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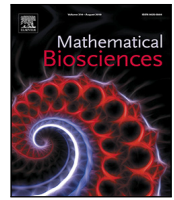Original Research Article

Optimization of additive chemotherapy combinations for an in vitro cell cycle model with constant drug exposures

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ABSTRACT

Proliferation of an *in vitro* population of cancer cells is described by a linear cell cycle model with n states, subject to provocation with m chemotherapeutic compounds. Minimization of a linear combination of constant drug exposures is considered, with stability of the system used as a constraint to ensure a stable or shrinking cell population. The main result concerns the identification of redundant compounds, and an explicit solution formula for the case where all exposures are nonzero. The orthogonal case, where each drug acts on a single and different stage of the cell cycle, leads to a version of the classic inequality between the arithmetic and geometric means. Moreover, it is shown how the general case can be solved by converting it to the orthogonal case using a linear invertible transformation. The results are illustrated with two examples corresponding to combination treatment with two and three compounds, respectively.

1. Introduction

Recent decades have seen an increased interest in combination therapies to treat cancer [1]. Mathematical modeling of cancer growth and treatment plays an important role in the discovery and development of pharmaceutical compounds, as well as in increasing our understanding of biological processes [2]. Mathematical tools and prediction techniques are especially suitable to support the combinatorial explosion associated with testing different pairs, or even triplets, of anticancer drugs simultaneously and at different dose levels [3].

The mammalian cell cycle is central to cancer growth and therefore also to our understanding of how to treat cancer. The cell cycle is typically divided into four or five stages: G_1, S, G_2, M, and also G_0. Here, G_1 and G_2 are gap, or growth, phases that separate the stages S, during which DNA synthesis takes places, and M, which is when mitosis occurs. The stage G_0 is a quiescent stage where a cell lays dormant in between cell cycles.

Chemotherapeutic compounds are often classified as cell cycle nonspecific, meaning that they target cells in all stages of the cell cycle, as well as quiescent cells, or cell cycle specific, meaning that they target cells in only one or a couple of the stages of the cell cycle [4]. Examples of cell cycle nonspecific drugs include platinum-based chemotherapies such as cisplatin and carboplatin. Examples of chemotherapeutic compounds that are cell cycle specific include enzymes such as asparaginase, which primarily target cells in the G_1 phase, antimetabolites such as 5-fluorouracil and gemcitabine, which target cells in the S phase,

topoisomerase including topotecan and irinotecan that target the G_2 phase, and taxanes such as paclitaxel as well as vinca alkaloids such as vinorelbine that primarily target cells in the M phase of the cell cycle [4].

Mathematical models that describe the growth of cancerous cells and tumors have been developed with varying complexity, taking into account many important biological features [5–7]. Perhaps the simplest model is described by a single difference or ordinary differential equation that captures growth that is either purely exponential, or which slows down or saturates as the tumor becomes large, *e.g.*, in the Gompertz model [8,9]. More advanced models use systems of differential equations, and partial differential equations can be used to describe spatial or age-related aspects of tumor growth [10–14].

In this paper, we consider a simple mathematical model of the cell cycle model with n stages. Early use of such a model to describe the growth of a population of cancer cells can be found in two papers by Takahashi [15,16]. Like many biological models based on ordinary differential equations, it can be recovered from a stochastic model where the time that a cell spends in each stage is assumed to be exponentially (or Erlang) distributed [17,18]. This assumption is consistent with some experimental results [19]. These models have been analyzed with respect to cell cycle kinetics without treatment [20–24], and have also been used to describe treatment with cell cycle specific drugs that target cells in certain stages of the cell cycle [25–29]. Similar models have also been used to describe combination treatments [30,31].
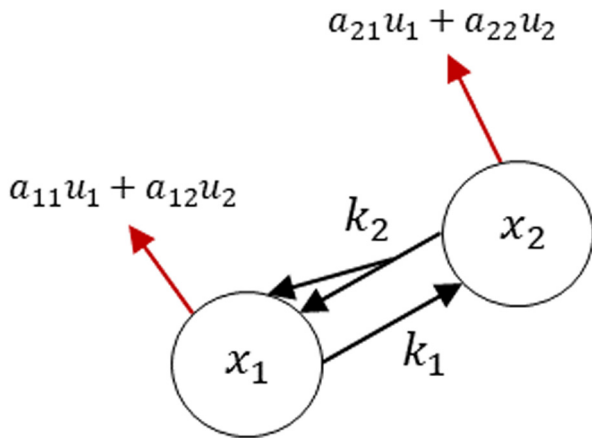
Fig. 1. Cell cycle model for a population of cancer cells with states x_1 and x_2, subject to combination therapy with two drugs, C_1 and C_2, with concentrations u_1 and u_2, respectively. Cells are transferred from state x_1 with rate k_1 and from x_2 back to x_1 with rate k_2 during which mitosis occurs. Drug C_1 acts cytotoxically on cells in state x_1 with rate a_{11} and on cells in state x_2 with rate a_{12}. Drug C_2 acts cytotoxically on cells in state x_1 with rate a_{12} and on cells in state x_2 with rate a_{22}.

Our analysis of the linear cell cycle model incorporates combination treatment with an arbitrary number of chemotherapeutic drugs, whose drug actions may be different for cells in different stages of the cell cycle. In comparison, earlier works using a similar model have only considered special cases with one or multiple specific drugs [7,15,30–32]. We first analyze the set exposure combinations that result in stability and refer to this as the shrinkage set. Then, we find an optimal treatment combination under the assumption of constant drug exposure by minimizing a weighted sum of the exposures subject to the constraint that the system is stable and therefore that the cancer cells will eventually be eradicated. The analysis rests on two main assumptions: (i) that cell killing is proportional to drug concentration, and (ii) that drug actions is additive, i.e., the model does not include general expressions for potential antagonistic or synergistic interactions.

Our results are illustrated using two examples. The first example combines the drugs 5-fluorouracil and vinorelbine, which act primarily on the S and M phases of the cell cycle, respectively. The second example incorporates a third compounds, irinotecan, which acts mainly on the G_2 phase of the cell cycle, and considers the problem of finding optimal triple combinations that result in a stable or regressing population of tumor cells.

We end the introduction with an analysis of the special case with a cell cycle model with two stages, subject to combination treatment with two chemotherapeutic compounds.

Consider a simple model of the cell cycle with two states x_1 and x_2. Cells in x_1 travel to x_2 with rate k_1. At x_2 mitosis occurs and the two daughter cells are transferred back to x_1 with rate k_2 where the cycle repeats. Two chemotherapeutic drugs, C_1 and C_2, induce cell death via apoptosis depending on the concentration of the drugs, denoted by u_1 and u_2, respectively. Assume that drug action is linear and that apoptosis is induced for cells in state x_j, due to drug C_i, with rate $a_{ij} \geq 0$. The model is illustrated in Fig. 1. Growth of the cell population subject to chemotherapeutic provocation with the two drugs is then described by the following system of differential equations

$$\dot{x}_1 = -(k_1 + a_{11}u_1 + a_{12}u_2)x_1 + 2k_2x_2, \; x_1(0) = x_{01},$$
$$\dot{x}_2 = k_1x_1 - (k_2 + a_{21}u_1 + a_{22}u_2)x_2, \quad x_2(0) = x_{02}, \tag{1}$$

where x_{01} and x_{02} are the number of cells in states x_1 and x_2 at time $t = 0$, respectively. Next, we investigate the stability of the system (1) to determine which combinations (u_1, u_2) lead to eradication of the cancer cell population. The system (1) has the system matrix

$$A := \begin{bmatrix} -(k_1 + a_{11}u_1 + a_{12}u_2) & 2k_2 \\ k_1 & -(k_2 + a_{21}u_1 + a_{22}u_2) \end{bmatrix}. \tag{2}$$
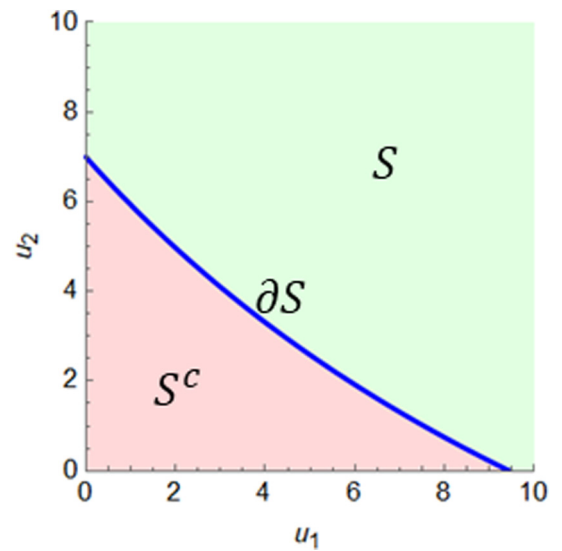

Fig. 2. Illustration of the shrinkage set S for the model (1) with parameter values $k_1 = 8, k_2 = 5, k_3 = 7, a_{11} = 0.1, a_{12} = 0.7, a_{21} = 0.6, a_{22} = 0.05$. The boundary set, ∂S, shown in blue, separates the concentration plane into a region of growth (red) and shrinkage (green). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

It is well-known that, for a 2×2-matrix, the system is stable if and only if tr $A < 0$ and det $A > 0$. We note that the condition on the trace always holds, and that the determinant is given by

$$\det A = a_{11}a_{21}u_1^2 + a_{12}a_{22}u_2^2 + (a_{12}a_{21} + a_{11}a_{22})u_1u_2$$
$$+ (a_{21}k_1 + a_{11}k_2)u_1 + (a_{12}k_2 + a_{22}k_2)u_2 - k_1k_2. \tag{3}$$

Eq. (3) describes a hyperbola in the u_1u_2-plane, since the discriminant, σ, is given by

$$\sigma = (a_{12}a_{21} + a_{11}a_{22})^2 - 4a_{11}a_{12}a_{21}a_{22}$$
$$= (a_{12}a_{21} - a_{11}a_{22})^2 > 0. \tag{4}$$

The hyperbola intersects the coordinate axes in two points, $(u_1^*, 0)$ and $(0, u_2^*)$, corresponding to the minimum concentration of either compound that, when given as monotherapy, results in stability. The determinant condition, det $A = 0$, for monotherapy becomes

$$\det A = a_{1j}a_{2j}u_j^2 + (a_{1j}k_2 + a_{2j}k_1)u_j - k_1k_2, \; j = 1, 2, \tag{5}$$

which is a second-order equation with exactly one positive root, given by

$$u_j^* = \frac{-(k_1a_{2j} + k_2a_{1j}) + \sqrt{(k_1a_{2j} + k_2a_{1j})^2 + 4a_{1j}a_{2j}k_1k_2}}{2a_{1j}a_{2j}}, \tag{6}$$

so that any $u_j > u_j^*$ leads to eradication. In general, we define the set

$$S := \{(u_1, u_2) \geq 0 : \det A \geq 0\}, \tag{7}$$

which we refer to as the shrinkage set. The boundary, ∂S, separates the first quadrant of the u_1u_2-plane into a region corresponding to stability and a shrinking cell population, and a region corresponding to instability and population growth. As noted above, ∂S is (part of) a hyperbola, and the set S is therefore convex. An illustration of this set is shown in Fig. 2.

We consider a linear objective function $F(u_1, u_2) := \alpha_1u_1 + \alpha_2u_2$, where the coefficients correspond to some cost, e.g., related to toxicity and side-effects, associated with the drugs, respectively. This leads to the convex optimization problem
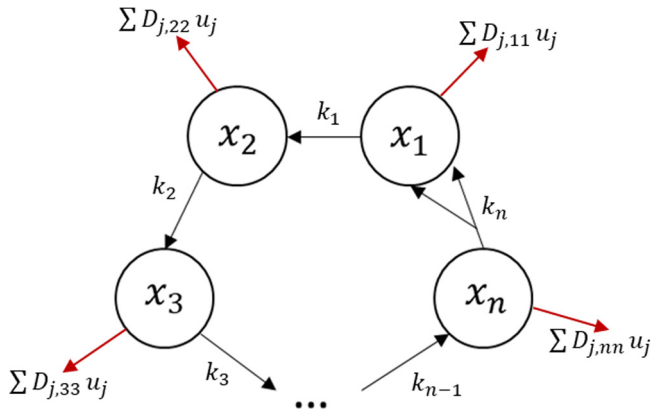
$$\min_{(u_1, u_2) \in S} F(u_1, u_2), \tag{8}$$

Fig. 3. Illustration of a linear cell cycle model with n states, $x_1, \ldots, x_n$, with transfer rates $k_1, \ldots, k_n$. During transfer between x_n and x_1 mitosis occurs and the cell splits into two daughter cells. Cell death due to the presence of cytotoxic compounds $C_1, \ldots, C_m$, with concentrations $u_1, \ldots, u_m$, is induced on cells in state x_i with total rate $\sum_j D_{j,ii} u_j$.

which can be solved using the standard technique with Lagrange multipliers.

2. Methods

Note: Throughout the rest of this paper, inequalities for vectors and matrices are to be interpreted element-wise, e.g., $A \geq B$ for matrices of equal dimensions, or $u \leq v$ for vectors of equal length.

We describe *in vitro* growth of a population of cancer cells with a general linear cyclic model with n states as a description of the cell cycle

$$\dot{x}_1 = 2k_n x_n - k_1 x_1, \quad x_1(0) = x_{01},$$
$$\dot{x}_i = k_{i-1} x_{i-1} - k_i x_i, \; x_i(0) = x_{0i}, \; i = 2, \ldots, n, \qquad (9)$$

where x_i represents the number of cells in the i:th stage, x_{0i} is the initial number of cells in stage i, and k_i are the transfer rates between stages. At the last stage, n, mitosis occurs and the two daughter cells are placed in state x_1 and re-enter the cell cycle. Unlike normal cells, we assume that the cancerous cells grow unimpeded, and never enter a quiescent stage G_0 to wait for external signals to commence proliferation.

The system (9) is linear and autonomous and can be written in matrix form

$$\dot{x} = Ax, \; x(0) = x_0, \qquad (10)$$

where $x = (x_1, \ldots, x_n)$ is the vector of states, $x_0 = (x_{01}, \ldots, x_{0n})$ is the initial vector, and the matrix A is given by

$$A = \begin{pmatrix} -k_1 & 0 & \ldots & 0 & 2k_n \\ k_1 & -k_2 & 0 & \ldots & 0 \\ 0 & k_2 & \ddots & & \vdots \\ \vdots & 0 & \ddots & \ddots & 0 \\ 0 & \ldots & 0 & k_{n-1} & -k_n \end{pmatrix}. \qquad (11)$$

It is easy to see that if $x_0 \geq 0$ the solution $x(t)$ remains in the non-negative orthant, O, for all $t \geq 0$. Take $x \in \partial O$. Then from (9) we have that $\dot{x}_i \geq 0$ for all i such that $x_i = 0$. Hence, the vector field Ax never points outside O and $x(t)$ is trapped inside.

We extend the cell cycle model (10) to account for multiple drug provocations, by introducing a control vector $u = (u_1, \ldots, u_m)$ where u_i represents the concentration of drug i, assumed to be non-negative and constant over time, which is plausible for an *in vitro* setting. We assume that drug action is linear and may be different for different stages in the cell cycle. Although drug action is in general sigmoidal, a linear approximation can often be justified for a range of exposure levels such that saturation effects are sufficiently small [33]. Moreover, we assume

additive effects for combinations of drugs that target the same stage of the cell cycle. This is a natural assumption given that we consider a general case and not specific compounds with known mechanisms. For each u_i, let D_i be a non-negative diagonal $n \times n$-matrix, whose entries $D_{i,jj}$ represent the efficiency of drug i at killing cells in state j. This model is illustrated in Fig. 3. The model is described by the following system of differential equations

$$\dot{x} = \left(A - \sum_{i=1}^{m} D_i u_i \right) x, \; x(0) = x_0. \qquad (12)$$

Eq. (12) is a linear system with respect to both states and drug concentrations. Define

$$A_u := A - \sum_{i=1}^{m} D_i u_i. \qquad (13)$$

Definition 1 (*Drug Action Matrix*)**.** We define the drug action matrix B to be the matrix whose entries are given by $b_{ij} = D_{j,ii}$.

The entries b_{ij} correspond to the drug action of drug j on state i. Using the drug action matrix, A_u can be written

$$A_u = A - \text{diag}(Bu). \qquad (14)$$

We investigate the asymptotic behavior of the solution $x(t)$ to (12) by considering the eigenvalues of A_u. In particular, we are interested in the case when all eigenvalues have negative real part, which implies that tumor will shrink over time and eventually be eradicated. First, we note that A_u can be written $A_u = N - \alpha_u I$, for some $\alpha_u \geq 0$, where N is a non-negative irreducible matrix. It follows from the classical Perron–Frobenius theorem (see, e.g., [34]) that A_u has a simple largest real eigenvalue λ_{max}, with a corresponding eigenvector v_{max} with positive components. Thus, the asymptotic growth rate will be determined by λ_{max} and the corresponding distribution of cells into the different states will be determined by v_{max}. Our objective is therefore to find those u such that λ_{max} becomes negative. We make the following definition

Definition 2 (*Shrinkage Set*)**.** For any square matrix A_u whose entries depend on a control vector u, define the *shrinkage set S* as the set of all non-negative u such that all eigenvalues of A_u have negative real part.

Before analyzing the shrinkage set for A_u we make the following observation.

Gerschgorin's circle theorem states that the eigenvalues of a matrix are contained inside the Gerschgorin discs in the complex plane [35]. Applying the theorem to the columns of A_u gives the discs $D(-k_i - \sum_j b_{ij} u_j, k_i)$, $i = 1, \ldots, n-1$ and $D(-k_n - \sum_j b_{nj}, 2k_n)$, where the first and second argument denotes the origin and radius of the disc, respectively. Only the last of these discs can intersect the positive half of the complex plane. Moreover, increasing the values of u does not change the radii of the Gerschgorin discs, but only pushes them further into the left half of the complex plane. If $\sum_j b_{ij} \geq k_n$ all discs are located in the left half of the complex plane, i.e., no eigenvalue of A_u has a positive real part. Applying the theorem to the rows of A_u gives the similar condition $\sum_j b_{nj} \geq 2k_n - k_1$. Note also that if there exists i such that $b_{ij} = 0$ for all j, i.e., there exists a state x_i that is not acted upon by any drug, then even if all other discs are pushed far into the left half of the complex plane, the corresponding Gerschgorin disc does not change and therefore provides a bound on how quickly the tumor can shrink.

The matrix A_u has non-negative off-diagonal entries and is thus a Metzler matrix (c.f. [36]). The additive inverse of such a matrix is known as a Z-matrix. Z-matrices have been studied extensively, as they can be written $\alpha I - N$, where α is a scalar, and N is a non-negative matrix. Metzler matrices and Z-matrices occur frequently in applications, e.g., in the study of linear compartment models [37]. What follows is a presentation of selected parts of the theory of Z-matrices, which are useful in establishing our main result.

Recall that a matrix is said to be positive stable if all of its eigenvalues have positive real part. A positive stable Z-matrix is known as an M-matrix. Hence, the question of stability for a Metzler matrix such as A_u in Eq. (13) can be recast as a question of whether its additive inverse is an M-matrix. In 1979, Berman and Plemmons published a collection of a large number of properties that are equivalent to a Z-matrix also being an M-matrix [38]. Some of these properties are useful to us and are summarized in the following theorem

Theorem 1 (*Berman and Plemmons, 1979*)**.** *Let $Z \in \mathbb{R}^{n \times n}$ be a Z-matrix. The following are equivalent to Z also being an M-matrix*

1. *Z is positive stable.*
2. *All eigenvalues of Z have positive real part.*
3. *$Zx \geq 0$ implies $x \geq 0$.*
4. *All principal minors of Z are positive.*
5. *The leading principal minors of Z are positive.*

Recall that a principal minor of a square matrix is the determinant of a submatrix obtained by removing a number of rows and the corresponding columns. The leading principal minors are the determinants of the submatrices obtained by successively removing rows and column, starting with the last row and column. Note that the determinant of a matrix counts as a principal minor to itself.

The sum of two Z-matrices is clearly a Z-matrix. However, it is well-known that the sum of two M-matrices need not be an M-matrix. Some results regarding convex combinations of M-matrices have been presented, c.f., Fan [39], Horn and Johnson [34], and Stipanović and Šiljak [40]. We shall use a result that follows immediately from a theorem originally proved by Cohen [41] although the version presented here is due to Friedland [42]

Theorem 2 (*Cohen, 1978*)**.** *Let M_1 and M_2 be two M-matrices such that $M_1 - M_2$ is a diagonal matrix. Then, for any $\alpha \in [0,1]$, the matrix $\alpha M_1 + (1 - \alpha) M_2$ is also an M-matrix.*

Note that this result follows from Theorem 1 when $M_1 - M_2 \geq 0$. An immediate consequence is the following proposition

Proposition 1. *Let M be a Metzler matrix, and D_i, $i = 1, \ldots, m$ be non-negative diagonal matrices. Then the shrinkage set S of $M_u := M - \sum_{i=1}^{m} D_i u_i$ is convex.*

Proof. Take $u, v \in S$ and $\alpha \in [0,1]$. Then $M_{\alpha u + (1-\alpha)v} = \alpha M_u + (1 - \alpha) M_v$. Since $-M_u$ and $-M_v$ and are M-matrices that differ only on the diagonal, It follows from Theorem 2 that $-\alpha M_u - (1 - \alpha) M_v$ is also an M-matrix, i.e., all of its eigenvalues have positive real part. Hence, all eigenvalues of $\alpha M_u + (1 - \alpha) M_v$ have negative real part and therefore $\alpha u + (1 - \alpha) v \in S$. □

Note that the proposition gives convexity for the shrinkage set of any compartment model where the control vector stimulates or induces output from the system. Note also that convexity is preserved if we replace the linear functions $(b_{ij} u_j)$ with any concave functions of u_j, e.g., saturable functions of Michaelis–Menten type [43].

Suppose now that we want the tumor described by (12) to be eradicated, i.e., the matrix A_u should be stable, while at the same time minimizing the metabolic strain induced by the drugs. A simple and common way to express this is based on the (weighted) total drug exposure $F(u)$ given by

$$F(u) := \sum_{i=1}^{m} \alpha_i u_i, = \alpha^T u, \tag{15}$$

where $\alpha \geq 0$ is a vector of weights that reflect the relative toxicity of the drugs u_i. Such objective functions have been used in various optimal control problems of cancer therapies, c.f. the book by Schättler and Ledzewicz [44].

3. Results

We begin by presenting the main results, with proofs, followed by two illustrative examples.

Lemma 1. *The shrinkage set for the matrix A_u given by Eq. (13) is the convex set given by*

$$S = \left\{ u \geq 0 : \prod_{i=1}^{n} \left(k_i + \sum_{j=1}^{m} D_{j,ii} u_j \right) > 2 \prod_{i=1}^{n} k_i \right\}. \tag{16}$$

Proof. We first note that convexity follows from Proposition 1. To find a formula for the shrinkage set S, it follows from Theorem 1 that we only need to study when the leading principal minors of $-A_u$ are positive. All leading principal minors except $\det(-A_u)$ are trivially positive since they are determinants of positive diagonal matrices. Therefore, S is determined by the condition $\det(-A_u) > 0$. An application of Leibniz's formula for determinants gives condition (16). □

Note that S can also be expressed using the drug action matrix B

$$S = \left\{ u \geq 0 : \prod_{i=1}^{n} \left(k_i + [Bu]_i \right) > 2 \prod_{i=1}^{n} k_i \right\}. \tag{17}$$

The Eq. (17) can be given a probabilistic interpretation. First, recall that a system such as (12) can be derived from a probabilistic model where the transfer time, T_i, from state x_i to the next state is an exponentially distributed random variable with parameter k_i. Similarly, cell death in state x_i due to drug j can be assumed to be exponentially distributed with parameter b_{ij}. By independence, the time until cell death for a cell in state x_i due to any of the drugs is exponentially distributed with parameter $\sum_j b_{ij} u_j$. We denote this time by τ_i. A cell in state i survives until the next state if $T_i < \tau_i$. The probability of this event is given by

$$\mathbb{P}(T_i < \tau_i) = \frac{k_i}{k_i + \sum_{j=1}^{m} b_{ij} u_j}. \tag{18}$$

Letting T be a random variable such that $T = 1$ if a cell survives the entire cell cycle, and $T = 0$ if the cell dies somewhere along the way. Then, by independence, the probability of surviving is given by

$$\mathbb{P}(T = 1) = \prod_{i=1}^{n} \frac{k_i}{k_i + \sum_{j=1}^{m} b_{ij} u_j}. \tag{19}$$

Since each completed cell cycle results in two new cells, the tumor is expected to shrink if $\mathbb{P}(T) < 1/2$, which gives the same condition as Eq. (17).

We first use this lemma to prove the following intuitively obvious fact about monotherapy

Proposition 2 (*Monotherapy*)**.** *Consider the monotherapy case with only one compound, whose tumor concentration we denote by u, acing on each of the states $x_1, \ldots, x_n$ according to the diagonal matrix D. Then, there exists exactly one value u^* such that any $u > u^*$ ensures stability of A_u.*

Proof. Define the polynomial P given by

$$P(u) := \prod_{i=1}^{n} \left(k_i + D_{ii} u \right) - 2 \prod_{i=1}^{n} k_i, \tag{20}$$

and note that $P(0) < 0$. By Lemma 1, A_u is stable precisely for those u for which $P(u) > 0$. Rewriting P on the form

$$P(u) = a_0 + a_1 u + \cdots + a_n u^n, \tag{21}$$

we note that $a_0 < 0$ and $a_i > 0$ for all $i \geq 1$. It follows from Descartes's rule of sign that P has exactly one positive root, u^*, and consequently $P(u) > 0$ for all $u > u^*$. □

Proposition 1 expresses the fact that for a single compound, there exists exactly one concentration u^* such that any concentration below it

will lead to tumor growth, whereas any concentration above will lead to tumor shrinkage. Finding an explicit formula for u^* is non-trivial, but it can be expressed using the trick from the proof of part (iii) of the next proposition involving combination therapy.

The next proposition considers the combination therapy case, with m drugs. We characterize the optimal solution, given a linear objective function in u and using the stability condition as a constraint. We optimize over the closed set $\bar{S}$, which will give an optimum $u^* \in \partial S$. Then, for any vector $\epsilon > 0$ we have that $u^* + \epsilon \in S$, which will ensure stability. We also assume, without loss of generality, that the drug action matrix B does not contain rows of all zeros, corresponding to states without drug action, since it is clear from Eq. (17) that the only influence from such states comes from the corresponding k_i, which can be simplified from Eq. (17).

Proposition 3 (*Combination Therapy*). *Let the shrinkage set S be defined by Eq. (17) and consider the optimization problem*

$$\min_{u \in \bar{S}} F(u) := \alpha^T u, \tag{22}$$

where $\alpha = (\alpha_1, \ldots, \alpha_m)$ is a vector of weights. We have the following cases depending on the drug action matrix B

(i) If B is rank-deficient with rank $n - k$ for some positive integer k, there exists an optimum u^* where at least k entries are zero.

(ii) If B is diagonal with positive diagonal entries, $u^* > 0$ if and only if

$$\|\theta\|_\infty < \sqrt[n]{2} \, GM(\theta), \tag{23}$$

where $\theta_i = \alpha_i k_i / b_{ii}$, and GM denotes the geometric mean. The optimum is then given by

$$u_i^* = \frac{k_i}{b_{ii}} \left(\sqrt[n]{2} \frac{GM(\theta)}{\theta_i} - 1 \right), \tag{24}$$

and the optimal value becomes

$$F(u^*) = n(\sqrt[n]{2} GM(\theta) - \bar{\theta}). \tag{25}$$

(iii) If B has full rank, the problem can be converted to case (ii) by a linear invertible transformation.

Proof. We first prove part (i). Note that, due to Lemma 1, the domain S can be written $S = \{u \geq 0 : g(u) \geq 0\}$ where

$$g(u) := \prod_{i=1}^{n} \left(k_i + [Bu]_i \right) - 2 \prod_{i=1}^{n} k_i. \tag{26}$$

Since the objective function F is linear, any optimum u^* must be located on the boundary $g(u) = 0$. If B is rank-deficient with rank $m - k$ for some positive integer k, then for each $v \in col(B)$ the solution set to $Bu = v$ is a k-dimensional affine space. If $u^* > 0$ is an optimum, then the m-dimensional tangent spaces of g and F at u^* must coincide and, since F is linear, be a level set to F. Now, since $Bu = Bu^*$ is a k-dimensional affine space, there must exist a point $\tilde{u}$, with k zeros, in this level set, that also satisfies $g(\tilde{u}) = 0$. This proves (i).

To prove (ii), note that when B is diagonal, the change of variables $y_i = b_{ii} u_i + k_i$ gives rise to the equivalent optimization problem, which also occurs, e.g., in one of the standard proofs of the inequality between the arithmetic and geometric means,

$$\min \quad \beta^T y, \tag{27}$$

$$\text{s.t.} \quad \prod_{i=1}^{n} y_i - 2 \prod_{i=1}^{n} k_i \geq 0, \tag{28}$$

$$\text{and } y_i \geq k_i, \quad i = 1, \ldots, n, \tag{29}$$

where $\beta_i = \alpha_i / b_{ii}$. This is a convex optimization problem, hence any local minimum must also be a global minimum (see, e.g., [45]). Since the objective function is linear and the optimum therefore is attained on the boundary, we can assume that Eq. (28) holds with equality. Define the Lagrangian

$$L(y, \lambda, \mu) := \beta^T y - \lambda \left(\prod_{i=1}^{n} y_i - 2 \prod_{i=1^n} k_i \right) - \sum_{j=1}^{n} \mu_i (y_i - k_i). \tag{30}$$

The Karush–Kuhn–Tucker conditions, which are necessary for optimality by the linear independent constraints qualification (see, e.g., [45]), are given by

$$\frac{\partial L}{\partial y_i} = \beta_i - \mu_i - \lambda \prod_{j \neq i} y_j \quad = 0, \tag{31}$$

$$\frac{\partial L}{\partial \lambda} = \left(\prod_{i=1}^{n} y_i - 2 \prod_{i=1}^{n} k_i \right) = 0, \tag{32}$$

$$\mu_i (y_i - k_i) \quad = 0, \tag{33}$$

$$\mu_i \quad \geq 0. \tag{34}$$

Let $K = \prod_{i=1}^{n} k_i$. Multiplying Eq. (31) with y_i and using Eq. (32) gives

$$y_i = \frac{2\lambda K}{\beta_i - \mu_i}. \tag{35}$$

Moreover, taking the product of Eq. (31) over all i and using Eq. (32) gives

$$\prod_{i=1}^{N} (\beta_i - \mu_i) = \lambda^n K^{n-1} \Rightarrow \lambda = \frac{\sqrt[n]{2} GM(\beta - \mu) GM(k)}{2K}. \tag{36}$$

Combining Eqs. (35) and (36) gives the solution

$$y_i^* = \frac{\sqrt[n]{2} GM(\beta - \mu) GM(k)}{\beta_i - \mu_i}. \tag{37}$$

Finally, if $u_i^* > 0$, then $\mu_i = 0$. Letting $\theta_i = \alpha_i k_i / b_{ii}$ gives

$$u_i^* = \frac{k_i}{b_{ii}} \left(\sqrt[n]{2} \frac{GM(\theta)}{\theta_i} - 1 \right). \tag{38}$$

The condition (23) follows by noting that it precisely the condition that ensures positivity in Eq. (38). This proves (ii).

Finally, we prove (iii) by constructing the desired linear invertible transformation. Note that the claim is obvious when B is invertible, since the change of variables $y = Bu$ gives case (ii) with new cost vector $\beta = B^{-T} \alpha$. If B has full rank, but does not have enough columns to span $\mathbb{R}^n$, B can be augmented with Euclidean unit vectors $e_{j_{m+1}}, \ldots, e_{j_n}$ such that

$\tilde{B} := [B, e_{j_{m+1}}, \ldots, e_{j_n}]$ is invertible. Letting the additional columns of $\tilde{B}$ correspond to control variables $u_{m+1}, \ldots, u_n$ with costs $\alpha_{m+1}, \ldots \alpha_n$ reduces the problem to the invertible case with the new variables $y = \tilde{B}u$ and costs $\beta = \tilde{B}^{-T} \alpha$, which gives the analog to Eq. (37)

$$y_i^* = \frac{\sqrt[n]{2} GM(\beta - \mu) GM(k)}{\beta_i - \mu_i}, \tag{39}$$

from which it follows

$$u_i^* = -k_i + \sum_{j=1}^{n} \tilde{B}_{ij}^{-1} \frac{\sqrt[n]{2} GM(\beta - \mu) GM(k)}{\beta_j - \mu_j}. \tag{40}$$

Finally, we note that by choosing the fictitious costs such that they satisfy the equations

$$\frac{k_i}{GM(\beta) GM(k)} = \sum_{j=1}^{n} \frac{\tilde{B}_{ij}^{-1}}{\beta_j}, \quad i = m+1, \ldots, n, \tag{41}$$

where $\beta = \tilde{B}^{-T} \alpha$ is the transformed cost vector, ensures that $u_i^* = 0$ for $i = m+1, \ldots, n$ so that the fictitious control variables do not appear in the solution. $\square$

Case (i) states that if we have more compounds than targets, then we do not lose anything by getting rid of some of the compounds. Case (ii) presents the diagonal case where each compound has a unique single target. This could also be called the orthogonal case, since it covers any orthogonal matrix B up to scaling and a reordering of the compounds. An important insight expressed in case (ii) is that there is intrinsic benefit from combining compounds with different targets, which is precisely due to the convexity of the shrinkage set established in Lemma 1 and Proposition 1. Case (iii) relates the general case back to case (ii) via a linear bijection.

We have the following obvious corollary to Proposition 3, case (ii), which can also be applied to case (iii), after a linear change of variables.

Corollary 1. *If $u_j^* = 0$ for some j, then equation (24) simplifies to*

$$u_i^* = \frac{k_i}{b_{ii}} \left(\sqrt[n-1]{2 \frac{\prod_{i \neq j} \theta_i^{n-1}}{\theta_i}} - 1 \right), \ i \neq j. \tag{42}$$

Proof. The proof is a direct calculation, letting $u_j^* = 0$ in Eq. (37) and solving for μ_j, then plugging this value back into Eq. (38) for $i \neq j$. $\square$

We can also consider, as a special case, the situation where all compounds are 'equal' in the sense that the θ_i are equal. This gives an estimate of the inherent benefits of targeting different states.

Corollary 2. *If $\theta_1 = \theta_2 = \cdots = \theta_n = \theta$, then the minimum value of the objective function, given by Eq. (25), becomes*

$$F(u^*) = \theta n (\sqrt[n]{2} - 1), \tag{43}$$

and in the limit as $n \to \infty$, we have $F(u^*) \to \theta \ln 2$.

Proof. The proof is a direct calculation. The limit follows, e.g., by an application of l'Hopital's rule. $\square$

For a single drug, $n = 1$, Corollary 2 gives the objective value $F(u^*) = \theta$, whereas in the limit as $n \to +\infty$ we have $F(u^*) = \theta \ln 2 \approx 0.7\,\theta$. Thus, Corollary 2 states that combination therapy with additive drugs that target different stages of the cell cycle can at most reduce the cost compared to single-agent treatment by 30%.

An example with a two-drug combination

We consider a cell cycle model with four states, $x_1, x_2, x_3,$ and x_4, corresponding to the stages $G_1, S, G_2,$ and M of the cell cycle, and transfer rates $k_1, k_2, k_3,$ and k_4. In order to obtain biologically plausible parameter values, we assume phase durations of 12, 6, 6, and $1/2$ hours for the phases $G_1, S, G_2,$ and M, respectively [46]. Since the average time in the i:th phase is given by $1/k_i$, we therefore set $k_1 = 1/12$, $k_2 = 1/6$, $k_3 = 1/6$, and $k_4 = 2$ h^{-1}. With these parameter values, the largest and only positive eigenvalue of the corresponding system matrix is given by $\lambda_{max} \approx 0.032$. The corresponding normalized eigenvector is $v_{max} \approx (0.55, 0.23, 0.20, 0.02)$. Thus, the initial proportion of cells in each phase is assumed to be given by v_{max}.

We now consider treatment with two cell cycle specific compounds that target different phases of the cell cycle. 5-fluorouracil is an antimetabolic agent that interferes with DNA and RNA synthesis, and therefore acts mainly on the S phase of the cell cycle, that is used to treat a variety of cancers including stomach cancer, colon cancer, pancreatic cancer and breast cancer [4]. Vinorelbine is a vinca alkaloid that interferes with microtubule assembly and therefore acts primarily on the M stage of the cell cycle [4]. Vinorelbine is used to treat different cancers including breast cancer and non-small cell lung cancer [47].

Let a denote the drug action parameter of 5-fluorouracil on cells in S phase, and let b denote the drug action parameter of vinorelbine on cell in M phase. Moreover, let α be the cost, or toxicity, or 5-fluorouracil and β be the toxicity of vinorelbine. Combinations of these compounds satisfy case (ii) of Proposition 3, i.e., the orthogonal case. Monotherapy with 5-fluorouracil requires exposure levels above k_2/a to achieve stability and a shrinking population of cancer cells, whereas the equivalent quantity for vinorelbine monotherapy is k_4/b. Note that since the M phase is much shorter than the S phase of the cell cycle, the potency parameter b would need to be much larger than a in order to achieve stability at similar exposure levels.

To compute biologically reasonable values for a and b we use reported *in vitro* geometric mean IC50 values across a large number of cell lines of 34.8 μM and 0.0217 μM for 5-fluorouracile and vinorelbine [48], and then use our model to find the corresponding a and b such that the cell population after 72 h of single-agent treatment is exactly half of the untreated cell population at the same time point. This yields the values $a = 19.4$ μM^{-1}h^{-1} and $b = 0.00115$ μM^{-1}h^{-1}.
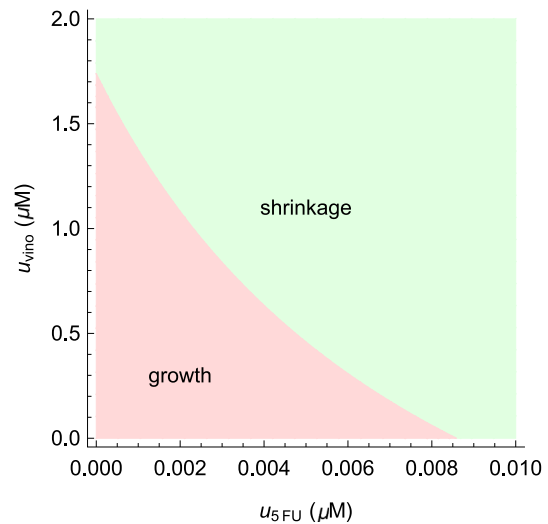

Fig. 4. Shrinkage set for combinations of 5-fluorouracil and vinorelbine. The green region indicates exposure combinations (u_{5FU}, u_{vino}) that will cause a reduction in the cancer cell population over time, whereas the red region indicates combinations that result in a growing cancer cell population. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The shrinkage set, defined in Lemma 1, is given by

$$(k_2 + au_{5FU})(k_4 + bu_{vino}) > 2k_2k_4. \tag{44}$$

This set is shown in Fig. 4. In terms of Eq. (23) we have that $\theta_{5FU} = \alpha k_2/a$ and $\theta_{vino} = \beta k_4/b$, and therefore $GM(\theta) = \sqrt{\alpha\beta k_2 k_4}/\sqrt{ab}$. It will be optimal to use only 5-fluorouracil if $\theta_{5FU} \geq 2\theta_{vino}$ and it will be optimal to use only vinorelbine if $\theta_{vino} \geq 2\theta_{5FU}$. Therefore if

$$\frac{\theta_{5FU}}{2} < \theta_{vino} < 2\theta_{5FU}, \tag{45}$$

or equivalently

$$\frac{\alpha k_2}{2a} < \frac{\beta k_4}{b} < \frac{2\alpha k_2}{a}, \tag{46}$$

it will be optimal to use both compounds and the optimum will be given by

$$u_{5FU}^* = \frac{k_2}{a} \left(\sqrt{2} \frac{\sqrt{\theta_{vino}}}{\sqrt{\theta_{5FU}}} - 1 \right), \tag{47}$$

and

$$u_{vino}^* = \frac{k_4}{b} \left(\sqrt{2} \frac{\sqrt{\theta_{5FU}}}{\sqrt{\theta_{vino}}} - 1 \right). \tag{48}$$

After inserting the values for $k_2, k_4, a,$ and b, Eq. (46) defines a condition on the costs α and β for which it will be optimal to use both compounds. This set in shown in Fig. 5. The light blue region shows the cost pairs (α, β) such that it will be optimal to use both compounds, whereas the white regions on either side indicate for which cost pairs it will be optimal to use only 5-fluorouracil or vinorelbine.

An example with a three-drug combination

We consider the same two compounds as in the previous example, 5-fluorouracil and vinorelbine, but this time also include irinotecan. Irinotecan is a topoisomerase I inhibitor, which stops the cell at the G2-checkpoint which eventually triggers apoptosis. Irinotecan is used to treat primarily colorectal cancers [49]. We assume that irinotecan acts on cell in the G2 phase with rate c, and let its cost be denoted by γ. The cell cycle model subject to triple combination treatment with 5-fluorouracil, vinorelbine, and irinotecan is shown in Fig. 6. To compute c we take the same approach as before and use the model to find
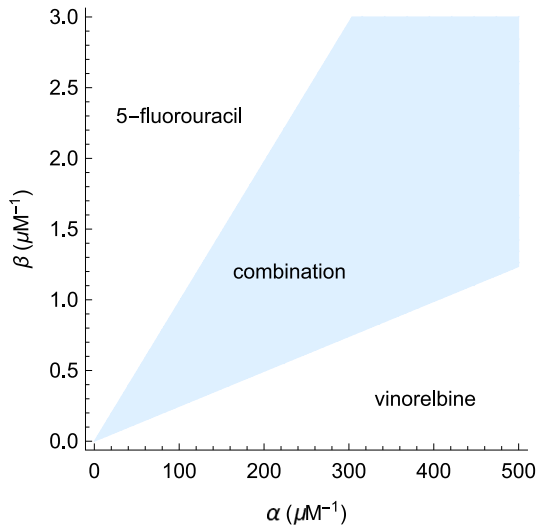
Fig. 5. Illustration of cost pairs (α, β), corresponding to 5-fluorouracil and vinorelbine respectively, for which it will be optimal to use monotherapy with 5-fluorouracil or vinorelbine (white regions), or combination therapy (light blue region). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
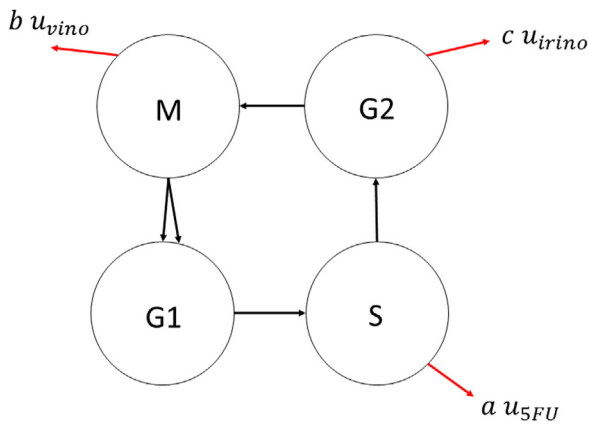

Fig. 6. Cell cycle model with four states G_1, S, G_2, and M, subject to provocation with the three drugs 5-fluorouracil, vinorelbine, and irinotecan, with exposures $u_{5FU}, u_{vino}, u_{irino}$, respectively. It is assumed that 5-fluorouracil acts on cells in the S phase with parameter a, vinorelbine acts on cells in phase M with parameter b, and irinotecan acts on cells in state G_2 with rate parameter c.

c such that the cell population after 72 h of irinotecan treatment at reported IC50 concentrations is exactly half the size of the untreated cell population. The reported geometric mean IC50 for irinotecan across a large number of cell lines is 13.8 μM [48], which corresponds to $c = 0.0029 \ \mu M^{-1}h^{-1}$.

The shrinkage set, defined in Lemma 1, for combinations of 5-fluorouracil, vinorelbine, and irinotecan is given by the condition

$$(k_2 + au_{5FU})(k_4 + bu_{vino})(k_3 + cu_{irino}) > 2k_2k_3k_4, \tag{49}$$

and is shown in Fig. 7.

Now let $\theta_{irino} = \gamma k_3/c$, and $GM(\theta) = \left(\theta_{5FU}\theta_{vino}\theta_{irino}\right)^{\frac{1}{3}}$. Then the conditions that ensure that it is optimal to use all three compounds are given by

$$\theta_{5FU} < \sqrt[3]{2}GM(\theta), \tag{50}$$

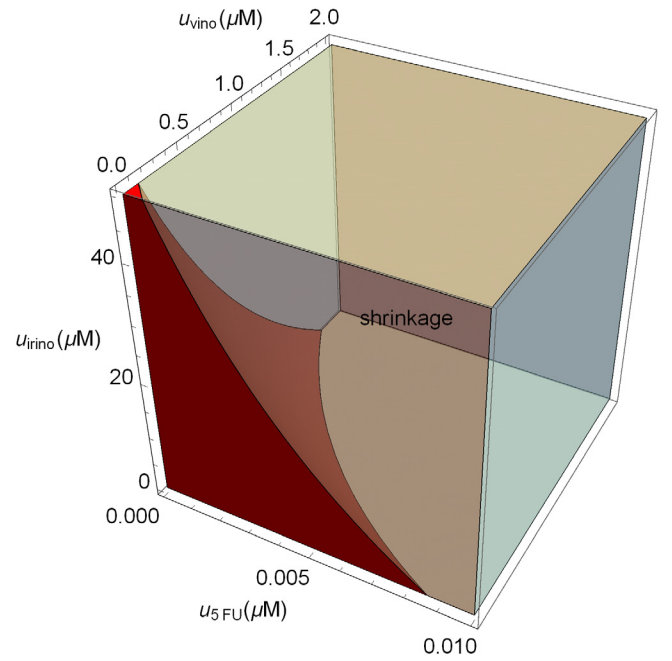$$\theta_{vino} < \sqrt[3]{2}GM(\theta), \tag{51}$$

Fig. 7. Shrinkage set for combinations of 5-fluorouracil, vinorelbine, and irinotecan. The green region indicates exposure combinations $(u_{5FU}, u_{vino}, u_{irino})$ that cause a reduction in the cancer cell population over time, whereas the red region indicates combinations that result in a growing cancer cell population. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
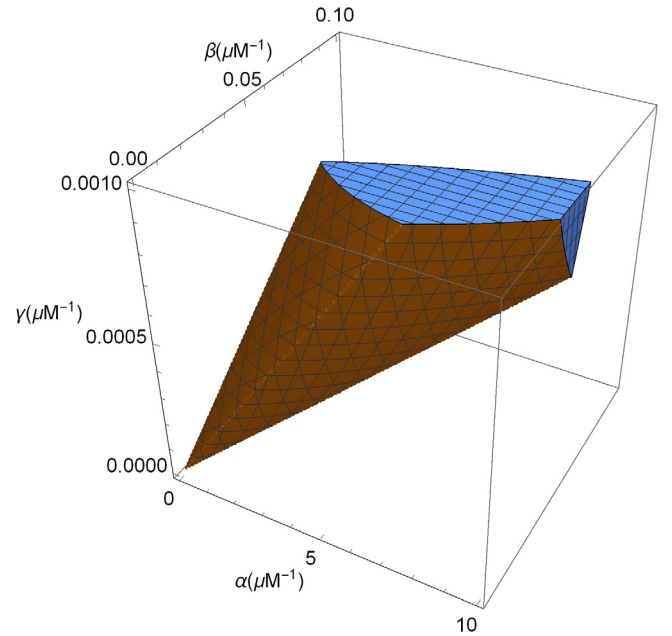

Fig. 8. Illustration of cost triples α, β, γ, corresponding to 5-fluorouracil, vinorelbine, and irinotecan, respectively, for which it is optimal to use all three compounds.

and

$$\theta_{irino} < \sqrt[3]{2}GM(\theta). \tag{52}$$

Using the estimated values for k_1, k_2, k_3 as well as a, b, c this gives rise to a region in cost space consisting of triples (α, β, γ) for which it is optimal to use all three compounds. This region is shown in Fig. 8. It is clear from the figure that in order for the optimum to use all three compounds we would need $\alpha \sim 10^2 \beta$ and in turn $\beta \sim 10^2 \gamma$.

4. Discussion

We analyze stability conditions for a linear cell cycle model subject to chemotherapeutic combination treatment. Such analyses are common for single-agent treatments and lead to the definition of threshold concentrations such that any concentration above the threshold results in a shrinking tumor or population of cancer cells [50] and have also been derived for combination treatments [32,51]. We prove the existence of such a concentration for our model in Proposition 2. However, finding an explicit formula for the general case where the single-agent treatment may act differently on different phases of the cell cycle is difficult. One way to approach this problem is to apply Proposition 3(iii), and the proof thereof, when there are more targets than compounds. For combination treatments, stability analysis leads to the definition of a shrinkage set consisting of all exposure combination that result in a shrinking cancer cell population. The geometry of the shrinkage set is interesting since it is related to the inherent benefits of combination therapy [51]. For the cell cycle model in this paper, where the anticancer agents are assumed to be additive, we show in Lemma 1 and Proposition 1 that the shrinkage set is always convex, and that this will be true for general compartment models subject to treatment with cell killing drugs.

We use the shrinkage set as a constraint to optimize combination treatments under the assumption of constant drug exposure. Constant exposure is achievable in an *in vitro* setting which is our primary consideration in this paper, but it is less feasible *in vivo*. Instead, constant exposure should be viewed as an approximation that is suitable for drugs that can be administered via continuous infusion, or which have frequent dosing and/or slow enough clearance such that exposure levels can be maintained at fairly stable levels. Our main result, given in Proposition 3, provides a formula for the optimal exposure combination as well as conditions for when one or several compounds are superfluous. We believe that these results can be useful when considering new combinations treatments with compounds for which cell cycle specificity is at least somewhat understood. Moreover, we believe that Corollary 2, which provides a bound of at most 30% cost reduction compared to single-agent treatment, to be a relevant result that can help determine whether additive combination treatments are feasible or whether synergistic compounds with drug interactions are necessary to achieve a desired cost reduction.

Optimization and optimal control in the more general case of cancer and cell cycle models with time-varying exposure have been analyzed in numerous papers including the works of Kimmel, Swierniak, Ledzewicz, and Schättler to name a few [44,52–56]. The objective to be minimized is typically a weighted L_1- or L_2-norm of the drug concentrations and tumor volume, combined with an endpoint penalty at a final time, which can be either fixed or free. Compared to the case with constant exposure, which is appropriate for the *in vitro* setting, optimal control problems are more suitable for the clinical setting [57]. Moreover, although optimal controls can be computed numerically for specific choices of parameter values, it is often very difficult to obtain explicit analytical solutions. One important result, which has been proved to hold for a general case of linear cancer models, states that optimal controls are always "bang–bang", i.e., at any time point the exposure level should be set either to a maximum allowed value, or to zero [44]. However, a complete determination of the switching times, when the exposure should be changed from its maximum value to zero or vice versa, is highly nontrivial.

The optimization problem with constant drug exposures that we consider in this paper is much simpler than the related optimal control problems and can therefore be analyzed analytically. Another difference is that whereas optimal control problems consider the full dynamics of the model as a constraint, we only consider the sign of the eigenvalues and therefore mainly consider the asymptotic behavior of the system. One important result by Clairambault et al. states that a linear model given by a Metzler matrix, with time-varying periodic coefficients, has worse stability properties than the corresponding system with time-averaged constant coefficients [58]. This provides justification for our restriction to only consider constant drug exposures.

The model considered in this paper describes an *in vitro* population of cancer cells. In order to describe *in vivo* tumor growth, the model would need to be modified to account for spatial heterogeneity in particular. This can be done using partial differential equations, or an ordinary differential equation approach such as the one by Evans et al. [59] who describe a tumor consisting of an outer shell and an inner core with the possibility to transfer cell mass between the two. See the paper by Checkley et al. for an example where the shell model was integrated into a cell cycle model [60].

5. Conclusions

This paper analyzes a simple *in vitro* model of a population of cancer cells that captures two important aspects of cancer treatment: (i) the cell cycle, and (ii) combination treatment with two or more chemotherapeutic compounds. We find an explicit condition for stability of the model, and show that the corresponding shrinkage set is convex, which is useful for understanding which exposure combinations may produce a stable or shrinking cancer cell population.

Moreover, our main result, Proposition 3, provides a condition for when compounds are redundant, and a solution formula for the case where all compounds are used. These results could potentially be useful for practical applications such as selecting compounds for additional experiments, as well as for more mathematical endeavors such as sensitivity analyses and to understand for which sets of parameter values a particular combination is optimal.

Finally, since our analysis is performed for general additive combination treatments, it has the potential to be used and reused to analyze many different chemotherapeutic scenarios. It could also be used as a baseline case for additivity, which may then help determine if a particular combination is synergistic or antagonistic.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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