A Model for the Resistance of Tumor Cells to Cancer Chemotherapeutic Agents

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ABSTRACT

A mathematical model of tumor resistance to chemotherapy based on a stochastic process of change is presented. The probability of no resistant cells is utilized as a fundamental quantity of interest, and the effects of various therapeutic strategies on it are explored. Situations where one or two drugs are available are treated in detail and extrapolation made to the n-drug case. The situation where two drugs may not be given simultaneously is examined, and it is found that sequential alternation of drugs satisfies certain optimality criteria when both drugs are equally effective. From this it is inferred that the simultaneous administration of all available active agents is optimal where this is permissible.

INTRODUCTION

Despite the development of anticancer chemotherapeutic agents which are successful in the therapy of animal tumors, the success of such drugs in the treatment of human neoplasms has been limited. Animal tumor models have indicated that one cause of such treatment failure is the acquisition of an inherent resistance to the treating agent, or agents, by the tumor cells. This resistance is then transferable at mitosis to the daughter cells, indicating a probable genetic origin for this form of insensitivity. Similar phenomena are seen in a variety of biological systems. The process was first examined from a mathematical perspective for bacteria displaying resistance to viral infection [1]. It was shown at that time that available evidence was compatible with the theory that these cells arose via random mutation and that such mutations occurred in the absence of the virus. The subject was extensively examined thereafter, in this and other contexts, by a number of authors [2–4]. Although much experimental evidence has accrued that cells which display inheritable resistance are the cause of treatment failure in experimental tumor systems

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[5] and that this arose via mutation to resistance in the absence of the drug [6], little attention has been paid to the therapeutic implications of this phenomenon.

More recently the consequences of this process of resistance in the treatment of human malignancy has been explored in the case where a single drug is available [7], and a rudimentary model for two drugs has been proposed [8]. We will present here a framework for viewing the problem and give detailed consideration to the situation in which two potent chemotherapeutic agents are available. The probability of no doubly resistant cells will then be viewed as a minimal condition for successful therapy. When two drugs may not be given simultaneously, this then leads to the consideration of strategies dictating the order in which they may be given. Some optimality criteria are proposed, and under the assumption that each of two agents is equally effective, strategies are found which satisfy these criteria.

THE MODEL

Consider the case where there are n different antitumor agents available, $T_1,\ldots,T_n$. A tumor cell can then be characterised as being in one of 2^n mutually exclusive states with respect to these agents, according to which therapies it is resistant to and which not. A cell will be defined to be resistant to an agent if it will survive administration of that agent at a stated dose with probability 1.

Let $R_{ij\cdots m}(t)$ be the number of cells at time t which are resistant to the drugs $\{T_i, T_j,\ldots,T_m\}$ and not resistant to any in $\{T_1,\ldots,T_n\}\setminus\{T_i, T_j,\ldots,T_m\}$, and refer to such cells as being in the state $R_{ij\cdots m}$. Those cells sensitive (not resistant) to all n drugs will be identified as members of R_0. For simplicity we will assume that the growth of unperturbed sensitive cells follows a pure birth process with birth rate λ per cell, and specify that at time 0 there were $R_0(0)$ sensitive cells present.

We will assume that at division each member of R_0 has a probability α_i $(i=1,\ldots,n)$ of giving rise to a single daughter cell which is resistant to the agent T_i at the dose given. From this it follows that the mutation rate α_i is a function of the drug, the concentration in which it is given, and the type of the tumor cells. Mutation rates between other substates will be indicated by $\alpha_{A,B}$, where A is the set indicating the initial resistant substate and B indicates the resultant substate. Also we will assume that $\alpha_{A,B} > 0 \Rightarrow B \setminus A$ contains one element and $A \subset B$. This implies that multiple resistance occurs by single steps and that no back mutation can occur.

We will now develop some results for the emergence of resistance to a single agent. We will assume that a single resistant cell divides repeatedly to form new resistant cells, and we may thus partition the resistant population into disjoint classes by the parent mutation which gave rise to them. It is then possible to utilize this structure to calculate the distribution of the number of

resistant cells [4] as follows. We will assume there are no resistant cells present at time 0, i.e., $R_i(0) > 0 \Leftrightarrow i = 0$. Define $M_i(t)$ to be the number of mutational events for treatment i prior to time t, that is, the number of transitions from R_0 to R_i. Let $W(u)$ be the growth process for resistant cells, which will be assumed to be independent and identical for each of the resistant subtypes, and let $\{\tau_{ji}\}$ $(j = 1,\ldots,M_i(t),\ i = 1,\ldots,n)$ be the times at which mutational events occur. Then [4]

$$R_i(t) = \sum_{j=1}^{M_i(t)} W(t - \tau_{ji}).$$

At each division of a sensitive cell there is a probability α_i that a cell resistant to agent i will be created, and thus each division may be viewed as a Bernoulli trial. In order to proceed we will make the further assumption that we may approximate $R_0(t)$ by $E[R_0(t)] = R_0(0)e^{\lambda t}$, so that we may calculate $R_i(t)$. As α_i is small and $E[R_0(t)]$ is large in situations of interest, we will approximate $M_i(t)$ using a Poisson distribution, and then $R_i(t)$ will satisfy the definition of a filtered Poisson process [9].

It follows from the general theory of filtered Poisson processes [9] that the probability generating function (p.g.f.) is given by

$$R_i(t,s) = \exp\left\{ \int_0^t m_i'(u)[W(t-u,s) - 1]\, du \right\}, \tag{1}$$

where $R_i(t,s)$ is the p.g.f. of $R_i(t)$, etc., and $m_i'(u)$ is the first derivative of the mean value function of the Poisson process.

If all initial cells from which the tumor originated are sensitive, then when there are $R_0(0)e^{\lambda t}$ sensitive cells present there have been $R_0(0)(e^{\lambda t} - 1)$ divisions, and thus the mean value of the Poisson process is

$$m_i(t) = E[M_i(t)] = R_0(0)\alpha_i(e^{\lambda t} - 1).$$

Experimental evidence [5] indicates that cells selected for resistance to a variety of agents are dynamically similar to the sensitive population from which they originated. Thus we choose $W(t)$ to be a birth process specified as follows:

$$E[W(t)] = e^{\lambda t}.$$

Then

$$W(t,s) = [1 - e^{\lambda t} + s^{-1}e^{\lambda t}]^{-1} \tag{2}$$

Use of (1) and integration then yields

$$R_i(t,s) = [1 - s + se^{-\lambda t}]^{R_0(0)\alpha_i e^{\lambda t}(s^{-1}-1)} \qquad \text{for} \quad i = 1,\ldots,n, \tag{3}$$

from which

$$E[R_i(t)] = R_0(0)\alpha_i \lambda t e^{\lambda t},$$

$$\mathrm{Var}\{R_i(t)\} = 2R_0(0)\alpha_i e^{2\lambda t}\left\{1 - \left(1 + \frac{\lambda t}{2}\right)e^{-\lambda t}\right\}.$$

Defining $P_i(t) = P\{R_i(t) = 0\}$ and taking $\lim_{s \to 0}$ of (3), we have

$$\begin{aligned} P_i(t) &= \exp\{-\alpha_i R_0(0)(e^{\lambda t} - 1)\} \\ &= \exp\{-\alpha_i E[R_0(t) - R_0(0)]\}. \end{aligned} \tag{4}$$

This could also be obtained by noting $P_i(t) = P\{M_i(t) = 0\}$ and conditioning on the value of $R_0(t)$, which leads to

$$(1 - \alpha_i)^{R_0(t) - R_0(0)}, \tag{5}$$

which is very similar to (4) for $\alpha_i \ll 1$, after conditioning (4) on $R_0(t)$.

SINGLE AGENT THERAPY, $n = 1$

In situations where only one drug is available, T_1, a necessary condition for cure is that the agent is capable of removing sensitive cells at a faster rate than they can regrow. If this condition is satisfied, then $P_1(t)$, the probability of no drug resistant cells, may be viewed as the maximal probability for successful treatment with that drug.

As may be expected, this probability shows an inverse relationship to tumor size, clearly indicating that early therapy (with a single agent) is more likely to be successful because of the smaller associated tumor size.

In a variety of tumor protocols, therapy is given in courses with intervening treatment free periods to allow the subject to recover from the associated effects on normal tissues. If each course brings about an instantaneous reduction in the number of sensitive cells followed by an intertreatment growth period, then the probability that there will be no cells resistant to this treatment at time t after a completed schedule of J courses is given by

$$P_1(t) = (1 - \alpha_1)\left\{\sum_{j=1}^{J}\left[R_0(t_j^-) - R_0(t_{j-1}^+)\right] + R_0(t) - R_0(t_j^+)\right\} \quad \text{for} \quad t \geq t_J, \tag{6}$$

where $R_0(t_j^+)$ is the number of sensitive cells present immediately after, and $R_0(t_j^-)$ is the number of sensitive cells immediately before, the jth cycle of therapy which takes place at time t_j.

In what follows we will assume that therapy is initiated when $R_0(t)$ (which is the size of the tumor for α_1 small) reaches some fixed size which will

occur at a random time t_1. For $t > t_1$, $R_0(t)$ is a random variable and thus $P_1(t)$ defined in (5) is conditional on $R_0(t)$. We thus seek to calculate the unconditional probability $P_1(t)$, that is, the mean value of $P_1(t)$ over the possible growth curves $\{R_0(t);\ t > t_1\}$. In order to calculate $R_i(t, s)$ we have assumed that the growth of $R_0(t)$ may be approximated by a piecewise exponential curve with discontinuities at times of therapeutic intervention. We expect this to be a reasonable approximation, since when $R_0(t)$ is large its growth will be close to exponential, and when $R_0(t)$ is small its effect on $R_1(t)$ will be small. In order to evaluate $P_1(t)$, we first consider the probabilistic nature of the process of drug induced cell death.

A substantial amount of evidence [5] exists to show that, for cells which are not resistant to certain drugs, the logarithm of the probability $q(D)$ that a cell will survive after exposure to the drug is linearly related to the dose D, i.e.,

$$\ln q(D) = \beta D,$$

where β (< 0) is a constant. We will refer to β as the cell survival parameter. Drugs with more complex properties will not be considered here, although they may be formally incorporated by allowing β to be a function of relevant parameters.

We now have all the information we require to compute $P_1(t)$. At this point we may also consider the quantity $P_0(t) = P\{R_0(t) = 0\}$. A necessary condition for cure is that $R_0(t) = 0$ and $R_1(t) = 0$ for some t, and thus we seek to maximize the components of the vector $\mathbf{P}(t) = [P_0(t), P_1(t)]$. We notice that $P_0(t)$ may be increased by giving the drug either for longer, or more frequently, or both, as long as the rate of cell growth does not outstrip the rate of cell death. However, this is not true for $P_1(t)$, which is monotonically decreasing in t if we only use agent T_1. Clearly the nature of Equation (6) indicates that for therapy to be successful, rapid depletion of sensitive cells is essential. Maintenance therapy strategies, where low dose therapies are continued over long periods of time, are unlikely to be successful except in cases where α_1 is very small. This also emphasizes (all other factors being equal) that the dose must be measured with respect to a time frame dictated by the growth rate of the tumor. The explicit calculation of $\mathbf{P}(t)$ for $n = 1$ follows as a special case of the procedure provided in the next section for $n = 2$.

THERAPY WITH TWO AGENTS, $n = 2$

By analogy, when two drugs are available we will seek to maximize each of the components of the following vector of unconditional probabilities:

$$\mathbf{P}(t) = [P_0(t), P_1(t), P_2(t), P_{12}(t)]$$

When both drugs may be given simultaneously, at full dose, it is preferable to use them in this way, as this results in enhanced kill of tumor cells at each cycle of therapy. Assuming independence of action of the two resistance processes, it is then possible to extend Equation (5) to allow for two resistant states. However, it is frequently found that active drugs have overlapping toxic affects on normal tissue, and it is impossible to give them together at full dose. It is therefore necessary to sequence the drugs and/or reduce their doses in order to obtain maximum effect. We will examine the effect of drug sequencing on $\mathbf{P}(t)$.

From the previous considerations for $n = 1$ it is clear that treatment should be given as frequently as allowed by the constraints outlined above. We will thus only consider strategies where treatment is given at the earliest times possible, as they will always be as good as or better than other strategies.

First we will extend the definition of the cell survival parameter in the natural way. Let β_{ik} be the cell survival parameter for a cell in R_k $(k = 0,1,2)$ treated with agent i $(=1,2)$. Notice $\beta_{ii} = 0$.

Define

$$\pi_{ik} = \exp(\beta_{ik} D_i),$$

where D_i is the dosage level (assumed constant) of agent i. Assuming that each cell behaves independently, then its probability of survival after administration of agent i is given by π_{ik} for members of $R_k(t)$ $(k = 0,1,2)$, and 1 for members of $R_{12}(t)$. Let $\pi_{ik}^{(s)}$ be the p.g.f. for the distribution of a cell surviving administration of an agent where the expected probability of survival is π_{ik}.

We will assume $R_1(0) = R_2(0) = R_{12}(0) = 0$ and condition on $R_0(t_1)$ being fixed. We may then proceed to calculate the p.g.f.'s of $R_1(t)$ and $R_2(t)$ as follows. For this purpose, as done previously, we will approximate the number of sensitive cells after the jth course of therapy by its expected value; thus

$$R_0(t_j^+) = R_0(t_j^-)\pi_{d(j)0} \tag{7}$$

where

$$d(j) = \begin{cases} 1 & \text{if } j\text{th course is } T_1, \\ 2 & \text{if } j\text{th course is } T_2. \end{cases}$$

Then the independence of the cells implies

$$R_i(t_j^+, s) = R_i\big(t_j^-, \delta_i(d(j))s + [1 - \delta_i(d(j))]\pi_{d(j)i}^{(s)}\big), \tag{8}$$

where

$$\pi_{ki}^{(s)} = 1 - \pi_{ki} + s\pi_{ki}, \qquad i = 1,2,$$

and $\delta_i(x)$ is the Kronecker delta between i and x. Also

$$R_{12}(t_j^+, s) = R_{12}(t_j^-, s).$$

Immediately prior to the application of therapy $j+1$, the expected size of the sensitive cell compartment will be

$$R_0(t_{j+1}^-) = R_0(t_j^+)e^{\lambda\Delta(j)} \tag{9}$$

where

$$\Delta(j) = t_{j+1} - t_j.$$

There will be two contributions to the new growth of singly resistant clones in (t_j, t_{j+1}), one arising from growth of cells initially resistant at t_j, the second from the transformation and growth of new mutations. Since these two contributions take place independently, the p.g.f. of the sum will be the product of the p.g.f.'s for the two parts.

Referring to the p.g.f. for the transformation and growth of new mutants in the interval (t_j, t_{j+1}) as $R_i(t_{j+1}, t_j, s)$ we have

$$R_i(t_{j+1}^-, s) = R_i(t_j^+, W(\Delta(j), s))R_i(t_{j+1}, t_j, s). \tag{10}$$

We may use (1) to calculate the p.g.f. $R_i(t_{j+1}, t_j, s)$ by using

$$m_i(t) = R_0(t_j)\alpha_i[e^{\lambda(t-t_j)} - 1] \qquad \text{for} \quad t \in [t_j, t_{j+1}). \tag{11}$$

This yields

$$R_i(t_{j+1}, t_j, s) = \{1 + se^{-\lambda\Delta(j)} - s\}^{\alpha_i R_0(t_{j+1}^-)(s^{-1}-1)}. \tag{12}$$

The recursive nature of Equation (10) along with Equations (8) and (12) thus permits calculation of $R_i(t, s)$ for $i = 1,2$. So that $R_i(t, s)$ will be defined for all t, we let $R_i(t_j, s) = R_i(t_j^-, s)$ for $j = 1, \ldots, J$. This then allows calculation of $R_i(t, s)$ for arbitrary t. Similarly we have

$$R_{12}(t_{j+1}^-, s) = R_{12}(t_j^+, W(\Delta(j), s))R_{12}(t_{j+1}, t_j, s)$$

Although $R_{12}(t, s)$ is of great interest, it is not easy to calculate, because Theorem (1) is not applicable as $R_1(t)$ and $R_2(t)$ are not differentiable.

This problem was avoided when calculating $R_i(t,s)$ $(i=1,2)$ by conditioning on $R_0(t_1^-)$ and using $E[R_0(t)]$ for $t > t_1$. Conditioning on $R_i(t_1^-)$ $(i=1,2)$ does not seem intuitively reasonable as it did for $R_0(t)$. However, we do not require $R_{12}(t,s)$ to evaluate $P_{12}(t) = P\{R_{12}(t)=0\}$. As noted previously, $P_{12}(t)$ is merely the probability that there are no mutations to double resistance. In the interval (t_j, t_{j+1}) the probability that no double mutations arise is $1-\alpha_{i,12}$ for every division by a member of R_i. We will now use this relationship to calculate $P_{12}(t)$.

Initially consider the R_1 compartment in the period (t_j, t_{j+1}). R_1 increases by two sources: growth of cells existing at t_j, and influx and growth of new mutants in (t_j, t_{j+1}). The number of divisions in existing cells equals the increase in the number of cells whose ancestors were in R_1. This may be modeled by considering a relabeled birth process B in which each new mutant will lead to a random number of divisions, $B(t)$, prior to t. Then

$$P\{B(t)=i\} = P\{W(t)=i+1\}$$

and

$$B(t,s) = s^{-1}W(t,s).$$

Now the p.g.f. of the number of divisions in (t_j, t_{j+1}) of cells in R_1 at t_j^+ is given by

$$R_1\big(t_j^+, B(\Delta(j),s)\big).$$

We can also calculate the p.g.f. of the number of divisions of resistant cells arising from new mutations in (t_j, t_{j+1}). We will refer to this quantity as $R_1'(t_{j+1}, t_j, s)$ [to indicate its relationship to $R_1(t_{j+1}, t_j, s)$], and it is calculated from Equation (1) using (11) and $B(t,s)$ in place of $W(t,s)$. This yields

$$R_1'\big(t_{j+1}, t_j, s\big) = \big[1 - s + se^{-\lambda\Delta(j)}\big]^{\alpha_1 R_0(t_{j+1}^-)s^{-2}(1-s)} \times \exp\big\{\alpha_1 R_0\big(t_j^+\big)(s^{-1}-1)\big(e^{\lambda\Delta(j)}-1\big)\big\}. \tag{13}$$

Now

$$P_{12}\big(t_{j+1}\big) = P_{12}\big(t_j\big)\prod_{i=1}^{2} R_i\big(t_j^+, B\big(\Delta(j), 1-\alpha_{i,12}\big)\big) R_i'\big(t_{j+1}, t_j, 1-\alpha_{i,12}\big) \tag{14}$$

Using the recursive nature of (14) and Equations (10), (12), and (13), it is

possible to calculate $P_{12}(t)$ for arbitrary t after noticing that for $t < t_1$

$$P_{12}(t) = R_1'(t,0,1-\alpha_{1,12})R_2'(t,0,1-\alpha_{2,12})$$
$$= \prod_{i=1}^{2}\left[\left\{\alpha_{i,12}+(1-\alpha_{i,12})e^{-\lambda t}\right\}^{R_0(0)\alpha_i\alpha_{i,12}(1-\alpha_{i,12})^{-2}e^{\lambda t}}\right.$$
$$\left.\times\exp\left\{R_0(0)\alpha_i\alpha_{i,12}(1-\alpha_{i,12})^{-1}(e^{\lambda t}-1)\right\}\right]. \quad (15)$$

To calculate $R_0(t,s)$ we can again use p.g.f.'s as follows:

$$R_0(t_{j+1}^-,s) = R_0\left(t_j^-,\pi_{d(j)i}\left(W(\Delta t(j)),s\right)\right). \quad (16)$$

This recursive relationship allows the calculation of $R_0(t,s)$ for any t. $P_i(t)$ $(i=0,1,2)$ may be calculated using Equation (10) or (16) evaluated at $s=0$.

A sample calculation is presented in Table 1, where each therapy is assumed to be given over a 21 day cycle [$\Delta(j)=21$ for $j=1,\ldots,J$], $R_0(0)=1$, the tumor doubling time is assumed to be 30 days [$\lambda=(\ln 2)/30$], the probability of any cell surviving one course of either therapy is 0.01 ($\pi_{ij}=0.01$, $i\neq j$), and all mutation rates are assumed to be 10^{-6} ($\alpha_1,\alpha_2,\alpha_{1,12},\alpha_{2,12}=10^{-6}$).

TABLE 1

Calculated Values for the First 10 Courses of Therapy[a]

Time	Therapy given in interval	Probability of no sensitive cells, $P_0(t)$	Probability that no cells are resistant to 1, $P_1(t)$	Probability that no cells are resistant to 2, $P_2(t)$	Expected tumor size	Probability of no double resistance in interval	Probability of no doubly resistant cells, $P_{12}(t)$
t_1^-		0.0	0.0	0.0	1.00×10^{10}	0.774	0.774
t_2^-	T_1	0.0	0.0	0.0	1.63×10^{8}	0.914	0.707
t_3^-	T_1	0.0	0.0	0.0	3.25×10^{6}	0.869	0.615
t_4^-	T_1	0.0	0.0	0.685	1.03×10^{6}	0.803	0.493
t_5^-	T_1	0.0	0.0	0.992	1.60×10^{6}	0.709	0.350
t_6^-	T_1	0.377	0.0	1.000	2.61×10^{6}	0.584	0.204
t_7^-	T_1	0.984	0.0	1.000	4.23×10^{6}	0.432	0.088
t_8^-	T_1	1.000	0.0	1.000	6.88×10^{6}	0.271	0.024
t_9^-	T_1	1.000	0.0	1.000	1.12×10^{7}	0.131	0.003
t_{10}^-	T_1	1.000	0.0	1.000	1.81×10^{7}	0.043	0.000
t_{11}^-	T_1	1.000	0.0	1.000	2.95×10^{7}	0.008	0.000

[a]Values given for each course represent the values immediately prior to the next course. The expected tumor size is given by $E[R_0(t)+R_1(t)+R_2(t)]$.

Table 1 illustrates the calculation for a strategy in which the first 10 cycles consist of T_1. As expected, this is seen to be very effective in eliminating cells in R_0 and R_2, so that at the end of 10 cycles the tumor is composed totally of R_1 and R_{12} cells. $P_{12}(t)$ is seen to decline steadily, so that at the end of the treatment period it is close to zero, indicating the likely existence of cells in R_{12} and consequently that use of T_2 at this point is unlikely to be ultimately successful.

SEQUENCING THERAPY FOR $n = 2$

An important question arises as to how we should sequence therapy. The probability of no doubly resistant clones developing prior to the institution of therapy is

$$(1-\alpha_{1,12})^{R_1(t)-M_1(t)}(1-\alpha_{2,12})^{R_2(t)-M_2(t)}$$

which for $\alpha_{1,12}, \alpha_{2,12} \ll 1$ is approximately equal to

$$\exp\{-\alpha_{1,12}[R_1(t)-M_1(t)]-\alpha_{2,12}[R_2(t)-M_2(t)]\}.$$

Clearly it is desirable to sequence therapy in such a manner that positive increments in the quantity

$$\alpha_{1,12}[R_1(t)-M_1(t)]+\alpha_{2,12}[R_2(t)-M_2(t)] \tag{17}$$

are minimized. In cases where both therapies may be given simultaneously this is clearly the optimum strategy, since R_0, R_1, and R_2 are simultaneously reduced. When they may not be given simultaneously we are faced with the choice of sequencing them and/or reducing doses to restrict toxicity to normal organ systems. We will now consider the case where it is desired to sequence two therapies whilst maintaining full dose of each, that is, subject to the restriction that simultaneous application is not permitted.

The algorithm for $P_{12}(t)$ may then be used to calculate the likely effect of various therapeutic strategies where therapies cannot be given simultaneously if values are known for the parameters $\pi_{10}, \pi_{20}, \pi_{12}, \pi_{21}, \alpha_1, \alpha_2, \alpha_{1,12}, \alpha_{2,12}$. Although this information is frequently available for experimental tumors, it is unlikely to be available for human tumors. It is therefore of interest to ask whether any deductions can be made from these considerations without explicit knowledge of the π's and the α's.

In general this does not seem to be the case, because of the complex nature of the relationships derived for $P_0(t)$, $P_1(t)$, $P_2(t)$, and $P_{12}(t)$. Analogously to the case $n = 1$, for cure to be possible the agents T_1 and T_2 must be applied in a way such that R_0, R_1, and R_2 cells are eliminated faster than they can repopulate. When therapies may not be given together, it is

clear that no therapeutic strategy will simultaneously maximize all components of $\mathbf{P}(t)$. In view of this we therefore attempt to maximize $P_{12}(t)$ $(t > t_J)$, as we can increase the number of cycles J so that $P_0(t)$, $P_1(t)$, and $P_2(t)$ are arbitrarily close to 1 $(t > t_J)$.

From the above we should require of an effective therapeutic strategy that $R_1(t)$ and $R_2(t)$ should be zero with large probability at completion. Now the finite nature of this process implies that if $R_1(t)$ and $R_2(t)$ are equal to zero with high probability, then $E[R_1(t)]$ and $E[R_2(t)]$ are small. This implies that reasonable strategies will have $E[R_1(t)+R_2(t)]$ small at completion, and from this it seems desirable to search for strategies which minimize $E[R_1(t)+R_2(t)]$, in the hope that they will be "good."

Alternatively, it is possible to take a more naive view of the whole process and use a quasideterministic approximation to the resistance process [9]. As might be expected, the probability of double resistance is then related to the expected numbers of singly resistant cells. This leads to the notion that the positive increments in the quantity

$$\alpha_{1,12}E[R_1(t)-M_1(t)]+\alpha_{2,12}E[R_2(t)-M_2(t)] \tag{18}$$

should be minimized during therapy. It is interesting that there exist strategies which simultaneously minimize the sum of positive increments in (18) and $E[R_1(t)+R_2(t)]$, when the two agents are of equal effectiveness. We will now examine this particular case and refer to agents of equal effectiveness as being equivalent.

Define two agents as being equivalent if they satisfy the following conditions:

(i) $\alpha_1 = \alpha_2$; $\alpha_{1,12} = \alpha_{2,12}$;

(ii) $\pi_{10} = \pi_{20}$; $\pi_{12} = \pi_{21}$;

(iii) identical intertreatment intervals, that is, $\Delta(j)$ constant for $j = 1,\ldots,J-1$.

Define a strategy consisting of J cycles of therapy S as $S = \{d(i)\}_{i=1}^{J}$, that is, the sequence in which agents are given. Strategies which do not apply an agent every $\Delta(j)$ units of time will not be considered, as they clearly will be inferior. Define a substrategy as $S_j = \{d(i)\}_{i=j}^{J}$, which is the strategy S after the jth cycle. The application of J cycles of therapy naturally partitions the lifetime of the tumor into $J+1$ disjoint periods. The independence of the process implies that we may consider separately resistant clones which arise in each of these periods.

Define

$$X(S_j,k) = \left| \sum_{i=j}^{k} \delta_1(d(i)) - \delta_2(d(i)) \right| \qquad \text{for} \quad 1 \leqslant j \leqslant k \leqslant J,$$

which represents the modulus of the number of times T_1 is given minus the number of times T_2 is given between the jth and kth cycles of therapy. Let

$$X_m(S_j) = \max\{X(S_j, k) : k \geqslant j \text{ and } k \leqslant J\};$$

then define

$$K(S_j) = \{k : X_m(S_j) = X(S_j, k)\},$$

that is, the set of cycles at which the modulus of the difference in the number of T_1's and T_2's is maximized commencing at j. We will be interested in the set of strategies

$$A_j = \{S_j : X_m(S_j) = 1\},$$

which is easily seen to be nonempty for all j.

Let $E_{ij}(t)$ be the size of the compartment of cells resistant to treatment i at time t which arose in the jth interval ($1 \leqslant j \leqslant J-1$). Define

$$C(S_j, t) = \sum_{i=1}^{2} \left(\sum_{k=j}^{J-1} \{E_{ij}(t_{k+1}^-) - E_{ij}(t_k^+)\} + E_{ij}(t) - E_{ij}(t_j^+) \right)$$
$$\text{for} \quad t \geqslant t_J, \tag{19}$$

that is the sum of the positive increments in the expected number of resistant cells originating in period j over the remaining treatment period. We are motivated to consider $C(S_j, t)$ because it is linearly related to the positive increments in (18) arising from mutations to resistance occurring in (t_j, t_{j+1}). This is because from equivalence $\alpha_{1,12} = \alpha_{2,12}$, and also, since $\alpha_1 = \alpha_2$ and $\pi_{10} = \pi_{20}$, increments in $M_1(t)$ and $M_2(t)$ are independent of the order in which the agents are given. Thus strategies which minimize (19) also minimize the corresponding element of (18).

Let

$$E_{1j}(t_j^-) = E;$$

then $E_{2j}(t_j^-) = E$ by equivalence. Let

$$c = \exp\{\lambda(t_{e+1} - t_e\}, \qquad e = 1, \ldots, J-1,$$

and

$$\pi = \pi_{12} = \pi_{21} < 1.$$

Define

$$n(k) = \sum_{i=j}^{k} \delta_1(d(i));$$

then

$$C(S_j, t) = E(c-1) \sum_{k=j}^{J-1} c^{k-j}\left[\pi^{n(k)} + \pi^{k-j+1-n(k)}\right] + Ec^{J-j}\left[\pi^{n(J)} + \pi^{J-j+1-n(J)}\right]\left(e^{\lambda(t-t_j)} - 1\right) \quad \text{for} \quad t > t_J.$$

Using this notation, it is now possible to show that $\Sigma_{j=1}^{J} C(S_j, t)$, and thus the positive increments in (18), are minimized by strategies which alternate therapy at each cycle.

THEOREM

$\Sigma_{j=1}^{J} C(S_j, t)$ is uniquely minimized by the two strategies which alternate therapy at each cycle.

This will be proven in three parts:

(i) For any $S_j \notin A_j$ we have $C(S_j', t) < C(S_j, t)$ for some $S_j' \in A_j$.
(ii) For $S_j, S_j' \in A_j$ we have $C(S_j, t) = C(S_j', t)$.
(iii) Let $S^1 = \{1,2,1,2,\ldots\}$, $S^2 = \{2,1,2,1,\ldots\}$. Then $S_j^1, S_j^2 \in A_j$ for $1 \leqslant j \leqslant J$.

Proof. (i): Let σ_k be the operator which transposes the kth and $k+1$st treatments. For arbitrary j assume that S_j minimizes $C(S_j, t)$. Assume, for the purpose of contradication, that

$$X_m(S_j) > 1.$$

Choose $k \in K(S_j)$. For $k < J$ we may assume without loss of generality that $d(k) = 1 \Rightarrow d(k+1) = 2$. Consider the strategy $\sigma_k S_j$. Then

$$C(S_j, t) - C(\sigma_k S_j, t) = E(c-1)c^{k-j} \times \left[\pi^{n(k)-1}(\pi - 1) + \pi^{k-j+1-n(k)}(1-\pi)\right].$$

Now

$$C(S_j, t) - C(\sigma_k S_j, t) \leqslant 0 \quad \Leftrightarrow \quad 2n(k) \leqslant k - j + 2.$$

But $2n(k) > k - j + 2$, since $X_m(S_j) \geqslant 2$, and this contradicts the assump-

tion. For $k = J$ consider the strategy S_j' where the last therapy given is changed, i.e.,

$$\delta_1(d'(J)) = 1 - \delta_1(d(J)).$$

Then one again obtains the same contradiction. By induction for any substrategy S_j such that $X_m(S_j) > 1$, then there exists a substrategy S_j' where $X_m(S_j) \leqslant 1$ satisfies $C(S_j', t) < C(S_j, t)$.

(ii): Let

$$S_j, S_j' \in A_j \quad \text{and} \quad S_j \neq S_j';$$

then

$$\begin{aligned} C(S_j, t) - C(S_j', t) &= E(c-1) \sum_{k=j}^{J-1} c^{k-j} \left[\pi^{n(k)} + \pi^{k+j+1-n(k)} - \pi^{n'(k)} - \pi^{k+j+1-n'(k)} \right] \\ &\quad + Ec^{J-j} \left[\pi^{n(J)} + \pi^{J-j+1-n(J)} - \pi^{n'(J)} - \pi^{J-j+1-n'(J)} \right] \\ &\quad \times \{ \exp[\lambda(t - t_J)] - 1 \} \end{aligned}$$

when $n'(k)$ is equivalent to $n(k)$ for substrategy S_j'. Now for $k - j + 1$ even,

$$n(k) = \frac{k-j+1}{2} = n'(k),$$

and for $k - j + 1$ odd,

$$n(k) = n'(k) \quad \text{or} \quad n(k) = k + j + 1 - n'(k),$$

because

$$X_m(S_j) \neq 1.$$

Thus

$$C(S_j, t) - C(S_j', t) = 0.$$

(iii): Consider $S^1 = \{1, 2, 1, 2, \ldots\}$, $S^2 = \{2, 1, 2, 1, \ldots\}$. Clearly $S_j^1, S_j^2 \in A_j$ for all j, and furthermore only they satisfy this property. Since these minimize $C(S_j, t)$ for each j, they thus minimize the sum

$$\sum_{j=1}^{J} C(S_j, t).$$

Thus we have that the alternating strategies S^1 and S^2 minimize the positive increments in (18), completing the proof.

To show that these strategies also minimize $E[R_1(t)+R_2(t)]$ is comparatively straightforward. It is easily seen that for $t > t_J$

$$E_{1_j}(t)+E_{2_j}(t) = Ec^{J-j}\left[\pi^{n(J)}+\pi^{J-j+1-n(J)}\right]e^{\lambda(t-t_J)}$$

is minimized by $n(J)=J/2$ for J even and $n(J)=(J\pm 1)/2$ for J odd, where $n(J)$ is required to be integral and $0<\pi<1$. Similar considerations to those used previously lead to the uniqueness of S^1 and S^2 in minimizing $E[R_1(t)+R_2(t)]$.

Although the idealization of equivalent agents may seldom be exactly realized in practice, many cases exist where it is approximately true. The incorporation of agents of varying effectiveness into a strategy based on estimates of their parameters would require great confidence both in this model and the data on which parameter estimates were based. However, when two equally effective agents are available, one does not have to rely on parameter estimates, and furthermore sequential alternation per se is unlikely to prove detrimental when the assumption of this model are not satisfied.

Unfortunately, the two optimization criteria satisfied by S^1 and S^2 do not necessarily imply that $P_{12}(t)$ is maximized over a fixed J cycles of therapy.

TABLE 2

Calculated Values of $\mathbf{P}(t)$ for a Strategy in Which T_1 and T_2 are Alternated for the First 10 Curves of Therapy

Time	Therapy given in interval	Probability of no sensitive cells, $P_0(t)$	Probability that no cells are resistant to 1, $P_1(t)$	Probability that no cells are resistant to 2, $P_2(t)$	Expected tumor size	Probability of no double resistance in interval	Probability of no doubly resistant cells, $P_{12}(t)$
t_1^-		0.0	0.0	0.0	1.00×10^{10}	0.774	0.774
t_2^-	T_1	0.0	0.0	0.0	1.63×10^{8}	0.914	0.707
t_3^-	T_2	0.0	0.0	0.0	2.65×10^{6}	0.996	0.704
t_4^-	T_1	0.0	0.0	0.0	52,847	0.997	0.702
t_5^-	T_2	0.0	0.0	0.0	1,020	1.000	0.702
t_6^-	T_1	0.377	0.0	0.428	275	1.000	0.702
t_7^-	T_2	0.984	0.281	0.428	8.7	1.000	0.702
t_8^-	T_1	1.000	0.281	0.971	7.0	1.000	0.702
t_9^-	T_2	1.000	0.957	0.971	0.23	1.000	0.702
t_{10}^-	T_1	1.000	0.957	0.999	0.18	1.000	0.702
t_{11}^-	T_2	1.000	0.999	0.999	6×10^{-3}	1.000	0.702

Characterizing the conditions under which S^1 and S^2 are optimal for $P_{12}(t)$ under equivalence is complex. A necessary condition is that $\pi c < 0.5$, as otherwise extinction of R_0, R_1, and R_2 cells may never occur no matter how large J is. This would seem to imply that the more effective the agents (that is, the smaller the values of the π's), the more likely it is that S^1 and S^2 will maximize $P_{12}(t)$. In a number of simulations S^1 and S^2 do prove to maximize $P_{12}(t)$ under equivalence. Table 2 gives the results for the previous example in which T_1 and T_2 are now alternated for the first 10 cycles. One other property of S^1 and S^2 is that if therapy is stopped arbitrarily prior to giving J cycles, then the resulting truncated strategies based on k cycles (say) are still optimal with respect to the original criteria. This is unique to S^1 and S^2.

MULTIAGENT THERAPY, $n > 2$

When n treatments are available the situation is quantitatively more complex and requires the simultaneous consideration of a large number of compartments. It is straightforward to see that any agents which may be given together, should be, since they will affect a greater proportion of the tumor's cells. Approximate solutions can be obtained using expected values for the various resistant compartments obtained from simple approaches based on ordinary differential equations. This would again lead to consideration of quantities generalizing that of (18). Computation of "best strategies" could then be made, given knowledge of the relevant parameters, although such a calculation is somewhat less precise than can be made for the two drug case. In general, however, it seems the withholding of a useful drug for the therapy of possible future treatment failure only increases the likelihood of such an event. This model would predict that cell growth inevitably leads to a diversity of resistance and that strategies which attempt to maximize the potential for cure do not permit unrestricted growth in individual cellular subpopulations. It seems intuitive that alternating strategies will satisfy analogous criteria to that proposed for $n = 2$ for any number of equivalent treatments. When n agents are available an alternating strategy will consist of cyclic application of each agent in turn until each has been given once etc. For n equivalent therapies there will be $n!$ alternating strategies.

Decisions regarding the inclusion of marginally effective agents in protocols with more effective drugs must rely on more explicit consideration of each individual case.

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