Drug resistance as a dynamic process in a model for multistep gene amplification under various levels of selection stringency

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Summary. 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 but that high levels of resistance and poor prognosis are strongly associated with gene or oncogene amplification. In this report we describe a probabilistic model for gene amplification in a tumor that grows under various drug protocols. The model is new in that it treats drug resistance as a dynamic process and examines specific assumptions about the underlying molecular events. Using this model, we specify the conditions for the emergence of drug-resistant mutants prior to selection as well as the relationship between the stringency of the selecting environment and the characteristics of the resultant cellular phenotype.

Introduction

DNA sequence amplification if frequently observed in tumors and transformed cells, and it has been suggested that tumor cells have an abnormal capacity to amplify DNA with high frequency [8, 18, 26, 44, 49, 50]. Gene amplification (GA) is known to contribute to the generation of drug resistance and to have prognostic significance in several types of cancer. Amplification of the dihydrofolate reductase (*dhfr*) gene confers resistance to the chemotherapeutic drug methotrexate (MTX) [37, 38, 45, 46], and that of the P-glycoprotein (*mdr1*) gene and the gene for both components of ribonucleotide reductase is correlated with multidrug resistance [8, 14, 18, 26, 28, 31, 32, 44, 50].

Whereas in the past, multidrug resistance has been thought to be uniquely determined by P-glycoprotein GA,

currently it becomes more and more apparent that only high levels of clinical multidrug resistance are associated with the amplification of this gene; lower levels may be attributable to other molecular mechanisms, e.g., transcriptional activation [36, 39]. The role of DNA amplification in clinical multidrug resistance has perhaps been somewhat overestimated due to the use of rodents as a model. It has recently been suggested that the propensity for DNA amplification and for other mutational events characterizes rodent but not normal human cell lines (Schimke et al., submitted for publication).

The role of P-glycoprotein GA in multidrug resistance has yet to be fully clarified, yet aggressive clinical behavior, resistance to chemotherapy, and poor prognosis have clearly been demonstrated to be associated with oncogene amplification [11–13, 35, 40, 41, 44, 47]. In particular, clinical resistance to cisplatin and 5-fluorouracil (F-Fura) was shown to be concomitantly associated with a 2- to 4-fold increase in DNA copy number for deoxythymidine monophosphate (dTMP) synthase and *dhfr* as well as with the amplification of *myc* ($2\times$), *h-ras* ($4\times$), and *c-fos* ($15\times$) oncogenes. These results suggest that amplification of genes and oncogenes that confer drug resistance may be involved in the development of cisplatin resistance [34].

Experimental and clinical data indicate that the emergence of drug resistance due to GA is a dynamic process involving several molecular events whose probability may be sensitive to environmental constraints. To obtain an understanding of these complex dynamics, it may be useful to investigate the effect of various assumptions about the underlying events within the framework of a mathematical model. This task is taken up in the present work.

Presented below is a model for GA that assumes forward (amplification) or backward (deletion) changes in the gene-copy number of one or many genes (see Stark et al. [43] for review). For purposes of simplicity, we assume that GA is intrachromosomal and associated with cell division, with one daughter cell having an altered number of gene-copies and the other being identical to the mother cell. The assumption of symmetric amplification has been

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(a)

(b)
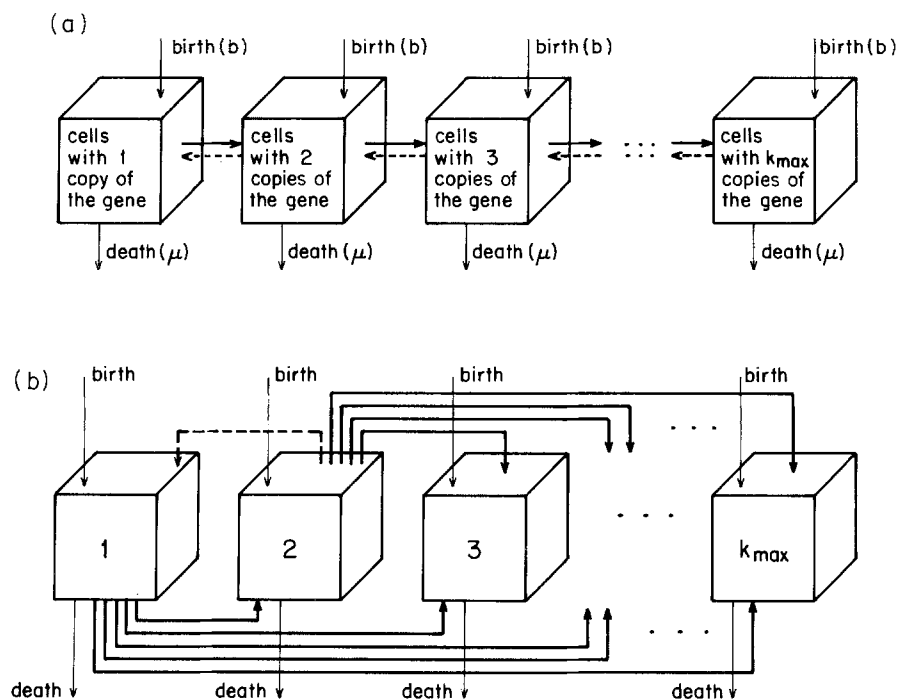

Fig. 1 a, b. Schematic representations of the *one-gene* GA process. (a) The *one-gene/one-copy* case accounting for an amplification process involving single-copy increments in one gene (see the GA model for further details). Parameters b and μ stand for the probability of cell proliferation and cell death, respectively. (b) The *one-gene/multicopy* case accounting for amplification increments of more than one copy per cell division

addressed elsewhere [25]; this assumption has no qualitative effect on the results presented below. In an investigation of a model in which gene amplification is initiated by unequal mitotic segregation of extrachromosomal DNA molecules (Harnevo and Agur, submitted for publication), we have specified the conditions under which such a process may accelerate the intrachromosomal GA dynamics described in the present report.

Our model assumes that GA can be spontaneous or drug-induced and that the number of gene copies that confer resistance may be dose-dependent; at higher concentrations, cells bearing a large number of copies may continue to be susceptible to the drug. This assumption relies on the observation that cells selected for resistance to a specific drug retain sensitivity to higher concentrations of the same drug, provided that resistance is conferred by a multistep process such as GA [37, 42]. Using this model, we investigate the effect of selection stringency on both genomic dynamics and treatment prognosis.

The GA model

The present model includes four general amplification processes. In the simplest process each step involves a single-copy increment in a single gene. In this case, denoted *one-gene/one-copy* (Fig. 1a), amplification is assumed to be initiated in one normal cell, i.e., in a cell that carries a single fuctional copy of this gene. This cell divides to yield two daughter cells, and there is some probability, α, that one of these cells will carry two copies of the gene. Division of the latter cell has the probability, α, of yielding one daughter cell bearing three copies of the gene and the probability, β, of losing a gene copy to yield a normal daughter cell carrying only one copy of the gene. The same process occurs independently in all cells such

that the cell population becomes heterogeneous with respect to the cellular gene-copy number. We allow for an upper bound on the number of gene copies in a cell; however, if this upper limit is very large, it will have no effect on the process. Cells divide with probability b, such that a fraction $1-b$ of the cells is quiescent, and they die with probability μ, which in the simplest form of the model is the same for all cells. In a more elaborate form of this process, we also allow for the possibility that cells bearing a large number of gene copies are less viable due to the higher fragility of larger chromosomes; in the latter case, μ becomes dependent on the gene-copy number. More elaborate GA dynamics are described in Fig. 1b, which illustrates the forward and backward amplification of one or more gene copies per cell division (denoted the *one-gene/multicopy* process).

In the amplification of two unlinked genes G_1 and G_2, denoted the *two-gene/one-copy* process (Fig. 2), a normal cell bears a single copy of the gene G_1 and a single copy of the gene G_2, and the above-described amplification process by single-copy increments occurs independently for each of the two genes. Thus, the cell population can carry any copy-number combination of genes G_1 and G_2. The fourth case of GA involves the amplification in two unlink genes (denoted the *two-gene/multicopy* process) of more than one gene copy per cell division.

To allow for the effect of cytotoxic drugs, we superimpose on the model an additional mortality factor. Thus, the drug's pharmacodynamics is described by (a) the proportion of sensitive cells eliminated by a given drug concentration; this proportion is denoted the fractional cell kill, d, and (b) the resistance threshold, i.e., the number of gene copies in a cell above which the cell is not affected by a given drug concentration. Two cases are studied with respect to the resistance threshold: (1) the threshold is independent of the fractional cell kill, d; and (2) the threshold
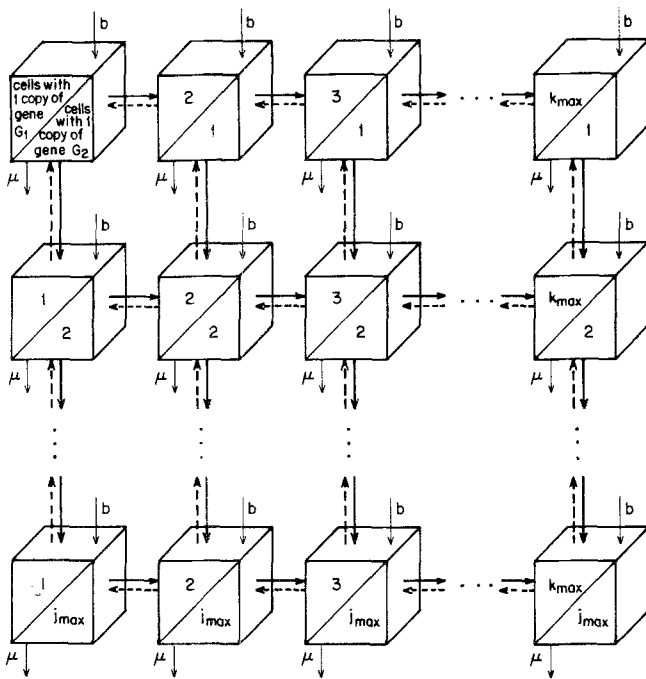
Fig. 2. Schematic representation of the *two-gene/one-copy* case for the amplification process of two genes, G_1 and G_2. Amplification occurs independently in each gene such that the population of cells originating from one normal cell can have any combination of 1 to k_{max} copies of G_1 and 1 to j_{max} copies of G_2
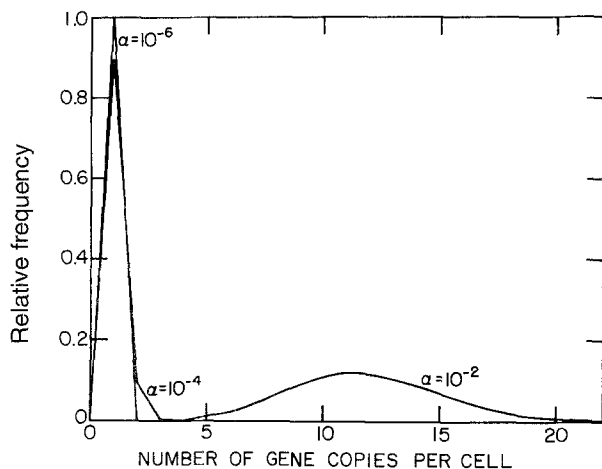

Fig. 3. The distribution of cells in a drug-free environment according to their gene-copy number for the *one-gene/one-copy* GA process. The relative frequency of cells in each copy-number compartment is calculated according to Eqs. 1 and 3. Distributions are depicted after 1000 generations of GA for amplification probabilities $\alpha = 10^{-2}$, 10^{-4}, and 10^{-6}

depends on d. These pharmacodynamic considerations render the computations quite elaborate. For example, when the amplification process involves two genes, the cell population should be divided into four "metacompartments" as follows:

1. Cells that are sensitive to both drugs (or drug combinations), i.e., cells whose gene-copy number lies below the resistance thresholds, k_c and j_c, of the genes G_1 and G_2, respectively

2. Cells that are sensitive to the first drug (or drug combination) but not to the second, i.e., cells bearing fewer than K_c copies of gene G_1 and more than j_c copies of gene G_2

3. Cells that are sensitive to the second drug (or drug combination) but not to the first, i.e., cells carrying more than k_c copies of gene G_1 and fewer than j_c copies of gene G_2

4. Cells that are resistant to both drugs (or drug combinations), i.e., cells bearing more than k_c and j_c copies of genes G_1 and G_2, respectively

The mathematical formulations for these four processes are described in detail in Appendix 1, whereas the general formula for the amplification of any number of genes in a cell can be trivially derived. Note that the present model can be degenerated to the one-gene model previously analyzed by Harnevo and Agur [25]. The formulation of the pharmacokinetic details is given in Appendix 2, whereas its use in computing the effect of GA dynamics on tumor size under a large range of protocols is described below.

Results

Using the model described above, we have computed the temporal changes in the mean number of tumor cells for a range of laboratory-estimated amplification probabilities [37, 42, 45] and for many different chemotherapeutic protocols. The time unit in our computations corresponds to one cell generation (one intermitotic interval); its transformation into calendar time therefore depends on the characteristic generation periods of individual cancers.

The distribution of cells according to their gene-copy number after 1000 cell generations of GA in a nonselective environment is presented in Fig. 3. This distribution appears to be strongly dependent on the amplification probability: for a realistic value of intrachromosomal GA probability, $\alpha = 10^{-4}$, the proportion of cells bearing amplified genes after 1000 generations of the process is roughly 1%; all of these cells carry two copies of the amplified gene. In contrast, if the GA probability is very large, $\alpha = 10^{-2}$, most of the cells will carry amplified genes, the average copy number being larger than 10. If the GA probability is very low, $\alpha = 10^{-6}$, the frequency of amplified genes will remain negligible after 1000 generations of the process. As demonstrated below, if the amplified gene confers drug resistance, selection stringency may have a strong effect on both the distribution of gene-copy numbers per cell and the treatment prognosis.

One-drug regimens

To study the effect of selection stringency on the evolution of drug resistance, we computed the changes in tumor size resulting from various drug protocols. Figure 4 compares the effect of two protocols: one in which the total drug concentration is given in large boluses (reflected in the fractional cell kill of $d = 0.99$) every seven cell generations, and one in which a roughly similar concentration is divided into many low-concentration ($d = 0.2$) doses that
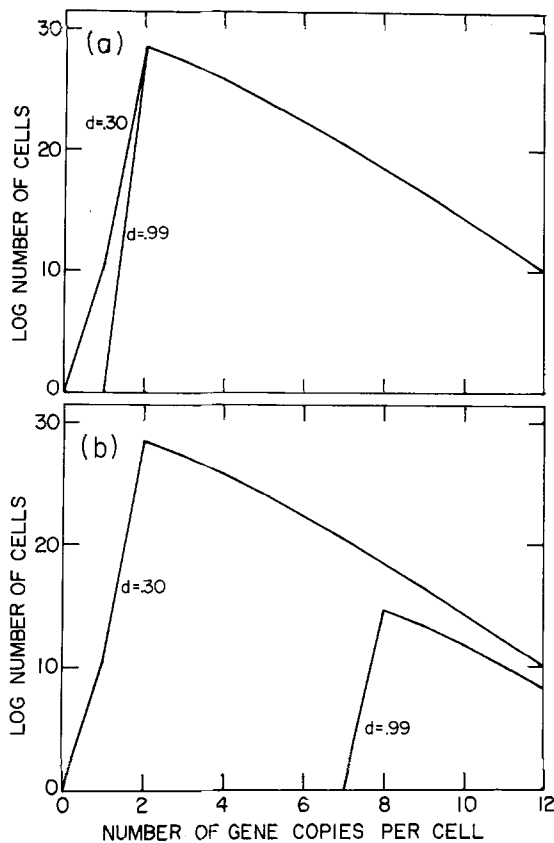
Fig. 4a, b. Effect of GA dynamics on treatment outcomes for the *one-gene/one-copy* case. Number of cells (log base 10 of the number of cells) is plotted as a function of gene-copy number for two drug protocols that differ in the fractional cell kill ($d = 0.3$ and $d = 0.99$). Treatment cycles are initiated after 500 and 900 cell generations; the number of doses used in each cycle are 100 for $d = 0.3$ and 30 for $d = 0.99$, and the amplification probability is $\alpha = 10^{-4}$. (a) Resistance threshold: $k_c = 1$ in both protocols. (b) Resistance threshold: $k_c = 1$ for $d = 0.3$ and $k_c = 7$ for $d = 0.99$
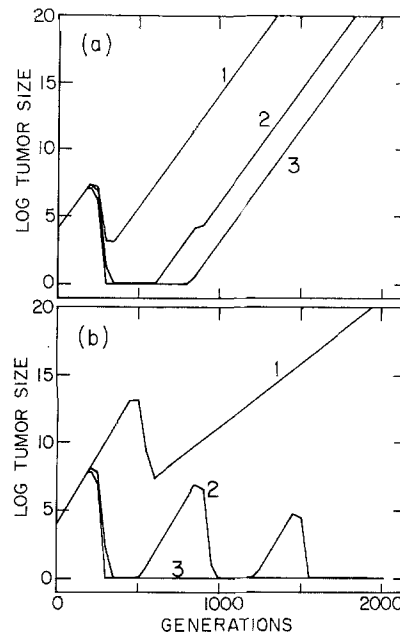

Fig. 5a, b. Effect of GA dynamics on treatment outcomes for the *one-gene/one-copy* case. Tumor size (log base 10 of the number of cells) is plotted as a function of time (cell generations) for three drug protocols that differ in fractional cell kill and in resistance threshold *1*, $d = 0.9$, and $k_c = 3$; *2*, $d = 0.95$ and $k_c = 6$; *3*, $d = 0.99$ and $k_c = 7$. Treatment cycles are initiated after 500 and 900 cell generations: the number of doses used in each cycle are 100, 35, and 30 for 1, 2, and 3, respectively; the amplification probability is $\alpha = 10^{-4}$. a Probability of natural cell mortality is constant. b Probability of natural cell mortality is copy-number-dependent

are applied at each cell generation. For this comparison, we computed the number of cells (log base 10) in each gene-copy-number compartment rather than calculating their relative frequency as shown in Fig. 3; the reason being that in the calculation of relative frequency, the drug's effect on the total cell population may cancel out its effect on the number of cells in particular compartments. The computations illustrated in Fig. 4a assume that all of the cells carrying more than one copy of the gene are resistant to any dose of the drug. Thus, the difference between the two protocols is reflected only in the fraction of normal cells eliminated. A dose that produces a high fractional cell kill ($d = 0.99$) when applied once weekly (if cell generation takes 1 day) for 30 weeks appears to cause somewhat higher mortality of normal cells than does a smaller dose ($d = 0.3$) applied every day for 43 weeks. Nevertheless, the overall effect on prognosis is expected to be similar since the differences between the two protocols in the total number of cells affected are small.

We also checked the assumption that higher drug concentrations may be associated not only with a larger rate of elimination of susceptible cells but also with a higher resistance threshold (Fig. 4b). As laboratory measurements for

the determination of relationships between the fractional cell kill, d, and the corresponding resistance thresholds, k_c, have not yet been obtained, we assumed arbitrary values for these relationships. Through these computations, we hoped to obtain a notion about the sensitivity of the prognosis to the drug schedule under the influence of a variety of such interactions. Our computations suggest that when drug resistance due to GA is involved, intermittent high-concentration dosing results in much greater mortality of tumor cells than does frequent low-concentration dosing (cf. Figs. 4b and 4a).

Figure 5 shows the changes observed in mean tumor size (log base 10) over time for various treatment protocols. These results suggest that the prognosis improves if the treatment causes a higher fractional cell kill per dose and if cell mortality is copy-number-dependent. From Fig. 5a it can be seen that an increase of as little as 5% in the fractional cell kill per dose may change the prognosis; if $d = 0.9$, all cells in the tumor become resistant during the course of the first treatment cycle; a second treatment cycle of the same protocol has no effect on the tumor, which reaches a detectable size in about 1000 cell generations (this corresponds to about 3 years if cell generation takes 1 day). In contrast, if the fractional cell kill is $d = 0.95$ and $d = 0.99$, a second treatment cycle considerably reduces the cell number and the tumor reaches a detectable size in about 1600 and 1850 cell generations, respectively (corresponding to more than 5 years if cell generation takes 1 day). The effect of drug concentration (as reflected in the fractional cell kill) on prognosis is even more pronounced
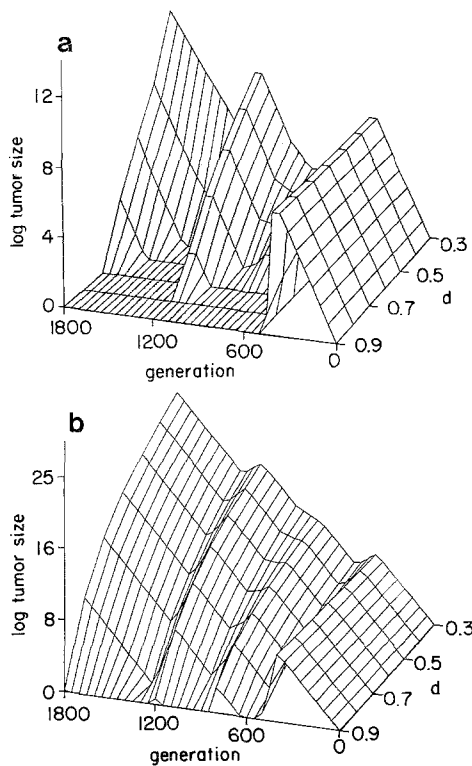
Fig. 6 a, b. Effect of the fractional cell kill in a combination of two non-cross-resistant drugs for the *two-gene/one-copy* case. GA probabilities are $\alpha_1 = 10^{-7}$ for the gene G_1 and $\alpha_2 = 10^{-5}$ for the gene G_2. Three treatment cycles are applied following the initial GA event at cell generations $n = 400$, $n = 800$, and $n = 1200$. Each cycle involves the alternating application of 2 drugs, each of which is used 10 times. The fractional cell-kill of the drug interacting with G_1 is $d = 0.3, 0.4, \ldots, 0.9$, corresponding to resistance thresholds of $k_c = 1,2,\ldots,7$, respectively. The parameters of the drug interacting with G_2 and its resistance threshold are (a) $d = 0.90$ and $j_c = 3$ and (b) $d = 0.50$, and $j_c = 1$
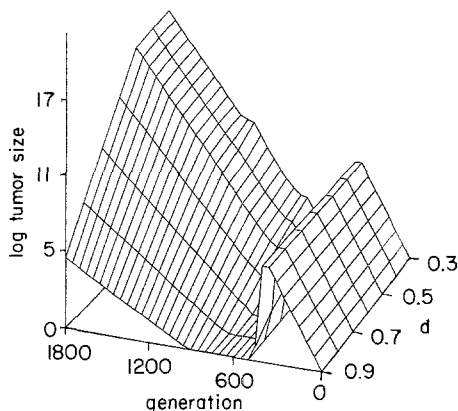

Fig. 7. Effect of the fractional cell kill in a combination of two non-cross-resistant drugs, for the *two-gene/multicopy* case. All parameters are the same as those described in Fig. 6 a

when cell mortality is copy-number-dependent (Fig. 5 b, see Eq. 7 for details), which limits the size of the resistant tumor; in this case, a fractional cell kill of $d = 0.95$ or $d = 0.99$ yields a complete response (2000 generations are calculated), whereas a value of $d = 0.9$ indicates a poor prognosis. As shown in Fig. 5, the mean tumor size may

appear to be initially reduced to zero and then to increase in later stages of the process, since in a probabilistic process the mean number of cancer cells may be much smaller than unity while the probability that there are cancer cells in the system remains positive. The variability in these means has been computed and discussed elsewhere [25].

Our computations indicate that chemotherapy appears to have little effect when GA involves multicopy increments and natural cell mortality is independent of the cellular number of gene copies. In this case, all residual tumors rapidly increase in size and become fully resistant. This finding holds true even for low-amplification probabilities (results not shown).

Two drug combinations

Computations of GA dynamics involving single-copy increments in two unlinked genes [33, 49] are presented in Fig. 6, in which alternating cycles of treatment with two non-cross-resistant drugs (or drug combinations) are imposed on the growing tumor and the resistance threshold is taken to be dose-dependent. The results illustrated in Fig. 6 are displayed in three-dimensional graphs, whereby every point on the manifold represents the log tumor size at a given cell generation for a given drug protocol. Such a concise representation of a large body of calculations has the advantage of enabling one to obtain a general view of the effect of drug concentration on the process. In Fig. 6 a, the fractional cell kill produced by one of the drugs is large ($d = 0.9$) and the effect of various values for the fractional cell kill obtained using the other drug is examined. In Fig. 6 b, the fractional cell kill produced by the first drug is low ($d = 0.5$). These results lead to the conclusion that alternating treatment with two non-cross-resistant drugs is almost always more effective than single-drug therapy. A comparison of Fig. 6 with protocols 1 and 2 in Fig. 5 a reveals that a two-drug combination yields a complete remission ($d = 0.90$ for each drug) as compared with the treatment failure obtained using a single-drug protocol involving a similar number of doses and a similar fractional cell kill. Moreover, an impressive remission is observed for the two-drug protocol, even when the fractional cell kill in the combination is much lower for one of the drugs ($d = 0.30$); when $d = 0.40$, the size of the residual tumor remains undetectable ($<10^9$) after 1800 cell generations. However, when the GA process involves multicopy increments (Fig. 7), a higher fractional cell kill is required to maintain the size of the tumor below the limit of detection.

Discussion

In recent years the problem of drug resistance in cancer has been mathematically attacked by Goldie and Coldman [20–23], Coldman and Goldie [16], Birkhead et al. [9, 10], Gregory et al. [24], and other investigators. 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 present model is new in that it treats the emergence of drug resistance as a dynamic process rather than as a single

event. Using this model, we focus on one of the many mechanisms that may lead to drug resistance, namely, on gene (or oncogene) amplification, and show how changes in the underlying assumptions affect the predictions about treatment efficacy. Unlike previous models, our GA model allows for the possibility of dose-dependent resistance. This assumption is based on the observation that cultured animal and human tumor cells selected for resistance to a specific drug regain their sensitivity when treated with higher concentrations of the same drug ([37, 38, 42]; also see the comment by Kuceck and Chan [30]). This dependence is expressed in our model by the gene-copy-number threshold for susceptibility that is associated with each drug dose.

Our results provide the formal basis for the heuristic conclusion that an effective treatment should entail a high drug concentration [5, 6, 19, 27, 29]. We show that under given conditions, a 5% difference in the fractional cell kill may completely alter the prognosis. Moreover, our results suggest that under one-copy GA 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 drug concentration divided into a few high-concentration doses applied at larger intervals, may result in a partial or a complete remission, depending on the actual value of the amplification probability and on the characteristic cell-cycle time of the tumor.

One implication of these theoretical results is that when a choice must be made between a protocol involving high-concentration doses given at extended intervals and one involving frequent low-concentration dosing, the former should be favored. However, as high drug doses may result in toxicity to the host, the problem of drug resistance should be considered in conjuction with that of drug selectivity. This task has been taken up by us [4] in an analysis of the optimal control of cancer growth aimed at the minimization of both drug resistance and drug toxicity to the host. This analysis supports the present conclusion concerning the superiority of intermittent high-concentration treatments. Another study has provided a formal method for predicting the effect on drug selectivity of cell-cycle-phase-specific drugs such as methotrexate, cytarabine, or zidovadine (AZT) [1–3, 15]. The results show that the elimination of somatic cells depends not only on the drug's pharmacokinetic and pharmacodynamic properties but also on the duration of the dosing interval per se as well as the life- cycle parameters, i.e., the duration of the drug-susceptible cell-cycle phase, the duration of the whole cycle, and the proliferation rate. This work suggests that drug toxicity to the host may be minimized when the dosing interval is an integer multiple of the average cycling time of the host's susceptible cells. This prediction has been verified in vitro and in vivo in mice treated with AZT or cytarabine [6, 7].

The GA model described in the present report is also new in that it allows for the possibility of doubly resistant cells. The general conclusion arising from the analysis of a two-gene amplification process is that the alternating application of a few non-cross-resistant drugs is more effec-tive than therapy with a single drug, even at concentrations that are somewhat lower than those used in the single-drug treatment. These findings are in agreement with previous empirical and theoretical studies reporting the higher efficacy of combination chemotherapy [16, 17, 21, 23].

The present results also suggest that treatment prognosis may be largely improved if cells bearing a large gene-copy number suffer higher mortality. Therefore, it may be interesting to examine the possibility of incorporating in the treatment an agent that increases the mortality of cells carrying highly amplified genomes. At present we are not aware of the existence of such an agent, but its potential use in improving treatment efficacy underlines the importance of a search in this direction.

The formal description of GA dynamics presented in this report enables a rigorous and systematic analysis of the emergence of drug resistance under a large range of constraints. That some of our predictions are supported by experimental and clinical observations suggests that other, less evident implications of this work should be seriously considered (e.g., that protocols involving intermittent high-concentration dosing are superior to those involving frequent low-concentration dosing). The present GA model can be used in studies examining the evolution of the system under a large range of parameter sets to obtain quantitative predictions concerning specific treatments.

Appendix 1: formulating the dynamics of gene amplification

The present gene-amplification model is an extension of the multicompartmental mathematical model for the amplification dynamics of one chromosomal gene [25]. Using this model, we consider a cell population that is heterogeneous with respect to the copy number of two *unlinked* genes (by unlinked we mean that amplifications at different loci are independent); the general case of m genes is a simple generalization of the following description. In our model the amplification process may occur at different rates, the probability of cell death may be constant or gene-copy-number-dependent, and tumor growth is taken to be independent of cell mass (i.e., young tumors, following surgery or during drug treatment) such that cell-population dynamics can be described by an exponential function.

We consider an amplification process initiated in one *normal* cell, i.e., a cell bearing one functional copy of the gene G_1 and one functional copy of the gene G_2. This cell can divide to yield one cell that is identical to its mother and one cell carrying k copies of the gene G_1 ($k \neq 1$). The probability of this event is $\alpha_I (1, k)$. Alternatively, the cell can divide to yield one cell that is identical to its mother and one cell in which the gene G_2 has undergone amplification into j copies ($j \neq 1$). The probability of the latter event is $\alpha_2 (1, j)$. The cell may also divide to yield two identical daughter cells with a probability of $1 - \left[\sum_{k=1}^{\infty} \alpha_1 (1, k) + \sum_{j=1}^{\infty} \alpha_1 (1, j) \right]$ which we denote as $1 - [\alpha_1(1) + \alpha_2(1)]$. Each of the newly generated cells then undergoes the same process again.

Denoting the variable number of copies of the gene G_1 as k and those of the gene G_2 as j, we can obtain the expected number of cells bearing k copies of gene G_1 and j copies of gene G_2 at the nth cell generation, $E (k, j, n)$, as follows:

$$E(1,1,n) = [1 + b - b(\alpha_1(1) + \alpha_2(1)]E(1,1,n-1),\qquad(1)$$

for $k, j = 1$, corresponding to cells carrying a single copy of each gene, where b represents the proportion of proliferating cells;

$$E(1,j,n) = [1 + b - b(\alpha_1(1) + \alpha_2(j)]\ E(1,j,n-1) +$$
$$b \sum_{i=1}^{j-1} \alpha_2(i,j)\ E\ (1,i,n-1)\qquad(2)$$

for $k = 1$ and $j \geq 2$, corresponding to cells bearing amplifications of gene G_2 only;

$$E(k,1,n) = [1 + b - b(\alpha_1(k) + \alpha_2(1)]E(k,1,n-1) +$$
$$b \sum_{i=1}^{j-1} \alpha_1(i,k)\ E\ (i,1,n-1)\qquad(3)$$

for $k \geq 2$ and $j = 1$, corresponding to cells carrying amplifications of gene G_1 only, and

$$E(k,j,n) = [1 + b - b(\alpha_1(k) + \alpha_2(j)]E(k,j,n-1) +$$
$$b \sum_{i=1}^{k-1} \alpha_1(i,k)E(i,j,n-1) +$$
$$b \sum_{i=1}^{j-1} \alpha_2(i,j)E(k,i,n-1),\qquad(4)$$

for $k \geq 2$ and $j \geq 2$, corresponding to cells bearing amplifications of both genes.

Taking into account the natural cell mortality, μ, we obtain the exact expectation using the formula

$$EM(k,j,n) = (1 - \mu)^n E(k,j,n),\ k,j = 1,2,\ldots,\qquad(5)$$

where $E(k,j,n)$ is computed correspondingly. Further analysis shows that when natural cell mortality is constant, the relative frequency of cells carrying a single copy of each gene (normal cells) tends toward zero:

$$\lim_{n\to\infty} RF_{1,1}(n) = \lim_{n\to\infty} \left(1 - \frac{b(\alpha_1(1) + \alpha_2(1))}{1+b}\right)^n = 0,\qquad(6)$$

with the rate of convergence depending on the amplification probabilities, α_1 and α_2, and on the number of cell generations, n. This analysis indicates that resistant cells will dominate the tumor when the amplification probabilities are large or when n is large. However, note that when α is small, the limit will not be reached within a relatively short time i.e., when n is small.

As the copy number of oncogenes and protooncogenes observed in human tumors is usually very limited [48], we assume that cell mortality can be copy-number-dependent, (in this approach, k may be any real number). To account for this assumption, we use the following logistic type function for cell mortality (and solution):

$$\frac{d\mu(k)}{dk} = \frac{1}{K}\ \mu(\kappa)[1 - \mu(\kappa)],\Rightarrow \mu(\kappa) = \frac{Me^{k/K}}{1 + Me^{k/K}},\qquad(7)$$

where K is the cell's carrying capacity for gene-copy number, $\mu(0) = \mu_0$ is the initial condition, and

$$M = \frac{\mu_0}{1 - \mu_0}.$$

This copy-number-dependent cell mortality creates a natural limit to the maximal gene-copy number in a cell and the process becomes nonlinear, with the number of cells in all copy-number compartments reaching an asymptote at a rate that is dependent on the different parameters and on the function $\mu(k)$ for $k = 1,2\ldots$. In the computations, we employ Eq. 7 for integer values of k. The significance of copy-number-dependent natural cell mortality is demonstrated in Results, in which drug protocols are superimposed on the models.

Appendix 2: formulating the effect of drugs

From the clinical point of view, cells in which the number of copies of the G_1 gene exceed a certain threshold, k_c, may be resistant to a given dose of drug A, and cells in which the number of copies of the G_2 gene exceed the threshold j_c may be resistant to a given dose of drug B [38, 39]. To account for these dose-dependent resistance thresholds, we refer to four compartments of cells as follows: (1) cells that are k- and j-sensitive, (2) cells that are k-resistant and j-sensitive, (3) cells that are j-resistant and k-sensitive, and (4) k- and j-resistant cells.

Equations $1 - 5$ are used for calculations of the distribution of cellular gene-copy numbers during the growth of the tumor. For evaluations of the effect of the drug on the sensitive cell compartments, we multiply the right-hand side of Eq. $1 - 4$ by the term $1 - d$, where d represents the fractional cell kill defined in the GA model.

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