

Article

The Study of Square Periodic Perturbations as an Immunotherapy Process on a Tumor Growth Chaotic Model

Dionysios Sourailidis ^{1,*}, Christos Volos ¹, Lazaros Moysis ¹, Efthymia Meletlidou ²
and Ioannis Stouboulos ¹

¹ Laboratory of Nonlinear Systems, Circuits & Complexity (LaNSCom), Physics Department, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece; volos@physics.auth.gr (C.V.); lmousis@physics.auth.gr (L.M.); stouboulos@physics.auth.gr (I.S.)

² Physics Department, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece; efthymia@auth.gr

* Correspondence: dsourail@physics.auth.gr

Abstract: In the present study, the simulation of an immunotherapy effect for a known dynamical system, that describes the process for avascular, vascular, and metastasis tumor growth based on a chemical network model, has been presented. To this end, square signals of various amplitudes have been used, to model the effect of external therapy control, in order to affect the population of immune cells. The results of the simulations show that for certain values of the amplitude of the square signal, the populations of the proliferating tumor cells in the vascular and metastasis stages have been reduced.

Keywords: bifurcation; chaos; cancer; immunotherapy; tumor growth model



Citation: Sourailidis, D.; Volos, C.; Moysis, L.; Meletlidou, E.; Stouboulos, I. The Study of Square Periodic Perturbations as an Immunotherapy Process on a Tumor Growth Chaotic Model. *Dynamics* **2022**, *2*, 161–174. <https://doi.org/10.3390/dynamics2020008>

Received: 26 January 2022

Accepted: 20 May 2022

Published: 6 June 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Cancer is a generic term assigned to a complex network of interactions of malignant cells, which no longer possess specialization and control over normal growth. This network of malignant cells could be classified as a nonlinear dynamical system, self-organized in time and space, definitely not in thermodynamic equilibrium, indicating high complexity, robustness, and versatility [1–3]. Also, the immune system's role is crucial in both the inhibition and the progression of cancer growth, resulting in the fact that the implementation of treatments that directly affect the immune response has in many cases proved to be extremely effective.

In the work of Page and Uhr [4], an investigation was conducted as to how tumor dormancy is induced by the interaction between antibody tumors. In another work [5], it is shown that any type of tumor developing in vivo has most of its cell proliferation constrained to the border. This may indicate that cell surface diffusion is the main mechanism responsible for growth in any type of tumor. Furthermore, another study [6] aimed to illustrate situations for which neither chemotherapy nor immunotherapy alone would suffice to control tumor growth but combining the therapies resulted in eliminating the entire tumor.

Nowadays, model-based approaches may help in the direction of a better characterization of cancer evolution and subsequent use of this knowledge for personalized treatment would increase the chance to overcome cancer treatment resistance. In numerous cases, various mathematical models have vastly contributed to understanding carcinogenesis and cancer evolution [7–13]. As a result, this makes the development of newer and more accurate models that describe the development of cancer in all its stages more compelling. Also, at this stage, it should be mentioned that three stages can be identified in tumor progression: avascular, vascular and metastatic [14].

At the first of these stages, in the avascular stage, the tumor obtains nutrients and “feeds” itself solely via the diffusion process, where nutrients already exist in the envi-

ronment [15–20]. In the second stage, the vascular phase, which takes place when the tumor evolution progresses more rapidly via the mechanism of angiogenesis (the process of producing new blood vessels), the malignant cancer cells secrete chemicals that can diffuse into the surrounding healthy tissues and trigger the growth of new capillary blood vessels [21–25]. The newly formed blood vessels infiltrate the tumor mass by supplying nutrients to it and leading to the tumor’s rapid growth. This manifests from the following process. When the tumor is furnished with ample nutrients, malignant cells divide (cellular mitosis). Consequently, the moment where the density of malignant cells in an area rises by a significant factor, the cells are compressed by their neighbors, so they aim to migrate to areas that are not as compressed. In the newly occupied areas, the process of division is repeated. Finally, in the third stage, the metastatic stage, malignant cells migrate through the blood vessels and/or the lymphatic system towards other parts of the body, leading to secondary tumors [26–30]. Therefore, this growth is the primary root of the host’s demise.

An important step in enriching the existing models of tumor growth is the inclusion of immunotherapy, which is the process of boosting the patient’s immune system response to cancer, so its growth is suppressed. However, capturing the effect of immunotherapy is a difficult task, due to its complex interaction with other agents in the body, like other organs. There have been studies conducted to this end [31,32], suggesting a model structure where the rate of first-order decline of tumor burden was assumed to depend on the amount of immune component and decrease while the tumor burden was increasing. This model structure was adopted to characterize, for example, the growth of prostate cancer by accounting for the dynamics of the immune system. Tumor cells were assumed to proliferate exponentially, and the number of cytotoxic T lymphocytes affected the cell decline rate.

To mitigate the effect of strong medicine on the patients’ other organs, like the kidneys, for example, immunotherapy usually consists of periodic drug regulation. This effect can be incorporated into a model of differential equations for tumor growth, by adding a periodic term to the equation describing the immune cells’ growth. Hence, different periodic terms can be used to model the effect of immunotherapy, like sinusoidal signals, square signals, triangular signals, or sawtooth signals. The differences in each periodic signal can yield different dynamics in the immune system response to tumor growth.

In the present work, the dynamical behavior of a tumor growth chaotic model illustrates an immunotherapy process by using square periodic perturbations in the density of immune cells. This approach is based on a well-known system proposed by Llanos-Perez et al. [33]. Their work stands on a chemical network, used to recommend a mechanism for tumor growth in its three stages. Llanos-Perez et al. laid the foundations for numerous researchers to conduct research banking on their work. At the outset, Betancourt-Mar et al. [34] developed it on existing thermodynamics formalism for the metabolic rate of human cancer cells. Montero et al. [35] worked on a biological approach to this problem. Guerra et al. [36] enriched the aforementioned work by including the epithelial-mesenchymal transition, which is a biologic mechanism permitting a polarized epithelial cell, which typically interacts with the basement membrane using its basal surface, to go through various biochemical changes enabling it to take over a mesenchymal cell phenotype, which encompasses boosted migratory capacity, invasiveness, and augmented resistance to apoptosis [37].

In addition, a work by Jaime et al. [38], built upon the work of Llanos-Perez et al., vastly improved the model with the aim of lessening malignant cell growth in relation to healthy cell growth. Their analysis concentrated on the stages of the vascular tumor growth and metastasis, for a tumor subject to periodic perturbations. The perturbations were inserted with the form of a sinusoidal function.

This approach constitutes the basis of this work, in which square signals, as perturbations, are used. This is done because a square signal is more realistic in the modeling of real immunotherapy processes. This process is examined further through the steady administration of an agent for a specific time period and then not administering it for the

same period of time. This is something that could be easily implemented in real conditions. Also, the study of the effect of a steady dose of treatment for a specific period of time is the main aim of this work. From the simulations, various phenomena from the nonlinear theory have been observed, including a period-doubling route to chaos, antimonotonicity, and crisis phenomena.

The outline of this work is described as follows. In Section 2, the mathematical model of tumor growth, which has been used, is presented and broken down. The dynamical behavior of the tumor growth system with square periodic perturbations, by using well-known tools from nonlinear theory, is investigated thoroughly in Section 3. Finally, Section 4 presents the conclusions of this work.

2. Mathematical Model Formulation

As mentioned, in 2016 Llanos-Pérez et al. [33] presented a novel model of tumor growth, which depicts the progression of cancer over the three basic stages, based on experimental evidence. In that study a chemical network model, where a classic chemical kinetics method utilizing the law of mass action has been adhered to. The model is described by the following mechanisms

- Step (a) $N + x \rightarrow 2x$
- Step (b) $2x \rightarrow y$
- Step (c) $y \rightarrow 2y$
- Step (d) $2y \rightarrow z$
- Step (e) $H + y + z \rightarrow 2z$
- Step (f) $2z \rightarrow y$
- Step (g) $H + x + z \rightarrow z$
- Step (h) $I + y \rightarrow ncp$
- Step (i) $I + z \rightarrow ncp$

where the variables x , y , and z , depict the propagating tumor cells in the avascular, vascular and metastatic phases, subsequently. Furthermore, parameter N serves as the population of normal cells, H is the population of the host cells, and I is the population of immune cells, which are the T lymphocytes together with the natural killer cells.

The chemical process consists of nine steps. Steps (a), and (b) correspond to the process of mitosis of proliferating tumor cells, and steps (b), (d), and (f) with the process of apoptosis. Steps (e), and (g) denote the action of host cells H . Steps (h), and (i) denote the effect of immune cells, where ncp is a non-cancerous-product. It is considered that the cells z are generated while cell apoptosis y takes place (step d), representing the occurrence of micrometastasis, which is the spread of cancer cells from their primary site, whereas the secondary tumors are too small to be clinically detected.

By considering the classic chemical kinetics method that applies the law of mass action, see [39], the resulting model of three differential equations is derived.

The aforementioned model of the chemical network described above is a three-dimensional system, outlining the avascular, vascular, and metastasis tumor growth.

$$\begin{cases} \dot{x} = x(2N - x) - Hxz \\ \dot{y} = y(4 - 0.14y) + 0.5x^2 - Iy - 0.5Hyx + 0.001z^2 \\ \dot{z} = -Iz + 0.07y^2 + 0.5Hyx - 0.002z^2 \end{cases} \quad (1)$$

In this work the numerical values of the constants are preserved, compared to the ones in the work of Llanos-Pérez et al. [33], which have been selected empirically with the purpose of having a larger abstraction and purity as possible, elicited from the derived chemical network model. System (1) has been studied further for its dynamics by Sourailidis et al. [40] and various phenomena surfaced, such as antimonotonicity and coexistence of attractors [34].

It is established that the immune system is a significant factor both in inhibiting and in the progression of tumor growth [41,42]. The utilization of therapies that directly affect

the immune system has been efficient [43], in addition to chemotherapies that positively influence the antitumor immune response [44]. Consequently, Jaime et al. have used a perturbation in the form of the term $A\sin^2(\omega t)$ to the population of immune cells I in order to simulate how immunotherapy or chemotherapy reflects on tumor growth [38]. Therefore, the set of equations in (1) becomes:

$$\begin{cases} \dot{x} = x(2N - x) - Hxz \\ \dot{y} = y(4 - 0.14y) + 0.5x^2 - [I_0 + A\sin^2(\omega t)]y - 0.5Hyz + 0.001z^2 \\ \dot{z} = -[I_0 + A\sin^2(\omega t)]z + 0.07y^2 + 0.5Hyz - 0.002z^2 \end{cases} \quad (2)$$

where A and ω are the control parameters. Parameter A denotes the amplitude of the perturbation and implies how intensely the treatment was conducted, while ω denotes the frequency of the external perturbation.

To extend upon the previous model of (2), a different signal than the one Jaime et al. inserted, namely a square signal, was used in order to be more feasible as an immunotherapy or chemotherapy approach. Therefore, system (2) takes the following form:

$$\begin{cases} \dot{x} = x(2N - x) - Hxz \\ \dot{y} = y(4 - 0.14y) + 0.5x^2 - [I_0 + S_i]y - 0.5Hyz + 0.001z^2 \\ \dot{z} = -[I_0 + S_i]z + 0.07y^2 + 0.5Hyz - 0.002z^2 \end{cases} \quad (3)$$

where S_i is mathematically described by the following equation and the time t is expressed in seconds (Figure 1).

$$S_i(t) = A\operatorname{sgn}\left(\sin \frac{2\pi t}{T}\right) = A\operatorname{sgn}(\sin 2\pi ft) = A\operatorname{sgn}(\sin \omega t) \quad (4)$$

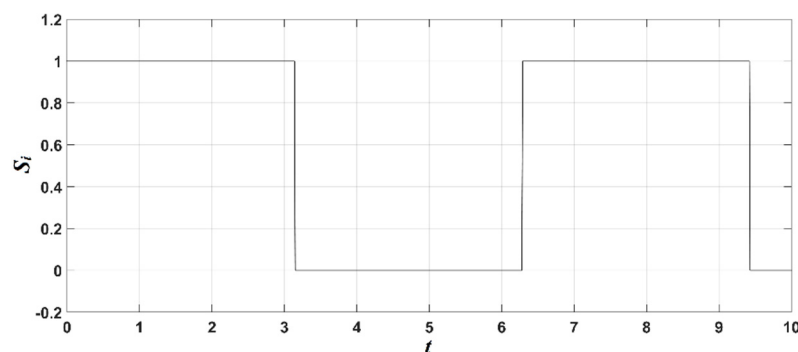


Figure 1. An example of a square signal, is described by Equation (4).

Furthermore, as in Ref. [38] parameters A and ω represent the amplitude of the perturbation (the intensity of the treatment) and the frequency of perturbation respectively. In this work the function $\operatorname{sgn}(x)$ is described as:

$$\operatorname{sgn}(x) = \begin{cases} 1, & x \geq 0 \\ 0, & x < 0 \end{cases} \quad (5)$$

The concept behind this suggestion is that the agent is administered at a steady rate for a specific amount of time, and then administration is halted for the same amount of time. This is believed to be an approach easier to implement and follow in clinical processes.

3. Dynamical Study of the System

As reported by Llanos-Perez et al. [33], the model of tumor growth of Equation (1) suggests a compelling behavior. To expand on this, as the population of immune cells I decreases, the system's complexity is increased. This can illustrate the epithelial-to-mesenchymal transition amid metastasis. For lower values of the critical value of the control parameter I

≈ 0.53 , associated with low immune surveillance, tumor cell populations exhibit chaotic behavior. The prevalence of metastatic cells z at chaotic dynamics has significant biological connotations. To begin with, the high sensitivity of the system to initial conditions suggests the inefficiency of making long-term predictions pertaining to the evolution of the disease. Moreover, the decreasing of the immunological parameter I might be able to provide a high degree of robustness. This would result in the level of success in the treatment of the tumor being low, particularly at the stage of metastasis.

Therefore, in this section, the effect of the suggested tumor growth model of Equation (3), with the square periodic perturbation is studied, through simulations by retaining values of parameter $N = 4$ and $I = 1$, for numerous values of H in the range $[3, 5]$, with initial conditions $(x_0, y_0, z_0) = (1, 0, 0)$. In order to investigate the dynamical behavior, system (3) is solved numerically with the use of the classical fourth-order Runge-Kutta integration algorithm. The time step is always $\Delta t = 0.001$ and the calculations take place utilizing variables and parameters in extended precision mode. For each parameter setting, the system is integrated for a long time period and the transient is discarded.

In order to study system's (3) dynamics and try to relate the system's dynamical behavior to biological phenomena concerning the effect of a treatment, the amplitude of the square periodic perturbation of Equation (4), namely the parameter A , is chosen as the bifurcation control parameter, while its frequency is $\omega = 1$. The bifurcation diagrams are computed by plotting the population of the tumor cells in the vascular phase y , when the system intersects the plane $x = 1$ with $dx/dt < 0$, while the control parameter A is increased in small steps.

From the observation of the bifurcation diagrams in Figure 2, interesting phenomena related to chaos theory can arise. Firstly, the route to chaos through a period-doubling sequence as the value of parameter A is decreased can be observed in all cases. Also, the interior crisis is shown as the range of the chaotic region suddenly increases, while the boundary crisis is observed when the chaotic attractor is abruptly destroyed as the parameter A is varied. Furthermore, period-3 windows have been found in all the bifurcation diagrams of Figure 2, which is a phenomenon always observed between chaotic regions.

From a biological point of view, bifurcation diagrams in Figure 2 reveal that as long as the intensity of treatment A (amplitude of perturbation) is high, the system is in a period-1 steady state. Therefore, at "high" values of treatment's intensity A , although there is a population of proliferating tumor cells, the system is in a stable condition. Also, for small ($H = 3$) or even bigger ($H = 5$) values of the population of the host cells, the system is driven to a period-1 state for the small amplitude of the perturbation A . For $H = 5$ the amplitude of the perturbation, for which the system enters in period-1, is only $A = 1.83$. This feature reveals the high complexity of the system. Furthermore, as the population of the treatment decreases the system's complexity increases by showing regions of chaotic or even periodic behavior.

In order to examine the difference in the phase portraits in $x - y$ plane, one of the cases, which has been examined through its bifurcation diagram, is selected. In more detail, Figure 3 shows the phase portraits for $N = 4$, $I = 1$, $H = 3.8$, and $\omega = 1$, as the population of the host cells is kept at $H = 3.8$, while the value of the intensity of the treatment A has taken values according to the bifurcation diagram of Figure 2c. Also, in all these plots, the first 1000 s of simulation are discarded in order to see the system's steady state. Concerning the system's dynamics, the route to chaos through the mechanism of period-doubling is observed in Figure 3a–d, as the value of A decreased. Furthermore, the period-3 attractor is observed in Figure 3e. Finally, after successive windows of chaotic and periodic behavior, which are illustrated in Figure 3f,g, system (3) enters to a region of extended chaotic behavior, in which the chaotic attractor is more complex.

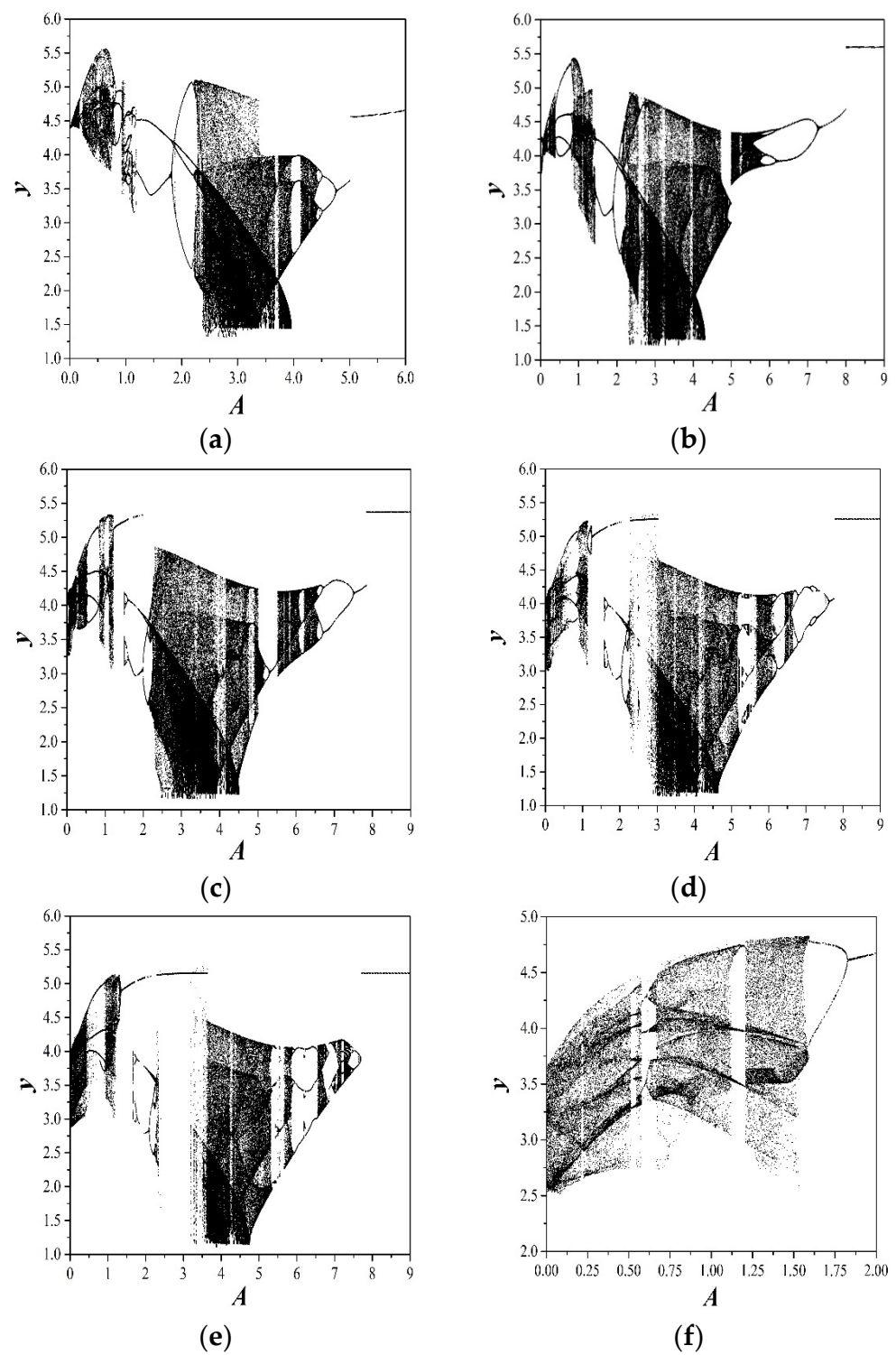


Figure 2. Bifurcation diagrams of y versus parameter A , for $N = 4$, $I = 1$, and $\omega = 1$, with (a) $H = 3$, (b) $H = 3.5$, (c) $H = 3.8$, (d) $H = 4$, (e) $H = 4.2$ and (f) $H = 5$.

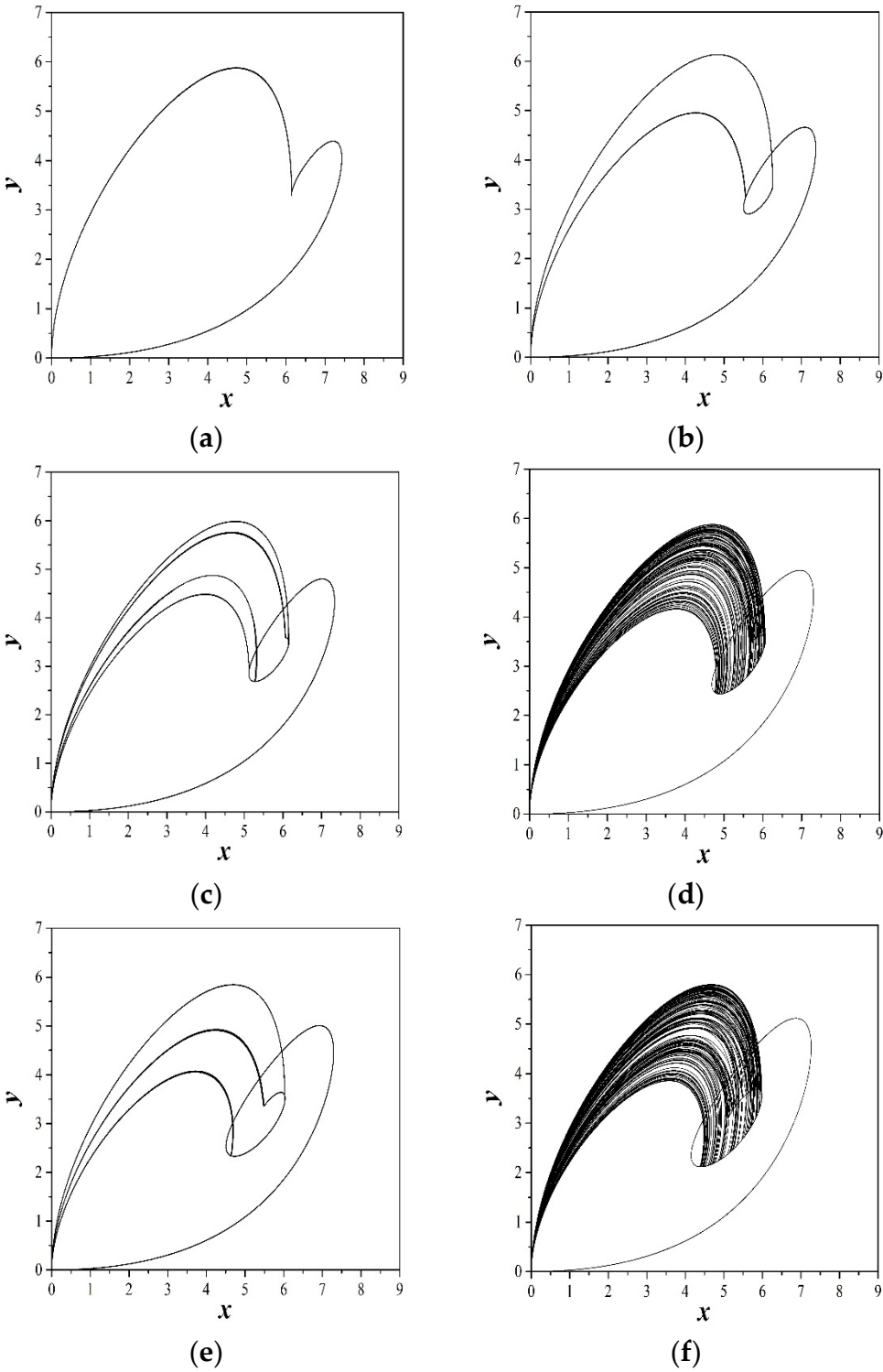


Figure 3. Cont.

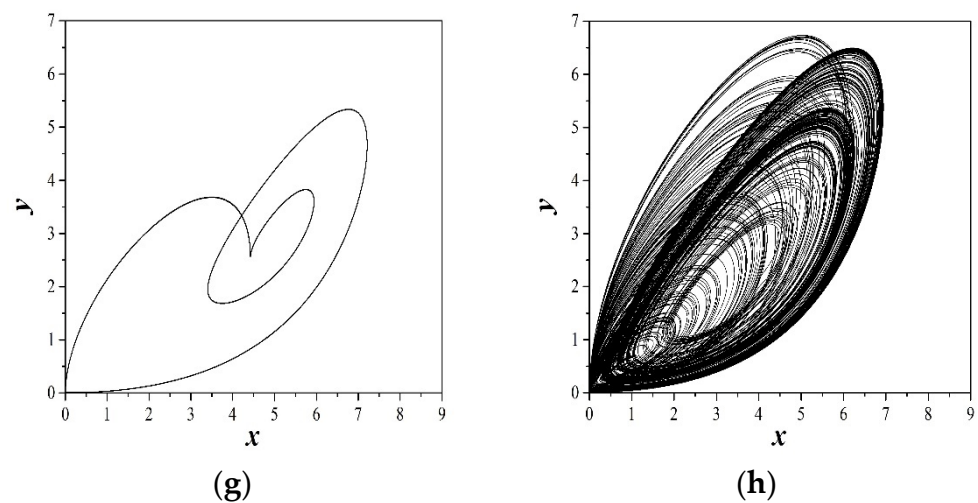


Figure 3. Phase portraits of y versus x , for $N = 4$, $I = 1$, $H = 3.8$ and $\omega = 1$, with (a) $A = 7.7$, (b) $A = 7$, (c) $A = 6.65$, (d) $A = 6.3$, (e) $A = 6.16$, (f) $A = 5.9$, (g) $A = 5.4$, (h) $A = 3$.

So, from this series of phase portraits in Figure 3, interesting phenomena concerning the behavior of the cancer model, which have been discussed earlier, are confirmed. As the intensity of treatment A decreases, the system increases its complexity. In more detail, the decrease in the intensity of treatment A could give a high degree of robustness, hence the level of success in the treatment of cancer may be low, especially at the stage of metastasis. This result suggests in a general way that the system evolves from periodic (less complex and robust) to chaotic states as the amplitude of perturbation decreases. Also, zones with periodic states are obtained, so that at certain treatment amplitudes, it is possible to reduce the complexity of the system. These results suggest that it is possible to optimize the conditions of implementation of the therapies to make them more efficient. Therefore, the proposed system (3), suggests that the treatment of a primary tumor from a microscopic level, avascular growth, to a macroscopic level, vascular phase, and the subsequent appearance of metastasis, is not a simple procedure, but it is a dynamic nonlinear, self-organized process, far from thermodynamic equilibrium, which exhibits a high degree of robustness, complexity, and hierarchy [33] and that in turn leads to the creation of new information and learning ability. Furthermore, in Figure 4 the time-series of y (proliferating tumor cells in a vascular phase), for the respective values of A , which has been depicted in Figure 3, is presented.

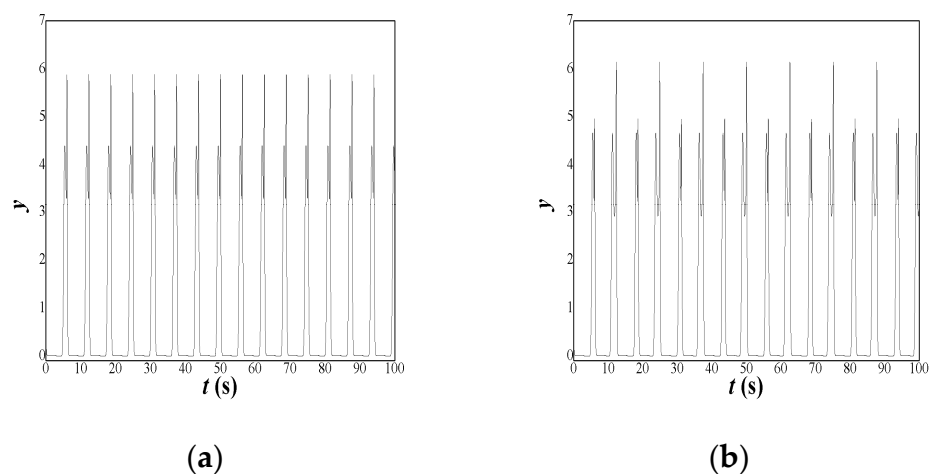


Figure 4. Cont.

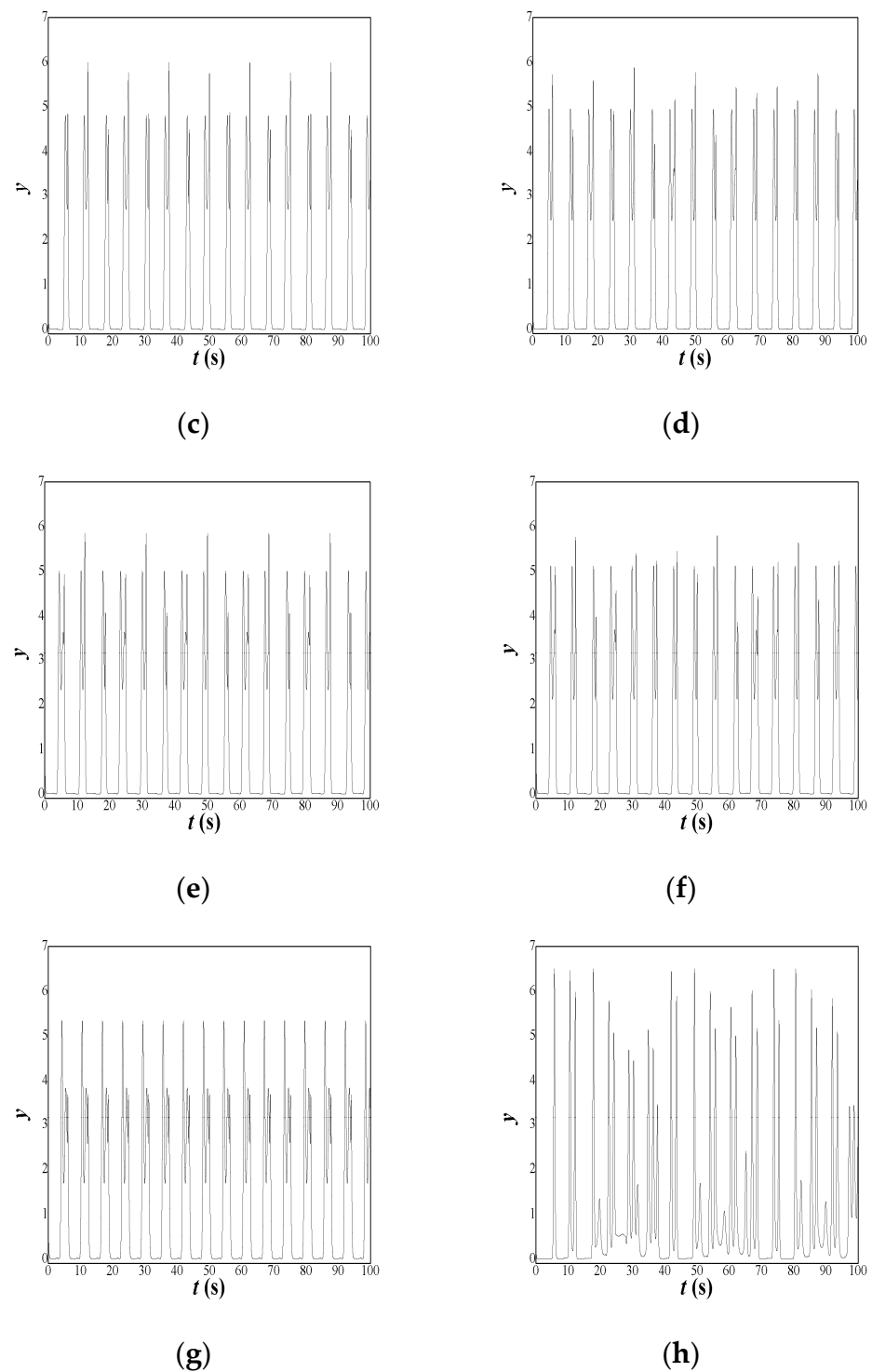


Figure 4. Time-series of y for $N = 4$, $I = 1$, $H = 3.8$ and $\omega = 1$, with (a) $A = 7.7$, (b) $A = 7$, (c) $A = 6.65$, (d) $A = 6.3$, (e) $A = 6.16$, (f) $A = 5.9$, (g) $A = 5.4$, (h) $A = 3$.

Finally, the emergence of coexisting attractors of the system is studied, by computing its continuation diagram. This diagram is analogous to the bifurcation diagram, with the only difference being that each time the continuation parameter is iterated, the initial condition of the system is taken as equal to the final state of the system from the previous simulation. Hence, in each iteration, the initial condition of the system changes, as opposed to the bifurcation diagram where it is kept steady. This is done in order to unmask any possible change in the dynamical behavior of the system with respect to initial conditions.

Indeed, as can be seen from the overlapping of the bifurcation and the continuation diagrams shown in Figure 5, for $H = 3.8$ the system showcases coexisting attractors roughly from $A = 1.23$ to $A = 7.85$. This indicates that the initial conditions can greatly affect the behavior of the system for this range of parameter A values. In more detail, system (3), in the aforementioned region presents a period-1 steady state, while from the bifurcation diagram of system (3) a great variety of phenomena has been observed, as it has been described using the phase portraits in Figure 3. This phenomenon is also very important for a biological point of view for the tumor model because the high sensitivity of the system to initial conditions implies the inability to make long-term predictions in relation to the evolution of the treatment.

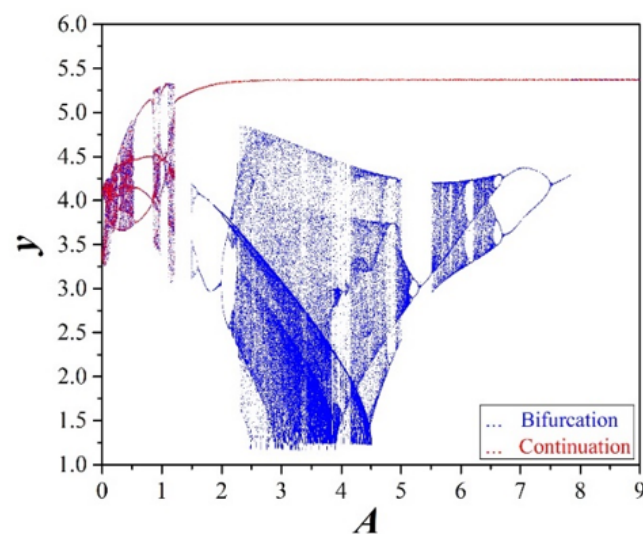


Figure 5. Overlapping bifurcation (blue color) and continuation (red color) diagram of y versus parameter A , for $N = 4$, $I = 1$ and $H = 3.8$.

The phase portrait of the coexisting attractor of that of Figure 3h, for $A = 3$, $N = 4$, $H = 3.8$, and $I = 1$, with initial conditions $(x_0, y_0, z_0) = (1, 5.36958, 6.67216)$, as well as the time-series of y , are shown in Figure 6. By the comparison of these figures with the respective phase portrait and time-series of Figures 3h and 4h, the great difference in system's (3) dynamical behavior has been confirmed. Therefore, the system can be in two different states (chaotic and period-1) for different values of initial conditions. This feature is very interesting because it implies that the same treatment could have different effects on the values of the initial populations of the tumor cells in the avascular, vascular and metastatic phases. In the aforementioned case and for the initial conditions of Figure 6, the system is in a period-1 steady-state also for small values of the intensity of treatment A .

Furthermore, by comparing the behavior of the undisturbed system of system (3), by setting $A = 0$, with the disturbed system (3) using parameter values, $N = 4$, $I = 1$, $H = 3.8$, $A = 3$, and $\omega = 1$, one can observe that the undisturbed system is in a period-2 steady-state behavior (Figure 7a) instead of a chaotic behavior (Figure 3h) or the coexisting period-1 state (Figure 6b), which system (3) presents. This suggests that the application of this kind of therapy causes differentiation in the system's behavior, due to its great sensitivity to parameter changes. Furthermore, as the value of treatment amplitude A increases, and the system of system (3) is also in a periodic state, like the case of $A = 5.4$ (Figure 4g), the amplitude of y has been reduced. These findings suggest that as the value of treatment amplitude A becomes higher, which implies more therapy, this results in lower peaks of the population of vascular tumors.

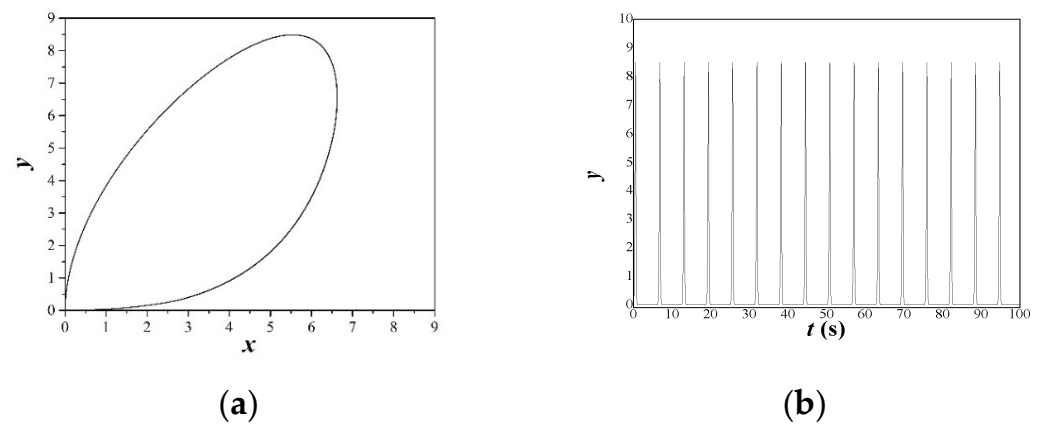


Figure 6. (a) Phase portrait of y versus x , and (b) time-series of y , for $N = 4$, $I = 1$, $H = 3.8$, $A = 3$ and $\omega = 1$, with initial conditions $(x_0, y_0, z_0) = (1, 5.36958, 6.67216)$.

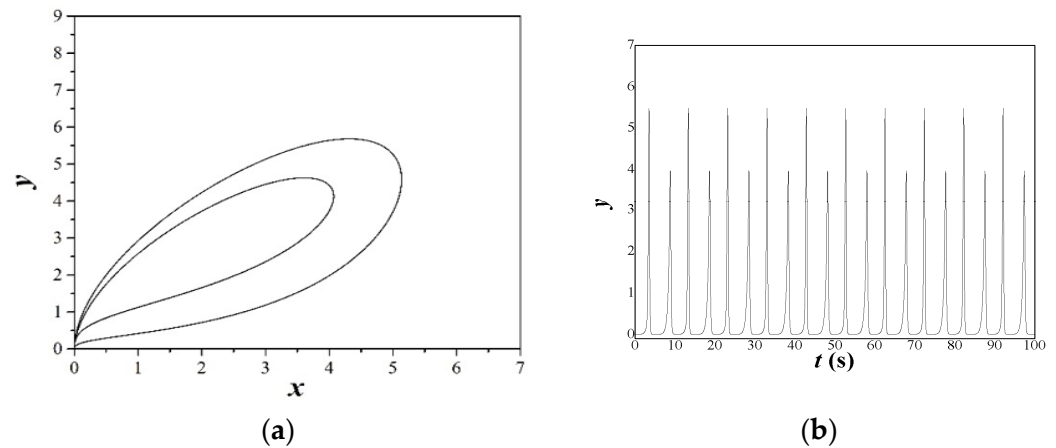


Figure 7. (a) Phase portrait of y versus x , and (b) time-series of y , for the undisturbed system ($A = 0$), with $N = 4$, $I = 1$, $H = 3.8$, $A = 3$ and $\omega = 1$.

Finally, in Figure 8 the time-series of the population of the tumor cells in the metastatic phase (z), which is a crucial phase in the progression of a tumor, for the case of undisturbed case, by setting in system (3) the parameter $A = 0$, and for the disturbed system (3), with parameter values, $N = 4$, $I = 1$, $H = 3.8$, $A = 3$, $\omega = 1$ and $(x_0, y_0, z_0) = (1, 0, 0)$, are depicted. By comparing these time series one can see that this kind of therapy may cause the system to become more convoluted. However, at certain treatment amplitudes A , like in the case of Figure 8b, it is possible to reduce the amplitude of the variable z except for some sporadic bursts. However, as the value of treatment amplitude A increases and the system of Equation (3) is also in a periodic state, like the case of $A = 5.4$ (Figure 9), the amplitude of the population of the tumor cells in the metastatic phase (z) has been significantly reduced. These results suggest that therapies can become more efficient by optimizing the conditions of the implementation.

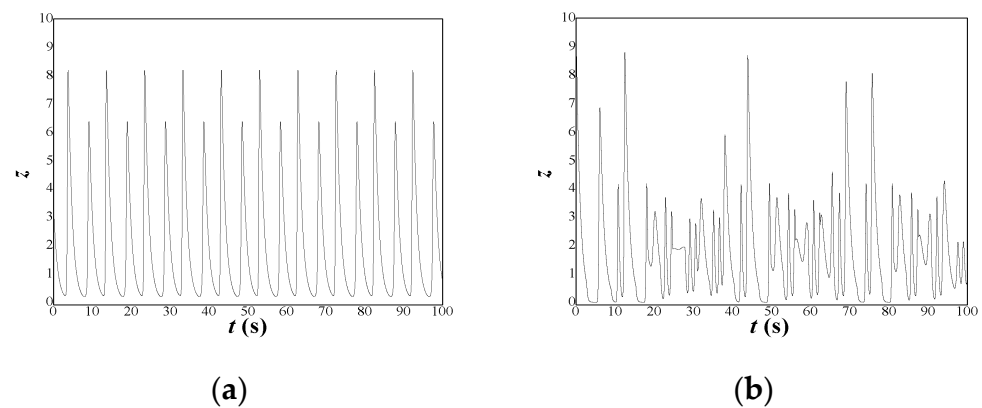


Figure 8. Time-series of z , (a) in the case of undisturbed system ($A = 0$), with $N = 4$, $I = 1$, $H = 3.8$ and (b) time-series of z , for system (3), with $A = 3$, $N = 4$, $I = 1$, $H = 3.8$ and $\omega = 1$.

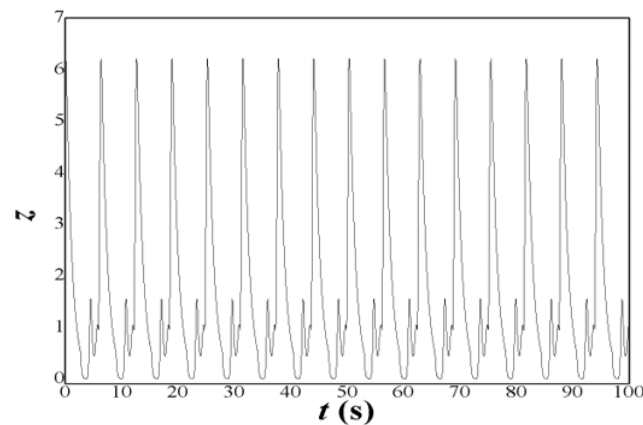


Figure 9. Time-series of z in the case of disturbed system of Equation (3), with $A = 5.4$, $N = 4$, $I = 1$, $H = 3.8$ and $\omega = 1$.

4. Conclusions

This work described the effect of immunotherapy on a dynamic model for tumor growth. The effect of immunotherapy was modeled by adding a square wave periodic term in the equation describing the immune cell growth. The dynamical analysis unmasked phenomena like the route to chaos through a periodic doubling sequence, crisis phenomena, and coexistence of attractors.

Comparing the results to the previous model (Ref. [38]) that considered the effect of immunotherapy as a sinusoidal function (system (2)), it is clear that such chaotic phenomena persist in all models but appear under different parameter values. This makes it clear that the way immunotherapy is modeled is crucial in modeling the progression of cancer growth. Furthermore, system (2) is driven from chaos to period-1 through a quasi-periodic behavior, while the proposed model of system (3) is driven from chaos to period-1 through a reverse period-doubling route as the value of treatment amplitude A is increased.

Also, the simulation results emerged that for certain values of the amplitude of the square wave periodic term, the populations of the proliferating tumor cells in the vascular and metastatic stages were reduced. This was made evident by observing the time series in Figure 4. It can be deduced from Figure 4h that at the lowest value of A used, the system was increasingly chaotic. This can result in it being harder to predict its future behavior, which is a desired trait since each type of tumor growth needs to be mitigated. Therefore, as the intensity of treatment A decreased, the system increased its complexity and gave a high degree of robustness, hence the level of success in the treatment of cancer may be low, especially at the stage of metastasis. This result suggests in a general way

that the system evolves from less complex and robust to chaotic states as the amplitude of perturbation decreases.

Thus, as future extensions of this work, the effect of frequency of the square signal on the system's behavior, as well as using different periods of administration between doses will be examined. Also, the average period between spikes is an interesting topic to consider next, as this measure could be indicative of how active the cell oscillations are. Furthermore, different types of periodic functions used to model immunotherapy, as well as combinations of them, will be considered. Finally, the fractional-order version of the model will be developed.

Author Contributions: Conceptualization, D.S. and C.V.; methodology, C.V.; software, C.V.; validation, D.S. and C.V.; formal analysis, D.S., C.V. and L.M.; investigation, D.S. and C.V.; resources, C.V.; data curation, C.V.; writing—original draft preparation, D.S. and C.V.; writing—review and editing, I.S. and E.M.; visualization, D.S.; supervision, C.V., I.S. and E.M.; project administration, D.S. and C.V.; funding acquisition, C.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

Acknowledgments: The authors are thankful to the anonymous reviewers for their constructive comments.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Wilkie, K.P. A Review of Mathematical Models of Cancer–Immune Interactions in the Context of Tumor Dormancy. In *Systems Biology of Tumor Dormancy*; Springer: Cham, Switzerland, 2013; pp. 201–234.
2. Hanahan, D.; Weinberg, R.A. Hallmarks of cancer: The next generation. *Cell* **2011**, *144*, 646–674. [[CrossRef](#)] [[PubMed](#)]
3. Bizzarri, M.; Giuliani, A.; Cucina, A.; D'Anselmi, F.; Soto, A.M.; Sonnenschein, C. Fractal Analysis in a Systems Biology Approach to Cancer. In *Seminars in Cancer Biology*; Academic Press: Cambridge, MA, USA, 2011; Volume 21, pp. 175–182.
4. Page, K.; Uhr, J. Mathematical models of cancer dormancy. *Leuk. Lymphoma* **2005**, *46*, 313–327. [[CrossRef](#)]
5. Brú, A.; Albertos, S.; Subiza, J.L.; García-Asenjo, J.L.; Brú, I. The universal dynamics of tumor growth. *Biophys. J.* **2003**, *85*, 2948–2961. [[CrossRef](#)]
6. De Pillis, L.G.; Radunskaya, A.E. Mathematical Models of Tumor–Immune System Dynamics. In *Modeling Tumor–Immune Dynamics*; Springer: New York, NY, USA, 2014; pp. 59–108.
7. Adam, J.A.; Bellomo, N. *A Survey of Models for Tumor–Immune System Dynamics*; Springer: Cham, Switzerland, 2012.
8. Deisboeck, T.S.; Wang, Z.; Macklin, P.; Cristini, V. Multiscale cancer modeling. *Annu. Rev. Biomed. Eng.* **2011**, *13*, 127–155. [[CrossRef](#)] [[PubMed](#)]
9. Dominic, W.; Natalia, K. *Computational Biology of Cancer: Lecture Notes and Mathematical Modeling*; World Scientific: Singapore, 2005.
10. Frank, S.A. *Dynamics of Cancer: Incidence, Inheritance, and Evolution*; Princeton, N.J., Ed.; Princeton University Press: Redwood City, CA, USA, 2007; Volume 1.
11. Nikulin, M.S.; Commenges, D.; Huber, C. (Eds.) *Probability, Statistics and Modelling in Public Health*; Springer: New York, NY, USA, 2006.
12. Tan, W.Y. *Stochastic Models for Carcinogenesis*; CRC Press: Boca Raton, FL, USA, 2020.
13. Voiculetz, N.; Balaban, A.T.; Niculescu-Duvaz, I.; Simon, Z. *Modeling of Cancer Genesis and Prevention*; CRC Press: Boca Raton, FL, USA, 1991.
14. Hoge, C.S.; Murray, B.T.; Sethian, J.A. Computational modeling of solid tumor evolution via a general Cartesian mesh/level set method. *Fluid Dyn. Mater. Processing* **2005**, *1–2*, 1–37.
15. Breward, C.J.W.; Byrne, H.M.; Lewis, C.E. The role of cell–cell interactions in a two-phase model for avascular tumour growth. *J. Math. Biol.* **2002**, *45*, 125–152. [[CrossRef](#)] [[PubMed](#)]
16. Byrne, H.M.; Chaplain, M.A.J. Necrosis and apoptosis: Distinct cell loss mechanisms in a mathematical model of avascular tumour growth. *Comput. Math. Methods Med.* **1998**, *1*, 223–235. [[CrossRef](#)]
17. Chaplain, M.A.J. Avascular growth, angiogenesis and vascular growth in solid tumours: The mathematical modelling of the stages of tumour development. *Math. Comput. Model.* **1996**, *23*, 47–87. [[CrossRef](#)]
18. Izquierdo-Kulich, E.; Rebelo, I.; Tejera, E.; Nieto-Villar, J.M. Phase transition in tumor growth: I avascular development. *Phys. A Stat. Mech. Its Appl.* **2013**, *392*, 6616–6623. [[CrossRef](#)]

19. Knighton, D.; Ausprunk, D.; Tapper, D.; Folkman, J. Avascular and vascular phases of tumour growth in the chick embryo. *Br. J. Cancer* **1977**, *35*, 347–356. [[CrossRef](#)] [[PubMed](#)]
20. Ward, J.P.; King, J.R. Mathematical modelling of avascular-tumour growth. *Math. Med. Biol. A J. IMA* **1997**, *14*, 39–69. [[CrossRef](#)]
21. Ribatti, D.; Vacca, A.; Dammacco, F. The role of the vascular phase in solid tumor growth: A historical review. *Neoplasia* **1999**, *1*, 293–302. [[CrossRef](#)] [[PubMed](#)]
22. Huang, Y.; Song, N.; Ding, Y.; Yuan, S.; Li, X.; Cai, H.; Shi, h.; Luo, Y. Pulmonary vascular destabilization in the premetastatic phase facilitates lung metastasis. *Cancer Res.* **2009**, *69*, 7529–7537. [[CrossRef](#)] [[PubMed](#)]
23. Keogan, M.T.; McDermott, V.G.; Paulson, E.K.; Sheafor, D.H.; Frederick, M.G.; de Long, D.M.; Nelson, R.C. Pancreatic malignancy: Effect of dual-phase helical CT in tumor detection and vascular opacification. *Radiology* **1997**, *205*, 513–518. [[CrossRef](#)] [[PubMed](#)]
24. Verstovsek, S.; Kantarjian, H.; Manshouri, T.; Cortes, J.; Giles, F.J.; Rogers, A.; Albitar, M. Prognostic significance of cellular vascular endothelial growth factor expression in chronic phase chronic myeloid leukemia. *Blood J. Am. Soc. Hematol.* **2002**, *99*, 2265–2267. [[CrossRef](#)] [[PubMed](#)]
25. Yao, J.C.; Phan, A.; Hoff, P.M.; Chen, H.X.; Charnsangavej, C.; Yeung, S.C.J.; Hess, K.; Ng, C.; Abbruzzese, J.L.; Ajani, J.A. Targeting vascular endothelial growth factor in advanced carcinoid tumor: A random assignment phase II study of depot octreotide with bevacizumab and pegylated interferon alfa-2b. *J. Clin. Oncol.* **2008**, *26*, 1316–1323. [[CrossRef](#)] [[PubMed](#)]
26. Chaffer, C.L.; Weinberg, R.A. A perspective on cancer cell metastasis. *Science* **2011**, *331*, 1559–1564.
27. Gupta, G.P.; Massagué, J. Cancer metastasis: Building a framework. *Cell* **2006**, *127*, 679–695. [[CrossRef](#)] [[PubMed](#)]
28. Nguyen, D.X.; Massagué, J. Genetic determinants of cancer metastasis. *Nature Rev. Genet.* **2007**, *8*, 341–352. [[CrossRef](#)]
29. Pani, G.; Galeotti, T.; Chiarugi, P. Metastasis: Cancer cell's escape from oxidative stress. *Cancer Metastasis Rev.* **2010**, *29*, 351–378. [[CrossRef](#)]
30. Poste, G.; Fidler, I.J. The pathogenesis of cancer metastasis. *Nature* **1980**, *283*, 139–146. [[CrossRef](#)] [[PubMed](#)]
31. Bunimovich-Mendrazitsky, S.; Halachmi, S.; Kronik, N. Improving Bacillus Calmette-Guerin (BCG) immunotherapy for bladder cancer by adding interleukin 2 (IL-2): A mathematical model. *Math. Med. Biol.* **2016**, *33*, 159–188. [[CrossRef](#)]
32. Kronik, N. Predicting outcomes of prostate cancer immunotherapy by personalized mathematical models. *PLoS ONE* **2010**, *5*, e15482. [[CrossRef](#)] [[PubMed](#)]
33. Llanos-Pérez, J.A.; Betancourt-Mar, J.A.; Cocho, G.; Mansilla, R.; Nieto-Villar, J.M. Phase transitions in tumor growth: III vascular and metastasis behavior. *Phys. A* **2016**, *462*, 560–568. [[CrossRef](#)]
34. Betancourt-Mar, J.A.; Llanos-Pérez, J.A.; Cocho, G.; Mansilla, R.; Martin, R.R.; Montero, S.; Nieto-Villar, J.M. Phase transitions in tumor growth: IV relationship between metabolic rate and fractal dimension of human tumor. *Phys. A* **2017**, *473*, 344–351. [[CrossRef](#)]
35. Montero, S.; Martin, R.; Mansilla, R.; Cocho, G.; Nieto-Villar, J.M. Parameters Estimation in Phase-Space Landscape Reconstruction of Cell Fate: A Systems Biology Approach. In *Systems Biology*; Humana Press: New York, NY, USA, 2018; pp. 125–170.
36. Guerra, A.; Rodriguez, D.J.; Montero, S.; Betancourt-Mar, J.A.; Martin, R.R.; Silva, E.; Bizzarri, M.; Cocho, G.; Mansilla, R.; Nieto-Villar, J.M. Phase transitions in tumor growth VI: Epithelial–Mesenchymal transition. *Phys. A* **2018**, *499*, 208–215. [[CrossRef](#)]
37. Kalluri, R.; Weinberg, R.A. The basics of epithelial-mesenchymal transition. *J. Clin. Invest.* **2009**, *119*, 1420–1428. [[CrossRef](#)]
38. Jaime, J.C.; Mesa-Álvarez, M.D.; Martin, R.R.; Betancourt-Mar, J.A.; Cocho, G.; Mansilla, R.; Nieto-Villar, J.M. Chronotherapy of cancer: Periodic perturbations in vascular growth and metastasis. *Biol. Rhythm Res.* **2018**, *50*, 495–504. [[CrossRef](#)]
39. Cardelli, L. From processes to ODEs by chemistry. In Proceedings of the Fifth IFIP International Conference on Theoretical Computer Science—TCS Springer, Boston, MA, USA, 7–10 September 2008; pp. 261–281.
40. Sourailidis, D.; Volos, C.; Moysis, L.; Stouboulos, I. Nonlinear phenomena and chaos in a tumor growth model. In Proceedings of the 2nd International Nonlinear Dynamics Conference, Rome, Italy, 16–19 February 2021.
41. Sonabend, A.M.; Ogden, A.T.; Maier, L.M. Medulloblastoma: Challenges for effective immunotherapy. *J. Neurooncol.* **2012**, *108*, 1–10. [[CrossRef](#)]
42. Meiliana, A.; Dewi, N.; Wijaya, A. Cancer Immunotherapy: A Review. *Indones. Biomed. J.* **2016**, *8*, 1–20. [[CrossRef](#)]
43. Humphries, C. Adoptive cell therapy: Honing that killer instinct. *Nature* **2013**, *504*, S13–S15. [[CrossRef](#)] [[PubMed](#)]
44. Ranjan, A.; Wright, S.; Srivastava, S.K. Immune consequences of penfluridol treatment associated with inhibition of glioblastoma tumor growth. *Oncotarget* **2017**, *8*, 47632–47641. [[CrossRef](#)] [[PubMed](#)]