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A MATHEMATICAL MODEL FOR M-PHASE SPECIFIC CHEMOTHERAPY INCLUDING THE G_0-PHASE AND IMMUNORESPONSE

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Abstract. In this paper we use a mathematical model to study the effect of an M-phase specific drug on the development of cancer, including the resting phase G_0 and the immune response. The cell cycle of cancer cells is split into the mitotic phase (M-phase), the quiescent phase (G_0-phase) and the interphase (G_1, S, G_2 phases). We include a time delay for the passage through the interphase, and we assume that the immune cells interact with all cancer cells. We study analytically and numerically the stability of the cancer-free equilibrium and its dependence on the model parameters. We find that quiescent cells can escape the M-phase drug. The dynamics of the G_0 phase dictates the dynamics of cancer as a whole. Moreover, we find oscillations through a Hopf bifurcation. Finally, we use the model to discuss the efficiency of cell synchronization before treatment (synchronization method).

1. **Introduction.** Chemotherapy treatment has demonstrated a definite capacity for controlling disseminated metastatic cancer and is widely used. Unfortunately, drugs in cancer chemotherapy kill normal as well as cancerous cells. Naturally it is desirable to kill as many cancerous cells as possible while sparing as many normal cells as possible. One way of accomplishing this goal is by taking advantage of the fact that many chemotherapeutic drugs are cycle-specific: they destroy cells only in specific phases of the cells' cycle. In the cell synchronization method, the cancerous cells are first synchronized by one drug. When nearly all the cancerous cells reach the desirable phase, they are treated with a second, cycle-specific drug. This kills the maximum number of cancer cells while sparing large numbers of normal cells. Some examples of such drugs are Cytosine Arabinoside (Ara-C), 5-fluorouracil and Prednisone, which work in the G_1 and S phases of the cell-cycle, and Vincristine, Paclitaxel and Bleomycin which work in the M phase of the cell-cycle [9, 15]. The

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cell is blocked from continuing in the cell cycle, and thus the drugs stop the cell proliferation and allow the immune system to attack and kill cancerous cells in a natural way [27].

A classical cell cycle model, the G_0-model, has been developed by Mackey [18]. Examples of the more recent work done with mathematical models of cycle-specific chemotherapy are by Webb [28] and Kheifetz *et al.* [12]. They develop linear and nonlinear age-structured models of cycle-specific chemotherapy. The advantage of shorter dosage periods are investigated in the case of the linear model. Another work of interest is by Birkhead *et al.* [1], in which a four-compartment linear system is developed to model the cycling, resistant and resting cells. Their results are limited to a few numerical calculations on four specific types of treatments. Swan [25] also examines cycle-specific chemotherapy in his review article. Particularly, he concentrates on age-structured models that take into account the age of the cells in each compartment of the cell cycle. He also studies an age-structured chemotherapeutic model of acute myeloid leukemia. The fact is that in the above articles only chemotherapy, not immunoresponse, is considered. Kirschner and Panetta [14] include the immune system in a mathematical model to study immunotherapy as an alternative to chemotherapy. In [27] Villasana and Radunskaya model the cycle-specific chemotherapy that includes the immune system but excludes the resting stage. In their paper, they study the interaction of tumor cells and drug with the immune system and show that the stability of fixed points may depend on the delay. De Boer *at al.* [7] represent a more specific model to study the macrophage T lymphocyte interactions that generate an antitumor immune response. With respect to cancer interaction with immune cells, the main difference between our work and theirs is that in their case they study a very specific type of lymphocyte which interacts with tumor cells, while in our case we consider a more general type of immune cells with main focus on the cycle-specificity of M-phase chemotherapy.

With respect to cancer interaction with immune cells, DeLisi and Resoigno [8] employ a simple deterministic predator-prey model to simulate immune surveillance in which immune cells and molecules are stimulated by a transplanted tumor. A specific model for T lymphocyte response to the growth of an immunogenic tumor is given by Kuznetsov *et al.* [17]. The model is used to describe the kinetics of B-lymphoma BCL_1 in the spleen of mice. Moreover, the model is applied to the analysis of immuno-stimulation of tumor growth, formation of a tumor dormancy and sneaking through of the tumor. With respect to growth kinetics of immune cells we use a model similar to that of Kuznetsov *et al.* [17]. With respect to cancer interaction with cycle-specific drugs, Kozusko *et al.* [16] develop a mathematical model to study the in-vitro cancer cell growth and response to treatment with the experimental antimitotic agent curacin A. They predict that curacin A will be quickly absorbed into cell phases and will express an effective control of cancer growth; that is, the cells will response with an increase in the rate of DNA synthesis, a decrease in the rate of mitosis and possibly an increase in the rate of apoptosis. In [4] Cojocaru and Agur provide a formal method for predicting the effect on treatment efficacy of cell-cycle-specific drugs, such as the cancer drug cytosine arabinoside (Ara-C). A comprehensive review of recent relevant results in mathematical modeling and control of the cell cycle and of the mechanisms of gene amplification (related to drug resistance), and estimation of the constructed models is given by Kimmel and Swierniak [13].

TABLE 1. Variables in model system

variable	meaning	unit
x	number of cancer cells in the interphase	cells
y	number of cancer cells in the mitotic phase	cells
z	number of cancer cells in the resting phase	cells
I	number of lymphocytes	cells
u	biomass of chemotherapy drugs	mg

The model we propose is an extension of the models above, in particular with respect to the quiescent G_0-phase. Our analysis shows that the G_0-phase is a critical factor for cancer treatment. Our model is based on a model the model developed by Villasana and Radunskaya [27]. However, we find that the model in [27] is questionable in the development of one of the model's delay terms, which will make solutions of the system negative in positive time. Therefore, we modify their model and include the immune system and the quiescent stage into the model. The main conclusion from our analysis is that a resting phase of tumor cells is the most important compartment for cancer treatment with an M-phase specific drug. This confirms the general understanding that cancer cells can avoid the chemotherapeutic agent in the resting compartment (see, for example, www.cancerhelp.org.uk). The surviving quiescent cells can contribute to further tumor growth when the chemotherapeutic effect has failed. For the analysis, here we study a single drug dose at time $t = 0$ only. However, in the numerical simulations, we also study multiple dosage protocols and find that multiple dosage protocols do not change the qualitative result. Thus the resting cells are the limiting factor for chemotherapy (according to our model). Additionally, we find that a time delay for the interphase can lead to stability switches. One implication is a scenario, where cancer cells can be controlled by arresting cells in the interphase. In these cases the arrested cells are subsequently killed by the immune response. In any case, the M-phase specific drug certainly reduces the overall tumor load of a patient, even though it might not cure cancer alone.

We present and develop the new model in section 1.1, whereas in section 1.2 we carry out a nondimensionalization to reduce the number of parameters.

The active cell compartment includes a time delay related to the transition of cells through the G_1, S and G_2 phases. We split our analysis according to this delay, studying the no-delay case in section 2 and the delay case in section 3. In both cases we investigate the stability of the cancer-free equilibrium in the cases of (i) no drug and no immune response, (ii) immune suppression without drug, and (iii) immune suppression with drug. In all cases we show that adminstration of an M-phase specific drug does not change the stability of the cancer-free equilibrium. However, cancer growth is significantly reduced by the drug, and in the case of a delay the drug can initiate or destroy oscillations. We prove a corresponding result on Hopf bifurcation in section 3.4. Moreover, in the delay case we prove the existence of a stability switch if the delay parameter is beyond a certain value. Furthermore, adminstration of an M-phase specific drug can lead to partial cell synchronization (expressed through oscillating solutions). In section 4, we illustrate our results with numerical simulations. The paper closes with a discussion in section 5.

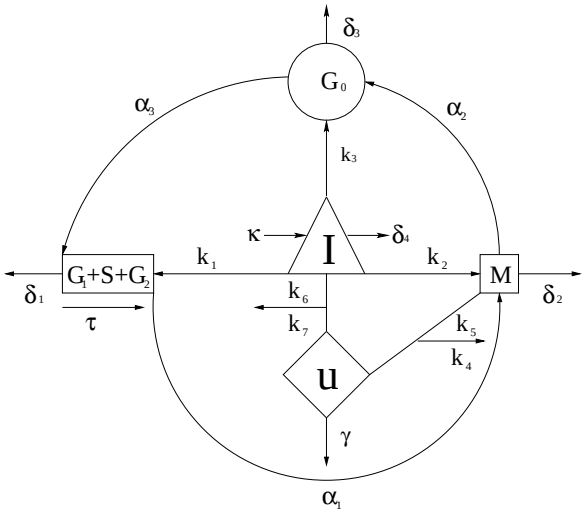

FIGURE 1. Arrow diagram of the cell cycle model (1) indicating immune response, I, and M-phase -specific drug, u.

1.1. **The model.** We take as our model of cancer treatment by chemotherapy a system of delayed differential equations, which takes the form

$$
\begin{aligned}
\dot{x}(t) &= \underbrace{\alpha_3 z(t)}_{\text{from resting phases}} - \underbrace{\alpha_1 x(t)}_{\text{flowing into mitosis phases}} - \underbrace{\delta_1 x(t)}_{\text{natural death}} \\
&\quad - \underbrace{k_1 I(t) x(t)}_{\text{destroyed by lymphocytes}} \\
\dot{y}(t) &= \underbrace{\alpha_1 x(t-\tau)}_{\text{from interphases}} - \underbrace{\alpha_2 y(t)}_{\text{to resting phases}} - \underbrace{\delta_2 y(t)}_{\text{natural death}} - \underbrace{k_2 I(t) y(t)}_{\text{destroyed by lymphocytes}} \\
&\quad - \underbrace{k_4 (1 - e^{-k_5 u(t)}) y(t)}_{\text{destroyed by drugs}} \\
\dot{z}(t) &= \underbrace{2\alpha_2 y(t)}_{\text{from mitosis}} - \underbrace{\alpha_3 z(t)}_{\text{to reproduce}} - \underbrace{\delta_3 z(t)}_{\text{natural death}} - \underbrace{k_3 I(t) z(t)}_{\text{destroyed by lymphocytes}} \\
\dot{I}(t) &= \underbrace{k}_{\text{constant growth source}} + \underbrace{\frac{\rho I(t)(x+y+z)^n}{a + (x+y+z)^n}}_{\text{growth due to stimulus}} - \underbrace{\delta_4 I(t)}_{\text{natural death}} \\
&\quad - \underbrace{(c_1 x(t) + c_2 y(t) + c_3 z(t)) I(t)}_{\text{combined with cancer cells}} - \underbrace{k_6 (1 - e^{-k_7 u(t)}) I(t)}_{\text{destroyed by drugs}} \\
\dot{u}(t) &= \underbrace{-\gamma u(t)}_{\text{exponential decay}} ,
\end{aligned}
$$

with initial conditions

$$x(t) = \phi_1(t),\, t \in [-\tau, 0]\,, y(0) = y_0,\, z(0) = z_0,\, I(0) = I_0,\, u(0) = u_0.$$

A schematic of this model is given in Figure 1, the meaning of each variable is listed in Table 1, and the interpretation of parameters is given in Table 2.

TABLE 2. Parameters in the model system

parameter	meaning	value	ref.
α_1	rate at which cells flow into the mitosis phase	$0-1$/day	[18, 27]
α_2	rate at which cells flow into the resting phase	$0-1$/day	[18]
α_3	rate at which cells leave from the resting phase to enter the cycle to reproduce	$0-1$/day	[18]
$c_i(i=1,2,3)$	losses due to the encounters with immune cells	$0.01\times10^{-6}-1\times10^{-6}$/cell day	[17, 27]
$\delta_i(i=1,2,3)$	proportions of natural death of x, y, and I	$0-1$/day	[17, 27]
δ_3	rate at which cells leave from the resting state to enter the blood	$0-0.056$/day	[18, 24]
ρ	proportion of the growth of lymphocytes due to stimulus by cancer cells	0.2/day	[14, 27]
a	speed at which the lymphocytes reach saturation level without stimulation	$0.5\times(0.1\times10^6\text{cell})^3$	[14, 27]
k	growth rate of the lymphocytes in the absence of cancer cells	0.15×10^6cell/day	[17, 27]
$k_i(i=1,2,3)$	rates at which lymphocytes destroy cells in different phases	$0.1\times10^{-8}-1\times10^{-8}$/cell day	[17, 27]
k_i $(i=4,6)$	proportions of drugs which eliminate cancer cells and lymphocytes	$0-1$/day	[22, 27]
k_i $(i=5,7)$	proportions of drugs which eliminate cancer cells and lymphocytes	$0.01\times10^{-2}-1\times10^{-2}$/mg	[[27]
γ	proportion of decay of the drugs	$0.1\times10^{-2}-1\times10^{-2}$/day	[27]
τ	resident time of cells in the interphase	$0-2$ days	[18, 27]

All constants are positive. By and large, cancer cells cannot differentiate into maturer forms of precursors. Consequently, we assume here that the cancer cells behave as a proliferative pool only. The cancer cells are self-renewing and consist of a resting compartment and an active compartment that is split into four phases due to cycle-specificity consideration here. In the resting state, the cancer cells leave at random to enter either the active compartment or the blood at fractional rates α_3 and δ_3. Cells in the blood are largely nonproliferative, and they are for the most part destined to die [23]. For the cancer population we consider here, stem cell influx is assumed to be negligible. The cancer cells reside in the cycle for a certain period of time τ before entering into the mitotic stage. Thus we have the term $x(t-\tau)$ in the system. The corresponding model in [27] has a negative delay term $-\alpha x(t-\tau)$ in the x-equation. This is problematic, because solutions might become negative and unphysiological oscillations occur. Besides the modified term $-\alpha x(t)$, our model includes the resting phase explicitly (z-equation), which was not studied in [27]. The terms $\delta_1 x(t)$, $\delta_2 y(t)$, $\delta_3 z(t)$, $\delta_4 I(t)$ in the model equations represent proportions of natural cell death or apoptosis, α_1, α_2 and α_3 represent the different rates at which cells flow between different phases or reproduce, the terms k_i and

c_i $(i = 1, 2, 3)$ represent losses from encounters of cancer cells with lymphocytes. We model the interaction of immune cells with cancer by the law of mass action. With respect to immune response function, the term $\frac{\rho I(t)(x(t)+y(t)+z(t))^n}{a+(x(t)+y(t)+z(t))^n}$ represents the nonlinear growth of the immune population due to stimulus by the cancer cells. Here we have chosen a Michaelis-Menten form for this term, following the literature (see, for example, [14, 17, 27]). We think it is reasonable, because proliferation of cancer-specific effector cells is stimulated by the presence of cancer cells but reaches a saturation level at cancer population. The saturation level depends on the health of the immune system, specifically on its ability to produce certain cytokines. In the absence of cancer cells ($x = y = z = 0$), the immune cells grow at a constant source rate k. Therefore, the recruitment function should be zero when there are no cancer cells and should increase monotonically toward a horizontal asymptote; this rational form reflects these characteristics in a simple, smooth function. The parameters ρ, a and n depend on the type of cancer being considered. With respect to high densities of drugs, we know that the drug interferes with cancer cells in mitosis, causing them to die naturally when they fail to complete the cycle [27]. Therefore we assume that once the drug encounters the cancer cell, the cancer cell is taken out of the cycle and can no longer proliferate. This is modeled by the term $-k_4(1 - e^{-k_5 u})y$ [4, 27], but there are other curves that describe a similar feature (see [22]). The drug decay is assumed to be exponential , and the coefficient γ incorporates both the elimination and absorption effects [27]. In [26] the effect of multiple applications of the drug is considered. Here we focus on a single drug dose treatment only. Multiple dosage protocols and other treatment options are beyond the scope of this paper. We also assume that the drug is harmful to the immune system and we leave a similar term in the I(t) equation. Biologically, this treatment term means that when no drugs are applied ($k_5 = 0$), there are no effects on the cancer cell population, since $1 - e^{-k_5 u} = 0$. Further, k_4 represents the intensity of the treatment. In this new model, we assume that the resting cells are not affected by the drugs but immune cells will attack them. This assumption derives from the fact that faster proliferating cells are more sensitive to the drugs, while the cells in the resting phase escape the action of cycle-specific cytotoxic agents [27]. For other assumptions for the model, the reader is referred to [27].

1.2. **Nondimensionalization.** As in [27], we nondimensionalize the system and write

$$\bar{t} = \frac{t}{\text{day}}, \quad \bar{x} = \frac{x}{x(0)}, \quad \bar{y} = \frac{y}{x(0)}, \quad \bar{z} = \frac{z}{z(0)}, \quad \bar{I} = \frac{I}{I(0)}, \quad \bar{u} = \frac{u}{u(0)}, \quad s = \frac{z(0)}{x(0)},$$

$$\bar{k}_1 = k_1 I(0), \quad \bar{k}_2 = k_2 I(0), \quad \bar{k}_3 = k_3 I(0), \quad \bar{k}_5 = k_5 u(0), \quad \bar{k}_7 = k_7 u(0),$$

$$\bar{a} = a/x^n(0), \quad \bar{c}_1 = c_1 x(0), \quad \bar{c}_2 = c_2 x(0), \quad \bar{c}_3 = c_3 z(0), \quad \bar{k} = k/I(0),$$

where $x(0) = y(0)$ are initial values. By renaming the variables $\bar{t}, \bar{x}, \bar{y}, \bar{z}, \bar{I}, \bar{u}$ to t, x, y, z, I, u respectively and the parameter values $\bar{k}, \bar{a}, \bar{k}_i, \bar{c}_j$ to k, a, k_i, c_j respectively, $i = 1 - 7$; $j = 1 - 3$. Then, none of the new parameters and variables have

dimensions. From this point on we will work with the nondimensionalized model:

$$\begin{aligned}
\dot{x}(t) &= s\alpha_3 z(t) - \alpha_1 x(t) - (\delta_1 + k_1 I(t))x(t) \\
\dot{y}(t) &= \alpha_1 x(t-\tau) - (\alpha_2 + \delta_2 + k_2 I(t))y(t) - k_4(1 - e^{-k_5 u(t)})y(t) \\
\dot{z}(t) &= 2s^{-1}\alpha_2 y(t) - (\alpha_3 + \delta_3 + k_3 I(t))z(t) \\
\dot{I}(t) &= k + \frac{\rho I(t)(x+y+sz)^n}{a+(x+y+sz)^n} - (\delta_4 + c_1 x(t) + c_2 y(t) + c_3 z(t))I(t) \\
&\quad -k_6(1 - e^{-k_7 u(t)})I(t) \\
\dot{u}(t) &= -\gamma u(t),
\end{aligned} \tag{1}$$

with initial conditions

$$x(t) = \phi_1(t),\, t \in [-\tau, 0]\,, y(0) = y_0\,, z(0) = z_0\,, I(0) = I_0\,, u(0) = u_0.$$

2. **Stability results for the nondelay case.** We first determine the type of dynamics that can arise in the system without the presence of the delay and then study the case with delay in section 3. A summary of the stability results appears in the discussion section, table 3. We begin by analyzing the simplest case: a drug-free model in a nondelay situation in the absence of an immune response.

2.1. **Drug-free model in the absence of an immune response.** In this subsection, we shall study the drug-free model in a nondelay case without an immune response. Necessary and sufficient conditions that guarantee the stability of the cancer-free equilibrium are obtained. Also, a necessary condition for cancer growth is obtained. In this case the equations are a simple set of ordinary differential equations:

$$\begin{aligned}
\dot{x}(t) &= -(\alpha_1 + \delta_1)x(t) + s\alpha_3 z(t) \\
\dot{y}(t) &= \alpha_1 x(t) - (\alpha_2 + \delta_2)y(t) \\
\dot{z}(t) &= 2s^{-1}\alpha_2 y(t) - (\alpha_3 + \delta_3)z(t),
\end{aligned} \tag{2}$$

with initial values

$$x(0) = x_0, y(0) = y_0, z(0) = z_0.$$

This is a linear system with the only equilibrium being $E_0(0,0,0)$. The Jacobian matrix about this equilibrium is

$$\begin{bmatrix}
-(\alpha_1 + \delta_1) & 0 & s\alpha_3 \\
\alpha_1 & -(\alpha_2 + \delta_2) & 0 \\
0 & 2s^{-1}\alpha_2 & -(\alpha_3 + \delta_3)
\end{bmatrix},$$

and the characteristic equation is

$$\lambda^3 + a_2\lambda^2 + a_1\lambda + a_0 = 0,$$

where

$$\begin{aligned}
a_2 &= \alpha_1 + \delta_1 + \alpha_2 + \delta_2 + \alpha_3 + \delta_3 \\
a_1 &= (\alpha_1 + \delta_1)(\alpha_2 + \delta_2) + (\alpha_2 + \delta_2)(\alpha_3 + \delta_3) + (\alpha_3 + \delta_3)(\alpha_1 + \delta_1) \\
a_0 &= (\alpha_1 + \delta_1)(\alpha_2 + \delta_2)(\alpha_3 + \delta_3) - 2\alpha_1\alpha_2\alpha_3 \\
&= \alpha_1\alpha_2(\delta_3 - \alpha_3) + (\alpha_3 + \delta_3)(\alpha_1\delta_2 + \alpha_2\delta_1 + \delta_1\delta_2).
\end{aligned} \tag{3}$$

Clearly, a_2, a_1 are positive and $a_2 a_1 > a_0$. By the Routh-Hurwitz criteria [6], necessary and sufficient conditions for λ to have negative real parts become $a_0 > 0$.

As a result, we have the following lemma.

LEMMA 2.1. *The cancer-free equilibrium E_0 of system (2) is locally asymptotically stable if and only if $a_0 > 0$.*

Biomedical interpretation

Note that G_0 acts as a control center to determine the rate of proliferation. α_3 represents the release rate at which cells in the resting phase at random enter into their cell cycles to reproduce cells, and δ_3 is the fractional rate at which cells are released randomly into the blood from the resting phase.

Assume $a_0 > 0$ such that a cancer is growing. To control the cancer growth and give a biomedical interpretation of this first result, we consider parameters that are beneficial for cancer eradication. In Lemma 1 the only condition for cancer decay is $a_0 > 0$. Hence for cancer to grow, a necessary condition is $\alpha_3 > \delta_3$, which means that the rate, δ_3, of leaving the resting state G_0 must be smaller than the transition rate α_3 from the resting compartment to the active compartment. If $\delta_3 > \alpha_3$ cancer will not grow (according to this model). It is interesting that already in this simple model the quiescent compartment G_0 controls the cancer dynamics. This fact will be confirmed by the more complex models later and has also been identified for radiation treatment in Dawson and Hillen [10].

2.2. **Drug-free model in the presence of immune suppression.** In this subsection, we will add the effect of immune suppression to study how lymphocytes will change the dynamical behavior of cancer cells when $\tau = 0$. New conditions for cancer growth or extinction that involve the immune suppression parameter terms will be obtained. When adding immune suppression, the system becomes

$$\begin{aligned}
\dot{x}(t) &= -(\alpha_1+\delta_1)x(t) + s\alpha_3 z(t) - k_1 x(t) I(t)\\
\dot{y}(t) &= \alpha_1 x(t) - (\alpha_2+\delta_2)y(t) - k_2 y(t) I(t)\\
\dot{z}(t) &= 2s^{-1}\alpha_2 y(t) - (\alpha_3+\delta_3)z(t) - k_3 z(t) I(t)\\
\dot{I}(t) &= k + \tfrac{\rho I(t)(x+y+sz)^n}{a+(x+y+sz)^n} - (c_1 x(t) + c_2 y(t) + c_3 z(t) + \delta_4) I(t).
\end{aligned} \tag{4}$$

Note that $E_1(0,0,0,k/\delta_4)$ is an equilibrium of this system with zero cancer level and a positive immune level. In general, there will be other fixed points, but this fixed point is of particular interest since it represents a cancer-free state. The Jacobian matrix about E_1 is

$$\begin{bmatrix}
-(\alpha_1+\delta_1+\frac{k_1 k}{\delta_4}) & 0 & s\alpha_3 & 0\\
\alpha_1 & -(\alpha_2+\delta_2+\frac{k_2 k}{\delta_4}) & 0 & 0\\
0 & 2s^{-1}\alpha_2 & -(\alpha_3+\delta_3+\frac{k_3 k}{\delta_4}) & 0\\
-\frac{c_1 k}{\delta_4} & -\frac{c_2 k}{\delta_4} & -\frac{c_3 k}{\delta_4} & -\delta_4
\end{bmatrix}.$$

Clearly, $\lambda = -\delta_4$ is an eigenvalue, and the remaining eigenvalues are given by the solutions to the characteristic equation

$$\lambda^3 + b_2\lambda^2 + b_1\lambda + b_0 = 0, \tag{5}$$

where

$$\begin{aligned}
b_2 &= \alpha_1+\delta_1+\alpha_2+\delta_2+\alpha_3+\delta_3+\tfrac{k}{\delta_4}(k_1+k_2+k_3)\\
b_1 &= (\alpha_1+\delta_1+\tfrac{k_1 k}{\delta_4})(\alpha_2+\delta_2+\tfrac{k_2 k}{\delta_4}) + (\alpha_2+\delta_2+\tfrac{k_2 k}{\delta_4})(\alpha_3+\delta_3+\tfrac{k_3 k}{\delta_4})\\
&+ (\alpha_3+\delta_3+\tfrac{k_3 k}{\delta_4})(\alpha_1+\delta_1+\tfrac{k_1 k}{\delta_4})\\
b_0 &= (\alpha_1+\delta_1+\tfrac{k_1 k}{\delta_4})(\alpha_2+\delta_2+\tfrac{k_2 k}{\delta_4})(\alpha_3+\delta_3+\tfrac{k_3 k}{\delta_4}) - 2\alpha_1\alpha_2\alpha_3\\
&= \alpha_1\alpha_2(\delta_3+\tfrac{k_3 k}{\delta_4}-\alpha_3) + (\alpha_3+\delta_3+\tfrac{k_3 k}{\delta_4})(\alpha_1\alpha_2\\
&+ \alpha_1(\delta_2+\tfrac{k_2 k}{\delta_4}) + \alpha_2(\delta_1+\tfrac{k_1 k}{\delta_4}) + (\delta_1+\tfrac{k_1 k}{\delta_4})(\delta_2+\tfrac{k_2 k}{\delta_4})).
\end{aligned} \tag{6}$$

Obviously, b_2, b_1 are positive and $b_2 b_1 > b_0$. By the Routh-Hurwitz criteria [6], necessary and sufficient conditions for λ to have negative real parts become $b_0 > 0$.

As a result, we have the following.

LEMMA 2.2. *For system (4), the equilibrium E_1 is locally asymptotically stable if and only if $b_0 > 0$.*

Biomedical interpretation

Note that $b_0 \geq a_0$. Hence even if without immune response a cancer grows ($a_0 < 0$), an immune response can be able to control cancer growth. In our model this occurs for example if $\alpha_3 > \delta_3$ and $\frac{k_3 k}{\delta_4} > \alpha_3 - \delta_3$. The parameter k represents the growth rate of lymphocytes, k_3 represents the rate at which lymphocytes destroy the cancer cells in the resting phase, and δ_4 is the the natural death rate of lymphocytes in the resting compartment.

In certain circumstances an increase in the number of lymphocytes might eventually increases the chance of cancer survival. This occurs if $b_0 \leq 0$, which implies that cancer cells outgrow the immune response ($\alpha_3 > \delta_3 + \frac{k k_3}{\delta_4}$). This behavior has in fact been observed by Prehn [23].

2.3. **Drug model with immune suppression.** Now we shall begin to consider the effect of an M-phase specific drug in the model along with the immune suppression when there is no delay, $\tau = 0$. We are interested in studying how the conditions for the cancer growth or extinction are varied when we apply drugs to the model. In this case the system considered becomes

$$\begin{aligned}
\dot{x} &= -(\alpha_1 + \delta_1)x + s\alpha_3 z - k_1 x I \\
\dot{y} &= \alpha_1 x - (\alpha_2 + \delta_2)y - k_2 y I - k_4(1 - e^{-k_5 u})y \\
\dot{z} &= 2s^{-1}\alpha_2 y - (\alpha_3 + \delta_3)z - k_3 z I \\
\dot{I} &= k + \tfrac{\rho I (x+y+sz)^n}{a+(x+y+sz)^n} - (c_1 x + c_2 y + c_3 z + \delta_4)I - k_6(1 - e^{-k_7 u})I \\
\dot{u} &= -\gamma u.
\end{aligned} \tag{7}$$

Under these circumstances, $E_2(0, 0, 0, k/\delta_4, 0)$ is an equilibrium of this system with zero cancer and drug levels and a positive immune level. Again, in general there are other fixed points, but this fixed point is of particular interest since it represents a cancer and drug-free state. The Jacobian matrix about E_2 is

$$\begin{bmatrix}
-(\alpha_1 + \delta_1 + \frac{k_1 k}{\delta_4}) & 0 & s\alpha_3 & 0 & 0 \\
\alpha_1 & -(\alpha_2 + \delta_2 + \frac{k_2 k}{\delta_4}) & 0 & 0 & 0 \\
0 & 2s^{-1}\alpha_2 & -(\alpha_3 + \delta_3 + \frac{k_3 k}{\delta_4}) & 0 & 0 \\
-\frac{c_1 k}{\delta_4} & -\frac{c_2 k}{\delta_4} & -\frac{c_3 k}{\delta_4} & -\delta_4 & \frac{k_6 k_7 k}{\delta_4} \\
0 & 0 & 0 & 0 & -\gamma
\end{bmatrix}.$$

Clearly, $\lambda = -\gamma$, $\lambda = -\delta_4$ are two eigenvalues. The remaining eigenvalues are the same as the solutions to characteristic equation (5); that is,

$$\lambda^3 + b_2\lambda^2 + b_1\lambda + b_0 = 0,$$

where b_2, b_1, b_0 are given by (6) in section 2.2. Using the same argument seen in section 2.2, we have the following lemma.

LEMMA 2.3. *For system (7), the cancer-free equilibrium E_2 is locally asymptotically stable if and only if $b_0 > 0$.*

Summary for the nondelay case

Comparing with Lemma 2 in section 2.2, Lemma 3 shows that the condition for the extinction of cancer cells in all phases remains the same, which implies that

the drug does not have any effects on the stability of the cancer-free equilibrium. This is because the cancer cells in the resting phase escape the action of the cycle-specific cytotoxic agents, and the drug was only given once. A numerical example for multiple dosages is given Figure 4. Therefore, we have the following.

(i) We find that including immune suppression in the model greatly helps to stabilize the system and inhibit the further growth of cancer cells (see the first term of b_0). This is reasonable, because some cancer cells are destroyed by lymphocytes, and increasing a_0 in terms of lymphocyte parameters makes conditions less favorable for cancer survival.

(ii) It follows from Lemma 2 that in certain situations lymphocytes lose the ability to recognize these cancer cells, and the cancer cells will continue to reproduce at a larger rate and eventually dominate the normal tissues.

(iii) Since the drug is assumed to be M-phase-specific, the cancer cells in G_0-phase escape the treatment. By the time they enter the cell cycle again and reach the M-phase, the drug has decayed and cancer growth is still possible. Hence, without delay, an M-phase specific drug has little or no effect. Note that here we consider only one administration of the drug at time $t = 0$. It is not surprising that, as the drug has faded out, the cancer is still able to grow, in particular if new viable cells are delivered from the quiescent state. Again, this confirms our initial statement that the quiescent compartment must be controlled to effectively eradicate cancer. More realistic drug treatment protocols can be included into our model and numerical simulations can be carried out as in Figure 4. For the analysis, however, we only study a single administration of the drug.

3. **Stability results in the presence of delay.** Now we determine the type of dynamics that can arise in the system in the presence of the delay. We begin by analyzing the simplest case: a drug-free model in a delay case in the absence of an immune response. We have seen in the previous sections that cells in the resting phase can evade the treatment and become viable as the treatment fades out. If the drug is M-phase specific, as we assume here, cells in the G_1, S, G_2-phases also avoid the drug. Hence if we include a time-delay for the transition through these phases, we expect even more cells to avoid treatment, which will or will not have a significant effect on the treatment success. In this section we consider a time delay for the phases G_1, S, G_2 and compare the results to those obtained without delay. Indeed, we will find stability switches due to a positive delay $\tau > 0$.

3.1. **Drug-free model with delay and no immune response.** In this subsection, we are interested in studying how the conditions for cancer growth or extinction are varied for positive values of the delay τ. When we add the effect of the delay in the model, we obtain

$$\begin{aligned} \dot{x}(t) &= -\alpha_1 x(t) - \delta_1 x(t) + s\alpha_3 z(t) \\ \dot{y}(t) &= \alpha_1 x(t-\tau) - (\alpha_2 + \delta_2) y(t) \\ \dot{z}(t) &= 2s^{-1}\alpha_2 y(t) - (\alpha_3 + \delta_3) z(t). \end{aligned} \tag{8}$$

Note that system (2) in section 2.1 corresponds to the special case when $\tau = 0$. As before the only equilibrium of this system is the cancer-free point $E_0(0, 0, 0)$. For the determination of stability in the case of delayed differential equations, we linearize the system about the equilibrium and consider exponential solutions that are characterized by the eigenvalues for exponents of these solutions. The characteristic equation for this system about the equilibrium E_0 is given by

$$\begin{vmatrix} \lambda+\alpha_1+\delta_1 & 0 & -s\alpha_3 \\ -\alpha_1 e^{-\lambda\tau} & \lambda+\alpha_2+\delta_2 & 0 \\ 0 & -2s^{-1}\alpha_2 & \lambda+\alpha_3+\delta_3 \end{vmatrix} = 0,$$

that is,

$$\lambda^3 + a_2\lambda^2 + a_1\lambda + a_0 + 2\alpha_1\alpha_2\alpha_3 - 2\alpha_1\alpha_2\alpha_3 e^{-\lambda\tau} =: P_1(\lambda) + Q_1(\lambda)e^{-\lambda\tau} = 0, \quad (9)$$

where a_0, a_1, a_2 are given by (3) in section 2.1.

There are many ways in which we can determine if there is a root λ of the characteristic equation with positive real part. Geometric arguments can be used to establish the stability of an equilibrium, such as those used by Mahaffy in [19], where the argument principle is used to count the number of zeros of characteristic equation (9) on the right hand side of the complex plane. However, in our case we will resort to some results by Cooke and van den Driessche in *Theorem* 1 of [5].

They define the function

$$F(y) = |P_1(iy)|^2 - |Q_1(iy)|^2,$$

and analyze the function $F(y)$, giving conditions under which equation (9) is stable as a function of τ. They also give conditions under which stability changes may occur as the delay τ is increased and show that in these cases the equilibrium is unstable for large enough τ. In short, Cooke and van den Driessche [5] proved the following: (a) Suppose that $F(y) = 0$ has no positive roots. Then if (5) is stable at $\tau = 0$, it remains stable for all $\tau \geq 0$, whereas if it is unstable at $\tau = 0$ it remains unstable for all $\tau \geq 0$. (b) Suppose that $F(y) = 0$ has at least one positive root and that each positive root is simple. Then as τ increases, stability switches may occur. There exists a positive $\bar{\tau}$ such that (9) is unstable for all $\tau > \bar{\tau}$. As τ varies from 0 to $\bar{\tau}$, at most a finite number of stability switches may occur. In addition, if $F'(y) \neq 0$, a stability switch does occur (Proposition 1 in [5]).

Following the steps in this theorem, it is straightforward to investigate the stability of the equilibrium and the conditions for cancer growth. In this case $F(y)$ is found to be

$$F(y) = y^6 + m_2 y^4 + m_1 y^2 + m_0,$$

where

$$\begin{aligned} m_2 &= a_2 - 2a_1, \\ m_1 &= {a_1}^2 - 2a_2(a_0 + 2\alpha_1\alpha_2\alpha_3), \\ m_0 &= {a_0}^2 + 4a_0\alpha_1\alpha_2\alpha_3. \end{aligned}$$

Let $y^2 = x$. Then $F(y)$ becomes

$$F_1(x) = x^3 + m_2 x^2 + m_1 x + m_0. \quad (10)$$

In order to examine the stability of the steady states, we employ a Lemma in [11] quoted here.

LEMMA 3.1. *Define*

$$\Delta = \frac{4}{27}{m_1}^3 - \frac{1}{27}{m_2}^2{m_1}^2 + \frac{4}{27}{m_2}^3 m_0 - \frac{2}{3}m_2 m_1 m_0 + {m_0}^2.$$

Suppose that $m_0 > 0$. Then:
(I) Necessary and sufficient conditions for the cubic equation (10) to have at least one simple positive root for x are either of the following:
(S1) $m_2 < 0$, $m_1 \geq 0$, ${m_2}^2 > 3m_1$, $\Delta < 0$ or

(S2) $m_1 < 0, \Delta < 0$.

(II) Necessary and sufficient conditions for the cubic equations (10) to have no positive real roots for x are one of the following:
(N1) $3m_1 \geq {m_2}^2$,
(N2) ${m_2}^2 > 3m_1$, $\Delta > 0$ *or*
(N3) ${m_2}^2 > 3m_1$, $\Delta \leq 0$, $m_2 > 0$, $m_1 > 0$.

Based on Lemma 1 and 4 and methods found in [5], we obtain the following stability theorems.

THEOREM 3.1. *For system (8), suppose that one of (N1), (N2), (N3) holds. Then 1) if $a_0 > 0$, the stability of equilibrium E_0 is independent of delay τ and it remains stable for all $\tau \geq 0$; and*
2) if $a_0 < 0$ and $m_0 > 0$, the stability of equilibrium E_0 does not depend on τ and it remains unstable for all $\tau \geq 0$.

It follows from Lemma 4 and Theorem 1 that there is a certain case in which the condition for cancer growth or extinction will remain unchanged even if we add the delay in the corresponding model. In such a case, we say the delay is harmless for the stability of the system.

THEOREM 3.2. *For system (8), assume either (S1) or (S2) holds. Then there exists a positive $\bar{\tau}$ such that*
(i) if $a_0 > 0$, the cancer-free equilibrium E_0 remains stable for $0 \leq \tau < \bar{\tau}$ and becomes unstable for all $\tau > \bar{\tau}$; and
(ii) if $a_0 < 0$ and $m_0 > 0$, the cancer-free equilibrium E_0 remains unstable for $0 \leq \tau < \bar{\tau}$ and becomes stable when $\tau > \bar{\tau}$.

Biomedical interpretation

Compared to the nondelay models discussed in section 2, the delay $\tau > 0$ can have two effects as expressed in Theorem 2.

In the nondelay case, Lemma 1, we found that cancer growth corresponds to $a_0 < 0$. Now, if in addition $m_0 > 0$ then there is a delay threshold $\bar{\tau}$ such that cancer would go extinct if the delay $\tau > \bar{\tau}$. Hence cell arrest in the interphase by another chemotherapeutic agent would be beneficial for treatment.

In another situation, we might find that $a_0 > 0$ but still cancer grows. This would corresponds to case (i) of Theorem 2 but now cancerous cells spend too much time in the interphase. In this case, as we see later, immune response and chemotherapy need to be considered.

3.2. **Drug-free model with $\tau > 0$ and immune suppression.** When we add the effect of the delay in the drug-free model with immune suppression, we obtain

$$\begin{aligned}
\dot{x}(t) &= -\alpha_1 x(t) - \delta_1 x(t) + s\alpha_3 z(t) - k_1 x(t) I(t) \\
\dot{y}(t) &= \alpha_1 x(t-\tau) - (\alpha_2 + \delta_2) y(t) - k_2 y(t) I(t) \\
\dot{z}(t) &= 2s^{-1}\alpha_2 y(t) - (\alpha_3 + \delta_3) z(t) - k_3 z(t) I(t) \\
\dot{I}(t) &= k + \tfrac{\rho I(t)(x+y+sz)^n}{a+(x+y+sz)^n} - (c_1 x(t) + c_2 y(t) + c_3 z(t) + \delta_4) I(t).
\end{aligned} \tag{11}$$

Again, $E_1(0, 0, 0, k/\delta_4)$ is an equilibrium and its analysis is similar to the case of section 3.1 though computations are complicated by more terms. This system has the same equilibria as the system described in section 2.2, but again we focus on the cancer free equilibrium E_1.

In the case of a positive delay, the characteristic equation for the linearized equation about the fixed point E_1 is given by:

$$\begin{vmatrix} \lambda+\alpha_1+\delta_1+\frac{k_1 k}{\delta_4} & 0 & -s\alpha_3 & 0 \\ -\alpha_1 e^{-\lambda\tau} & \lambda+\alpha_2+\delta_2+\frac{k_2 k}{\delta_4} & 0 & 0 \\ 0 & -2s^{-1}\alpha_2 & \lambda+\alpha_3+\delta_3+\frac{k_3 k}{\delta_4} & 0 \\ \frac{c_1 k}{\delta_4} & \frac{c_2 k}{\delta_4} & \frac{c_3 k}{\delta_4} & \lambda+\delta_4 \end{vmatrix} = 0\,.$$

Clearly, $\lambda = -\delta_4$ is an eigenvalue and the remaining eigenvalues are given by the solutions to the characteristic equation

$$\begin{aligned} H(\lambda) &= P_2(\lambda) + Q_2(\lambda)e^{-\lambda\tau} \\ &= \lambda^3 + b_2\lambda^2 + b_1\lambda + b_0 + 2\alpha_1\alpha_2\alpha_3 - 2\alpha_1\alpha_2\alpha_3 e^{-\lambda\tau}, \end{aligned}$$

where b_0, b_1, b_2 are given by (6) in section 2.2.

Geometric arguments using the argument principle can be used to establish the stability of a given fixed point by counting the number of zeros of $H(\lambda)$ on the right-hand side of the complex plane [19]. The argument is based on the relative orientation of $H(\lambda)$ compared to $P_2(\lambda)$ as we traverse a given contour. Unfortunately, the theorem developed in [19] cannot be used directly in our case because the hypotheses are not satisfied, but the argument can be modified, and we can thereby deduce conditions on the parameter space which ensure stability. However, these conditions are not easy to satisfy in reality. Therefore, we will apply the same methods as in section 3.1 to study the stability.

Define

$$G(y) = |P_2(iy)|^2 - |Q_2(iy)|^2.$$

Then

$$G(y) = y^6 + n_2 y^4 + n_1 y^2 + n_0, \tag{12}$$

where

$$\begin{aligned} n_2 &= b_2 - 2b_1 \\ n_1 &= {b_1}^2 - 2b_2(b_0 + 2\alpha_1\alpha_2\alpha_3) \\ n_0 &= {b_0}^2 + 4b_0\alpha_1\alpha_2\alpha_3. \end{aligned}$$

Let $y^2 = x$. Then $G(y)$ becomes

$$G_1(y) = x^3 + n_2 x^2 + n_1 x + n_0 \tag{13}$$

To analyze the stability we use again *Lemmas* 4 with m_i replaced by n_i, $i = 1, 2, 3$.

Based on *Lemmas* 2 and 4, we obtain the following theorems.

THEOREM 3.3. *For system (11), suppose one of (N1), (N2), or (N3) is satisfied for $m_i = n_i$, $i = 1, 2, 3$. Then*
1) if $H(\lambda)$ is stable with $\tau = 0$ (i.e. $b_0 > 0$), it remains stable for all $\tau \geq 0$,
2) if $H(\lambda)$ is unstable with $\tau = 0$ (i.e. $b_0 < 0$) and $n_0 > 0$, it remains unstable for all $\tau \geq 0$.

Theorem 3 implies that there exists a certain case in which the condition for cancer growth or extinction of all cancer cells will remain unchanged even if we add the delay in the corresponding model with immune suppression. In such a case, we say the delay is harmless for the stability of the system.

THEOREM 3.4. *For system (11), assume either (S1) or (S2) for $m_i = n_i$, $i = 1, 2, 3$ holds. Then there exists a positive $\hat{\tau}$ such that*
(i) if $b_0 > 0$, the cancer-free equilibrium E_1 remains stable for $0 \leq \tau < \hat{\tau}$, and becomes unstable for all $\tau > \hat{\tau}$.
(ii) if $b_0 < 0$ and $n_0 > 0$, the cancer-free equilibrium E_1 remains unstable for $0 \leq \tau < \hat{\tau}$, and becomes stable for $\tau > \hat{\tau}$.

Biomedical interpretation

As mentioned earlier, in the context of cancer models, stability switching as the delay is varied is very important, because many cycle-specific drugs retain the cells or trap them in a given phase, thus increasing the time a cell spends in a particular compartment. For an example, if spindle assembly is blocked, then the point where the M state becomes unstable moves to a much higher mass/DNA value. As a consequence, mitosis becomes a stable state, and cells entering into the M phase will be stuck there [21].

This analysis shows that care must be taken when trapping the cells in a compartment since the ultimate effect may be adverse: the cancer-free fixed point may switch from a stable equilibrium to an unstable one (see (i) of Theorem 4). This would mean that when the immune response is blocked, the system would not move toward the disease-free state. On the other hand, it is possible to increase or decrease the resident time during the interphase to "unlock" a fixed point from its instability and to push it toward the stable range (see (ii) of Theorem 4).

3.3. **Drug model when $\tau > 0$ with immune suppression.** When we add the administration of a delay, we obtain our full model (1). Again $E_2(0, 0, 0, k/\delta_4, 0)$ is an equilibrium and its analysis is similar to that shown in section 3.2. The system has the same equilibria as the system described in section 2.3, but again we focus on the cancer-free equilibrium E_2.

In this case, the characteristic equation for the linearized equation about a fixed point E_2 is given by

$$\begin{vmatrix} \lambda + \alpha_1 + \delta_1 + \frac{k_1 k}{\delta_4} & 0 & -s\alpha_3 & 0 & 0 \\ -\alpha_1 e^{-\lambda\tau} & \lambda + \alpha_2 + \delta_2 + \frac{k_2 k}{\delta_4} & 0 & 0 & 0 \\ 0 & -2s^{-1}\alpha_2 & \lambda + \alpha_3 + \delta_3 + \frac{k_3 k}{\delta_4} & 0 & 0 \\ \frac{c_1 k}{\delta_4} & \frac{c_2 k}{\delta_4} & \frac{c_3 k}{\delta_4} & \lambda + \delta_4 & \frac{-k_6 k_7 k}{\delta_4} \\ 0 & 0 & 0 & 0 & \lambda + \gamma \end{vmatrix} = 0$$

Obviously, $\lambda = -\delta_4$, $\lambda = -\gamma$ are two eigenvalues. The remaining eigenvalues are given as the solutions to the characteristic equation

$$F(\lambda) = \lambda^3 + b_2\lambda^2 + b_1\lambda + b_0 + 2\alpha_1\alpha_2\alpha_3 - 2\alpha_1\alpha_2\alpha_3 e^{-\lambda\tau} = 0,$$

where b_2, b_1, b_0 are given by (6) in section 2.2 and the stability analysis of the equilibria is the same as for $H(\lambda)$ in section 3.2. Thus we state the appropriate theorems here.

THEOREM 3.5. *For system (14), suppose one of (N1), (N2), (N3) with $m_i = n_i$, $i = 1, 2, 3$ holds. Then*
1) if $b_0 > 0$, then E_2 remains stable for all $\tau \geq 0$; and
2) if $b_0 < 0$ and $n_0 > 0$, then E_2 remains unstable for all $\tau \geq 0$.

THEOREM 3.6. *For system (14), assume either (S1) or (S2) with $m_i = n_i$, $i = 1, 2, 3$ holds. Then there exists a positive $\hat{\tau}$ such that*
(i) if $b_0 > 0$, the cancer-free equilibrium E_2 remains stable for $0 \leq \tau < \hat{\tau}$, and becomes unstable for all $\tau > \hat{\tau}$; and
(ii) if $b_0 < 0$ and $n_0 > 0$, the cancer-free equilibrium E_2 remains unstable for $0 \leq \tau < \hat{\tau}$, and becomes stable for $\tau > \hat{\tau}$.

Biomedical interpretation

The interpretation of this result is the same as above. Under conditions (ii) a cell arrest in the interphase is beneficial for treatment. For conditions (i) a long delay in the interphase enables cells to avoid treatment and re-enter the M-phase. The next question, again, is the question of multiple treatments, which we will not study here; however, a numerical solution for multiple dosages is given in Figure 4.

3.4. **Hopf bifurcation.** With the aid of Theorem 1 in [5], it is also straightforward to check for possible Hopf bifurcations for the full model (1), when we increase the delay τ. The importance of Hopf bifurcations in this context is that at the bifurcation point a limit cycle is formed around the fixed point, resulting in stable periodic solutions. The existence of periodic solutions is relevant in cancer models because it implies that the cancer levels may oscillate around a fixed point even in the absence of any treatment. Such a phenomenon has been observed clinically and is known as "Jeff's Phenomenon" [11]. Periodic oscillations also indicate cell synchronization within the cell cycle. In this section, we will prove that such Hopf bifurcations can occur. Now consider a general characteristic equation for system (1):

$$\lambda^3 + r_2\lambda^2 + r_1\lambda + r_0 - s_0 e^{-\lambda\tau} = 0. \tag{14}$$

Let $\lambda = u + iv$,$(u, v \in R)$, and rewrite (14) in terms of its real and imaginary parts as

$$\begin{aligned} u^3 - 3uv^2 + r_2(u^2 - v^2) + r_1 u + r_0 &= s_0 e^{-u\tau}\cos(v\tau) \\ 3u^2 v - v^3 + 2r_2 uv + r_1 v &= -s_0 e^{-u\tau}\sin(v\tau). \end{aligned} \tag{15}$$

Let $\bar{\tau}$ be such that $u(\bar{\tau}) = 0$. Then the above equations reduce to

$$\begin{aligned} -r_2\bar{v}^2 + r_0 &= s_0\cos(\bar{v}\bar{\tau}) \\ -\bar{v}^3 + r_1\bar{v} &= -s_0\sin(\bar{v}\bar{\tau}). \end{aligned} \tag{16}$$

It follows by taking the sum of squares that

$$\bar{v}^6 + ({r_2}^2 - 2r_1)\bar{v}^4 + ({r_1}^2 - 2r_2 r_0)\bar{v}^2 + {r_0}^2 - {s_0}^2 = 0. \tag{17}$$

Suppose that $\bar{v}_1$ is the largest positive simple root of equation (17). Then with this value of $\bar{v}_1$, (16) determines a $\bar{\tau}_1$ uniquely such that $u(\bar{\tau}_1) = 0$ and $v(\bar{\tau}_1) = \bar{v}_1$. To apply the Hopf bifurcation theorem as stated in Marsden & McCracken [20], we state and prove the following theorem.

THEOREM 3.7. *Suppose that equation (17) has at least one simple positive root and $\bar{v}_1$ is the largest such root. Then $iv(\bar{\tau}_1) = i\bar{v}_1$ is a simple root of equation (14) and $u(\tau) + iv(\tau)$ is differentiable with respect to τ in a neighborhood of $\tau = \bar{\tau}_1$.*

Proof. To show that $iv(\bar{\tau}_1) = i\bar{v}_1$ is a simple root, equation (14) can be written as $f(\lambda) = 0$ where

$$f(\lambda) = \lambda^3 + r_2\lambda^2 + r_1\lambda + r_0 - s_0 e^{-\lambda\tau}. \tag{18}$$

Any double root λ satisfies

$$f(\lambda) = 0,\ f'(\lambda) = 0,$$

where

$$f'(\lambda) = 3\lambda^2 + 2r_2\lambda + r_1 + \tau s_0 e^{-\lambda\tau}. \tag{19}$$

Substituting $\lambda = i\bar{v}_1$ and $\tau = \bar{\tau}_1$ into (19) and equating real and imaginary parts if $i\bar{v}_1$ is a double root, we obtain

$$\begin{aligned} -r_2\bar{v}_1^2 + r_0 &= s_0\cos(\bar{v}_1\bar{\tau}_1) \\ -\bar{v}_1^3 + r_1\bar{v}_1 &= -s_0\sin(\bar{v}_1\bar{\tau}_1), \end{aligned} \tag{20}$$

and

$$\begin{aligned} r_1 - 3\bar{v}_1^2 &= -\bar{\tau}_1 s_0\cos(\bar{v}_1\bar{\tau}) \\ 2r_2\bar{v}_1 &= \bar{\tau}_1 s_0\sin(\bar{v}_1\bar{\tau}). \end{aligned} \tag{21}$$

Now, equation (16) can be written as $F(\bar{v}_1) = 0$, where

$$F(v) = (-r_2v^2 + r_0)^2 + (-v^3 + r_1v)^2 - (s_0)^2 \tag{22}$$

$$F'(v) = 2(-r_2v^2 + r_0)(-2r_2v) + 2(-v^3 + r_1v)(-3v^2 + r_1). \tag{23}$$

By substituting (20) and (21) into (22), (23), we obtain

$$F(\bar{v}_1) = F'(\bar{v}_1) = 0.$$

Note that $\bar{v}_1$ is a double root of $F(\bar{v}_1) = 0$ and that $F(\bar{v}_1) = F'(\bar{v}_1) = 0$, which is a contradiction as we have assumed that $\bar{v}_1$ is a simple root of (17). Hence $i\bar{v}_1$ is a simple root of equation (14), an analytic equation. By using the analytic version of the implicit function theorem (Chow & Hale [3]), we can see $u(\tau) + iv(\tau)$ is defined and analytic in a neighborhood of $\tau = \bar{\tau}_1$. □

Next, to establish Hopf bifurcation at $\tau = \bar{\tau}_1$, we need to verify the transversality condition

$$\frac{du}{d\tau}|_{\tau=\bar{\tau}_1} \neq 0.$$

By differentiating equations (16) with respect to τ and setting $u = 0$ and $v = \bar{v}_1$, we obtain

$$\begin{aligned} A\tfrac{du}{d\tau}|_{\tau=\bar{\tau}_1} + B\tfrac{dv}{d\tau}|_{\tau=\bar{\tau}_1} &= -s_0\bar{v}_1\sin(\bar{v}_1\bar{\tau}_1) \\ -B\tfrac{du}{d\tau}|_{\tau=\bar{\tau}_1} + A\tfrac{dv}{d\tau}|_{\tau=\bar{\tau}_1} &= s_0\bar{v}_1\cos(\bar{v}_1\bar{\tau}_1), \end{aligned} \tag{24}$$

where

$$\begin{aligned} A &= r_1 - 3\bar{v}_1^2 + s_0\bar{\tau}_1\cos(\bar{v}_1\bar{\tau}_1) \\ B &= -2r_2\bar{v}_1 + s_0\bar{\tau}_1\sin(\bar{v}_1\bar{\tau}_1). \end{aligned}$$

Solving for $\frac{du}{d\tau}$, $\frac{dv}{d\tau}$ form (23) with the help of (16), we have

$$\frac{du}{d\tau}|_{\tau=\bar{\tau}_1} = \frac{\bar{v}_1^2[3\bar{v}_1^4 + 2({r_2}^2 - 2r_1)\bar{v}_1^2 + {r_1}^2 - 2r_2r_0]}{A^2 + B^2}. \tag{25}$$

Let $z = \bar{v}_1^2$. Then equation (17) reduces to

$$\Phi(z) = z^3 + ({r_2}^2 - 2r_1)z^2 + ({r_1}^2 - 2r_2r_0)z + {r_0}^2 - {s_0}^2.$$

Hence

$$\frac{d\Phi}{dz} = 3z^2 + 2({r_2}^2 - 2r_1)z + {r_1}^2 - 2r_2r_0.$$

Since $\bar{v}_1^2$ is the largest positive single root of equation (17), then

$$\frac{d\Phi}{dz}|_{z=\bar{v}_1^2} > 0.$$

Therefore,

$$\frac{du}{d\tau}|_{\tau=\bar{\tau}_1} = \frac{\bar{v}_1^2}{A^2 + B^2}\frac{d\Phi}{dz}|_{z=\bar{v}_1^2} > 0.$$

TABLE 3. Parameter values in Figure 2

Parameter	value	parameter	value
α_1	$1\,\text{day}^{-1}$	k_1	$0.1\text{x}\,10^{-7}\,\text{cell}^{-1}\text{day}^{-1}$
α_2	$0.6\,\text{day}^{-1}$	k_2	$0.4\,\text{x}\,10^{-8}\,\text{cell}^{-1}\text{day}^{-1}$
α_3	$0.9\,\text{day}^{-1}$	k_3	$0.1\,\text{x}\,10^{-8}\,\text{cell}^{-1}\text{day}^{-1}$
δ_1	$0.11\,\text{day}^{-1}$	k_4	$0.25\,\text{day}^{-1}$
δ_2	$0.28\,\text{day}^{-1}$	k_5	$0.25\,\text{x}\,10^{-3}\,\text{mg}^{-1}$
δ_3	$0.1\,\text{x}\,10^{-4}\,\text{day}^{-1}$	k_6	$0.3\,\text{x}\,10^{-1}\,\text{day}^{-1}$
δ_4	$0.3\,\text{day}^{-1}$	k_7	$0.5\,\text{x}\,10^{-2}\,\text{mg}^{-1}$
c_1	$0.2\,\text{x}\,10^{-6}\,\text{cell}^{-1}\text{day}^{-1}$	ρ	$0.2\,\text{day}^{-1}$
c_2	$0.8\,\text{x}\,10^{-7}\,\text{cell}^{-1}\text{day}^{-1}$	γ	$0.3\,\text{x}\,10^{-2}\,\text{day}^{-1}$
c_3	$0.108\,\text{x}\,10^{-6}\,\text{cell}^{-1}\text{day}^{-1}$	k	$0.15\,\text{x}\,10^{6}\,\text{cellday}^{-1}$
a	$0.5\,\text{x}\,(0.1\,\text{x}\,10^{6}\,\text{cell})^3$		

We summarize the preceding details in the following theorem.

THEOREM 3.8. *Suppose that (17) has at least one simple positive root and $\bar{v}_1$ is the largest such root. Then a Hopf bifurcation occurs as τ passes through $\bar{\tau}_1$ for system (1). On the other hand, if (17) has no positive real roots, then the disease-free fixed point is locally asymptotically stable for all values of τ for system (1).*

4. **Numerical results.** In this section, we will use the original mathematical model (1) to determine numerical solutions for the cancer population. To do this, we first find reasonable estimates for the values of the parameters from [27]. For the cancer cells, we will use the estimates for the parameters of $\tau = 14\,hr(0.6\,\text{days})$, $x(0) = y(0) = 0.1$ x 10^6 cells, $z(0) = 0.2$ x 10^6, $\alpha_1 = 0.84\,\text{day}^{-1}$, $\alpha_2 = 0.9\,\text{day}^{-1}$, $\alpha_3 = 0.024\,\text{day}^{-1}$, $\delta_1 = 0.11\,\text{day}^{-1}$, $\delta_2 = 0.67\,\text{day}^{-1}$, $\delta_3 = 0.056\,\text{day}^{-1}$.

First, we consider the nondelay case. The MATLAB simulations in Figure 2 (a) show that cancer without delay, with immune suppression, grows exponentially in the absence of drugs, in which the total cancer biomass $N(t) = x(t) + y(t) + z(t)$ reaches $25\,\text{x}\,10^6$ cells after 60 days. When we apply the drug to the model, the cancer-free equilibrium is still unstable (see Lemma 3), however; cancer grows much slower and only reaches $15\,\text{x}\,10^6$ cells in 60 days. Hence, although from the linear analysis the stability of the cancer-free equilibrium does not change, the drug reduces cancer growth by about 40%.

In the introduction we mentioned the cell synchronization method, where cancer cells are synchronized within the cell cycle prior to the application of the M-phase specific drug. We test this treatment strategy with our model. In Figure 3 we investigate two different delays ($\tau = 0$ in Figure 3 (A) and $\tau = 2$ in Figure 3 (B)), where we assume that all cancer cells are in the same stage of the cell cycle. We observe the strongest treatment effect if the drug is given at the time all cells are in the M-phase (dotted line in Figure 3 (B)).

Finally, as an example, we consider a typical dose protocol in which the drugs are delivered periodically. To reduced the toxic effect of the chemotherapeutic agent on healthy tissue, the administration of the drug is split between treatment days and treatment holidays. For example [2], patients receiving the drug Vincristine on days ,1,2,3,4, have a rest on days 5 to 27 and have another four days of treatment and so on. In Figure 4 (b) we show show a simulation of the first 50 days, where all

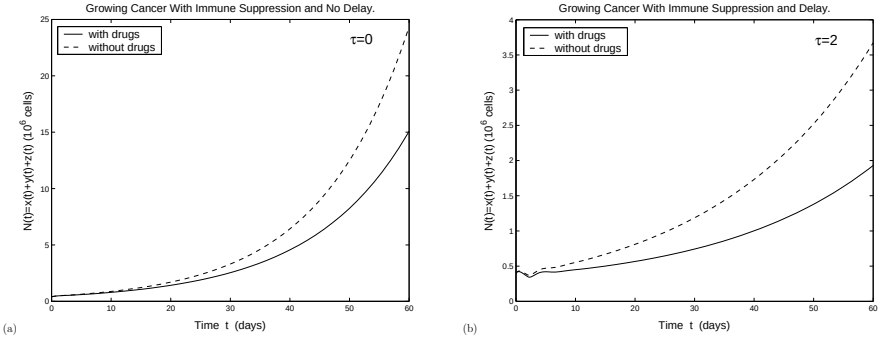

FIGURE 2. (a) In the absence of delay, solutions for models (4) and (7) in the case of no drugs and drugs applied respectively. (b) In the case of delay, solutions for models (11) and (14) in the case of no drugs and drugs applied respectively. When drug is applied, cancer grows much slower than in the untreated case. Here $x(0) = y(0) = 0.1\,\mathrm{x}\,10^6$ cells, $z(0) = 0.2\,\mathrm{x}\,10^6$ cells, $I(0) = 2\,\mathrm{x}\,10^6$ cells, $u(0) = 8$.

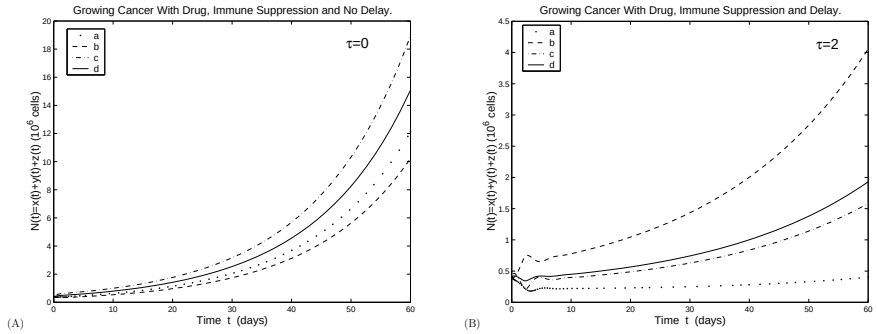

FIGURE 3. Solutions for models (11) and (14) with the same parameter values as in *Figure* 2 but with different initial values. In the case of (A), (a) with $x(0) = 0$, $y(0) = 0.4\,\mathrm{x}\,10^6$ $z(0) = 0$; (b) $x(0) = 0.4\,\mathrm{x}\,10^6$ $y(0) = 0$, $z(0) = 0$; (c) $x(0) = 0$, $y(0) = 0$, $z(0) = 0.4\,\mathrm{x}\,10^6$; (d) $x(0) = 0.1$, $y(0) = 0.1$, $z(0) = 0.2\,\mathrm{x}\,10^6$. In the case of (B), the initial values are the same as for case (A) but with a delay of 2 days.

parameter values are the same as in (a) of Figure 3 (B). Figure 4 (b) shows that the overall effect of multiple dosage protocols is slightly reduced compared to a full dose delivery at day 1 (Figure 4 (a)). However, side effect are reduced as well. More detailed and more specific treatment protocols could be tested numerically. The qualitative results about the stability of the cancer-free equilibrium are unchanged.

5. **Discussion.** In this paper we study the effect of a cell-cycle -specific drug on the growth of cancer as well as interactions with the immune response. The cancer cells are split into three compartments related to the cell cycle, the M-phase, the G_0-phase and the interphase (G_1, S, G_2). We also study the effect of a time delay during the passage of cells through the interphase. Immune cells are assumed to

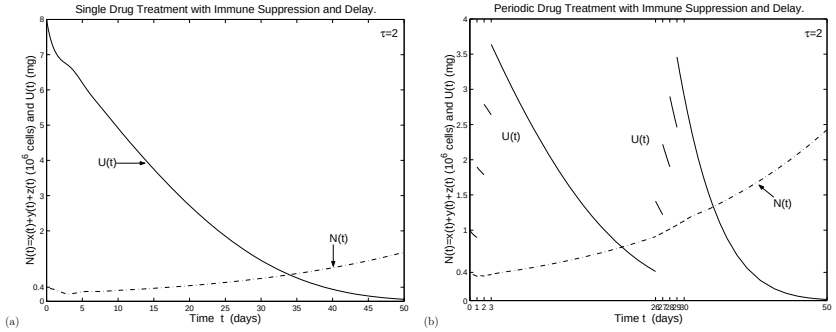

FIGURE 4. Solutions for models (14) with the same parameter values and initial values as in (a) of $Figure\ 3\ B$ but with a different drug delivery term where $\dot{u}(t) = f\delta_0(t) + f\delta_1(t) + f\delta_2(t) + f\delta_3(t) + f\delta_{27}(t) + f\delta_{28}(t) + f\delta_{29}(t) + f\delta_{30}(t) - \gamma u(t) - \xi y(t)u(t)$. In the presence of periodic treatment, the drug is delivered in the first and last four days of a month with one dose each day, a total dose of 8 doses for a month, the same amount as in the single treatment where the drug is initially delivered in 8 doses at the beginning. That is, $f = 1\text{mg}\,\text{day}^{-1}$ for the periodic drug delivery and $f = 0$ for single drug delivery. In both cases $\xi = 0.4\text{cell}^{-1}\,\text{day}^{-1}$.

TABLE 4. A summary of the main theoretical stability results for system (1)

Section	Delay	Immune Sup.	Drug	Result	Cancer
Section 2.1	No	No	No	$a_0 < 0$	grows
Section 2.2	No	Yes	No	$b_0 < 0$	grows
Section 2.3	No	Yes	Yes	$b_0 < 0$	grows
Section 3.1	Yes	No	No	1) $a_0 < 0$ and $\tau < \bar{\tau}$	grow
				2) $a_0 > 0$ and $\tau > \bar{\tau}$	grows
Section 3.2	Yes	Yes	No	1) $b_0 < 0$ and $\tau < \hat{\tau}$	
				2) $b_0 > 0$ and $\tau > \hat{\tau}$	grows
Section 3.3	Yes	Yes	Yes	1) $b_0 < 0$ and $\tau < \hat{\tau}$	
				2) $b_0 > 0$ and $\tau > \hat{\tau}$	grows

interact with cells from all phases and the chemotherapy drug only affects M-phase cells and immune cells.

We break down the stability analysis of the cancer-free equilibrium into the special cases as illustrated in Table 4.

It is easy to see from Table 4 that the drug does not change the stability of the systems, since cancer cells in the resting stage escape the action of drugs. However, as we see in the numerical simulation, cancer growth is significantly reduced through the M-phase specific drug. Now we can answer the questions we listed at the beginning. In the absence of any treatments, we see that cancer growth mainly depends on the death rate of cells in the resting phase and the reproduction rate at which cells in the resting phase go into the cell cycle (see Lemma 1). Cancer will

begin to grow if the reproduction rate is greater than the death rate of cancer cells in the resting phase. In that case, without treatment cancer will grow, accumulate and eventually become fatal to the body. Cell cycle duration is an important factor which can give rise to oscillation of solutions. When including the cell cycle time into consideration, we determined those situations where the cell cycle time delay is harmless and in which cases, stability switches occur and thus periodic solutions exist.

When disease develops, the immune system is a natural force to fight the disease. Taking the immune suppression into account in the model, we show that it will greatly help to inhibit the growth of cancer cells (see Lemma 2), especially, if the lymphocytes are rapidly producing and are very effective in combining with cancer cells in order to destroy them. There is little chance for the cancer cells to reach maturity. Unfortunately, this may happen only at the beginning of the disease, and we may depend on drugs to inhibit the growth of cancer cells if the disease gets worse and the lymphocytes lose their ability to fight these abnormal cells.

Our model presented here is relatively simple, but it gives interesting information about the dynamics of the system. The inclusion of a quiescent phase into consideration does give us a deep insight into the mechanism of disease development and helps us to understand how the resistant population contributes to the eradication of the disease. Our simulations are consistent with our analysis.

Here we discuss a few features we consider significant that have not been included in our model. For example the inclusion of another delay in the cell cycle might be pertinent as we separate the phases of the cycle to be more precise about the model action of the drugs on the different phases. It is possible that these delays may be a function of the drug. The immune system is a very complicated entity, and in this paper we have merely touched the surface of the interactions and processes involved in the immune system response. A more careful study and detailed modelling of the interaction is another avenue of possible future research. In our analysis, we study the dynamics of the model for a single administration of the chemotherapeutic agent. From this analysis, we see that the quiescent compartment plays an essential role. We speculate that a similar analysis for an G_1, S-phase specific drug, such as considered in Kimmel and Swierniak [13] would also emphasize the G_0-phase for cancer control. For more quantitative predictions, a realistic drug-treatment protocol needs to be included, and numerical simulations need to be done. We give one those example in Figure 4.

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