Metamodelling tumor–immune system interaction, tumor evasion and immunotherapy

Alberto d’Onofrio*

Epidemiology and Biostatistics Division, European Institute of Oncology, Via Ripamonti 435, Milano I-20141, Italy

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Abstract

The tumor–immune system competitive interaction is very complex, being nonlinear and, to some extent, evolutive. Furthermore, the tumor itself is not a single well defined disease, but, more correctly, a wide family of diseases, characterized by some important common features, but also by many great differences between them. For this reason, we introduced in [A. d’Onofrio. A general framework for modeling tumor–immune system competition and immunotherapy: Mathematical analysis and biomedical inferences. Physica D 208 (2005) 220–235], and in other works, a new mathematical way of studying the interaction of tumors with immune system and immunotherapy. Our approach is not based on new specific models, but on meta-models (i.e. family of models), which are studied with the help of the qualitative theory of differential equations. We review here our work on this field, by illustrating our main results, in view of their possible biomedical applications, and extending them.

Keywords: Tumor–immune system; Immunotherapy; Qualitative theory of differential equations

1. Introduction and main mathematical and biological results

The generic word “cancer” in reality denotes an entire family of high-mortality diseases [1,2] each differing from the other, but all characterized by a remarkable lack of symptoms [1,2] and by time courses that may be classified, in a broad sense, as nonlinear since it reflects macroscopically a considerable number of intra-cellular and inter-cellular phenomena which are strongly nonlinear and time-varying. As a consequence the behavior of “cancer” is anti-intuitive. In our opinion, this inherent nonlinearity is the main reason why, despite the enormous strides in prevention [4] and cure [1,2], “cancer” is one of the leading causes of death worldwide [5], and, unfortunately, is likely to remain so for many years to come [6].

For these reasons, as previously stressed by Bellomo and Maini in [7], we strongly believe that methods of modern mathematical physics, and in particular the theory of finite and infinite dimensional dynamical systems, may play an important role in oncology of the XXIst century, both from a theoretical point of view and also in the clinical practice, by means of appropriate model-based decision support systems. We hope that these software systems might become a day by day tool for oncologists as well as the ECG devices are for cardiologists. We would like to stress that we are

* Tel.: +39 0257489377.
E-mail address: alberto.donofrio@ieo.it.

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not speaking of a far misty future. In fact, we believe that, even today, some software based on physical–mathematical models might also be cautiously used in clinical practice in a limited but relevant field: discriminating between treatments which would have high probability of failures.

Among the many phenomena of interaction involving tumor cells (TCs), in this article we shall focus on the interactions with the cells of the immune system [2,8] (IS), which are, broadly speaking, of the Volterrian type [9,26]. Furthermore, the responses of tumor cells (TCs) to these interactions are characterized by a considerable evolutionary ability via changes by means of mutations to enhance their survival in a hostile environment [10,11].

The tumor–IS interaction takes place because TCs are characterized by a vast number of genetic and epigenetic events leading to the appearance of specific antigens, called neoantigens, triggering antitumoral actions by the IS [12]. These observations provided a theoretical basis [13] to the old empirical hypothesis of immune surveillance, i.e. that the IS may act to eliminate tumors [14]. The story of cancer immunobiology is, in fact, very old, but only in recent years, thanks to new molecular techniques (and to large epidemiological studies) a sufficient amount of evidence has been accumulated in favor of this hypothesis [8].

The competitive interaction between TCs and the IS involves a considerable number of events and molecules, and as such is extremely complex and, as a consequence, the IS is not able to eliminate a neoplasm in all cases, which may escape from IS control. Of course, a dynamic equilibrium may also be established, such that the tumor may survive in a microscopic steady state (MISS), which is undetectable by diagnostic equipment [15]. However, consider a tumor which is constrained by the IS in a MISS. Over a long period of time (a significant fraction of the mean life span in men, according to [8]), the neoplasm may develop multiple strategies to circumvent the action of the IS [16,12,8,17], which, in the long term, may allow it to evade immune surveillance and to re-commence growing to its carrying capacity [15]. The tumor has adapted itself to survive in a hostile environment, in which antitumor immune response is activated [15]. In other words the immunogenic phenotype of the tumor is “sculpted” [8] by the interaction with the host’s IS. For this reason, the theory of IS–T interaction has been called immunoediting theory by Dunn et al. [8].

Finally, the study of the interaction tumor–immune system led to the proposal and implementation of an interesting therapeutic approach: the immunotherapy [1,2], consisting in stimulating the IS in order to better fight, and hopefully eradicate, a cancer. In particular, in this paper I will be referring to generic immunostimulations, for example via cytokines. The basic idea of immunotherapy is simple and promising, but the results obtained in medical investigations are globally controversial [23–25], even if in recent years there has been evident progress. One of our main aims will be to illustrate how mathematics may help us to better understand better this kind of therapy, and its clinical trials.

Coming to the mathematical way to model the above interactions, the basic idea of the ecological modelling of TCs–IS interaction of Ref. [26,15] is simple: TCs and effector cells (ECs) of IS are seen as two competing populations. TCs are mainly the prey of the ECs, whose proliferation is stimulated, in turn, by the presence of TCs. However, TCs also induce a loss of ECs; and there is an influx of ECs, whose intensity may depend on the size of the tumor [26]. Based on this simple scheme and on its generalizations, many works have appeared using a finite dimensional approach based on specific models with constant or tunable parameters [27–34] (and references therein).

However an approach based on a specific model is in contrast with the polymorphic nature of cancer, and it does not allow easily to catch the general features of the TC–IS interaction.

Here we will analytically study this topic within the mathematical framework of the approach, introduced by us in [26,15,35]. This approach is based on meta-models (where meta-model means “a family of models”) and, in our opinion, is a natural and effective way to capture the common features of a such a wide family of diseases as tumors represent. Note that another very interesting way of studying the immuno-oncological dynamics is the infinite dimensional kinetic modeling introduced by Bellomo and coworkers [36–38].

In this work we shall review and also extend the metamodels proposed in our previous papers [26,15,35]. In particular, we give a new and more general result on global eradication of the disease under immunotherapy, and we extend the study of tumor evasion to the case in which the tumor-induced proliferation depends on both TC and effectors.

2. A metamodel for tumor–immune system interaction

Analyzing the best known finite dimensional models [28,29,27,31,33], we note that their main features are the following:

- Existence of a tumor free equilibrium;
Depending on the values of parameters, there is the possibility that the tumor size may tend to $+\infty$ or to a macroscopic value;

- Possible existence of a “small tumor size” equilibrium, which coexists with the tumor free equilibrium;
- Constance of the influx of effector cells;
- Variable proliferative profile of effectors, depending on the tumor burden;
- Constant of tumor-size dependent death rate of effectors.

An “accessory” feature is the existence of limit cycles [27]. From this rough summary, one may understand that the puzzling results obtained up to now by immunotherapy [23] may be strictly linked to the complex dynamical properties of the immune system–tumor competition. In general, it happens that the cancer-free equilibrium coexists with other stable equilibria or with unbounded growth, so that the success of the cure depends on the initial conditions, and – even theoretically – it is not always granted.

Summarizing and generalizing the above cited biological features and models, in [26] we introduced the following general family of models:

\[
\begin{align*}
x' &= x(f(x) - \phi(x)y) \\
y' &= \beta(x)y - \mu(x)y + \sigma q(x) + \theta(t)
\end{align*}
\]  

(1) (2)

where:

- $x$ and $y$ are the “sizes” (total number, densities or $a$-dimensional quantities) of, respectively, tumor cells and of ECs of IS;
- $0 < f(0) \leq +\infty$, $f'(x) \leq 0$ and in some relevant cases we shall suppose that it exists an $0 < K \leq +\infty$ such that $f(K) = 0$, $\lim_{x \to 0^+} x f(x) = 0$. In such a way, $f(x)$ summarizes many widely used models of tumor growth rates, such as the Exponential model: $f(x) = \alpha > 0$ [39], the Gompertz: $f(x) = \alpha \log(A/x)$ [39, 40] and its generalizations [39,40], the Logistic model: $f(x) = \alpha(1 - x/A)$ [40], the Hart–Schochat–Agur: $f(x) = C * x^{-\gamma}$, $0 < \gamma < 1$ [41], the von Bertalanffy: $f(x) = \alpha(x^{-1/3} - b)$ [40,42], the Guiot et al.’s model: $f(x) = \alpha(x^{-3/4} - b)$ [43], the linear growth model by Bru and coworkers [44] which may be written as follows: $f(x) = C x^{-1/3}$ (note that it may be considered a particular case of the von Bertalaffy model and of the Hart–Schochat–Agur model) etc.
- $\phi(x) > 0$, $\phi(0) = 1$, $\phi'(x) \leq 0$ and $x \phi(x) \to l \leq +\infty$;
- $\beta(x) \geq 0$, $\beta(0) = 0$ and $\beta'(x) \geq 0$;
- There is a tumor-induced loss of ECs [16] $\mu(x) > 0$, which is and increasing function $\mu'(x) > 0$;
- $q(x)$ is such that $q(0) = 1$ (as a consequence $\sigma = Q(0)$) and it may be non-increasing or also initially increasing and then decreasing, i.e. we may assume that either the growth of tumor decreases the influx of immune cells or that, on the contrary, it initially stimulates the influx). Note that it has been experimentally observed that in some cases cancer progression may cause generalized immunosuppression (i.e. $q'(x) < 0$ for $x \gg 1$). See [45] and references therein.

For the sake of simplicity we define the following function $\Psi(x) = \mu(x) - \beta(x)$ and write:

\[
\begin{align*}
x' &= x(f(x) - \phi(x)y) \\
y' &= -\Psi(x)y + \sigma q(x) + \theta(t).
\end{align*}
\]  

(3) (4)

The function $\Psi(x)$ is assumed to be positive, otherwise it is assumed to be positive in $[0, x_1) \cup (x_2, +\infty)$ with $\Psi(x_1) = \Psi(x_2) = 0$. We may assume that it has an absolute minimum in $[0, +\infty)$. We may use $\Psi(x)$ to classify the tumors depending on their degree of aggressiveness against the IS:

- $\Psi(x) > 0$: in such a case the ability of destroying immune cells is never won by the stimulation effect on the IS (i.e. $\beta(x) < \mu(x)$ everywhere in the feasible set), therefore the tumor may be indicated as “highly aggressive”/“lowly immunogenic”;
- Variable sign $\Psi(x)$: since in such a case the destruction of cells may be compensated by the stimulation effect, we will refer to such tumors as “lowly aggressive”/“highly immunogenic”.

\[ \text{A. d’Onofrio / Mathematical and Computer Modelling 47 (2008) 614–637} \]
The above model includes as particular cases the models [28,29,31,33]. For instance, the Stepanova model [28] is such that $f(x) = \alpha$, $\phi(x) = 1$, $\beta(x) = \beta_1 x$, $q(x) = 1$ and $\mu(x) = \mu_0 + \mu_1 x^2$; the de Vladr–Gonzalez model [33] is similar, but $f(x) = \alpha \log(K/x)$.

In the numerical examples of the next sections, we shall refer to Kuznetsov’s model [29,30] in which the growth rate is logistic $f(x) = \alpha(1 - x/K)$, $\phi(x) = 1$ and:

$$
\beta(x) = \frac{\beta_0 x}{m + x}, \quad \mu(x) = \mu(0) + \mu_1 x, \quad q(x) = 1.
$$

We chose this model since it is theoretically interesting and since its authors published some fitting of the parameters, based on real data (B-lymphoma $BL_1$ in mice).

Note that Nani and Freedman proposed an interesting model of adoptive cellular immunotherapy in which generic functions are used [46]. However, their approach differs from ours since in their model the proliferation of cells of the immune systems is not stimulated by cancer cells. In other words in the Nani and Freedman model the interaction tumor cells–immune system is only destructive for immune cells. Furthermore, in their model the “loss rates” are proportional (in our notation we might write $\mu(x) = \mu(0) + \text{const} \ast \phi(x)$).

In the absence of treatment, system (3) and (4) admits the existence of a cancer free equilibrium $CF = (0, \sigma/\Psi(0))$. If $f(0) < +\infty$, we have that if $\sigma > \sigma_{cr} = \alpha \Psi(0)f(0)/\phi(0)$ $CF$ is locally asymptotically stable (LAS), unstable if $\sigma < \sigma_{cr}$. Biologically, $\sigma > \sigma_{cr}$ means that the immune system works very well and that it is able to destroy small tumors. On the contrary $\sigma \approx 0$ means that there is immuno-depression.

2.1. Is Gompertzian growth compatible with the hypothesis of immuno-surveillance?

If $f(0) = +\infty$, as in the Gompertzian case (used, for example, in [33]) and in other tumor growth models, then $CF$ is unstable anyway (as previously stressed for the particular model [33]) because in such a case the right derivative of $xf(x)$ at $x = 0$ is $+\infty$.

In the light of [33] and of our generalization, this implies that the immune system would never be able to totally suppress even the smallest tumor cell aggregates, which is a very strong biological inference since it would imply the impossibility of the immune surveillance.

This instability result deserves some comments because it has deep medical–therapeutical implications: the impossibility to completely recover from any type of tumors whatsoever. On the contrary, it is commonly held that the immune system may be able, in some cases, to kill a relatively small aggregate of cancer cells. In the background of all cancer therapies (which are of finite duration) there is the implicit hypothesis that the drug will kill the vast majority of the malignant cells and that the relatively few residual cells may in some cases be killed by the immune system [47]. Accepting this hypothesis, the equilibrium CF should have the possibility to be at least LAS and, as a consequence, for small $x$ the function $f(x)$ should be bounded.

The most important unbounded law of growth, the Gompertz, introduced in oncology in [48,49], is largely used in bio-mathematical literature and in theoretical biology and medicine. Research works showed that the Gompertzian model fits data well from experimental and in vivo tumors [50–56] and theoretical justifications were proposed in Refs. [57–61].

However, the doubling time of a population of cells cannot be lower than the minimal time needed by a cell to divide, which is obviously non-null. This biological constraint is in contrast with the unboundedness of $f(x)$ in the Gompertz and other models, as stressed by Wheldon [39]. More recently, Castorina and Zappala in [62,63] used methods of statistical mechanics to derive a model similar to the Gomp-Ex model, which was previously proposed on biological grounds in [64,39].

Furthermore, Marusic and coworkers, using real data, performed a comparison of many models [40], showing that Gompertz’s model fitted their data slightly less well than the Plantadosi model [65], which has finite $f(0)$. Furthermore, in their fittings, it was not possible to discriminate between the pure Gompertz model and the Gomp-Ex model. In fact, Demicheli and coworkers used Gomp-Ex model to fit in vitro and in vivo data obtaining results strongly supporting this model [66].

Summarizing, the results by de Vladr and Gonzalez (and our extensions) are very valuable, but they may be read in a dichotomic way:

- A tumor is permanent: the immune system is never able to completely eradicate even the smallest tumor.
Since there is relevant experimental evidence that the immune system is able in some cases to eliminate small tumors [8] (as we will see in following sections, the ability to eradicate the disease or not depends on initial conditions), the properties of the de Vlader–Gonzalez model (and of our extension) may be seen as a further evidence that Gompertzian and other models characterized by \( f(0) = +\infty \) are not appropriate for very small tumors, in agreement with [39,62,63,44].

2.2. Macroscopic equilibria

Other multiple non null equilibria may be found by finding the positive intersection of the two nullclines:

\[
y_C(x) = \frac{f(x)}{\phi(x)}, \quad y_f(x) = \frac{\sigma q(x)}{\Psi(x)},
\]

(6)

(7)

The characteristic polynomial of the Jacobian, calculated at a given equilibrium point \((x_e, y_e)\), is:

\[
\lambda^2 + \left( \Psi(x_e) - x_e\phi(x_e)\Psi'(x_e) \right) \lambda + \Psi(x_e)x_e\phi(x_e)\left( -\pi'(x_e) + y'_f(x_e) \right) = 0.
\]

(8)

So the LAS condition is:

\[
y'_C(x_e) < \frac{\Psi(x_e)}{x_e\phi(x_e)} \quad \text{and} \quad y'_f(x_e) > y'_C(x_e).
\]

(9)

Note that the first part of the AND condition is automatically fulfilled when \( y'_C(x) \leq 0 \) (because \( x_e \) cannot lie in an interval where \( \Psi(x) < 0 \)), whereas the second part has a straightforward geometrical interpretation.

More generally, if \( y'_C(x) \leq 0 \) by applying the Dulac–Bendixon theorem with multiplicative factor \( 1/(xy\phi(x)) \) one obtains that the presence of limit cycles is not possible, since:

\[
\text{Div} \left( \frac{1}{xy\phi(x)}(x'(x, y), y'(x, y)) \right) = \frac{\alpha y'_C(x)}{y} - \sigma \frac{q(x)}{x\phi(x)y^2} < 0.
\]

(10)

3. A meta-model with tumor-size dependent proliferation

In this section we shall implement the biologically rooted modifications to the family of models of Ref. [26], that were proposed in the introduction, and also other modifications.

The first modification involves the uptake rate of TCs. We shall allow this function to be a non-monotone function [67,68] of the tumor burden and a nonlinear function of the number of ECs. The latter feature is considered in order to take into account possible cooperative and/or competitive effects between ECs. We shall show that when the effectors-related saturation level of the TC uptake is sufficiently high, there is no substantial qualitative changes in the tumor dynamics by comparison with the case in which the dependence on the ECs is linear. This leads to the following metamodel:

\[
\begin{cases}
x' = x(f(x) - \phi(x)\pi(y)), \\
y' = \beta(x)y - \mu(x)y + \sigma q(x) + \theta(t).
\end{cases}
\]

(11)

We shall call the meta-model composed by Eq. (11) as “Model I”.

The biological meanings (and the mathematical properties) of the functions appearing in Model I are the following:

- The predation function \( \pi(y) \) is no longer linear, as in [26,15], but it is a generic growing function: \( \pi'(y) \geq 0 \), and it is such that \( \phi(+\infty) \leq +\infty \). The nonlinear behavior of \( \pi(y) \) has been introduced to model cooperative and/or competitive inter-effectors interactions;
- The functional response\([69,67,68]\) \( U(x) \) of tumor cells is defined as follows: \( U(x) = x\phi(x) \), where: \( \phi(x) > 0 \), \( 0 < \phi(0) < +\infty \), \( \phi'(x) \leq 0 \). The strict positivity of \( \phi(0) \) is required to allow the possibility of immune surveillance, as we will show in Section 5. Note that we do not set the further constraint: \( x\phi(x) \to l \leq +\infty \). As a consequence, the functional response may be non-monotone [67,68].
Remark. Note that the existence of a $K$ such that $f(K) = 0$ implies that the set $\Omega = [0, K] \times [0, +\infty)$ is positively invariant.

Another feature in modeling the tumor–IS competition, leading to a new meta-model, concerns the proliferation ability of IS cells in response to tumors. It is reasonable, in fact, to suppose that the proliferation of ECs increases as the tumor burden increases [26]. However, does the stimulation depend on the absolute number of TCs or, more likely, does it depend on the relative abundance of the two competing populations? From this second viewpoint, for example, a very large baseline population of IS cells has no particular need to be stimulated in its proliferation. On the contrary, a quite small baseline population of ECs needs to proliferate greatly in the presence of a tumor burden which is low in absolute numbers, but which is big in comparison with $y$. So, in accordance with an interesting specific model proposed in the literature by Waniewski and coworkers [73], we propose a second meta-model in which the proliferation of ECs is seen as a generic increasing function of the ratio

\[
\frac{x}{y}.
\]

Finally, we further extend our study by modeling the proliferation as a generic function of both TCs and ECs. The dependence on TCs is increasing, that on ECs is decreasing.

From a biological point of view, an appreciable result of this work is the demonstration that if the relationship tumor–IS is such that the proliferation of ECs depends on the above ratio, then the tumor cannot be considered highly immunogenic according to the definition given in Ref. [26], since for large sizes of ECs the proliferation is negligible. In other words, in such a case the IS is somewhat less efficient, since there are no intervals in tumor size such that the tumor induced proliferation rate overcomes the tumor-induced loss of ECs. Instead, whenever the proliferation is a more general function of both the tumor cells and ECs, there are no qualitative differences with the case in which the proliferation depends solely on tumor size: some tumors will be highly immunogenic, other less immunogenic.

Another point of bio-mathematical interest is that we shall show that these three general meta-models are such that limit cycles are possible, according to clinical observations [27,70–72]. Regular persistent oscillations are fundamental in physiology [69], so it is of some interest to show in a theoretical framework that they also occur in such a particular pathological states as tumors. Moreover, we shall also provide some conditions for the uniqueness and global attractiveness of the limit cycles, which we will apply to biologically realistic modifications of a well known model by Kuznetsov and coworkers [29]. The uniqueness of limit cycles is an interesting mathematical topic, but its biological importance should not be underestimated: a unique and globally attractive limit cycle means that from all initial conditions in the biologically meaningful set the system will ultimately reach the LC; and that whatever perturbation which can also move the state very far from the cycle is not able to destroy it: the biological system does not only restart cycling with the same period it had before the perturbation, but, and this is biologically highly significant, with the same law of temporal variation (apart from a phase variation). To better understand the practical biomedical implications of these facts, remember that immunotherapies or chemotherapies may be considered among the most important examples of such perturbations.

Immunotherapies, at the current state of the art, have a finite time length, so it is important to find conditions which guarantee the globally asymptotically stable (GAS) eradication of the disease. However, one might think that a suboptimal target would be to lead the tumor in the region of convergence of a microscopic steady state, which might be perfectly controlled by the IS. However, it must be remembered that the Volterrian interaction TCs–IS is also evolutionary: tumor cells evolve their ability to evade the control and, as a consequence, no microscopic steady state may be considered permanent. Instead, a tumor free steady state is the unique equilibrium which is effectively permanent. This is another, more subtle, but no less biologically relevant, reason for the relevance of the study of the GAS of the tumor free equilibrium. In Section 7, we shall provide a “natural” condition for the global eradicability of a tumor, both via natural immune surveillance and by constant immunotherapy.

Here, following the above biological considerations, and extending Refs. [73,74], we propose a second meta-model in which the proliferation of ECs is seen as a generic increasing function of the ratio $x$ and $y$:

\[
\begin{align*}
    x' &= x(\alpha f(x) - \phi(x)\pi(y)), \\
    y' &= (P(x,y) - \mu(x))y + \sigma q(x) + \theta(t),
\end{align*}
\]  

\[ (12) \]
with the following constraints:
\[ P(0, y) = 0, \quad \frac{\partial P}{\partial x} > 0, \quad \frac{\partial P}{\partial y} \leq 0. \] (13)

For example, Waniewski \[73,74\] proposed this functional relationship depending on the ratio \( x/y \):
\[ \beta(x/y) = \beta_\infty \frac{\left( \frac{x}{y} \right)^a}{1 + \left( \frac{x}{y} \right)^a} = \beta_\infty \frac{x^a}{x^a + y^a}. \] (14)

More generally, in Ref. \[75\] has been introduced this proliferation function:
\[ P(x, y) = A \frac{x}{1 + mx + ny}. \] (15)

Of course, from a strictly mathematical point of view, model (12), which we shall call “Model II”, includes also model I.

4. Null-clines and their properties

The equilibria of the above defined families of models are determined by the equations \( x' = 0 \) and \( y' = 0 \).

The equation \( x' = 0 \), which is the same for all the families of models, yields \( x = 0 \) (which we will examine later), or:
\[ xf(x) - U(x)\pi(y) = 0 \Rightarrow y = Y_C(x) = \pi^{-1}(y_c(x)), \] (16)

where \( y_C(x) := f(x)/\phi(x) \). We will call \( Y_C(x) \) as the \( C \) null-cline, whereas we will call \( y_C(x) \) the baseline \( C \)-curve, which is defined as the curve which would be the \( C \) null-cline if it was \( \pi(y) = y \). Note that one may rewrite the tumor-dynamics equations of Models I and II in the following technically useful form:
\[ x' = U(x)(y_c(x) - \pi(y)). \] (17)

Clearly, if \( \pi(+\infty) > \max_{x \in [0,K]} y_c(x) \), then \( Y_C(x) \) is bounded, otherwise it has vertical asymptotes, located at the solutions of the equation:
\[ \pi(+\infty) = y_c(x). \] (18)

For the sake of simplicity, in the next sections we shall consider \( \pi(+\infty) > \max_{x \in [0,K]} y_c(x) \), whereas the case \( \pi(+\infty) \leq \max_{x \in [0,K]} y_c(x) \) will be shortly studied in the last section.

**Remark.** When \( \pi(+\infty) > \max_{x \in [0,K]} y_c(x) \), the shape of \( Y_C(x) \) has the same properties of \( y_c(x) \), thus, on the properties of the dynamical system in study, the nonlinearity and saturation of \( \pi(y) \) does not imply qualitative differences in comparison with the case \( \pi(y) = y \).

For which concerns the \( y' = 0 \), this equation determine a function, \( Y_I(x) \) which we will call the I null-cline.

Coming to model II, for all \( x > 0 \) the value \( Y_I(x) \) is obtained by solving the equation:
\[ P(x, y) = \mu(x) - \frac{\theta + \sigma q(x)}{y}, \] (19)

where:
- \( P(x, y) \) is a positive and \( y \)-decreasing function
- \( \mu(x) - (\theta + \sigma q(x))/y \) is a \( y \)-increasing function (a hyperbola) negatively unbounded as \( y \to 0^+ \), which is positive for \( y > y^*(x) = \mu(x)/(\theta + \sigma q(x)) \).

If \( \lim_{y \to +\infty} P(x, y) = 0 \), Eq. (19) has a unique solution \( y \in (y^*(x), +\infty) \) and it no \( \hat{x} < +\infty \) exists such that
\[ \lim_{x \to \hat{x}} Y_I(x) = \infty. \] (20)
Note that the boundedness of $Y_I(x)$ also holds if we relax the assumption (ii), and change it with the less restrictive:

$$\lim_{y \to +\infty} P(x, y) < \mu(x) \quad \text{for all } x > 0. \quad (21)$$

Thus, we may define the following function:

$$\Psi_\infty(x) = +\mu(x) - \lim_{y \to +\infty} P(x, y), \quad (22)$$

which generalize function $\Psi(x)$. If $\Psi_\infty(x)$ is strictly positive, the I null-cline is bounded (and, biologically, the tumor may be classified as “lowly immunogenic”/“highly aggressive”), instead when $\Psi_\infty(x)$ has variable sign, then the I null-cline is unbounded and, obviously, its asymptotes are the zeroes of $\Psi_\infty(x)$ (and, biologically, the tumor may be classified as “highly immunogenic”/“lowly aggressive”). For the sake of the biological simplicity, we shall assume that this function has only one or two zeroes, as we assumed for $\Psi(x)$.

**Remark.** It is worth noting that for $P(x, y)$ for which for all finite $x$ it is $\lim_{y \to +\infty} P(x, y) = 0$, for example if $\beta$ is as in (15), the tumor is classified as “lowly immunogenic”.

**Remark.** Note that the boundedness of $Y_I(x)$ implies that the set $\{(x, y) | 0 \leq y \leq Y_I^{\text{Max}}\}$ is positively invariant. Moreover, if it exists a $K$ such that $f(K) = 0$, a positively invariant set is $Q = [0, K] \times [0, Y_I^{\text{Max}}]$.

Both for Models II and I, the I null-cline has the property that:

$$Y_I(x) > \frac{\sigma q(x)}{\mu(x)} - \frac{\sigma q(x)}{\mu(x) - P(x, 0)}. \quad (23)$$

Model II, when $P(x, 0) < \mu(x)$ is also such that:

$$Y_I(x) < \frac{\sigma q(x)}{\mu(x)} - \frac{\sigma q(x)}{\mu(x) - P(x, 0)}. \quad (24)$$

Models with $\beta$ is in (15), for small $x$ have this behavior:

$$Y_I(x) = \frac{\sigma q(0)}{\mu(0)} \left( 1 + \left( \frac{\beta'(0) + \sigma q'(0)}{\sigma q(0)} - \frac{\mu'(0)}{\mu(0)} \right) x \right) + O(x^2). \quad (25)$$

Note that the effect of the constant therapy on the I null-cline is to increase it, which may be easily seen geometrically. In fact:

$$\frac{\partial}{\partial \theta} Y_I(x; \theta) = \frac{Y_I(x; \theta)}{\theta + \sigma q(x) - (Y_I(x; \theta))^2 \frac{\partial}{\partial y} P} > 0. \quad (26)$$

Note also that, in the case of unbounded $Y_I$, since $\theta$ does not change $\Psi_\infty(x)$, the positions of the vertical asymptote remain unchanged.

Finally, in the following sections it will be useful to find, in given subsets of $\mathbb{R}_+$ (e.g. for $x \in [0, K]$), the maximum value of $Y_C(x)$ and the maximum value of $Y_I(x)$, when this function is bounded. The maximum points of the these two functions in the given sets will be indicated as follows: $(x_C^M, Y_C^{\text{Max}})$ and $(x_I^M, Y_I^{\text{Max}})$.

### 5. Equilibria and their local stability

#### 5.1. Non-null disease equilibria

Single or multiple equilibrium points may be determined by the intersections of the null-clines in $\mathbb{R}^2_+$.

Given the simple structure of the meta-models, it results that the stability properties of these equilibria can be easily related to the geometrical properties of $Y_C(x)$ and $Y_I(x)$:
Proposition 5.1. For an equilibrium point \( E = (x_e, y_e) \), the condition for the local asymptotic stability (LAS) are:

\[
Y'_I(x_e) > Y'_C(x_e) \quad \text{and} \quad U(x_e)\pi'(y_e)Y'_C(x_e) < C_2,
\]

where \( C_2 \) is positive value defined as:

\[
C_2 = \begin{cases} 
    \Psi(x_e), & \text{Model I} \\
    -\frac{\partial B}{\partial y}(x_e, y_e) & \text{Model II.}
\end{cases}
\]

The first condition tells us that at \( E \), the tangent line at the \( I \) null-cline must have a higher angular coefficient. The second condition is automatically fulfilled when \( Y'_C(x) \leq 0 \), otherwise indicates that the angular coefficient of the tangent line of \( Y_C \) at \( E \) must be negative or, if positive, it must not be too high.

5.2. Tumor free equilibrium

In absence of therapy, for \( x = 0 \) there is a disease-free equilibrium:

\[
TF = \left( 0, \frac{\sigma q(0)}{\mu(0)} \right),
\]

which, when \( \sigma = 0 \), degenerates to \((0, 0)\).

Proposition 5.2. In the absence of immunotherapy \( (\theta = 0) \), if \( \sigma > 0 \) and \( Y_I(0) > Y_C(0) \), i.e.:

\[
\frac{\sigma q(0)}{\mu(0)} > \pi^{-1}(y_e(0)),
\]

then the tumor free equilibrium \( TF \) is locally asymptotically stable. If \( \sigma = 0 \), then the degenerate tumor free equilibrium \( TF = (0, 0) \) is always unstable.

The equilibrium \( TF \) is such that the \( y \)-axis is its stable manifold, both in the “degenerate” \( (\sigma = 0) \) and in the “regular” \( (\sigma > 0) \) case. In fact, all orbits having starting point on the \( y \)-axis \( (x(0), y(0)) = (0, y_o) \) are such that \( x(t) = 0 \) and:

\[
y' = -\mu(0)y + \sigma q(0) \Rightarrow y(t) \rightarrow \frac{\sigma q(0)}{\mu(0)}.
\]

Finally, we point out here that we supposed that \( \phi(0) > 0 \). In fact, if we would allow \( \phi(0) = 0 \), a consequence would be the impossibility of immune surveillance since:

Lemma 5.3. In the absence of immunotherapy \( (\theta = 0) \), if \( \phi(0) = 0 \) then the tumor free equilibrium \( TF \) is always unstable.

6. Limit cycles: Biological and mathematical analysis

In the medical literature there are some examples of observations of persistent oscillations in oncological diseases \([27,70,71]\), in some case with very long periods \([72,27]\). In \([26]\), we also studied the limit cycle dynamics present in the case of \( q(x) = 0 \), i.e. of immuno-depressed patients. However, Kirschner and Panetta \([27]\), via numerical simulations of their three-dimensional model, showed that periodic solutions are possible both for no influx and for positive influx.

In \([26]\), we showed that a necessary condition for the presence of periodic orbits (or, more generally, of closed orbits such as homoclinic or loops of heteroclinic) is that the function \( \phi(x) \) is not constant in a way such that \( y'_C(x) \) has variable sign. This is true also in models in which the proliferation depends on the burden of effectors, since by applying to models I and II the Dulac’s criterion with multiplicative function \((xyU(x)) \) it immediately follows that:

Proposition 6.1. If \( y_C(x) \) is decreasing, there are no closed orbits for meta-models I and II.
On the contrary, if the C null-cline has variable sign and the equilibrium point is in the zone where \( y_C'(x) > 0 \).

**Proposition 6.2.** Both for models I and II, let there be, other than TF, a unique equilibrium point \( E_Q = (x_e, y_e) \). If \( E_Q \) is unstable with \( a_o > 0 \), then there is at least one LAS limit cycle.

By transforming model II in an appropriate Lienard system, it is also possible to assess if the arising limit cycle is unique.

**Example.** The requirement of the above proposition does not lead to biologically unrealistic constraints. In fact, modifying the model [29] by assuming:

\[
\phi(x) = \frac{\phi_o}{1 + \frac{x}{L}},
\]

i.e. supposing that the uptake function \( U(x) = x\phi(x) \) follows a Michaelis–Menten saturation function. Numerical calculations showed that there is at least a LAS limit cycle for biologically plausible values of the adimensional parameters \( K \) and \( L \) (dimensional unit: \( 10^6 \) cells). For example when \( K = 210 \), easy numerical computations show that it has to be \( 0 < L < 97 \) and that in large portion of the interval \( (0, 97) \) the limit cycle is unique.

### 7. Globally stable eradication

In this section, we are going to study the possibility of elimination of a neoplasm, either naturally, thanks to immune surveillance, or by means of appropriate immunotherapy. We have previously analyzed the conditions for the local stability of the TF equilibrium, which, under constant therapy, may be written as follows: there exists a value \( \theta_{\text{LAS}} \geq 0 \) such that for \( \theta > \theta_{\text{LAS}} \) TF is LAS. Thus, it is reasonable to investigate if there is a second value \( \theta_{\text{GAS}} \geq \theta_{\text{LAS}} \geq 0 \) such that for \( \theta > \theta_{\text{GAS}} \) the disease-free equilibrium is GAS. Moreover, for periodically varying therapies, at least in the case \( \pi(y) = y \), we would like to recover similar criteria. This second task, is, as it is intuitive, far less easy, and we will give sufficient GAS criteria, which sometimes will be quite sharp.

Let us start with an observation: in the practical applications, it is not possible to have rigorously constant therapy. In the best case, we may idealize a constant therapy as periodic function \( \theta(t) \), \( \theta(t_0) = \theta_{\text{max}} \) with \( (\theta_{\text{max}} - \theta_{\text{min}})/\theta(t) \ll 1 \).

We give here a proposition on the global eradicability of a tumor, which extends the slightly less general Proposition 6.1 of [35]:

**Proposition 7.1.** If \( \theta(t) \geq 0 \) is such that

\[
y_I''(x) = Y_I(x; \theta_{\text{min}}) > Y_C(x),
\]

(where \( Y_I(x; \theta_{\text{min}}) \) is biologically defined) then TF is GAS, i.e. under therapy modeled by \( \theta(t) \) the tumor is eradicated independently from initial tumor burden (and value of \( y(0) \)). If \( \theta(t) = 0 = \theta_{\text{m}} \), the meaning of the fulfillment of (33) is that the immune surveillance is able to eliminate the disease, from whatever initial condition.

**Proof.** The proof is virtually identical to the proof of the less general proposition 6.1 of [35]. \( \square \)

**Corollary 7.2.** Let us define

\[
T = \{ \theta \geq 0 | 0 = \min(0, y_I(x; \theta) - y_C(x)) \}, \quad \theta_{\text{cr}} = \min(T).
\]

Applying Proposition 7.1 it follows that \( \theta_{\text{min}} \geq \theta_{\text{cr}} \Rightarrow \text{TF is GAS} \).

**Remark.** When \( \theta(t) \) is constant it is \( \theta_{\text{cr}} = \theta_{\text{GAS}} \), otherwise it is: \( \theta_{\text{cr}} > \theta_{\text{GAS}} \).

In some important cases, it is possible to study the global behavior of the family [26, 35]:

1. If the tumor is aggressive and \( f(x) = 1 \) and \( y_C'(x) \geq 0 \), if it is \( Y_I(x) < y_C(x) \) then \( x(t) \rightarrow +\infty \);
2. If the tumor is aggressive, it exists \( K \) such that \( f(K) = 0, y_C'(x) < 0 \) and there is a unique LAS equilibrium point \( S = (x_e, y_e) \), then \( S \) is GAS.
3. If the tumor is aggressive and \( y'_C(x) \) is non-constant and there is a unique LAS equilibrium point \( S = (x_e, y_e) \), if it holds also that
\[
y_C^{Max} > Y_I^{Max}
\]
then \( S \) is GAS.
4. If the tumor is aggressive and there does not exist a \( K \) such that \( f(K) = 0, y'_C(x) < 0 \) and there is a unique LAS equilibrium point \( S = (x_e, y_e) \), then \( S \) is GAS.
5. If \( y'_C(x) < 0 \), there is \( K \) such that \( f(K) = 0 \), there is a unique LAS equilibrium point \( S \) then \( S \) is GAS.
6. If there is no \( K \) such that \( f(K) = 0 \), \( y'_C(x) < 0 \) and there is a unique LAS equilibrium point \( S \) then \( S \) is GAS.
7. If tumor is aggressive and \( q(x) = 0 \) then \( Y(x(0), y(0)) \) it is \( y(t) \rightarrow 0^+ \). Furthermore, in accordance with the growth law \( f(x) \), either the tumor tends to an equilibrium value or it grows unbounded.
8. If tumor is aggressive and \( f(x) = 1 \) and \( \phi(x) = \text{const} = \varphi \), and there are two equilibria \( S = (x_e, y_e) \) (LAS) and \( U = (x_u, y_u) \) (unstable) and there is a separatrix curve \( y = \Sigma(x) \) which does not join \( S \) to \( U \), then there are two sets \( A \) and \( B \) such that if \( (x(0), y(0)) \in A \) then \( (x(t), y(t)) \rightarrow S \), whereas if \( (x(0), y(0)) \in B \) then \( x(t) \rightarrow +\infty \).
9. Let tumor is aggressive, \( y'_C(x) \leq 0 \) and it exists \( K \) such that \( y_C(K) = 0 \). Let there be 4 equilibria CF (unstable), \( S_l = (x_e, y_e) \) (LAS), \( U = (x_u, y_u) \) (unstable) and \( S_r = (x_e, y_e) \) (LAS), and let there be a separatrix curve \( y = \Sigma(x) \) which does not join \( S_l \) or \( S_r \) to \( U \), then there are two sets \( A \) and \( B \) such that if \( (x(0), y(0)) \in A \) then \( (x(t), y(t)) \rightarrow S_l \), whereas if \( (x(0), y(0)) \in B \) then \( (x(t), y(t)) \rightarrow S_r \).
10. For variable sign \( Y'_C(x) \), let there be three equilibria: TF LAS, \( U \) Unstable and MA LAS, and \( 0 < x_U < x_{MA} \). Let us call \( \Sigma(x) \) the separatrix passing for \( U \), and \( (x_o, y_o) \) a generic point. If \( Y'_C(x) \leq 0 \) for \( x \geq x_{MA} \) then:
   - If \( y_o > \Sigma(x_o) \) then \( (x(t), y(t)) \rightarrow TF \)
   - If \( y_o < \Sigma(x_o) \) then \( (x(t), y(t)) \rightarrow MA \)
   - If \( y_o = \Sigma(x_o) \) then \( (x(t), y(t)) \rightarrow U \)
   (the third statement is naturally obvious).

**Remark.** The configurations studied in the last proposition above may be induced by an immunotherapy that is not sufficiently intense to eradicate the disease. Their biological meaning is that the therapy is effective only when the initial conditions belong to the region of convergence of TF: the outcome of the therapy is, thus, state-dependent, which is a major practical problem, since the initial conditions are not known or known with large confidence intervals. However, from these propositions one may infer that if at the end of a chemotherapy (when, hopefully, it should be \( x \ll 1 \)) the immune system levels are sufficiently high, starting a mild immunotherapy might increase the probability of eradication, because it is sufficiently probable that the starting point of the patient lies in the region of convergence of TF.

### 7.1. Tumor evasion

Of the three outcomes of the TCs–IS competition (i.e. elimination, escape and equilibrium [8]), the elimination and equilibrium were analyzed in the literature by means of Lotka–Volterra-like biophysical models and, as we illustrated in this article, metamodels [26,15]. However, the escape has been investigated more closely in the recent immunobiological literature by stressing phenomena which were, to the best of our knowledge, recently considered by mechanistic studies in this field only by Kutsnetsrov and Knott in their seminal stimulating paper [30], with an approach apparently similar to ours (we shall discuss the similarities and differences in the text). Summarizing, we may thrichotomize the escape as follows:

- A tumor has low immunogenicity, so that it may grow towards a macroscopic equilibrium steady state size (MASS), which is globally stable;
- A tumor is not eliminated, but it is sufficiently immunogenic, so that the IS may be able to control its growth, which reaches a MISS. However, over a long period of time, the neoplasm develops multiple strategies to circumvent the action of the IS [16,12,8,17] and which, in the long term, allows it to evade immune surveillance and to restart growing up to its carrying capacity. The tumor has adapted itself to survive in a hostile environment, in which antitumor immune response is activated. For example, it may develop mechanisms to spread inducing only a moderate level of immunity [12]. In other words the immunogenic phenotype of the tumor is “sculpted” by the interaction with the host’s IS. For this reason, the theory of IS–T interaction has been called immunoediting theory.
in [8]. We note here that, if we see the TCs as prey of the predatory effectors, this ability to change their behavior correlates with the fact that in ecological systems there is remarkably more evidence of evolutionary responses in prey than in predators [18];

- An external major immunodepressive event occurs (e.g. a post-transplantation immunosuppressive therapy), which may transform an undetected tumor into a non equilibrium state, belonging to the Region Of Convergence (ROC) of a MASS with an outbreak.

Clearly, the second modality of escape of the above list is markedly different from the other two, both from a biological and a system theory point of view. Therefore, we propose here to call (2) tumor “evasion” (from the IS control) and to reserve the term “escape” to the modalities (1) and (3).

In this article, we propose to model, in first approximation, the described framework of behavioral strategies interrelated with phenotype changes by means of the Lotka–Volterra models with adaptively changing interaction strength [18–22], particularly with adiabatically varying parameters [19,21]. In the TCs–IS interaction, the adaptation of the prey and the consequential temporal variation of parameters are aimed at maximizing the final size reached by the tumor. In the same time, as we will see, the associate region of convergence is maximized, in the sense that it covers, at the end of the process, all the biologically meaningful state space. Thus, the transition from an apparent disease-free state to a sudden cancer explosion may be viewed in terms of catastrophic transition from a MISS to a phase of growth.

Summarizing, tumor development may experience 4 periods: a (very) long starting phase (i.e. the carcinogenesis), followed by a short phase of competition with the immune system and a long phase of tumor dormancy. Note that during this third phase “tumor cells may employ multiple immunoevasive strategies to elude the powerful integrated innate and adaptive antitumor immune responses” [8]. As a consequence, if the patient (who does not know that he/she has a tumor) does not die before, there may be a tumor outbreak.

8. Metamodeling the loss of equilibrium

In [26], we proposed a simple metamodel of TCs–IS interaction, in which the phenomenon of evasion was not taken into the account, but which were sufficiently detailed to describe other complexities of TCs–IS competition and, in terms of these complexities, to explain some contradictory results of clinical trials of immunotherapy. However, in the framework of [26], a tumor equilibrium is “forever”, which, according to the medical literature, is not true in all cases.

Following our previous biological analysis, we extend the “model I” by allowing the functional interactions (e.g. the tumor induced proliferation of effectors) to depend on some parameters as follows:

\[
\begin{align*}
  x' &= x \left( f(x) - \frac{\phi}{L(x; a)} y \right) = xf(x) - U(x)y \\
  y' &= P(x, y; b_1, b_2)y - \mu(x; c)y + \sigma q(x) + \theta(t),
\end{align*}
\]

where: \( U(x; a) = x \frac{\phi}{L(x; a)} \) is the tumor cells uptake by the effectors, with: \( L(0; a) = 1 \), and:

\[
(\partial / \partial x)L(x; a) \geq 0.
\]

\( P(x, y; b_1, b_2) \) is the tumor-stimulated proliferation rate of IS effectors, which will depend on two (or more) generic parameter \( b_1 \) and \( b_2 \); \( \mu(x; c) \) models as usual the tumor-induced loss of effectors [16], and depends on a positive parameter \( c \).

The parameters \( \phi, a, b_1, b_2 \) and \( c \), which we introduce in this section, shall be modeled as adiabatically varying parameters.

They have the following biological meanings: \( \phi \) is the baseline uptake of tumor cells: \( U(x) \approx \phi x \) for small \( x; a \) may be considered as linked to the start of the saturation effects (e.g. when \( L(x; a) = 1 + ax \)) or, in case of non-monotone uptake, the maximum uptake (e.g. when \( L(x; a) = 1 + ax^2 \)); \( b_1 \) is related to the baseline value of \( \beta \) (\( \beta(x; b_1, b_2) \approx b_1 x \) for \( x \ll 1 \)); \( b_2 \) to the start of the saturation in \( \beta(x; b_1, b_2) \) (e.g. when \( \beta(x; b_1, b_2) = b_1 x / (1 + b_2 x) \)); \( c \) is related to the baseline killing of IS effectors (\( \mu(x; c) \approx \mu(0) + cx \) for \( x \ll 1 \)).
Accordingly, we assume that:
\[
\frac{\partial}{\partial a} L(x; a) \geq 0
\]
\[
\frac{\partial}{\partial b_1} P(x, y; b_1, b_2) \geq 0
\]
\[
\frac{\partial}{\partial b_2} P(x, y; b_1, b_2) \leq 0
\]
\[
\frac{\partial}{\partial c} \mu(x; c) \leq 0.
\]

For sake of simplicity we shall consider that the variation of parameters are uncorrelated or that they have relationships of cooperative kind. However, in reality, there may be some strategies with contradictory outcomes, such as those linked to Fas/FasL pathway, which not only contributes to the immune privilege but can also enhance the antitumor response [16].

The parameter-dependent C-nullcline is such that:
\[
Y_c(x; a, \phi) = L(x; a) \frac{f(x)}{\phi} \geq \frac{f(x)}{\phi},
\]
and the parameter-dependent I-nullcline is implicitly defined as follows:
\[
(P(x, Y_i(x, y; b_1, b_2, c); b_1, b_2) - \mu(x; c))Y_i(x, y; b_1, b_2, c) + \sigma_q(x) + \theta = 0.
\]

From their intersection, we may determine the equilibria and their local, and in some case global, stability properties.

When the tumor is sufficiently immunogenic, so that the proliferation rate may become greater than the TCs killing rate, the curve \(Y_i(x; b_1, b_2, c)\) is unbounded and it has two or one vertical asymptotes.

Note that:
\[
(\partial/\partial a)Y_c(x; a, \phi) \geq 0,
\]
\[
(\partial/\partial \phi)Y_c(x; a, \phi) \leq 0,
\]

i.e. an increasing of \(a\) or a decreasing of \(\phi\) tends to move up the \(Y_c\) nullcline.

Finally, by applying the theorems on implicitly defined function to Eq. (37) (and using its properties), one obtains after some calculations that:
\[
(\partial/\partial c)Y_i(x; b_1, b_2, c) = -\frac{\partial \mu}{\partial c} \frac{Y_i(x; b_1, b_2, c)}{\mu(x; c)},
\]
\[
(\partial/\partial b_i)Y_i(x; b_1, b_2, c) = \frac{\partial P}{\partial b_i} \frac{Y_i(x; b_1, b_2, c)}{Y_i(x; b_1, b_2, c)} \frac{\sigma_q(x) + \theta}{Y_i(x; b_1, b_2, c)} + y \left(-\frac{\partial P}{\partial y}\right) i = 1, 2,
\]

which implies that:
\[
(\partial/\partial c)Y_i(x; b_1, b_2, c) < 0,
\]
\[
(\partial/\partial b_1)Y_i(x; b_1, b_2, c) > 0,
\]
\[
(\partial/\partial b_2)Y_i(x; b_1, b_2, c) < 0.
\]

Summarizing, an increase of the values of \(b_2\) or of \(c\) tends to move down the \(I\)-nullcline, as well as a decrease of \(b_1\).

8.1. Bifurcation analysis

Let us now consider the above parameters as constant in time and let us perform a bifurcation analysis.

In case of aggressive tumors, if we, for example, assume as bifurcation parameter \(\phi\), we have that, in the most complex case, the system has 4 equilibria: the unstable equilibrium TF and three other equilibria: a MISS, a MASS and, between them, a fourth unstable equilibrium point \(U\). Varying the bifurcation parameter, also the equilibria vary their locations, and we may determine a critical value \(\phi_{cr}\) at which a global bifurcation happens: \(m\) and \(U\) collapse for
Fig. 1. Bifurcation analysis of a Kuznetsov’s system \([29]\): \(f(x) = 1.636(1 - x/K), L(x; a) = 1, \ beta(x) = 1.131x/(20.19 + x), \mu(x, c) = 0.347 + 0.0311x, q(x) = 1, \) and \(K = 100, \sigma = 0.6. \) Ordinate unit is \(10^6 \) cells. Numerical data adapted from \([26]\), with carrying capacity chosen by the following criteria of graphical beauty. The tumor has low immunogenicity. The baseline nullclines are shown by a solid line. The effect of the variation of parameter \(\phi\) on the intersections of the \(Y_i\) nullcline with the \(Y_c(x; a, \phi)\) nullcline (dashed lines) is a hysteresis bifurcation. In fact, for \(\phi = 1\), there are three equilibria: a MISS, a MASS (near to the theoretical carrying capacity) and an unstable equilibrium \(U\) between them. Decreasing \(\phi\), the equilibria MISS and \(U\) tend to approach and there is a threshold value \(\phi_{cr}\) such that they collapse. A further decrease of \(\phi\) implies that there is an unique GAS MASS.

\[f(x) = 1.636(1 - x/K), L(x; a) = 1, \beta(x) = 1.131x/(20.19 + x), \mu(x, c) = 0.347 + 0.0311x, q(x) = 1, K = 100, \sigma = 0.6. \] Ordinate unit is \(10^6 \) cells.

\[
\phi = \phi_{cr}\text{ and, for } \phi < \phi_{cr}, M \text{ becomes unique and GAS (see Fig. 1). Similar results holds, as it can be readily seen, by varying the other parameters.}
\]

In case of unbounded \(f(x)\), immune surveillance is not possible and as a consequence only MASSes or MISSes are possible. However in the light of the immunoediting theory \([8]\) and of our biological and modelistic analysis, the Evasion mechanism would imply a greater probability of developing detectable tumors during the life span, as a consequence of the fact that no elimination is possible and therefore in all cases the non escaping neoplasms would tend to a MISS. This might offer further evidence against this class of \(f(x)\), other than the new and traditional classes considered in \([26]\).

In case of highly immunogenic tumors, the nullcline \(Y_i(x; b_1, b_2)\) has two vertical asymptotes, and negative variations in the predation coefficient \(\phi\) or in \(\alpha\), would never lead to the disappearing of the MISS. On the contrary, variations in the parameters of \(\gamma(b_1\) and \(b_2)\) or in the loss rate \(\mu(x)(c)\) would lead to the insurgence of a GAS MASS (see Fig. 2). In all cases there may be – for some particular models – a phase of insurgence of oscillations.

Since the parameters vary adiabatically, when the values are far from the critical values, we may approximate the dynamics of the system as a sequence of slowly varying equilibria. Once the critical value is passed, there is a relatively sudden catastrophic transition towards an equilibrium near the carrying capacity, as shown in Fig. 3.
Kutnetsov and Knott in [30] proposed some numerical simulation of model [29], which they modified by allowing slow variation of parameters. We have been, of course, deeply influenced by the reading of that paper; however our approach is markedly dissimilar, both biologically and mathematically. In fact, in this paper we build up a new theory of the loss of T–IS equilibrium, which is based on the most recent works both in immunobiology and theoretical ecology of prey–predator interaction. Furthermore our research is not based on a specific model, but on the general metamodel approach introduced by us recently [26], which add generality to our results. Finally, our work is general also since it based on a generic qualitative analysis via bifurcation theory and the numerical simulations which we showed have been used simply to better illustrate the results obtained in the bifurcation analysis.

8.2. Further mathematically based biological inferences on tumor evasion

Since \( p(t) \) varies very slowly, the permanence in the MISS may be very long, with a length comparable to the mean life span. As a consequence, the described phenomenon might further elucidate the reasons underlying the cases of very late tumor recurrences after apparently successful surgical and/or pharmacological therapies. In fact, let us consider a therapy starting at \( t = t_i \) and ending at \( t = t_f \), with \( p(t_f) \approx p(t_i) \). At \( t = t_f \), in the worst case the state of the system \((x(t_f), y(t_f))\) belongs to the ROC of the MASS, in the best case \( x(t_f) = 0 \), but in many cases it may be that the state belongs to the ROC of a MISS. We recall also that [26], if we assume that the cancer may have a unbounded growth rate function \( f(x) \), the MISS is the only one which may be reached by any non surgical therapy whatsoever. So, the patient has apparently experienced a total remission, but if the tumor is able to develop efficient evasion strategies, the disease will, unfortunately, reappear as a late recurrence. Furthermore, because of the long time elapsed since the first occurrence of the disease, there will be a non null probability of an erroneous diagnosis of second cancer independent of the previous one. Thus, within the framework of these results and of the considerations given at the end of [26], the delivery of an immunotherapy might be an option to be seriously considered in order to avoid this late effect and increasing the probability of total remission.

Finally, in the light of the analysis of this article, we would like to discuss briefly the hypothesis stated in [8] that “the immune system can facilitate tumor progression, at least in part, by sculpting the immunogenic phenotype of tumors as they develop”. In our opinion, if we limit to the immune-system triggered variation of the tumor immunogenicity properties, we cannot say that this is true. On the contrary, as we have seen, the effect of the immune system on the tumor dynamics is to stop, block or at least considerably decelerate tumor progression. Mathematically, this may been seen clearly since:

\[
x' = x \left( f(x) - \frac{\phi}{L(x; a)} y \right) \leq xf(x),
\]

which implies that

\[
x(t) \leq z(t)
\]
where:
\[ z' = f(z), \quad z(0) = x(0) \]
and \( z(t) \) is the “virtual” tumor dynamics in absence of immune system.

However, a different answer might be possible if evidence were offered that among the strategies to circumvent the immune control there is also the increase of TCs proliferation.

### 9. Immunotherapies: Theoretical and numerical analysis

**Remark.** In the next subsections some asymptotic analyses of therapies shall be conducted. The meaning of the underlying \( t \rightarrow +\infty \) limits is the following: the therapies are administered for a time interval \([0, t_f]\) which is finite but sufficiently long to guarantee that the number of cancer cells is zero or that other targets have been reached. On the contrary, we suppose here that the interval during which the therapy is administered is sufficiently small with respect to the typical times needed for tumor evasion, in order to neglect this phenomenon in our theoretical and numerical analysis.

#### 9.1. Continuous infusion therapy

For \( f(0) < +\infty \), all the considerations we have done in the absence of therapy hold also in case of Continuous Infusion Therapy. In fact, we may consider the influx as changed from \( \sigma q(x) \) to \( \theta + \sigma q(x) \), so that there is this therapy induced tumor free equilibrium:

\[ TF = \left( 0, \frac{\theta + \sigma q(0)}{\mu(0)} \right), \quad (38) \]

and:

**Proposition 9.1.** In the presence of constant immunotherapy \((\theta)\), if:

\[ \frac{\theta + \sigma q(0)}{\mu(0)} > \pi^{-1}(y_c(0)), \quad (39) \]

then the therapy-induced tumor free equilibrium \( TF \) is LAS.

\[ \sigma + \theta_m > \sigma_{cr}, \quad (40) \]

Because of the co-presence of other equilibria, the above criterion is not global, i.e. the immunotherapy is not able to guarantee the disease eradication from whatever initial values \((x(0), y(0)))\).

However, observing that since \( \partial Y_I/\partial \theta > 0 \) it follows:

\[ y_I^{\text{with therapy}}(x) > y_I^{\text{no therapy}}(x) \quad (41) \]

it happens that, roughly speaking, the stable equilibrium size of the cancer becomes smaller and the unstable equilibria greater, so that the basin of attraction of the unbounded solution is reduced.

Let us consider now some typical situations in case of \( y_C'(x) < 0 \):

- Non aggressive tumor. In such a case, in absence of therapy there may be in the most complex case 4 equilibria: CF (unstable), a small tumor equilibrium \( E_{\text{micro}}^o \) (LAS), a macroscopic equilibrium \( E_{\text{MACRO}}^o \) (LAS) and an intermediate unstable equilibrium \( E_{U}^o \), as in Fig. 4-subplot 1. \( E_{\text{micro}}^o \) is determined by the intersection between \( y_C(x) \) and the branch \( y_I^o(x) \), \( E_{\text{MACRO}}^o \) and \( E_{U}^o \) by the intersection between \( y_C(x) \) and \( y_I^o(x) \). Increasing \( \theta \) there are new equilibria. For \( \theta > \theta_{cf} = y_c(0) - y_I^o(0) \) CF becomes at least LAS and \( E_{\text{micro}}^o \) disappear. On the right, as a consequence of the elementary properties of continuous decreasing functions, increasing \( \theta \) the equilibria move and it is \( x_{Eu}(\theta) > x_{Eu}(0), x_{EMACRO}(\theta) < x_{EMACRO}(0) \), and there exists \( \theta_e \in (0, y_I^o(x_{Eu}) - y_C'(x_{EMACRO})) \) such that for \( \theta > \theta_e \) \( E_{\text{MACRO}}^o \) and \( E_{U}^o \) disappear. Summarizing, when \( \theta > \theta = \max(\theta_{cf}, \theta_e) \) then CF is GAS (Fig. 4-subplot 3).

If \( \theta_{cf} < \theta \) then for \( \theta_{cf} < \theta < \theta_{cf} \) \( E_{\text{micro}}^o \) is GAS (Fig. 4-subplot 2), whereas when \( \theta_{cf} < \theta \) for \( \theta_{cf} < \theta < \theta_e \) CF is LAS and coexists with \( E_{U}^o \) and \( E_{\text{MACRO}}^o \) (Fig. 5).
Fig. 4. Illustration of the effect of a CIT on a typical configuration in a lowly aggressive tumor. The case is shown in which \( \theta_r < \theta_{cf} \). \( y_f(x) \) is plotted as a solid line, whereas \( y_c(x) \) is dashed. The equilibria are plotted as black points and they are labeled \( U \) when unstable, otherwise \( S \). First subfigure: in the absence of therapy there are four equilibria among which is CF. Second subfigure: with a therapy with \( \theta_r < \theta < \theta_{cf} \) CF is unstable and coexists with a microscopic tumor equilibrium which is GAS. Third subfigure: for a high dose therapy \( \theta > \theta_{cf} \) CF becomes GAS.

Fig. 5. Illustration of the effect of a CIT in a low aggressive tumor for \( \theta_{cf} < \theta_r \) and \( \theta_{cf} < \theta < \theta_r \). Symbols as in Fig. 4.

- Aggressive tumors. In such a case, in the absence of therapy there may in the most complex case be one macroscopic equilibrium: \( E_{\text{Macro}}^\nu \) (GAS) and, of course, CF (unstable). Increasing \( \theta \) two further equilibria may appear. The analysis is similar to the previous one (cf. Figs. 6 and 7) and we may find a \( \bar{\theta} \) such that for \( \theta > \bar{\theta} \) CF is GAS. Note that when the tumor is aggressive it is very likely that \( \bar{\theta} \) is “extremely high”: \( \bar{\theta} \gg \sigma \);

- Aggressive tumors with \( \Psi'(x) < 0 [76] \). In such a case, in the absence of therapy there may in the worst case be one macroscopic equilibrium: \( E_{\text{Macro}}^\nu \) (GAS) and, of course, CF (unstable). Increasing \( \theta \), if when \( y_f(0) = y_c(0) \) it is \( y_f'(0) < y_c'(0) \) then we may find two values \( \theta_{cf} \) and \( \bar{\theta} > \theta_{cf} \) such that for \( \theta_{cf} < \theta < \bar{\theta} \) CF is LAS and there is the birth of a third unstable equilibrium \( E_u \). Finally for \( \theta > \bar{\theta} \) CF is GAS. Note that if when \( y_f(0) = y_c(0) \) it is \( y_f'(0) > y_c'(0) \) then \( \theta_{cf} = \bar{\theta} \).
Fig. 6. Illustration of the effect of a CIT in an aggressive tumor for increasing values of the CIT.

Fig. 7. Illustration of the effect of a CIT in an aggressive tumor, similar to Fig. 6, but with LAS CF coexisting with two other equilibria ($\theta_{cf}$ “low”).

Remark. If $Y'_c(x)$ has variable sign, there may be further equilibria, but similar bifurcation analysis may be done. Note, however, that in this case, sometimes an increase of $\theta$ may cause loss of stability and, accordingly to Proposition 6.2, the birth of limit cycles, i.e. there may be therapy-induced Hopf’s bifurcations.

When $f(0) = +\infty$ the total elimination cannot be achieved by immunotherapy alone. Furthermore, even the suboptimal target of reducing the cancer to a microscopic size in many relevant cases cannot be achieved for therapies of finite duration, however they may be long. In fact, let it be $\Psi(x) > 0$ (aggressive tumor) and let there be a unique GAS macroscopic equilibrium $E_{MACRO}$. By applying a CIT with $\theta$ sufficiently high there is a unique GAS microscopic equilibrium. However, when the therapy ceases $\theta$ falls to zero and the cancer restarts growing macroscopically, since $E_{MACRO}$ is again GAS. We note in brief that if the original equilibrium is microscopic (e.g. micrometastasis) the effect of the therapy is simply to create another and temporary microscopic equilibrium.

Let us suppose that there are three co-existing equilibria: $E^{\circ}_{micro}$ (LAS), $E^{\circ}_{U}$ (unstable and through which a separatrix $\Sigma^{\circ}$ passes) and $E^{\circ}_{MACRO}$ (LAS). Applying a CIT with $\theta > \tilde{\theta}$ there is an unique GAS microscopic equilibrium. Thus at the end of the therapy (at $t = t_f$) depending on the position of $P_f = (x(t_f), y(t_f))$ relatively to $\Sigma^{\circ}$, we have that either $(x(t), y(t)) \rightarrow E_{micro}$ or $(x(t), y(t)) \rightarrow E_{MACRO}$. 
We note that $\theta$ acts as a global bifurcation parameter, and we point out that these behaviors may be observed in case of bounded $f(0)$ when therapy is applied for an insufficient time.

9.2. Periodic scheduling

In the case of periodic therapy, it turns out that it is possible to have a tumor free periodic solution:

$$\text{TF}^* = (0, y^*(t))$$

(42)

where $y^*(t)$ is the asymptotic periodic solution of the ODE $y' = -\mu(0)y + \sigma q(0) + \theta(t)$. Moreover:

**Proposition 9.2.** In the case of periodic therapy, if:

$$\frac{1}{T} \int_0^T \pi(y^*(t)) dt > y_c(0),$$

(43)

then the tumor free periodic solution $\text{TF}^* = (0, y^*(t))$ is LAS.

Furthermore, if $\pi(y) = y$, it is easy to show that the condition (43) becomes:

$$\sigma + \overline{\theta(t)} > \sigma_{cr}.$$

Two basic models of periodic therapy may be:

$$\theta_r(t) = \frac{G}{1 - \exp(-cT)} \exp(-c \text{Mod}(t, T)), \quad \theta_m = \frac{G}{cT},$$

(44)

which represent a boli-based delivery. The “shape” of $\theta_r(t)$ depends on $c$ and the corresponding asymptotic periodic eradication solution is given by $(\alpha, z_r(t))$:

$$z_r(t) = \sigma \psi(0) + \frac{G}{\psi(0) - c} \left( \frac{E - \psi(0) \text{Mod}(t, T)}{1 - E - cT} - \frac{E - \psi(0) \text{Mod}(t, T)}{1 - E - \psi(0) T} \right).$$

9.3. Numerical simulations

Concerning periodic immunotherapy, the local and global analytical results of the previous sections are interesting and they are general since they were derived for the general family of models. However, there are many points which need further study, which has to be simulation-based and, as a consequence, on a specific model.

A point of particular practical relevance is the influence of the shape of the therapy term $\theta(t)$ in its outcome. In particular we refer to the comparison between a constant infusion therapy and a boli-based therapy. In fact, in other kinds of anticancer therapies, namely in anti angiogenic therapy, the shape of the therapy may be critical in determining whether or not the cancer will be eradicated [3].

We performed a set of simulations of immunotherapy on the basis of the specific model proposed by Kuznetsov et al. [29], in which:

$$f(x) = 1.636(1 - 0.002x), \quad \phi(x) = 1, \quad \beta(x) = \frac{1.131x}{20.19 + x}, \quad \sigma q(x) = 0.1181$$

$$\mu(x) = 0.00311x + 0.3743,$$

(which implies that: $\sigma_{cr} \approx 0.612$ and $\sigma^* \approx 1.44 \gg \sigma$), and:

$$t^\text{true} = 9.9 t^\text{adim} \, \text{days}, \quad (X, Y) = 10^6 (x, y) \, \text{cells}.$$

Note that, as it may be seen easily, the tumor is not aggressive. In fact the term $\mu(x)$ is relatively small: $\mu'(x) = 0.00311 \ll 1$. We also performed simulations in a case of a more aggressive tumor, for which we set $\mu(x) = 10(0.00311x) + 0.3743$.

In our simulations we assumed $\sigma + \theta_m > \sigma_{cr}$ which means that the mean value of the therapy, if given as CIT, would ensure the LAS of the disease free equilibrium. Since for each $T$ the mean value is constant; this means that
Fig. 8. Non aggressive tumor: phase portrait of model \([29]\) in the absence of therapy. There are two LAS equilibria, whose basins of attraction are separated by the separatrix line (plotted with a thick line). The nullcline \(y_C(x)\) is plotted with short dashes, the nullcline \(y_I(x)\) and its vertical asymptotes are plotted with long dashes.

Fig. 9. Non aggressive tumor: phase portrait of model \([29]\) in the presence of constant therapy with \(\sigma + \theta_m = 1.1 \sigma_{cr}\). There is a tumor-free equilibrium \(CF = (0, 1.799)\), which is globally stable. The nullcline \(y_C(x)\) is plotted with short dashes, the nullcline \(y_I(x)\) and its vertical asymptotes are plotted with long dashes. Note that the orbits stemming from initial points characterized by low \(y(0)\) are characterized by an initial fast growth of the tumor size, followed by a regression to 0.

in the limit \(c \rightarrow +\infty\) the therapy \(\theta_r(t)\) tends to become impulsive, but the amount of therapy delivering remains unchanged.

We found that:

• In the absence of therapy: non-aggressive tumor has two stable equilibria: one slightly less than the carrying capacity and the other corresponding to a small tumor (see phase portrait in Fig. 8). For the highly aggressive tumor there is one GAS equilibrium slightly less than the carrying capacity;

• With constant therapy: the non-aggressive tumor has a cancer-free equilibrium, which results to be GAS (Fig. 9). Note that the orbits stemming from initial points characterized by low values of the number of immune system cells are characterized by an initial rapid growth of the tumor size, followed by a regression to 0. Biologically, the therapy might seem to help the tumor growth, instead of fighting it. For the highly aggressive tumor, the cancer free equilibrium is LAS, but there is also a high size LAS equilibrium \([10]\);

• In the presence of periodic therapy with \(\theta_r(t)\), for both types of tumors the phase portrait is roughly similar to that of the constant therapy: the cancer-free periodic solution remains GAS for the non aggressive tumor (Fig. 11). For the aggressive tumor there is the coexistence of the cancer free solution with a solution fluctuating around high values of the cancer size (near the equilibrium of the constant therapy). The two basins of attraction for the aggressive tumor remain unvaried with respect to those of the constant therapy (Fig. 12).

• For \(\theta_r(t)\) the dependence of the qualitative properties of the system on the parameter \(c\) is not critical.
Fig. 10. Aggressive tumor: phase portrait of model [29] in presence of constant therapy. There is a tumor free equilibrium $C_F = (0, 1.799)$ and another LAS equilibrium, whose basins of attraction are divided by a separatrix line (plotted with a thick line). The nullcline $y_C(x)$ is plotted with short dashes, the nullcline $y_I(x)$ and its vertical asymptotes are plotted with long dashes.

Fig. 11. Non aggressive tumor: phase portrait of model [29] in presence of periodic therapy $\theta_r(t)$ with $T = 0.202$ (=2 days) and $1/c = 0.1T$. There is a tumor free equilibrium $(0, z(t)) \approx (0, 1.799)$ which remains GAS. The nullcline $y_C(x)$ is plotted with short dashes, the nullcline $y_I(x)$ and its vertical asymptotes are plotted with long dashes.

Fig. 12. Aggressive tumor: phase portrait of the model [29] in the presence of periodic therapy with $T = 0.202$ (=2 days) and $1/c = 0.5T$. The basins of attraction of the tumor free equilibrium $CP^* = (0, z(t)) \approx (0, 1.799)$ and of the macroscopic size equilibrium remain near unchanged with respect to the CIT scheduling (the basin of $C_F$ is slightly greater than in the CIT). The nullcline $y_C(x)$ is plotted with short dashes, the nullcline $y_I(x)$ and its vertical asymptotes are plotted with long dashes.

- Both with CIT and with periodic therapy $y(t)$ may reach values considerably higher than the physiological value $\sigma/\mu(0)$, which might model some serious side effects of immunotherapies due to the excess of immunocompetent cells [1,2].
Finally, we would like to illustrate some qualitative medical inferences from the investigations that we have proposed here. The main problem of immunotherapy is that, as it is clear from our analysis and simulations, in general, eradication may be possible but is dependent on the initial conditions \((x(0), y(0))\). However, the IC are in medical practice unknown or known with very large confidence intervals (cf. [77] for the cancer cells at the start of radiotherapy). This makes it impossible to plan an anticancer therapy based solely on this therapy. This is a peculiarity of immunotherapy, since there are other kinds of anticancer cures for which a globally stable eradication is possible [3]. However, in our simulations we have seen that in some particular cases the model [29] predicts that globally stable eradication is possible also in case of immunotherapy, but that it depends on the “degree of aggressiveness” of the cancer, i.e., on the framework of the model [29], on the parameter \(\mu_1\). However, \(\mu_1\) is difficult to be estimated (as a range) and, in particular, on single patients. If in the future it might be possible, the option to use immunotherapy as main strategy, for relatively small “non aggressive” tumors, could be seriously considered. Furthermore, we showed that the behavior of the system does not depend on the amplitude of fluctuations of \(\theta(t)\), so that the option of continuous intravenous infusion is not, dynamically, better than the bolus-based therapy. This result may be of interest, since continuous intravenous infusion may cause major practical problems to the patients. Finally, in case of disease aggressive towards the immune system, since our simulations indicated that all the positive quadrant is GAS towards a macroscopic disease in absence of therapy and low \(\sigma\), whereas in the presence of therapy the eradication is possible in an adequate basin (see Fig. 10), we may infer that a conventional therapy should be followed by immunotherapy to increase the probability of total remission.

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References


