

Using Control Theory to Make Cancer Chemotherapy Beneficial from Phase Dependence and Resistant to Drug Resistance

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Abstract

Two major obstacles against successful chemotherapy of cancer are (1) the cell-cycle-phase dependence of treatment, and (2) the emergence of resistance of cancer cells to cytotoxic agents. One way to understand and overcome these two problems is to apply optimal control theory to mathematical models of cell cycle dynamics. These models should include division of the cell cycle into subphases and/or the mechanisms of drug resistance. We review our 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 parameters of the constructed models.

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1 Introduction

In this paper we are concerned with three issues:

1. The inner structure of the cell cycle and the cell-cycle-phase specificity of some chemotherapy agents.
2. The dynamics of emergence of resistance of cancer cells to chemotherapy, as understood based on recent progress in molecular biology.
3. Estimation of quantitative parameters of the cell cycle, drug action and cell mutation to resistance.

The main purpose of the paper is to outline our own views on the issues involved. The paper is in large part a critical survey of published work by us and others. Wherever appropriate, we give credit to others, without attempts at an exhaustive review.

The philosophy of this paper is related to our professional experience. The first author has spent almost ten years in a cancer research institute trying to develop models of the cell cycle for the purpose of estimation of cell-cycle-phase specific action of anticancer drugs. More recently, he has investigated gene amplification as the mechanism of resistance of cancer cells. The other author has been involved for two decades in attempts to develop a satisfactory theory of optimal control of bilinear systems resulting from a description of chemotherapy action using ordinary differential equations. The cell-cycle-phase specificity is essential for the initial period of chemotherapy, when at issue is the most efficient reduction of the cancer burden. This seems to be of practical importance mainly in nonsurgical cancers such as for example leukemias. Emergence of clones of cancer cells resistant to chemotherapy is important in treatment and prevention of systemic spread of disease. This comprises potential treatment of metastasis and all variants of adjuvant chemotherapy.

Mathematical modeling of cancer chemotherapy has had more than four decades of history. It has contributed to the development of ideas of chemotherapy scheduling, multidrug protocols, and recruitment. It has also helped in the refinement of mathematical tools of control theory applied to the dynamics of cell populations [39]. However, regarding practical results it has been, with minor exceptions, a failure. The reasons for that failure are not always clearly perceived. They stem from the direction of both biomedicine and mathematics: important biological processes are ignored and crucial parameters are not known, but also the mathematical intricacy of the models is not appreciated.

In this paper, we would like to outline several directions of research which may play a role in improving the situation and realizing the obvious potential existing in the mathematical approach. Because of recent progress in methods of monitoring cancer cell populations, new insights and more precise measurements became possible. This, together

with a progress in mathematical tools, has renewed hopes for improving chemotherapy protocols.

Cell-cycle-phase specificity of some cytotoxic drugs is important since it makes sense to apply anticancer drugs when cells gather in the sensitive phases of the cell cycle. It can be approached by considering dissection of the cell cycle into an increasing number of disjoint compartments, with drug action limited to only some of them. We provide a classification of several simplest models of this kind. Mathematical problems encountered include singularity and non-uniqueness of solutions of the optimization problems.

The emergence of resistance to chemotherapy has been first considered in a point mutation model of Coldman and Goldie (e.g.[30], [47]) and then in the framework of gene amplification by Agur and Harnevo (e.g. [52], [53], [54]). The main idea is that there exist spontaneous or induced mutations of cancer cells towards drug resistance and that the scheduling of treatment should anticipate these mutations. The point mutation model can be translated into simple recommendations, which have even been recently tested in clinical trials. The gene amplification model was extensively simulated and also resulted in recommendations for optimized therapy. We present a model of chemotherapy based on a stochastic approach to evolution of cancer cells. Asymptotic analysis of this model results in some understanding of its dynamics. This, in our opinion, is the first step towards a more rigorous mathematical treatment of the dynamics of drug resistance and/or metastasis. Optimization of the chemotherapy in this case may be viewed as the progress in creating chemotherapy resistant to drug resistance.

There is no doubt that the parameters of spontaneous cancer cells populations existing *in vivo* in humans differ considerably from those of the test tube “transformed” cells and from those of the induced animal tumors. However, much information regarding cell-cycle-phase specificity of anticancer agents has been obtained using *in vitro* experimental models. We present some of these results which do not seem to be sufficiently well known. We also discuss some approaches to estimation of cell cycle parameters of human tumors. Finally we discuss estimation of the rates of mutations leading to drug resistance.

2 Modeling the Cell Cycle

The cell cycle is composed of a sequence of phases traversed by each cell from its birth to division. These phases are: G_1 , or the growth phase; S , or the DNA synthesis phase; G_2 , or the preparation for division phase; and M , or the division phase. After division, the two daughter cells usually re-enter G_1 . It may however happen that one or both daughters deviate from this path and become dormant or resting, or in other words, they enter the quiescent G_0 phase. From there after a variable and usually rather long time cells may reenter the cell cycle in G_1 [9].

This idealized scheme is confounded in solid tumors by the existence of a geometric gradient of availability of oxygen and nutrients. This causes a stratification in viability of cells: usually, cycling cells are located near the surface or near blood vessels, further layers are occupied by dormant cells, while the deepest regions form a necrotic core. This may lead to self-limiting growth phenomena, which may be described by biologically based non-linear models including Pearl-Verhulst, Cox-Woodbury-Meyers, Michaelis-Menton equations (see e.g. [115], [114], [90], [116], [113]), or Gompertz-type equations ([50], [108], [110], [142], [148], [117]). It is interesting to note that Gompertz proposed his model for demographic purposes [48], and its biological meaning is difficult for justification, but the gompertzian growth is a good approximation of a number of experimental data for tumor growth. We do not consider this structure in our models. Instead we build a set of models of cell cycle kinetics composed of compartments (see e.g. [59], [133]) each of them containing a phase or a cluster of phases.

The transit times through all the phases of the cell cycle are variable, particularly in malignant cells. Usually it is assumed that most of this variability is concentrated in the G_1 phase (and in G_0 whenever it exists). The simplest models arise if the transit times through each compartment are assumed exponentially distributed.

Denote by $N_i(t)$ the average number of cells in the i -th compartment at time t , and by $x_i^+(t)$ and $x_i^-(t)$, the average flow rates of cells into and out of this compartment, respectively. Then,

$$\dot{N}_i(t) = x_i^+(t) - x_i^-(t), \quad (1)$$

and

$$x_i^-(t) = a_i N_i(t), \quad (2)$$

where a_i is the parameter of the exponential distribution, equal to the inverse of the average transit time. If the preceding compartment is numbered $i - 1$, then

$$\dot{N}_i(t) = -a_i N_i(t) + a_{i-1} N_{i-1}(t). \quad (3)$$

for $i = 2, 3, \dots, n$ where n is a number of compartments. The boundary condition for the obtained set of equations is given by:

$$\dot{N}_1(t) = -a_1 N_1(t) + 2a_n N_n(t). \quad (4)$$

Therefore, under the exponentiality assumption, the unperturbed dynamics of cell cycle, i.e. the number of cells in various cell cycle compartments versus time, in the absence of external stimuli, is expressed by a system of ordinary linear differential equations. We consider three types of perturbations of the cell cycle [118], [128]:

Cell Killing. At time t , only a fraction $u(t)$ of the outflux from compartment i contains viable cells ($0 \leq u(t) \leq 1$). The remaining cells are dead and no longer considered part of the system.

$$\dot{N}_i(t) = -a_i N_i(t) + a_{i-1} N_{i-1}(t), \quad (5)$$

$$\dot{N}_{i+1}(t) = -a_{i+1} N_{i+1}(t) + u(t) a_i N_i(t), \quad (6)$$

The reproductively dead cells may however continue to progress through the cycle for some time, thus confounding estimates of cell proliferation.

Cell Arrest. At time t , the outflux from compartment i is reduced to a fraction $v(t)$ of the normal value ($0 < v_m \leq v(t) \leq 1$). The remaining cells are arrested in compartment i .

$$\dot{N}_i(t) = -v(t) a_i N_i(t) + a_{i-1} N_{i-1}(t), \quad (7)$$

$$\dot{N}_{i+1}(t) = -a_{i+1} N_{i+1}(t) + v(t) a_i N_i(t). \quad (8)$$

The complete arrest is not possible, and it is why v_m is always strictly positive.

Alteration of the Transit Time. The parameter of the exponential distribution of the transit time through compartment i is changed by factor $y(t) > 0$. Depending on whether $y(t)$ is less or greater than 1, this is equivalent to respectively extending or reducing the mean transit time. In the latter case it is used in the so called recruitment of dormant cells to the proliferation cycle. The mathematical description has the form:

$$\dot{N}_i(t) = -y(t) a_i N_i(t) + a_{i-1} N_{i-1}(t), \quad (9)$$

$$\dot{N}_{i+1}(t) = -a_{i+1} N_{i+1}(t) + y(t) a_i N_i(t), \quad (10)$$

Formally, these equations are identical as those describing cell arrest. This effect is caused by the exponentiality assumption.

Since our models describe an average behaviour of considered subpopulations the compartments which they represent are sometimes called "probabilistic" or "statistical" ones. "Deterministic" description of the continuously dividing population should be described by partial differential equations (for example of von Förster [43]), integro-differential or integral equations (for example [65]). In this case the one independent variable represents the chronological time while the other age or size. In our models the age is simply discretized and the dynamics from one stage to the other is averaged. The combined approach is to use both "probabilistic" and "deterministic" compartments to model the cell cycle as for example in [34, 35].

The first class of drug actions is represented by G_2/M specific agents, which include the so-called spindle poisons like Vincristine, Vinblastine or Bleomycin which destroy a mitotic spindle [25] and Taxol [42] or 5-Fluorouracil [26] affecting mainly cells during their division. Killing agents also include S specific drugs like Cyclophosphamide [42] or Methotrexate - MTX [96] acting mainly in the DNA replication phase, Cytosine Arabinoside - Ara-C, rapidly killing cells in phase S through inhibition of DNA polymerase by competition with deoxycytosine triphosphate [32]. Among the blocking drugs used to

arrest the cells immediately before or during DNA synthesis we can mention antibiotics like Adriamycin, Daunomycin, Dexorubin, Idarubicin which cause the progression blockage on the border between the phases G_1 and S by interfering with the formator of the polymerase complex or by hindering the separation of the two polynucleotide strands in the double helix [4]. Another blocking agent is Hydroxyurea - HU [84], [35] which is found to synchronize cells by causing brief and invisible inhibition of DNA synthesis in the phase S and holding cells in G_1 . The recruitment action was demonstrated [5] for Granulocyte Colony Stimulating Factors - G-CSF, Granulocyte Macrophage Colony Stimulating Factors - GM-CSF, Interleukin-3 - Il-3, specially when combined with Human Cloned Stem Cell Factor - SCF.

This classification of anticancer agents is not quite sharp and there is some controversy in the literature concerning both the site and the role of action of some drugs. For example, although mostly active in specific phases Cyclophosphamide and 5-Fluorouracil kill cells also in other phases of the proliferation cycle that enables to encounter them to cycle specific agents [25], [21]. On the other hand some antimetabolic agents like curacin A [77] act by increasing the S phase transition (blocking) and decreasing the M phase transition.

Killing agents which we consider in our model are applied in the G_2/M phase which makes sense from a biological standpoint for a couple of reasons. First, in mitosis M the cell becomes very thin and porous. Hence, the cell is more vulnerable to an attack while there will be a minimal effect on the normal cells. Second, chemotherapy during mitosis will prevent the creation of daughter cells.

While the killing agent is the only control considered in the two-compartment model below, in the three-compartment model in addition a blocking agent is considered which slows down the development of cells in the synthesis phase S and then releases them at the moment when another G_2/M specific anticancer drug has maximum killing potential (so-called synchronization [22]). This strategy may have the additional advantage of protecting the normal cells which would be less exposed to the second agent (e.g. due to less dispersion and faster transit through G_2/M) [34], [2]. This cell cycle model includes separate compartments for the G_0/G_1 , S and G_2/M phases.

One of the major problems in chemotherapy of some leukemias is constituted by the large residuum of dormant G_0 cells which are not sensitive to most cytotoxic agents [26], [57], [83]. Similar findings for breast and ovarian cancers were reported, e.g. in [42, 28]. As indicated by these authors the insensitivity of dormant cells to the majority of anticancer drugs and percentage of tumor mass resting is a fact which, if ignored, leads not only to clinical problems but also to some erroneous theoretical considerations. Experiments with Ara-C [32], indicated that while double injected during cell cycle or combined with Adriamycin or anthracyclines led to serious reduction of leukemic burden without an evident increase of negative effect on normal tissues. This therapeutic gain was attributed to the specific recruitment inducing effect of Ara-C on leukemic cells in the dormant phase

It became possible to efficiently recruit quiescent cells into the cycle using cytokines [132] (substances playing a role in the regulation of normal hemopoiesis) like G-CSF, GM-CSF, and especially Il-3 combined with SCF. Then, a cytotoxic agent like Ara-C or anthracyclines may be used. The other three compartment model below uses separate compartments for the G_0 , G_1 and $S + G_2/M$ phases and includes such a recruiting agent. Moreover, it enables also analysis of the alteration of the transit time through G_0 phase due to the feedback mechanism that recruits the cells into the cycle when chemotherapy is applied. In a similar way we may model other types of manipulation of the cell cycle as for example the use of triterpenoids to inhibit proliferation and induce differentiation and apoptosis in leukemic cells [75].

The important assumption which is satisfied in all our models is that the control systems which they represent are *internally positive* [60] i.e.:

- (+) The first orthant of the control system is positively invariant, that is for any admissible control and for positive initial states, the state remains positive for all times $t > 0$.

Thus the obvious modelling state-space constraints that the state is positive, need not be included in our model explicitly and the analysis simplifies. A simple sufficient condition for (+) to hold (for example, see [60]) is that:

- (M) all the system matrices for all admissible controls are so-called M -matrices, i.e. have negative diagonal entries, but non-negative off-diagonal entries.

This condition is natural and will be satisfied for any compartmental model whose dynamics is given by balance equations where the diagonal entries correspond to the outflows from the i -th compartments and the off-diagonal entries represent the inflows from the i -th into the j -th compartment, $i \neq j$. It is satisfied for each of the models described here. More generally, if condition (+) were violated, this is a strong indication that the modelling is inconsistent.

3 Control Problems with Cell-Cycle-Phase Dependence

The classical control theoretic design problem may be stated as follows. Let the dynamic properties of a system be described by its state, and the external actions i.e. control and disturbances be given by input variables. Moreover assume that we are given a target set of required system states or outputs. Find control actions which enable reachability

of the desirable region. If we are able to describe a disease by a finite number of dynamically changing parameters we are also able to formulate the control problem in the sense mentioned above. In the models considered here the problem of finding an optimal cancer chemotherapy protocol is formulated as an optimal control problem over a finite time-interval, the fixed therapy horizon. The state variable is given by the average number of cancer cells and the control is the effect of the drug dosages on the respective subpopulation. The goal is to maximize the number of cancer cells which the agent kills, respectively minimize the number of cancer cells at the end of the therapy session, while keeping the toxicity to the normal tissues acceptable. The latter aspect is modelled implicitly by including an integral of the control over the therapy interval in the objective so that minimizing controls will have to balance the amount of drugs given with the conflicting objective to kill cancer cells.

From the first attempts at cell-cycle-phase dependent chemotherapy, one of the central ideas was that of *synchronization* [79]. In one version, the concept includes using an agent to arrest cells before they enter a sensitive phase. After enough of them accumulate, they are released into the sensitive phase and then targeted by a killing agent. This tactic is employed in the first of our three-compartments models. However, synchronization may be achieved, at least in theory, using only one agent, by periodic administration (see e.g. [35], [140]). At appropriate frequency and dosage, maximum efficiency would be achieved (the *resonance*). This tactic may be combined with attempts at sparing the normal cells by taking advantage of the difference in cell cycle duration of cancer and normal cells. This problem was considered in a number of papers by Agur and coworkers (e.g. [2], [3], [29]). Their line of reasoning is based on the so called Z-method in which the crucial parameter is the elimination coefficient Z measuring the treatment efficacy defined by

$$Z = 1 - \frac{T_m}{T_h}, \quad (11)$$

where T_m is the elimination time of the malignant population and T_h is the one of critical host population. Agur *et al.* [3] find that treatment efficacy is a nonmonotonic function of the relation between the cell generation time and the period of drug administration with maxima occurring when the critical host cell cycle length is a multiple of the chemotherapeutic period. The results in the papers imply that short drug-pulses at appropriate intervals may be more efficient than a drug administered at arbitrary intervals or a continuous slowly released drug. Under the condition that the cell cycle parameters of malignant cells have a relatively large variation, the drug protocol could be determined by the host temporal parameters alone and should reduce cytotoxicity even in the case of similar mean cell cycle times for cancer and normal tissues.

3.1 Single Compartment, Single Killing Agent

In the simplest model it is assumed that the cytotoxic agent is not cell-cycle-phase specific [72]. Therefore, whole cell cycle is modeled as a single compartment. The corresponding single differential equation has the form,

$$\dot{N}(t) = -aN(t) + 2u(t)aN(t), \quad N(0) = N_0, 0 \leq u(t) \leq 1. \quad (12)$$

Control variable $u(t)$ assumes values $u(t) = 1$ when the drug is not administered, $u(t) = 0$ when the maximum dose is used, and $0 < u(t) < 1$ in all other cases.

This bilinear model is used to find the optimal control which minimizes the performance index,

$$J = rN(T) + \int_0^T [1 - u(t)] dt. \quad (13)$$

In biological terms, the effect of the optimal control is minimization of the number of cancer cells at the end of the assumed therapy interval $[0, T]$, combined with minimization of the cumulative negative effects of the drug upon the normal tissues; r is a weighing coefficient.

This optimization problem is mathematically so simple that it can be explicitly solved. Substituting the solution of equation (12) into (13) yields,

$$J = rN(T) + \frac{1}{2}T + \frac{1}{2a} \ln \left[\frac{N_0}{N(T)} \right], \quad (14)$$

and its minimum value is obtained for

$$N(T) = \frac{1}{2ar}, \quad (15)$$

if the following inequality is satisfied,

$$0 \leq \frac{1}{2}T - \frac{1}{2a} \ln(2arN_0) = T_1 \leq T. \quad (16)$$

This inequality results from the constraints imposed on the control variable. In this case, any control satisfying the relationship,

$$\int_0^T u(t)dt = \frac{1}{2}T + \frac{1}{2a} \ln \left[\frac{N_0}{N(T)} \right] = \frac{1}{2}T - \frac{1}{2a} \ln(2arN_0), \quad (17)$$

is optimal.

In [121] we explain the nonuniqueness of the solution by its total singularity. Moreover in [120] we have shown that extensions of the first order model assuming Bellman's model of pharmacokinetics for anticancer drug (see [16], [31]), or simultaneously considering the drug effect on cancer and normal proliferating cells, do not enable us to avoid the singularity of optimal control.

3.2 Two Compartments, Single G₂M - Specific Killing Agent

This is probably the simplest situation in which it is possible to contemplate the effects of phase specificity [119], [123]. Compartment 1 consists of the G₁ and S phases and compartment 2 of the G₂ and M phases. The corresponding system of two differential equations has the form,

$$\begin{aligned}\dot{N}_1(t) &= -a_1 N_1(t) + 2ua_2 N_2(t), & N_1(0) &= N_{10} > 0, \\ \dot{N}_2(t) &= -a_2 N_2(t) + a_1 N_1(t), & N_2(0) &= N_{20} > 0.\end{aligned}\tag{18}$$

The performance index has the form analogous to (14),

$$J = \sum_{i=1}^2 r_i N_i(T) + \int_0^T [1 - u(t)] dt,\tag{19}$$

and its interpretation is identical as before.

If the optimal control is of the bang-bang type, it can be found from the maximum principle [100] by minimizing the so called hamiltonian function:

$$H = p_1(-a_1 N_1 + 2ua_2 N_2) + p_2(-a_2 N_2 + a_1 N_1) + 1 - u,\tag{20}$$

that results in:

$$u(t) = \begin{cases} 0; & 2a_2 N_2(t)p_1(t) > 1, \\ 1; & 2a_2 N_2(t)p_1(t) < 1, \end{cases}\tag{21}$$

where $p = (p_1, p_2)^T$ is the costate vector defined by the conjugate equations,

$$\begin{aligned}\dot{p}_1(t) &= a_1(p_1(t) - p_2(t)), & p_1(T) &= r_1, \\ \dot{p}_2(t) &= a_2(p_2(t) - 2p_1(t)u(t)), & p_2(T) &= r_2,\end{aligned}\tag{22}$$

Since the control system satisfies condition (M), then it follows from the adjoint equation that for any admissible control the first orthant in costate-space is negatively invariant under the flow of the adjoint system, i.e. if $p_i(T) > 0$ for all $i = 1, 2$, then $p_i(t) > 0$ for all times $t \leq T$. In this case, since $N(0)$ and $p(T)$ have positive components, it follows that all states N_i and costates p_i are positive over $[0, T]$.

The case $2a_1 N_2 p_2 = 1$ leads to the singular control problems which cannot be excluded using only the first order necessary conditions .

The standard method to solve the problem is to find a numerical solution of the two point boundary value problem (TPBVP) which may be performed using Mohler's STVM [87], [103], semianalytical shooting algorithm [123] or gradient type methods [37], [38]. Among the other methods used to solve optimal control problems arising in chemotherapy scheduling we should mention control parametrization techniques developed by Teo and

Martin (see [86], [85]). Numerical studies do not exhibit the whole complexity of the problem. By finding invariance properties of the solutions to the TPBVP on the torus and formulating a special symmetry relation we have been able to classify [125] all the solutions to TPBVP problems. The analysis has indicated the irregularity of the optimal control problem [126], arising from multiplicity of solutions [99], existence of periodic trajectories [122] and existence of singular solutions [121]. The classification of complete trajectories enables to avoid a major disadvantage of a penalty method which has been used in formulation of the performance index i.e. no systematic way of choosing the value of weighting vector. Since final values of the costate vector are weighting parameters in the performance index the analysis of solutions for all possible boundary conditions allows for consideration of their sensitivity to the value chosen for r . The regions of r for which the multiple solutions of the optimal control problem may appear can also be easily assigned [128]. To avoid them, additional constraints may be imposed for the process of reducing the tumor burden. One of the reasonable requirements is that the tumor-population decreases faster than a given rate as proposed by Sundareshan and Fundakowski ([111], [112]) for multicompartamental models. This constraint may be for example satisfied for the periodic solutions [127].

Moreover recently singularity of optimal arcs was excluded with the use of high-order necessary conditions for optimality and sufficient conditions for optimal bang-bang strategies were found which enable to determine whether controls found by the use of Pontryagin maximum principle are at least locally optimal [80]. More precisely singular controls are calculated by differentiating the switching function in time until the control variable explicitly appears in the derivative, then finding the control which makes it equal to 0. For a single-input system which is linear in the control it is known [78] that the order of this derivative must be even, say $2k$, and k is called the order of the singular arc on the interval I . It is a necessary condition for optimality of a singular arc of order k , the so-called generalized Legendre-Clebsch condition [78], that

$$(-1)^k \frac{\partial}{\partial u} \frac{d^{2k}}{dt^{2k}} \frac{\partial H}{\partial u} \geq 0. \quad (23)$$

Note that the term $\frac{\partial H}{\partial u}$ in (23) represents the switching function for the problem. This framework directly applies to the 2-compartment model which has a scalar control. Elementary and direct calculations show that in this case singular arcs are of order 1 and that

$$\frac{\partial}{\partial u} \frac{d^2}{dt^2} \frac{\partial H}{\partial u} = 4a_1 a_2 > 0 \quad (24)$$

violating the Legendre-Clebsch condition. To develop sufficient conditions for local optimality field-theoretic concepts have been used. Essentially, if the flow of the system is a diffeomorphism away from the switching surfaces and if it crosses the switching surfaces

transversally, then using the method of characteristics a differentiable solution to the Hamilton-Jacobi-Bellman equation can be constructed [92]. This then implies optimality of the flow. The transversality condition:

$$\left| \frac{d}{dt}(N_2(t_k)p_1(t_k)) \right| + 2a_2N_2(t_k)S(t_k) > 0 \quad (25)$$

should be checked at each crossing of the switching surfaces at time t_k . To find $S(t_k)$ a matrix discrete Riccati type equation should be solved iteratively for the moments of control switchings. The sufficient conditions lead to yet another numerical algorithm for the optimal protocols design based on backward integration of the combined flow along the characteristics.

3.3 Three Compartments, Cell Arrest in S and Killing in G₂M

One of the conceivable strategies of protocol optimization, exploiting drug specificity, is to arrest cancer cells in the S phase [22], [49], and then release them at the moment when another G₂M specific anticancer drug has the maximum killing potential. This strategy may have the additional advantage of protecting the normal cells which would be less exposed to the second agent (e.g. due to less dispersion and faster transit through G₂M). The cell cycle model includes separate compartments for the G₁, S and G₂M phases [119], [123].

The control problem is to find $u(t) \in [0, 1]$ and $v(t) \in [v_m, 1]$ such that

$$\begin{aligned} \dot{N}_1(t) &= -a_1N_1(t) + 2u(t)a_3N_3(t), & N_1(0) &= N_{10} > 0, \\ \dot{N}_2(t) &= -v(t)a_2N_2(t) + a_1N_1(t), & N_2(0) &= N_{20} > 0, \\ \dot{N}_3(t) &= -a_3N_3(t) + v(t)a_2N_2(t), & N_3(0) &= N_{30} > 0. \end{aligned} \quad (26)$$

and the index

$$J = \sum_{i=1}^3 r_i N_i(T) + \int_0^T [1 - u(t)] dt, \quad (27)$$

is minimized. The bang-bang solution found from the maximum principle has the following form

$$u(t) = \begin{cases} 0; & 2a_3N_3(t)p_1(t) > 1 \\ 1; & 2a_3N_3(t)p_1(t) < 1 \end{cases} \quad (28)$$

$$v(t) = \begin{cases} v_m; & p_2(t) < p_3(t) \\ 1; & p_2(t) > p_3(t) \end{cases} \quad (29)$$

where the costate vector satisfies the following set of equations,

$$\begin{aligned}\dot{p}_1(t) &= a_1(p_1(t) - p_2(t)), & p_1(T) &= r_1, \\ \dot{p}_2(t) &= a_2(p_2(t) - p_3(t))v(t), & p_2(T) &= r_2, \\ \dot{p}_3(t) &= a_3(p_3(t) - 2p_1(t))u(t), & p_3(T) &= r_3,\end{aligned}\tag{30}$$

The arising TPBVP may be once more treated numerically [38] by the gradient method in the way similar as for two-compartmental models. Analytical treatment becomes much more complicated since the problem could not be projected into the plane. But also in this case it is possible to eliminate singular controls as not optimal and formulate sufficient conditions for local optimality of bang-bang strategies [81]. In this case the generalized Legendre-Clebsch condition (23) still applies to the first control u if we freeze the second control v . Assuming v is constant, it can be shown that a singular control u must be of order 2, but again (23) is violated. Direct, but longer calculations verify that

$$\frac{\partial}{\partial u} \frac{d^4}{dt^4} \frac{\partial H}{\partial u} = -12a_1a_2a_3^2v(a_1 + a_2v)p_1(t)N_2(t) < 0.\tag{31}$$

Furthermore, if the control v is singular on an interval I , then it can easily be seen that u also must be singular on I . In this case it is a necessary condition for optimality, the so-called Goh condition [78], that on I we have

$$\frac{\partial}{\partial v} \frac{d}{dt} \frac{\partial H}{\partial u} \equiv 0.\tag{32}$$

However, a direct calculation gives

$$\frac{\partial}{\partial v} \frac{d}{dt} \frac{\partial H}{\partial u} = 2a_2a_3p_1(t)N_2(t) > 0\tag{33}$$

violating the Goh-condition. Derivation of the sufficient conditions of local optimality for bang-bang strategies follows the similar line as for the two-compartment model. Of course transversality conditions should be checked for both switching controls and to do this we are led to a system of discrete Riccati equations which should be iterated in all considered switching moments for both control variables. An additional assumption, formulated only for technical reasons, is that there are no simultaneous switchings for both controls.

3.4 Three Compartments, Cell Recruitment from G_0 and Killing in G_2M

One of the major problems in chemotherapy of some leukemias is constituted by the large residuum of dormant G_0 cells which are not sensitive to most cytotoxic agents. It became

recently possible to efficiently recruit these cells into the cycle using cytokines [5], [132], substances playing a role in the regulation of normal hemopoiesis. Then, a cytotoxic agent may be used. To model such a system, we use separate compartments for the G_0 , G_1 and $S + G_2M$ phases, numbered 0, 1 and 2 [128].

The control problem is to find $u(t) \in [0, 1]$ and $y(t) \in [1, y_m]$ such that

$$\begin{aligned} \dot{N}_0(t) &= -ya_0N_0(t) + 2b_0u(t)a_2N_2(t), & N_0(0) &= N_{00} > 0, \\ \dot{N}_1(t) &= -a_1N_1(t) + ya_0N_0(t) + 2b_1u(t)a_2N_2(t), & N_1(0) &= N_{10} > 0. \\ \dot{N}_2(t) &= -a_2N_2(t) + a_1N_1(t), & N_2(0) &= N_{20} > 0. \end{aligned} \quad (34)$$

where b_0 and b_1 are the probabilities of the daughter cell entering after division G_0 and G_1 , respectively. The index to be minimized is

$$J = \sum_{i=0}^2 r_i N_i(T) + \int_0^T [1 - u(t)] dt. \quad (35)$$

An interesting special case is $N_{00} > 0, N_{10} = N_{20} = 0, r_0 > 0, r_1 = r_2 = 0$, i.e. all the cells concentrated in G_0 at the onset of the therapy, the principal purpose of the therapy being minimization of their eventual number based on presumption that this will yield the eventual demise of the whole population.

The bang-bang solution found using the maximum principle has the following form

$$u(t) = \begin{cases} 0; & 2a_2N_2(t)(b_0p_0(t) - b_1p_1(t)) > 1 \\ 1; & 2a_2N_2(t)(b_0p_0(t) - b_1p_1(t)) < 1 \end{cases} \quad (36)$$

$$y(t) = \begin{cases} 1; & p_1(t) > p_0(t) \\ y_m; & p_1(t) < p_0(t) \end{cases} \quad (37)$$

where the costate vector satisfies the following set of equations,

$$\begin{aligned} \dot{p}_0(t) &= y(t)a_0(p_0(t) - p_1(t)), & p_0(T) &= r_0, \\ \dot{p}_1(t) &= a_1(p_1(t) - p_2(t)), & p_1(T) &= r_1, \\ \dot{p}_2(t) &= a_2[p_2(t) - 2u(t)(b_0p_0(t) + b_1p_1(t))], & p_2(T) &= r_2, \end{aligned} \quad (38)$$

The arising two-point boundary value problem (TPBVP) expressed by equations (34) and (36)-(38) is formally similar to the TPBVP expressed by equations (26) and (28)-(30) and leads to the same mathematical problems. In this case the analysis of singular arcs is slightly more cumbersome [124]. For constant y we have:

$$\frac{\partial}{\partial u} \frac{d^2}{dt^2} \frac{\partial H}{\partial u} = 4a_1a_2b_1 > 0 \quad (39)$$

violating the Legendre-Clebsch condition. These calculations therefore exclude the optimality of singular controls u when y is constant. It might still be possible, however, that y is singular and not constant over any subinterval $J \subset I$. In this case u also must be singular on I . For this example the Goh condition is actually satisfied but after some simple but lengthy calculations we have found that it is possible only for $u = 0.5$ and leads to constant N_i s and p_i s and in consequence to constant y but it, in turn, implies violation of the Legendre-Clebsch condition. In [124] it is shown also that the sufficient conditions for bang-bang strategies in this case and in more general class of multicompartment models could be derived similarly as for the previously considered cases. In this case the numerical results can be obtained by the same gradient method [127].

The interesting finding [128] is that our results do not change at least in qualitative sense if instead of modeling and minimizing cancer population we rather decide to model and maximize population of cells in critical normal tissues while maximizing the cumulative negative cytotoxic effect.

4 Evolution of Resistance by Gene Amplification

4.1 Biological Background

The amount of DNA per cell remains constant from one generation to another because during each cell cycle the entire content of DNA is duplicated and then at each mitotic cell division the DNA is evenly apportioned to two daughter cells. However, recent experimental evidence shows that for a fraction of DNA, its amount per cell and its structure undergo continuous change.

One way the genome of cancer cells may rapidly evolve is by an increase in copy number of specific genes, referred to as *gene amplification*. Gene amplification can be enhanced by conditions that interfere with DNA synthesis and is increased in some mutant and tumor cells. Increased number of gene copies may produce an increased amount of gene products and, in tumor cells, confer resistance to chemotherapeutic drugs. Amplification of oncogenes has been observed in many human tumor cells and also may confer a growth advantage on cells which overproduce the oncogene products (for an overview see surveys by Stark [109] and Windle and Wahl [146]).

In the classical experiments of Schimke and his coworkers [23, 61], the anticancer drugs served to select for cells with amplified genes. In some of cell lines, when the selective agent was removed, the cells with amplified genes gradually disappeared from the population. The stochastic mechanism leading to this reversal is discussed in more detail further in this subsection. It was observed that in such cases the amplified genes were located on extrachromosomal fragments of DNA called *Double Minute Chromosomes (DM's)*. In other cases, the amplification was stable, ie. persisted after the selective agent had been

removed. In such cases, the amplified genes usually are located on elongated chromosome arms. The most regular of these elongated arms exhibit a regular band structure (the so called *Homogeneously Staining Regions* or *HSR's*), but other less regular structures are also observed. They are either caused by reintegration of extrachromosomal genes as proposed by Windle, Wahl and coworkers [145], or they arise by a separate mechanism as proposed by Stark and coworkers [107]. Mathematical models show that depending on circumstances each of the two variants of stable amplification is plausible [7], [70] (see also a critique by Harnevo and Agur [54]).

4.2 Probabilistic Modeling of Unstable and Stable Gene Amplification

4.2.1 Unstable Gene Amplification

Summary of Observations. In some populations of cells with double minute chromosomes, both the increased drug resistance and the increase in number of gene copies are *reversible*. The classical experiment confirming this includes transferring the resistant cell line into drug-free medium, [23, 61], where cells gradually lose resistance to the drug by losing extra gene copies. In these experiments, the dihydrofolate reductase (DHFR) gene was amplified after exposing murine 3T6 cells [23] or mouse sarcoma S-180 cells [61] to Methotrexate (MTX).

The population distribution of numbers of gene copies per cell can be estimated by flow cytometry after staining gene products. In the experiments mentioned, [61], two features of these distributions are notable. (1) As expected, the proportions of resistant cells (with amplified genes) decrease with time. (2) Less obvious, the shape of the distribution of the number of gene copies limited to the resistant cell subpopulation seems to remain stable during the loss of resistance.

The Branching Random Walk and Other Models. A mathematical model of unstable drug resistance should take into account (1) stochastic changes in number of gene copies from one generation to another and (2) the stochastic variability in cell lifetimes. One stochastic process which accomodates both (1) and (2) is a random walk superimposed on the time-continuous branching process [6] of cell proliferation, ie. a *branching random walk* [71]. We consider a population of abstract particles of types $j = 0, 1, 2, \dots$:

1. The lifespans of all particles are independent identically distributed exponential random variables with mean $1/\lambda$.
2. At the moment of death, a particle of type $j \geq 1$ produces two progeny particles each belonging to type $j + 1$ with probability b , to type $j - 1$ with probability d ,

and to type j with probability $1 - b - d$. A particle of type $j = 0$ produces two progeny of type 0.

3. The process is initiated at time $t = 0$ by a single particle of given type i .

The simplest models of gene amplification in [68] and [71] assume the above process. Cells with 2^{j-1} gene copies are said to belong to type j (with 0 gene copies, to type 0). The parameters b and d are the probabilities of gene *amplification* and *deamplification*, respectively.

One of the properties of Markov processes with absorbing states is the possibility of existence of the quasi-stationary distributions. In intuitive terms, the unabsorbed part of the probability mass of the process, while constantly shrinking, approaches a limit if it is properly normed. The Yaglom theorem for subcritical branching processes [6] can be quoted as an example. It is this property that explains the apparent stability of distributions of gene copy number per cell in the resistant subpopulation, placed in the non-selective medium.

Indeed, let us assume the time-discrete equivalent of the model outlined above (as in [68]). Let us denote by X_n the *type* (see the definition above) of the cell in the n th generation of a randomly selected lineage ($n \geq 0$). Then, $\{X_n, n \geq 0\}$ is a time-discrete Markov chain with the following transition matrix

$$\begin{bmatrix} 1 & 0 & 0 & 0 & 0 & \dots \\ d & (1-b-d) & b & 0 & 0 & \dots \\ 0 & d & (1-b-d) & b & 0 & \dots \\ 0 & 0 & d & (1-b-d) & b & \dots \\ 0 & 0 & 0 & \ddots & \ddots & \dots \\ \dots & \dots & \dots & \dots & \dots & \dots \end{bmatrix}. \quad (40)$$

$\{X_n, n \geq 0\}$ is a random walk with an absorbing boundary at 0. Let us denote by p_i^n the probability that the cell type is i in the n th cell generation,

$$p_i^n = \Pr\{X_n = i\}. \quad (41)$$

We consider the limit properties, as n tends to infinity, of the gene extinction probability p_0^n , and of the set of conditional probabilities,

$$c_i^n = \Pr\{X_n = i | X_n \neq 0\} = p_i^n / (1 - p_0^n). \quad (42)$$

that the cell type is i , provided the gene is not extinct. We limit ourselves to the most important subcritical case, $d > b$, and assume $X_0 = 1$. Let $v_n(s) = \sum_{i \geq 1} c_i^n s^i$ be the conditional probability generating function (*p.g.f.*) of X_n , given $X_n \neq 0$ and let $E_\infty =$

$\lim_{n \rightarrow \infty} \sum_{i \geq 1} 2^{i-1} c_i^n$ be the expected number of gene copies as $n \rightarrow \infty$. We have the following result (Theorem 1 in [68]).

Suppose that $d > b$. Then

$$(1 - p_0^n) \sim K h^n / \sqrt{n^3}, \quad (43)$$

as $n \rightarrow \infty$, where $h = 1 - (\sqrt{d} - \sqrt{b})^2$, $K = [1 - (\sqrt{d} - \sqrt{b})^2]^{3/2} \sqrt[4]{d} / [2\sqrt{\pi}(\sqrt{d} - \sqrt{b})^2 \sqrt[4]{b^3}]$, and

$$v_n(s) \rightarrow v(s) \equiv s \left(\frac{\sqrt{d} - \sqrt{b}}{\sqrt{d} - s\sqrt{b}} \right)^2, \quad (44)$$

and consequently,

$$E_\infty = v(2)/2 = \left(\frac{\sqrt{d} - \sqrt{b}}{\sqrt{d} - 2\sqrt{b}} \right)^2, \quad (45)$$

as $n \rightarrow \infty$.

This result can be derived from Theorem 2 and Lemma 3 in [95]. As stated above, it assures existence of the limit distribution of the number of gene copies per cell in selective conditions.

4.2.2 Stable Amplification

Summary of Observations. In the experimental system of Windle, Wahl and co-workers [145], the amplification of the DHFR gene was observed in a Chinese Hamster Ovary (CHO) cell line, which contained only a single DHFR gene. Cells were challenged by MTX. Amplified genes residing on extrachromosomal elements were observed in cell cultures 8-9 generations later, while predominantly chromosomally amplified genes were seen after about 30 generations (only these two time points were investigated). This can be interpreted as an indication that some extrachromosomal elements containing amplified gene copy numbers are eventually reintegrated into chromosomes.

Mathematical Model and Its Predictions. In the model devised to reproduce these observations [70], the basic indivisible unit which serves as the template for the production of additional gene copies is *the amplicon*, which contains at least one copy of the target gene. The size of such structures could range from submicroscopic to an entire arm of a chromosome and they may be circular or linear. The *acentric (replicating) element (ARE)* is understood to be an extrachromosomal molecular structure containing one or more amplicons but no centromere. A centromere is required for regular segregation to daughter cells. *The reintegrated element (RE)* is the ARE after it has reintegrated into a chromosome.

The following processes are considered in the model: (a) change in the number of ARE's per cell, (b) change in the number of amplicons per ARE, and (c) reintegration of ARE's into chromosomes.

Types of elements: ARE's containing $i = 1, 2, \dots$ amplicons, and RE's containing $i = 1, 2, \dots$ amplicons. In each cell generation, with probability a , the ARE containing i amplicons replicates to yield a product with $2i$ amplicon copies. The catenated replication product then dissociates producing two acentric molecules. This process results in a pair of molecules containing, respectively, j and $2i - j$ amplicons, where $j = 1, \dots, 2i - 1$. It is assumed that the probability of each pair $(j, 2i - j)$ is the same, equal to $1/(2i - 1)$. The molecules segregate so that they both go to the same daughter cell with probability q , and go to different daughter cells with probability $1 - q$. With probability g , the ARE with i amplicon copies replicates to yield a product with $2i$ amplicon copies, but this replication product does not dissociate. It then goes with equal probability to one of the two daughters. With probability $c = 1 - (a + g)$, per cell generation, the ARE containing i copies of the amplicon, integrates into a chromosome with a centromere and then replicates and segregates with the chromosome. This results in each daughter cell containing an equal number of RE copies. The probability of reintegration is $c = 1 - (a + g)$.

We may formally define the following random variables:

- $X_n^i(\omega)$, the number of ARE's with i copies of the amplicon, in the n -th cell generation,
- $Y_n^i(\omega)$, the number of RE's with i copies of the amplicon, in the n -th cell generation.

The sequence $\{(X_n^1, Y_n^1), (X_n^2, Y_n^2), \dots\}, n = 0, 1, 2, \dots\}$, is a *multitype Galton-Watson process with a denumerable infinity of particle types*. [66].

Modeling the expected values of the process enables reproducing the main features of Wahl's experiments: (1) The initial increase in number of acentric elements per cell, and the number of amplicon copies per acentric element. (2) Subsequent decrease of the number of ARE's per cell, as they become reintegrated. (3) Eventual emergence of a population of cells containing only integrated elements with a spectrum of amplicon copy numbers at one or more chromosomal locations.

5 Control Under Evolving Resistance

5.1 Clonal Resistance / Simulation of Gene Amplification

Resistance to antineoplastic drugs has been a major impediment to the successful treatment of cancer. Recent studies suggest that several mechanisms are responsible for the

emergence of drug resistance and that high levels of resistance and poor prognosis are strongly associated with gene or oncogene amplification.

In recent years the problem of drug resistance in cancer has been mathematically attacked by many authors. The first series of models were devised by Coldman and Goldie [30] (for an overview see the book by Wheldon [142]). Underlying these models was the assumption that drug resistance in cancer results from a single mutational event whose probability is constant and independent of external constraints. The model was generalized to describe evolution of resistance to a number of agents. If it is assumed that multiple resistance is the most important thing to avoid in the course of chemotherapy, then the resulting recommendation is to alternate treatments effective against strains resistant to single agents, as frequently as possible [46].

Harnevo and Agur [53] introduce a model which treats the emergence of drug resistance as a dynamic process rather than a single event. Using this model, based on their previous works [52], they focus on gene amplification as one of the mechanisms that may lead to drug resistance, and show how changes in the underlying assumptions affect the predictions about treatment efficacy. The mathematical modeling results suggest that under gene amplification dynamics with high amplification probability, protocols involving frequent low-concentration dosing may result in the rapid evolution of large fully resistant residual tumors; the same total doses divided into high-concentration doses applied at larger intervals may result in partial or complete remission. This last recommendation is an alternative to that of Coldman and Goldie.

Another suggestion is that treatment prognosis may be largely improved if cells bearing a large number of gene copy number have high mortality. Therefore, it may be interesting to examine the possibility of incorporating in the treatment an agent (hypothetical, at present) that increases the mortality of cells carrying highly amplified genomes.

5.2 Mathematical Model and Optimization of Control Under Evolving Resistance

In this subsection we present an infinite system of differential equations which may be used to model controlling a cell population with evolving drug resistance caused by gene amplification or other mechanisms. The model is general enough to accommodate different interpretations (see further on). The model is motivated by a representation in the terms of the branching random walk [71], but it also can be understood as a mathematical variation of the model used by Harnevo and Agur in [53].

The hypotheses are as follows: We consider a population of cells of types $i = 0, 1, 2, \dots$. Cells of type 0 are sensitive to the agent, whereas the types $i = 1, 2, \dots$ consist of resistant cells of increasing level of resistance (for example, with increased number of DHFR or CAD gene copies per cell).

1. The lifespans of all cells are independent identically distributed exponential random variables with means $1/\lambda_i$ for cells of type i .
2. A cell of type $i \geq 1$ may mutate in a short time interval $(t, t + dt)$ into a type $i + 1$ cell with probability $b_i dt + o(dt)$ and into type $i - 1$ cell with probability $d_i dt + o(dt)$. A cell of type $i = 0$ may mutate in a short time interval $(t, t + dt)$ into a type 1 cell with probability $\alpha dt + o(dt)$, where α is several orders of magnitude smaller than any of b_i s or d_i s.
3. The chemotherapeutic agent affects cells of different types differently. It is assumed that its action results in fraction u_i of ineffective divisions in cells of type i .
4. The process is initiated at time $t = 0$ by a population of cells of different types.

The postulated relationship for the rate α of the primary amplification event can be written as follows

$$\alpha \ll \min(d_i, b_i), \quad i \geq 1. \quad (46)$$

In view of the subcriticality of the process in Subsection 4.2.1, it seems reasonable to assume

$$d_i > b_i, \quad i \geq 1. \quad (47)$$

If we denote $N_i(t)$ the expected number of cells of type i at time t , we obtain the following infinite system of differential equations:

$$\left\{ \begin{array}{l} \dot{N}_0(t) = [1 - 2u_0(t)]\lambda_0 N_0(t) - \alpha N_0(t) + d_1 N_1(t), \\ \dot{N}_1(t) = [1 - 2u_1(t)]\lambda_1 N_1(t) - (b_1 + d_1)N_1(t) + d_2 N_2(t) + \alpha N_0(t), \\ \dots \\ \dot{N}_i(t) = [1 - 2u_i(t)]\lambda_i N_i(t) - (b_i + d_i)N_i(t) + d_{i+1} N_{i+1}(t) + b_{i-1} N_{i-1}(t), \quad i \geq 2, \\ \dots \end{array} \right. \quad (48)$$

Also, the following relationships between b_i s and d_i s seem to be justified by the intuition that cells overloaded with amplified gene copies may acquire new copies with more difficulty and lose them easier:

$$d_{i+1} \geq d_i, \quad b_{i+1} \leq b_i, \quad i \geq 1. \quad (49)$$

As postulated by Schimke (see eg.[23, 61]), cells with more copies of the drug resistance gene may proliferate slower, ie.

$$\lambda_{i+1} \leq \lambda_i, \quad i \geq 0. \quad (50)$$

In the simplest case, in which the resistant cells are totally insensitive to drug's action, and if we ignore differences between parameters of cells of different type, i.e.

$$u_0 = u, u_i = 0, i \geq 1, \text{ and } b_i = b, d_i = d, \lambda_i = \lambda, \quad i \geq 0,$$

the system (48) assumes the following form:

$$\left\{ \begin{array}{l} \dot{N}_0(t) = [1 - 2u(t)]\lambda N_0(t) - \alpha N_0(t) + dN_1(t), \\ \dot{N}_1(t) = \lambda N_1(t) - (b + d)N_1(t) + dN_2(t) + \alpha N_0(t), \\ \dots \\ \dot{N}_i(t) = \lambda N_i(t) - (b + d)N_i(t) + dN_{i+1}(t) + bN_{i-1}(t), \quad i \geq 2, \\ \dots \end{array} \right. \quad (51)$$

Note that in this model d, b denote respective intensivities and not probabilities as it was in matrix (40). Model (51) may be used to find the optimal control which minimizes an appropriate performance index, eg.

$$J = \sum_{i \geq 0} r_i N_i(T) + \int_0^T u(t) dt, \quad (52)$$

Necessary conditions for optimal control could be found using the maximum principle in its abstract version (see e.g [91]). They are formally similar to those obtained for respective finite dimensional problems. To solve them efficiently, finite approximation of the system should be used. The other possibility conferred for example in [131] is to reconfigure the model (51) into the equivalent integro-differential form.

The model (48) can describe dynamics of any cell population stratified into a sequence of subcompartments (types) with different kinetics, with fluxes of cells between these subcompartments. For example, the amplified gene may not confer resistance but allow the cell to proliferate faster (or to alter the pattern in a more complex way, see eg. [67]). This might be the case if it is an oncogene. Then we might assume all u_i s equal and $\lambda_{i+1} \geq \lambda_i$.

Systems of the type (48) and (51) are not as straightforward as finite dimensional systems of differential equations. However, at least in simpler cases, their asymptotic

behavior can be characterized quite precisely. As an example, let us consider the following system,

$$\left\{ \begin{array}{l} \dot{N}_1(t) = \lambda N_1(t) - (b+d)N_1(t) + dN_2(t), \\ \dots \\ \dot{N}_i(t) = \lambda N_i(t) - (b+d)N_i(t) + dN_{i+1}(t) + bN_{i-1}(t), \quad i \geq 2, \\ \dots \end{array} \right. \quad (53)$$

This is a model of population of cells in which the sensitive cells are instantly annihilated, and there is no influx of new resistant cells. Let us denote $N(t) = \sum_{i \geq 1} N_i(t)$. We have the following result obtained using the methods of [71]. Suppose that $N_i(0) = \delta_{i1}$ and $d \neq b$. Then

$$N(t) = e^{\lambda t} - e^{\lambda t} \sqrt{d/b} \int_0^t \frac{I_1(2\sqrt{bd}\tau)}{\tau} e^{-(b+d)\tau} d\tau. \quad (54)$$

where $I_1(t)$ is the modified Bessel function of order 1 [1]. Moreover,

$$\begin{aligned} N(t) \sim & \left[1 - \frac{\min(b, d)}{b} \right] e^{\lambda t} \\ & + \frac{d}{2\sqrt{\pi} \sqrt[4]{(bd)^3 (\sqrt{d} - \sqrt{b})^2}} t^{-3/2} e^{[\lambda - (\sqrt{d} - \sqrt{b})^2]t}, \end{aligned} \quad (55)$$

as $t \rightarrow \infty$.

This result may be derived using Laplace transform machinery. Denote Laplace transforms of $N_1(t)$ and $N(t)$ by $\hat{N}_1(s)$ and $\hat{N}(s)$, i.e. $\hat{N}_1(s) = \int_0^\infty N_1(t) e^{-st} dt$, $\hat{N}(s) = \int_0^\infty N(t) e^{-st} dt$. Then:

$$\hat{N}_1(s) = \frac{s - \lambda + b + d - \sqrt{(s - \lambda + b + d)^2 - 4bd}}{2bd}. \quad (56)$$

$$\hat{N}(s) = -\frac{s - \lambda + b + d - \sqrt{(s - \lambda + b + d)^2 - 4bd}}{2b(s - \lambda)} + \frac{1}{s - \lambda}. \quad (57)$$

Note that (see [36]).

$$(s + b + d) - \sqrt{(s + b + d)^2 - 4bd}$$

is the Laplace transform of

$$(2\sqrt{bd}/t)I_1(2\sqrt{bdt}) \exp[(-b-d)t],$$

Using this we obtain time functions $N_1(t)$ (see below) and $N(t)$ (see(54)) by performing inverse Laplace transforms of (56) and (57):

$$N_1(t) = \frac{I_1(2\sqrt{bdt})}{t\sqrt{bd}} e^{[\lambda-(b+d)]t}, \quad (58)$$

Using the equations (58) and (54) we can analyze the behavior of functions $N_1(t)$ and $N(t)$ as t approaches infinity. The formulae for the asymptotic expansions of $I_1(t)$ and $\int_0^t \frac{I_1(2\sqrt{bd\tau})}{\tau} e^{-(b+d)\tau} d\tau$ given in Lemma 1 and Lemma 2 in the paper [71] (obtained via the Laplace method for integrals [24]) lead to the asymptotic expansions for $N_1(t)$:

$$N_1(t) \sim \frac{1}{2\sqrt{\pi} \sqrt[4]{(bd)^3}} t^{-3/2} e^{[\lambda-(\sqrt{d}-\sqrt{b})^2]t}, \quad (59)$$

and $N(t)$ (see(55)). From (59) and (55), the condition that both $N_1(t)$ and $N(t)$ converge exponentially to zero, as $t \rightarrow \infty$, is:

$$\sqrt{d} - \sqrt{b} > \sqrt{\lambda}. \quad (60)$$

If (60) is not satisfied, then we have two possibilities. If $\sqrt{d} - \sqrt{b} < \sqrt{\lambda}$, then the solution diverges exponentially to infinity. If $\sqrt{d} - \sqrt{b} = \sqrt{\lambda}$, then both N_1 and N still converge to zero. However, the convergence is not exponential [73]. Let us notice that the term at $e^{\lambda t}$ in the asymptotic expansion disappears if $d > b$. This separates the behavior in the supercritical case from that in the subcritical case. In the former, the resistant population grows exponentially. In the latter, it decays only if $\sqrt{d} - \sqrt{b} > \sqrt{\lambda}$. The same reasoning could be repeated for initial conditions of the form $N_i(0) = \delta_{ik}$ for $k > 1$ leading to similar expression for $N(t)$ and similar asymptotic behaviors (see [129]). Since the system is linear the condition (60) is necessary and sufficient for eradication of the resistant subpopulation for any initial conditions with final support. If λ is considered the only parameter affected by control, this means that unless somehow accessed by cytostatics, the resistant subpopulation may maintain itself even in the subcritical case. The analysis presented in [98] leads to a conclusion that being stable for any finite initial condition, the solution to (53) can diverge if we allow initial conditions with infinitely many nonzero elements. The factor that determines stability of the solution in this case is that the rate of decay of successive elements of the initial vector must be faster than $[b+d-\lambda-\sqrt{(b+d-\lambda)^2-4bd}]^{-1}$ [73]. Biologically, the results for initial conditions with infinite support can be interpreted as follows: Suppose that a significant subpopulation of

resistant cells reached large number of gene copies. Then this population is a persistent source of proliferating malignant cells, much more difficult to eradicate than it would be the case under a finite mutation model [129].

Asymptotic analysis of this model results in some understanding of its dynamics . This, in our opinion, is the first step towards a more rigorous mathematical treatment of the dynamics of drug resistance and/or metastasis . In this case the system is decomposed onto two parts one which includes only the sensitive subpopulation modeled by the first equation of (51) and the remaining infinite dimensional part of this model which describes the drug resistant subpopulation. The results on the asymptotic behavior of the drug resistant subpopulation not supplied from the sensitive compartment, combined with the Laplace transforms machinery and theory of closed loop systems with positive feedback (Nyquist theorem) enable analysis of an asymptotic behaviour of the whole population modeled by (51) for the case of constant dosage drug administration. More precisely using Nyquist theorem [147] for systems with irrational transfer functions we found [98] that the conditions which ensure asymptotic eradication of the overall cancer population by continuous constant drug dosage in subcritical case are given by (60), and

$$u > \frac{\alpha}{d(-b + d - \lambda + \sqrt{(b + d - \lambda)^2 - 4bd})} + \frac{1}{2}. \quad (61)$$

Moreover the representation of the cancer population in the form of the closed loop system with positive feedback enables reformulation of the infinite dimensional model of dynamics into the integro-differential form [130]. This in turn leads to formulation of optimization problem for protocols design in the presence of drug resistance [131] in the form treatable by an abstract Pontryagin maximum principle in the version given by [10]. In the simplest case when the therapy is initiated when there are no resistant clones yet and weights $r_i = r$ for $i = 0$ and r_1 elsewhere we have the following reformulation of the model (51) and the performance index (52):

$$\begin{aligned} \dot{N}_0(t) &= d\alpha \int_0^t \phi(t-s)N_0(s)ds - \alpha N_0(t) + (1 - 2u(t))\lambda N_0(t), & t > 0 \\ N_0(t) &= N_0(0), & t \leq 0 \end{aligned} \quad (62)$$

$$J = rN_0(T) + \int_0^T [\alpha r_1 N(T-t)N_0(t) + u(t)]dt, \quad (63)$$

where $\phi(t)$ is given by formula (58) for $N_1(t)$ and $N(t)$ by formula (54). Using the abstract maximum principle we obtain the following necessary conditions for optimal control $u(t)$ and costate $p(t)$ [131]:

$$u(t) = \begin{cases} 0; & 2\lambda N_0(t)p(t) < 1 \\ 1; & 2\lambda N_0(t)p(t) > 1 \end{cases} \quad (64)$$

$$\dot{p} = - \left[d\alpha \int_t^T \phi(s-t)p(s)ds + p(t)((1-2u)\lambda - \alpha) + \alpha r_1 N(T-t) \right], \quad p(T) = r \quad (65)$$

This analysis does not take into account singular solutions for which in this case we have no results dealing with their elimination. The gradient method proposed for two and three compartment models [37], [38] may be however used with some technical modifications [105] to find optimal bang-bang and suboptimal periodic solutions. The models in this section are based on the hypothesis that the process of gene amplification can be described by a branching random walk, as in [71]. A more realistic process, including proliferation of gene copies between cell divisions, as well as random segregation of gene copies between daughter cells, is described in [66].

6 Remarks on Estimation of Parameters

6.1 Estimation of Cell Cycle Parameters and Drug Action in Cultured Cells

Much work has been done on estimation of cell cycle transit times and of the fractions of cells arrested and/or killed by drugs in cultured normal and transformed cells. The most systematic series of experiments and measurements known to us has been carried out in the 1980's in the laboratory of Darzynkiewicz, Traganos and their coworkers in the Memorial Sloan-Kettering Cancer Center and in the New York Medical College. A number of cultured cell lines and a variety of drugs in various concentrations were evaluated by these researchers using flow cytometric techniques.

The following account is based on reviews [33] and [138] which include numerous original references. The stathmokinetic or “metaphase arrest” technique consists of blocking cell division by an external agent (usually a drug, eg. vincristine or colchicine). The cells gradually accumulate in mitosis, emptying the postmitotic phase G_1 and with time also the S phase. Flow cytometry allows precise measurements of the fractions of cells residing in different cell cycle phase. The pattern of cell accumulation in mitosis (M) depends on the kinetic parameters of the cell cycle and is used for estimation of these parameters. Exit dynamics from G_1 and transit dynamics through S and G_2 and their subcompartments can be used to characterize very precisely both unperturbed and perturbed cell cycle parameters. A true arsenal of methods have been developed to analyze the stathmokinetic data. Application of these methods allow quantification of the cell-cycle-phase action of many agents.

One of the interesting findings was the existence of *aftereffects* in the action of many cytotoxic agents. The action of these drugs may extend beyond the span of a single cell

cycle. For example, cells blocked in the S-phase of the cell cycle and then released from the block, may proceed apparently normally towards mitosis but then fail to divide, or divide but not be able to complete the subsequent round of DNA replication. In some experiments it was possible to trace the fates of individual cells and conclude that their nuclear material divided but the cytoplasmic contents failed to separate.

The consequence of the aftereffects is that it may be difficult to infer the long-term effects of cytotoxic drugs based on short term experiments like the stathmokinetic experiment. One way of testing this assertion is to carry out both types of experiments, short term and long term, subjecting cells to the action of the same concentration of the same drug. We may then estimate the parameters of the cell cycle and of drug action based on the short-term experiment, substitute them into a mathematical model and try to predict the results of the long-term experiment. This program has been carried out in a study by Kimmel and Traganos [74]. Using two concentrations of an experimental anticancer agent CI-921, it was found that while cell cycle estimates based on stathmokinetic experiments did not differ for these two concentrations, the effects of continuous 24-hour exposure to the drug were completely different. Only the effects of low concentration continuous exposure were predicted by the mathematical model using estimates from stathmokinetics.

Analogous aftereffects following irradiation of cells were discovered by Kooi and co-workers [76].

Another long term program of estimation based on flow cytometry has been carried out by Bertuzzi and Gandolfi and their collaborators at IASI in Rome and European Institute of Oncology. For many years their interest has been concentrated mainly on reducing errors of estimates using procedures like regularization, but also simulations and modeling of cytotoxic action on the cell cycle (see eg. [20], [18]). Moreover their estimation procedures is also used for modelling the cell kinetic characteristics of *in vivo* experimental tumors [18]. Recently they have also been concentrated on the modeling of tumor cords (see e.g. [17], [19]). Estimation of parameters for such models results in better understanding of nongenetic reasons of drug resistance.

6.2 Estimation of Cell Cycle Parameters in Cells from Human Tumors

Recently, much research has been carried out on estimation of cell kinetic parameters of cells in human tumors *in vivo*. Basically, the procedure consists of injecting the tumor with a labeling compound selectively incorporated by cells synthesizing DNA and then, after removal of the tumor, of following the “relative movement” of the labeled cells through the S-phase. The method was introduced by Begg and co-workers [14]. They followed-up with a series of application papers [15], [55], [13]. Their main interest is on the potential of pre-treatment cell kinetic parameters to predict outcome in cancer

patients treated by radiotherapy. One of the findings is that pretreatment cell kinetic measurements carried out using flow cytometry only provide a relatively weak predictor of outcome after radiotherapy e.g. [56, 11], [12]. One of possible reasons of this negative result is the change of the parameters during radiotherapy and the effect of breaks in the therapy for the cell cycle parameters. In our collaborative research with colleagues from MCS Institute of Oncology in Gliwice we have observed such phenomena while analysing similar material (neck and head cancers) [136, 135]. It is difficult to extend these results for chemotherapeutic effects.

A series of mathematical refinements and applications of the Begg's method have been introduced by R.A. White of the Biomathematics Department at the M.D. Anderson Institute in Houston [143], [144]. Of a number of papers by other authors, we quote one devoted to cell cycle kinetics of leukemias [101]. There are also available many papers on parameter estimation of cell cycle kinetics for experimental human tumors transplanted in mice or other animals (see e.g. previously mentioned [18], where results for human ovarian carcinoma transplanted in mice are presented).

New possibilities in cell cycle parameter estimation both *in vitro* and *in vivo* are now established by DNA microarray technology. By processing the data on expression of thousands of genes in different time samples one can identify the dynamics behavior of the analysed cell populations. There have been a number of bioinformatical and biomathematical tools developed to cope with such analysis. Among them we refer to the one based on Singular Value Decomposition which seems to give especially promising results (see, [104] and references therein).

6.3 Estimation of Rates of Emergence and Evolution of Resistance

Based on gene amplification studies, there exist three phases in the evolution of resistance process:

- The relatively rare *primary event*, ie. the establishment of the founder cell of the resistant clone containing at least one unstable copy of the target gene (the probability of this event, per cell division, corresponds to the ratio α/λ in our Equ. (48)).
- Subsequent *amplification* and *deamplification* events, occurring at high rates compared to α/λ , resulting from instability of the amplified gene (the probabilities of these events, per cell division, correspond to the ratios b_i/λ and d_i/λ in Equ. (48)).
- Possible *stabilization* of the resistant phenotype, by integration of the amplified gene in the chromosomal structures (no counterpart in Equ (48)).

The numerical values of the probabilities of gene amplification and deamplification can be estimated based on data in [23] and [61]. The probabilities of deamplification (d) are of the order of 0.10 in both cases, while the probabilities of amplification (b) are about 5 times lower. The process is strongly subcritical. This means among others that in the absence of selection, the amplified phenotype disappears from the population. It can be revived by rare *primary events*, such as amplification of extrachromosomal genes following a deletion of the target gene from the chromosome arm (see further on). The primary tool with which the primary rate was estimated, is the Luria-Delbrück’s fluctuation analysis [82]. It consists of finding an experimental distribution of the number of mutant (ie. resistant) colonies in cell populations cultured for a number of generations, and fitting it to the theoretical distribution derived based on a branching process-type model of proliferation and mutation. A number of researchers carried out this procedure for mutation to drug resistance (by gene amplification and other means) [137], [88], [89], [139], obtaining estimates of the mutation probabilities, per cell division, in the range from 10^{-8} to 10^{-6} , with generally higher estimates for tumorigenic than for “normal” cells. The data from the above papers were re-analyzed in a paper by Kimmel and Axelrod [69], using a two-stage model of mutation. Although the estimates of primary event probabilities remain mostly unchanged, the probabilities of second stage forward and backward mutation are much higher, comparable to the estimates of amplification and deamplification probabilities obtained in [68] and in [71] (of the order of 0.02 and 0.10, respectively, as mentioned above).

The classical explanation for the loss of resistance in cells with amplified DNA in extrachromosomal elements is that in the absence of selective pressure cells with extra gene copies grow slower and are outgrown by the sensitive cells [61]. Our model assumes a purely stochastic mechanism. A combination of two mechanisms is likely. For further comments, see [68].

Estimates of the reintegration probabilities are provided in the study by Kimmel, Axelrod and Wahl [70] where fitting the model to data from [145] makes possible to estimate its parameters a , g , c , and q . The best fitting values are $a = 0.780$, $g = 0.195$, $c = 0.025$, and $q = 0.9$. The rate of integration c and the probability of cosegregation q are biologically important parameters. A high rate of cosegregation might have clinical implications in the sense of making some cells more sensitive to chemotherapy.

7 Discussion

In this paper we discuss the cell-cycle-phase dependence of cytotoxic drug action and drug resistance in the context of optimization of cancer chemotherapy.

Attempts at optimization of cancer chemotherapy using optimal control theory have a

long history ([113] is a review by Swan). The methods include the maximum principle both in the discrete [8], [64] (with parameters taken from [51], [63]) and continuous versions [116], [103], for a variety of models and performance indices. The idea has been criticized many times (see e.g. [134], [142]). Only simplest concepts have won attention in the medical world. These include the clonal resistance model [46] and the kinetic resistance theory by Norton and Simon [93].

The simplest cell-cycle-phase dependent models of chemotherapy can be classified based on the number of compartments and types of drug action modeled. In all these models the attempts at finding optimal controls are confounded by the presence of singular and periodic trajectories, and multiple solutions but recently singular trajectories are excluded and sufficient conditions for strong local optimality are found for a class of bang-bang strategies. Moreover, efficient numerical methods have been developed. In simpler cases, it is possible to provide exhaustive classification of solutions. We have reviewed analytic and computational methods which are available. All these attempts have to be viewed with caution, because of the existence of *aftereffects* in the action of many cytotoxic agents. The action of these drugs may extend beyond the span of a single cell cycle. For example, cells blocked in the S-phase of the cell cycle and then released from the block, may proceed apparently normally towards mitosis but then fail to divide, or divide but not be able to complete the subsequent round of DNA replication. If such effects are substantial, they are likely to disrupt or complicate the resonances. As indicated for example in [96], [97], the aftereffects due to accumulation of drugs (in this case MTX) result in great interindividual differences of the effectiveness of treatment.

The consequence of the aftereffects is that it may be difficult to infer the long-term effects of cytotoxic drugs based on short term experiments like the stathmokinetic experiment. One way of testing this assertion is to carry out both types of experiments, short term and long term, subjecting cells to the action of the same concentration of the same drug. We may then estimate the parameters of the cell cycle and of drug action based on the short-term experiment, substitute them into a mathematical model and try to predict the results of the long-term experiment. Constructing mathematical models including aftereffects is possible but leads to notational and computational complications. Essentially, part of cells released from the direct action of the drug, are redirected into a different cell cycle in which a large part of them are either permanently arrested or die. This leads to models with increased dimensionality. It seems, however, that it still is possible to place the models in the general class (P) of multicompartmental models discussed in [124].

Concerning the emergence of drug resistance, we have presented the problem in the framework of gene amplification, although much of what is written may apply to different mechanisms which are reversible and occur at high frequency. We have defined a mathematical model which can be used to pose and solve an optimal chemotherapy prob-

lem under evolving resistance. We have shown some results regarding dynamics of this model and techniques used to find solutions for optimization of chemotherapy protocols. Analysis of variants of this model should give insight into possible scheduling strategies of chemotherapy in the situations when drug resistance is a significant factor. It is possible, for example, using the decomposition technique presented in the paper to include both effects of drug resistance and phase specificity or partial resistance of different subpopulations both in singleagent or multiagent chemotherapy(see [106]).

All possible applications of the mathematical models of chemotherapy are contingent on our ability to estimate their parameters. There has been a progress in that direction, particularly concerning precise estimation of drug action in culture and estimation of cell cycle parameters of tumor cells *in vivo*. Also, more is known about the mutation rates of evolving resistant cell clones.

The traditional area of application of ideas of cell synchronization, recruitment and rational scheduling of chemotherapy including multidrug protocols, is in treatment of leukemias. It is there where the cell-cycle-phase dependent optimization is potentially useful.

The emergence of resistant clones is a universal problem of chemotherapy. However, it seems that its most acute manifestation is the failure to treat metastasis. A part of this problem is the imperfect effectiveness of adjuvant chemotherapy as the tool to eradicate undetectable micrometastases. In view of toxicity of anticancer drugs, optimal scheduling is potentially useful in improving these treatments. Yet another challenge discussed recently in modeling of cancer chemotherapy is related to antiangiogenic therapy (see e.g. [94, 141, 41, 44]). Although the process of vascularization is strongly distributed (see e.g. [58], [27], [45]) some simple two or three compartmental models have been also proposed ([102, 40]). The advantage of using antiangiogenic therapy is in resistancy to drug resistance [62]. It is due to the fact that it is directed against non-malignant endothelial cells which are genetically stable. Methodology described in our paper can be efficiently extended for this class of nonlinear models. Moreover decomposition for finite dimensional controlled part and infinite dimensional uncontrolled part used by us in analysis and optimization of drug resistance evolution and therapy may be applied to the more complicated models of angiogenesis with distributed parameter compartments.

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