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# Qualitative analysis of controlled drug resistance model - inverse Laplace and semigroup approach

by

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**Abstract:** In this paper we study some properties of infinite models of the controlled evolution of drug resistance. We combine asymptotic techniques used in previous studies of similar models with methods of control theory and of semigroup theory. It enables us to find conditions for stability of the model both when the sensitive population is annihilated and when there exists a permanent influx from the sensitive compartment into the drug resistant one. The conditions are expressed in terms of relationships between amplification and deamplification ratios as well as average life times of cells and intensity of anticancer drug action.

**Keywords:** stability, infinite dimensional systems, biomedical models, branching processes

## 1. Introduction

### 1.1. 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 in which the genome of cancer cells may rapidly evolve is by increasing the copy

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, 1993, and Windle and Wahl, 1992). In the classical experiments of Schimke and his coworkers, Brown, Beverly, Schimke (1981), Kaufman, Brown, Schimke (1981), the anticancer drugs served to select 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 section. 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, i.e. 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 and Wahl (1992), or they arise by a separate mechanism as proposed by Stark (1993). Mathematical models show that depending on circumstances each of the two variants of stable amplification is plausible, Axelrod, Baggerly, Kimmel (1993), (see also a critique by Harnevo, Agur, 1992).

## 1.2. Probabilistic modeling of unstable and stable gene amplification

### 1.2.1. 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, Brown, Beverly, Schimke (1981), Kaufman, Brown, Schimke (1981), confirming this, includes transfering the resistant cell line into drug-free medium, 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 or mouse sarcoma S-180 cells 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, 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.

tance 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 of cell proliferation, ie. a *branching random walk*. We consider a population of abstract particles of types $j = 0, 1, 2, \ldots$:

1. 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.
2. The process is initiated at time $t = 0$ by a single particle of given type $i$.

The simplest models of gene amplification in Kimmel, Axelrod (1990) 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. The moment of death mentioned in point 1. represents in this case the moment of cell division. 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, Athreya, Ney (1972), 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.

**Model versus data.** The numerical values of the probabilities of gene amplification and deamplification can be estimated based on data in Brown, Beverly, Schimke (1981), Kaufman, Brown, Schimke (1981). 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, in particular, 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 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. Our model assumes a purely stochastic mechanism.

### 1.2.2. Stable amplification

**Summary of observations.** In the experimental system of Windle, Wahl (1993), the amplification of the DHFR gene was observed in a Chinese Hamster

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, Kimmel, Axelrod, Wahl (1992), 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 AREs per cell, (b) change in the number of amplicons per ARE, and (c) reintegration of AREs into chromosomes. Types of elements: AREs containing $i = 1, 2, \ldots$ amplicons, and REs containing $i = 1, 2, \ldots$ amplicons. In each cell generation, with probability $\tilde{a}$, 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 $j = 1, \ldots, 2i - 1$. 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 $\delta$, and go to different daughter cells with probability $1 - \delta$. With probability $\tilde{b}$ 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 $\tilde{c} = 1 - (\tilde{a} + \tilde{b})$, 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. Thus $\tilde{c}$ is the probability of reintegration.

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 our previous papers, Świerniak, Kimmel, Polański (1996), and Kimmel, Świerniak, Polański (1998), we have analyzed the time-continuous branching random walk models of gene amplification. The evolution of the drug resistance of tumor cells is modeled, as in Kimmel, Stivers (1994), using infinite system of

tant subpopulation of tumor cells in the case when the sensitive population is anihilated and the process has been initialized by the nonzero resistant subpopulation of type 1. Moreover, the case of initial conditions with infinitely many nonzero elements has been considered. It represents the situation in which a significant subpopulation of resistant cells has reached a large number of gene copies and becomes a persistent source of proliferating malignant cells.

## 2. Model of drug resistance

We consider a population of neoplastic cells stratified into subpopulations of cells of different types, labeled by numbers $i = 0, 1, 2, \ldots$. If the biological process considered is gene amplification, then cells of different types are identified with different numbers of copies of the drug resistance gene and differing levels of resistance. Cells of type 0, with no copies of the gene, are sensitive to the cytostatic agent. Due to the mutational event the sensitive cell of type 0 can acquire a copy of gene that makes it resistant to the agent. Likewise, the division of resistant cells can result in the change of the number of gene copies. The resistant subpopulation consists of cells of types $i = 1, 2, \ldots$. The probability of mutational event in a sensitive cell is of several orders smaller than the probability of the change in number of gene copies in a resistant cell. Since we do not limit the number of gene copies per cell, the number of different cell types is denumerably infinite. The hypotheses are as follows:

1. The lifespans of all cells are independent exponentially distributed 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 $\alpha$ is several orders of magnitude smaller than any of $b_i$s or $d_i$s, i.e.
$$\alpha << \min(d_i, b_i), \quad i \geq 1. \tag{1}$$
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.

If we denote by $N_i(t)$ the expected number of cells of type $i$ at time $t$, and we assume the simplest case, in which the resistant cells are insensitive to drug's action, and there are no differences between parameters of cells of different type

has the following form:

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

It is worth noting that parameters $b$ and $d$ in this model denote probability intensities and not the probabilities as in subsection 1.2, but we use the same notation since that their meaning is relevant.

## 3. Stability of simplified model

Systems of the type (2) 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:

$$\begin{cases} \dot{N}_1(t) = \lambda N_1(t) - (b+d)N_1(t) + dN_2(t), \\ \quad \dots \\ \dot{N}_i(t) = \lambda N_i(t) - (b+d)N_i(t) + dN_{i+1}(t) + bN_{i-1}(t), i \geq 2, \\ \quad \dots \end{cases} \tag{3}$$

This is a model of a population in which the sensitive cells are instantly annihilated, and there is no influx of new resistant cells. Let us take $N(t) = \sum_{i\geq 1} N_i(t)$. Suppose that $N_i(0) = \delta_{i1}$ and $d \neq b$. Denote Laplace transforms of $N_i(t)$ and $N(t)$ by $\hat{N}_i(p)$ and $\hat{N}(p)$, respectively, $\hat{N}_i(p) = \int_0^\infty N_i(t)e^{-pt}dt$, $\hat{N}(p) = \int_0^\infty N(t)e^{-pt}dt$. Then we have (for $i = 1$):

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

and

$$\hat{N}(p) = -\frac{p-\lambda+b+d-\sqrt{(p-\lambda+b+d)^2-4bd}}{2b(p-\lambda)} + \frac{1}{p-\lambda}. \tag{5}$$

This result can be found by considering the generating function of the Laplace transforms of functions $N_i(t)$:

$$\hat{\mathcal{N}}(p,s) = \sum_{i\geq 1} \hat{N}_i(p)s^i, \ s \in [0,1], \tag{6}$$

We also take

$$\mathcal{N}_0(s) = \sum_{i\geq 1} N_i(0)s^i. \tag{7}$$

By performing necessary manipulations in the system (2), we obtain

$$\hat{\mathcal{N}}(p,s)\left(p-\lambda+b+d-bs-\frac{d}{s}\right) = \mathcal{N}_0(s) - d\hat{N}_1(p), \tag{8}$$

or

$$\hat{\mathcal{N}}(p,s) = \frac{s[\mathcal{N}_0(s) - d\hat{N}_1(p)]}{-bs^2+(p-\lambda+b+d)s-d}. \tag{9}$$

From the analyticity of $\hat{\mathcal{N}}(p,s)$, we conclude that the numerator of (9) has to be equal to 0 if $s = s_1(p)$, where $s_1(p)$ is the root of the denominator which satisfies $s_1(p) \in (0,1]$ when $p \in [\lambda, \infty)$:

$$s_1(p) = \frac{p-\lambda+b+d-\sqrt{(p-\lambda+b+d)^2-4bd}}{2b}. \tag{10}$$

Therefore, we have

$$\hat{N}_1(p) = \frac{\mathcal{N}_0[s_1(p)]}{d}. \tag{11}$$

Taking $\mathcal{N}_0(s) = s$ as assumed and substituting (11) into (9) we obtain, if $s = 1$,

$$\hat{\mathcal{N}}(p,1) = -\frac{p-\lambda+b+d-\sqrt{(p-\lambda+b+d)^2-4bd}}{2b(p-\lambda)} + \frac{1}{p-\lambda}. \tag{12}$$

$\hat{\mathcal{N}}(p,1)$ is the Laplace transform of $N(t) = \sum_{i\geq 1} N_i(t)$. Note that

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

is the Laplace transform of

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

where $I_1(t)$ is the modified Bessel function of order 1

We have the following result from Kimmel, Świerniak, Polański (1998) obtained using the methods of Kimmel, Stivers (1994), based on the inverse

THEOREM 3.1 *Under previously made assumptions:*

$$N(t) = e^{\lambda t} - e^{\lambda t}\sqrt{d/b}\int_0^t \frac{I_1(2\sqrt{bd}\tau)}{\tau} e^{-(b+d)\tau} d\tau. \tag{13}$$

*Moreover,*

$$N(t) \sim \left[1 - \frac{\min(b,d)}{b}\right] e^{\lambda t} + \frac{d}{2\sqrt{\pi}\sqrt[4]{(bd)^3}(\sqrt{d}-\sqrt{b})^2} t^{-3/2} e^{[\lambda-(\sqrt{d}-\sqrt{b})^2]t}, \tag{14}$$

*as* $t \to \infty$.

Let us note 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 $\sqrt{d} - \sqrt{b} > \sqrt{\lambda}$. If $\lambda$ 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 asymptotic behavior of the resistant subpopulation was analyzed for the case where the initial condition contained only one nonzero element $N_1(0) = 1$, while $N_i(0) = 0$, $i > 1$. It is possible to extend that approach to the case of two or more non-zero elements. The number of nonzero initial conditions must be, however, finite.

Now we will allow infinitely many elements $N_i(0)$ not equal to 0. We will formulate the stability analysis problem in the terms of spectral properties of an appropriate operator.

Let us assume the following conditions which guarantee, based on the previous results, that the solution starting from $N_1(0) = 1$, $N_i(0) = 0$, $i > 1$ decays exponentially to zero, as $t \to \infty$:

$$d > b, \tag{15}$$

$$\sqrt{d} - \sqrt{b} > \sqrt{\lambda}. \tag{16}$$

It seems most appropriate to choose the initial condition from space $l_1$ of the absolutely summable infinite sequences with the norm

$$|\mathbf{N}| = \sum_{i \geq 1} |N_i|. \tag{17}$$

However, the $l_1$-norm may grow to infinity for some solutions.

This suggests formulating the problem in a different space, included in $l_1$, which imposes additional conditions on the rate of decay of $N_i$'s. Let us write system (3) in the form

where $\mathbf{N}(t)$ belongs to a Banach space $B$ and $\mathbf{A}$ is now a linear operator mapping $B$ into itself. The form of $\mathbf{A}$ is implied by system of equations (3) and may be written as

$$\mathbf{A} = (\lambda - b - d)\mathbf{I} + d\mathbf{F} + b\mathbf{P}, \tag{19}$$

where $\mathbf{I}, \mathbf{F}, \mathbf{P}$ are identity, left and right shifting operators respectively. We will consider $B$ being the space $l_1^R$ of infinite sequences summable exponentially with base $R > 1$, i.e.

$$\mathbf{N} \in l_1^R \iff |\mathbf{N}|_R = \sum_{i \geq 1} |N_i| R^i < \infty. \tag{20}$$

The $l_1^R$ spaces are Banach spaces with norms given by (20). The elements of sequences that belong to $l_1^R$ are, generally, complex numbers. It can be verified that $\mathbf{A}$ maps each of the $l_1^R$ spaces into itself, and that it is a bounded linear operator.

It is well known that the asymptotic behavior of the norm of solution is related to the spectral properties of the (bounded) operator $\mathbf{A}$ (see e.g. Bensoussan, DaPrato, Delfour, Mitter, 1992),

$$|\mathbf{N}(t)|_R \xrightarrow{\exp} 0 \iff \sup\{\Re(\mu) : \ \mu \in \sigma_R(\mathbf{A})\} < 0, \tag{21}$$

where $\sigma_R(\mathbf{A})$ denotes the spectrum of $\mathbf{A}$ generally different in each of the $l_1^R$ spaces and $\Re(\mu)$ is a real part of complex number $\mu$.

To examine this spectrum, we write the following equation,

$$\mathbf{L} = (\mu\mathbf{I} - \mathbf{A})\mathbf{N}, \tag{22}$$

where $\mathbf{L}, \mathbf{N} \in l_1^R$. Calculating the generating functions for both sides of Eqn. (22) we obtain,

$$\mathcal{L}(s) = [-bs + (\mu - \lambda + b + d) - \frac{d}{s}]\mathcal{N}(s) + dN_1, \tag{23}$$

or

$$\begin{aligned} s\mathcal{L}(s) - sdN_1 &= [-bs^2 + (\mu - \lambda + b + d)s - d]\mathcal{N}(s) \\ &= -b[s - s_1(\mu)][s - s_2(\mu)]\mathcal{N}(s), \end{aligned} \tag{24}$$

where $\mathcal{N}(s)$ and $\mathcal{L}(s)$ are generating functions of sequences $\mathbf{N}$ and $\mathbf{L}$. The location of the roots $s_1(\mu)$ and $s_2(\mu)$ (compare with (10)) decides whether $\mu$ belongs to the spectrum or to the resolvent of operator $\mathbf{A}$. We have:

THEOREM 3.2 *Let us set, without loss of generality,* $|s_1(\mu)| \leq |s_2(\mu)|$.

*Then:*

1. *If* $|s_1(\mu)| < R$ *and* $|s_2(\mu)| > R$, *then* $\mu$ *belongs to the resolvent set of* $\mathbf{A}$.

3. *In the remaining cases, $\mu$ belongs to the non-point part of the spectrum of* $\mathbf{A}$.

Carrying out appropriate evaluations, we obtain:

1. $\sup\{\Re(\mu) : \mu \in \sigma_R(\mathbf{A})\} \geq 0$ iff $R \in [1, s_1(0)]$,
2. $\sup\{\Re(\mu) : \mu \in \sigma_R(\mathbf{A})\} < 0$ iff $R \in (s_1(0), s_2(0))$,
3. $\sup\{\Re(\mu) : \mu \in \sigma_R(\mathbf{A})\} \geq 0$ iff $R \in [s_2(0), \infty)$.

The above three conditions classify the properties of the system (3) in Banach spaces $l_1^R$ with different values of $R$. The system is exponentially stable ($\sup\{\Re(\mu) : \mu \in \sigma_R(\mathbf{A})\} < 0$) for the values of the base parameter $R$ in the range $R \in (s_1(0), s_2(0))$. Then, the Banach spaces $l_1^R$ with $R \in (s_1(0), s_2(0))$ are stable state spaces for the system (3). Choosing initial conditions from these spaces results in solutions converging to zero. It might seem surprising that further increase of the value $R$ ($R \in [s_2(0), \infty)$) results in the loss of the exponential stability property. However, one should remember that exponential stability is expressed "relative" to the norm in $l_1^R$ which changes with $R$.

The same result may be found using the theory of semigroups. In this case it is convenient to perform all considerations in $l_1$ space for the modified operator $\tilde{\mathbf{A}} = (\lambda - b - d)\mathbf{I} + d\mathbf{F}/R + b\mathbf{P}R$. Moreover from the theorem due to Sklyar-Shirman-Lyubich-Phong-Arendt-Batty (see Arendt, Batty, 1988) it results that in the case when

$$\sqrt{d} - \sqrt{b} = \sqrt{\lambda} \tag{25}$$

the semigroup generated by $\mathbf{A}$ decays when time increases but not necessarily exponentially as it was in the case when (16) held. Moreover, if (16) is satisfied then for $R = s_1(0)$ the semigroup is asymptotically stable and for $R = s_2(0)$ it is not asymptotically stable.

## 4. Stability of the model of evolution of drug resistance

The analysis of the asymptotic behavior of the resistant subpopulation was carried out under the assumption that there was no external cell influx. However, the techniques of Laplace transformation enable including such possibility. Assuming that the initial condition for (1) is zero, $N_i(0) = 0, i = 1, 2.....$ and using the calculations similar to those previously performed in Świerniak, Kimmel, Polański (1996), we find that the function $N_1(t)$ is a convolution of two functions: $\alpha N_0(t)$, and the free solution for the first state variable $N_1(t)$ of equation (2) (being also the impulse transfer function of the system) in the case analysed in theorem 1. Equivalently, using the Laplace transforms $\hat{N}_0(p) = \int_0^\infty N_0(t)e^{-pt}dt$, $\hat{N}_1(p) = \int_0^\infty N_1(t)e^{-pt}dt$, we have

$$\hat{} \qquad p - \lambda + b + d - \sqrt{(p - \lambda + b + d)^2 - 4bd}$$

In other words,

$$\alpha \frac{p - \lambda + b + d - \sqrt{(p - \lambda + b + d)^2 - 4bd}}{2bd}$$

is the transfer function of the system with input $N_0$ and output $N_1$.

The equations of the asymptotic model without cell influx have the same form as a part of the drug resistance model (1). Consequently, we can "cut" the model equations (1) into two parts. The first part is the single differential equation that describes the population of sensitive cells. Taking into account the cell flows between two parts of the model we conclude that we may consider the drug resistance evolution model as a feedback system. We confine our analysis to the case of the constant dosage of a cytotoxic agent. Then we may treat the value $u$ that appears in the first equation of (1) as a constant parameter. Analyzing the first equation of the model with input function $N_1(t)$ and output $N_0(t)$ we can calculate that

$$\hat{N}_0(p) = \frac{d}{p + \alpha - (1 - 2u)\lambda} \hat{N}_1(p). \tag{27}$$

With the transfer function of the sensitive subpopulation compartment and the transfer function of the resistant compartment one can represent the flow chart of the system as the feedback loop .

The loop transfer function for the system is

$$K(p) = \frac{\alpha[p - \lambda + b + d - \sqrt{(p - \lambda + b + d)^2 - 4bd}]}{2b[p + \alpha - (1 - 2u)\lambda]}. \tag{28}$$

The frequency response of (28) is:

$$K(j\omega) = K(p) \mid_{p=j\omega} . \tag{29}$$

Note that the feedback loop is a positive one. Then the Nyquist type theorem for infinite dimensional systems states that the feedback loop is stable if both systems defined by transfer functions are stable, and

$$\sup_{\omega} | K(j\omega) | < 1. \tag{30}$$

By the analysis of the relation (28), it can be verified that the supremum in (30) is achieved for $\omega = 0$, with the condition that both transfer functions define stable systems. As a result we can state the following conditions of exponential stability of the drug resistance model (1):

$$\sqrt{d} - \sqrt{b} > \sqrt{\lambda}. \tag{31}$$

(Stability condition for resistant population).

$$\alpha > 0.5 + \frac{\alpha}{} \tag{32}$$

(Stability condition for the feedback loop). The stability condition of the sensitive compartment ( $u > 0.5 - \frac{\alpha}{2\lambda}$ ) is included in the condition (32). Condition (32) makes sense only if (31) is satisfied. Inequality (32) gives the smallest value $u$ which ensures elimination of cancer cell population. This value increases if both $d-b-\lambda$ and $\sqrt{d}-\sqrt{b}-\sqrt{\lambda}$ are small, i.e. the denominator in (32) is close to zero. The condition that both $d-b-\lambda$ and $\sqrt{d}-\sqrt{b}-\sqrt{\lambda}$ are close to zero is satisfied if either $b$ or $\lambda$ is small.

## 5. Discussion

In this paper we have studied the properties of evolution of a model of drug resistance in the framework of gene amplification, although much of what is written may apply to different mechanisms which are reversible and occur at high frequencies. We have defined a mathematical model which can be used to pose and solve a chemotherapy problem under evolving resistance. Analysis of the variants of this model should give insight into possible scheduling strategies of chemotherapy in the situations when drug resistance is a significant factor. The model we analyzed is defined by infinite systems of linear differential equations. The solution to that system describes the expected numbers of cells of different types. Assuming constant parameters, we obtained analytical closed-form results. The study of the model of the resistant subpopulation without the external influx reveals that subcriticality of the amplification process ($b < d$ ) is not sufficient for extinction of the resistant cells. The population of resistant cells becomes extinct only if stronger condition $\sqrt{d}-\sqrt{b} > \sqrt{\lambda}$ is satisfied.

The further analysis leads to a conclusion that while being stable for any finite initial condition, the solution can diverge if we allow initial conditions with infinitely many nonzero elements. The factor that determines stability of the solution in this case is the rate of decay of successive elements of the initial vector. The rate of decrease must be faster than $[b+d-\lambda-\sqrt{(b+d-\lambda)^2-4bd}]^{-1}$. Moreover, the analysis of the case in which the resistant compartment is persistently supplied by the sensitive compartment has enabled to find the minimal intensity of the cytotoxic drug action which ensures stability of system behaviour providing that the drug resistant population satisfies previously discussed conditions. Using a model with infinite number of cell types is a useful idealization. The number of gene copies which determines the possible number of cell types, can be very large in tumor cells. In view of this, our approach is justified in the way similar to that of many probabilistic models of cell populations where infinite tails of distributions are assumed. For the infinite dimensional model studied in this paper it was possible to obtain analytical results concerning stability of evolution of populations of cancer cells. 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

scheduling is potentially useful in improving these treatments.

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