e_4})\right] s + o(\eta) \\ &= f(x_0(\bar{t})) - f(x_0(\bar{t}) + \bar{u}(0)\mathbf{e_4}) + o(\eta). \end{aligned}$$

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References

Boczkowski, D., Nair, S.K., Nam, J.H., Lyerly, H.K., Gilboa, E., 2000. Induction of tumor immunity and cytotoxic T lymphocyte responses using dendritic cells transfected with messenger RNA amplified from tumor cells. Cancer Res. 60(4), 1028–1034.

Dietz, A.B., Litzow, M.R., Gastineau, D.A., Vuk-Pavlovic, S., 2001. Engineering dendritic cell grafts for clinical trials in cellular immunotherapy of cancer: example of chronic myelogenous leukemia. Croat Med. J. 42(4), 428–435.

Folland, G.B., 1999. Real Analysis Modern Techniques and Their Applications. Wiley, New York.

Garavello, M., Piccoli, B., in press Hybrid necessary principle. SIAM J. Control Optim.

Goldsby, R.A., Kindt, T.J., Osborne, B.A., 2000. In: W.H. Freeman. Kuby Immunology, 4th edn. New York.

Kirschner, D., Panetta, J.C., 1998. Modeling immunotherapy of tumor-immune interaction. J. Math. Biol. 37, 235–252.
Perelson, A., Weisbuch, G., 1997. Immunology for physicists. Rev. Mod. Phys. 69, 1219–1267.
Piccoli, B., Sussmann, H.J., 2000. Regular synthesis and sufficiency conditions for optimality. SIAM J. Control Optim. 39, 359–410.
Pontryagin, L.S., Boltianski, V., Gamkrelidze, R., Mitchtchenko, E., 1961. The Mathematical Theory of Optimal Processes. Wiley, New york.
Preziosi, L. (Ed). 2003. Cancer Modeling and Simulation. Chapman & Hall/CRC (UK), London, June 26.