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Resonance in Periodic Chemotherapy: A Case Study of Acute Myelogenous Leukemia

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The effects of periodic chemotherapy administration are evaluated within the context of a G_0 model of the cell cycle. Parameters are estimated for normal bone marrow cells and malignant cells in acute myelogenous leukemia (AML). This model explicitly includes the resting G_0 phase and the feedback mechanism that recruits the cells back into the cell cycle. Periodic chemotherapy administration can induce resonance within our model under *high* cell kill rate where the average cell cycle times may change during the course of treatment, and therapeutic benefits from these resonances cannot be solely based on cell cycle times in untreated tissue. The depletion rate under chemotherapy and the regrowth rate may differ between the cell populations, and our analysis suggests that this favors the tumour cells. We were able to distinguish between the effects of cycle-non-specific, *S*-phase-specific and *M*-phase-specific drugs, and found that these can show differences in sharpness and location of the resonance phenomenon. We conclude that resonance chemotherapy (chronotherapy) is unlikely to be efficacious in the treatment of AML.

1. Introduction

Chemotherapy is often the only means to treat patients with acute myelogenous leukemia, but it is toxic because of many side-effects, and does not usually offer a lasting cure (Lee *et al.*, 2000). Chemotherapy can kill malignant tumour cells in one or more of the proliferative phases of the cell cycle, but it is also a potent killer of normal proliferative cells in bone marrow where hematopoietic cells are produced. The goal of chemotherapy is, therefore, to maximally kill the malignant cells and minimally kill the normal cells. To do this, one must consider differences between normal and malignant cells.

In some tumours, the cell cycle times of normal and malignant cells differ. Chronotherapy studies the timing of drug administration in order to achieve optimal therapeutic success (resonance) for disease treatment. The basic idea behind this resonance is as follows. Given a cell population in which all cells divide at the same age, a single dose of chemotherapy kills cells in a specific age range. These cells will not produce any daughter cells and if the next dose is administered where these daughter cells would have been susceptible

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to chemotherapy, a minimal kill is observed. Therefore, administration of chemotherapy with a period commensurate with the normal cell cycle time is hypothesized to minimize the normal cell kill.

1.1. THE CELL CYCLE

Self-replication of cells takes place through several different phases in the *cell cycle*. These phases are: G_1, S (DNA synthesis), G_2 and M (mitosis). After completing cell division (M), the daughter cells can recycle, decycle temporarily (and remain in a dormant state (G_0) until environmental conditions stimulate their reentry into the cell cycle), or they can leave the cell cycle and progress along a course that leads to reproductive cessation, terminal differentiation, and eventual death (Alison & Sarraf, 1997; Burns & Tannock, 1970). Some decycled stem cells find themselves in G_0 and do not cycle until marrow depletion causes them to reenter the cycle and proliferate in order to replenish the marrow (Baserga, 1981; Burns & Tannock, 1970). The time between two mitoses is called the cell-cycle time which may vary widely depending on the duration of the G_0 (Baserga, 1981) and G_1 phases (Burns & Tannock, 1970).

In the context of periodic chemotherapy administration, a relevant question is whether there is a well-defined cell cycle time or inter-mitotic interval, T_{IM}. The time spent in the S, G_2, and M phases is measurable and well defined. Variations in T_{IM} stem primarily from the time spent in G_0 and G_1.

1.2. CLINICAL CHEMOTHERAPEUTIC TREATMENT OF AML

Chemotherapeutic agents are often phase specific and target cells in one or more of the cell cycle phases e.g. S-phase specific. Most often, remission induction chemotherapy in treatment of AML will consist of a combination of S-phase-specific drugs such as cytarabine and an anthracycline (adriamycin/doxorubicin, daunorubicin, idarubicin (Foon & Casciato, 1995; Sather *et al*., 1978)). A typical administration schedule is as follows. Cytarabine is administered continuously for a week and the anthracycline is administered by a large, rapid bolus injection three times over a three-day period. The administration schedule is typically not periodic but, in rare cases, a high dose of Ara-C is periodically administered as a bolus injection every 12 hr (Beutler *et al*., 1995) when the patients are in relapse.

Ara-C (cytosine arabinoside, cytarabine)
100 mg m^{-2} intravenous (IV) as a continuous infusion for 7–10 days
and
adriamycin (doxorubicin)
30 mg m^{-2} IV days 1–3 (Sather *et al*., 1978)

Cytarabine
100 mg m^{-2} by continuous IV infusion for seven days
and
Idarubicin
12 mg m^{-2} IV push on either days 1, 2 and 3 or days 5, 6 and 7
or
daunorubicin 45–60 mg m^{-2} may be substituted for idarubicin (Foon & Casciato, 1995)

1.3. OVERVIEW

In Section 2, we review the experimental efforts that have been made to examine the effects of periodic chemotherapy administration as well as the theoretical (mathematical) treatments that have been carried out to test these ideas. Much of this previous work has concluded that properly designed periodic chemotherapy can be more effective than constant chemotherapy. However, all of these studies have also ignored several key elements of the mammalian cell cycle and its regulation that may play a pivotal role in altering this conclusion. In Section 3, we present the formulation of a G_0 cell cycle model that we feel avoids these omissions, and analyse the steady states of this model for their stability. Section 4 extends this model to the situation in which exogenous chemotherapy is being administered and briefly examines both conventional and periodic chemotherapy protocols within the context

of this extension. We use the best available parameters derived for both normal human bone marrow and bone-marrow cells typical of acute myelogenous leukemia. The result of this study is that in every case the protocols are more destructive to the normal cell population than they are to the leukemic population. Section 5, the core of this work, examines the possibility of chemotherapeutic-induced resonance within the bone-marrow cells in the context of the role of the feedback in the system, phase-specific drugs and the intensity of the chemotherapy. We conclude with a brief summary in Section 6. The appendices detail the parameter estimation procedures that we have followed.

2. Previous Work

Optimal chemotherapy scheduling has been investigated in the past in a number of experimental and theoretical studies.

To our knowledge, Dibrov and his coworkers (Churikova *et al*., 1986; Dibrov *et al*., 1988, 1986, 1998, 1984, 1985; Vtiurin *et al*., 1987) were the first to carry out extensive experimental work investigating the treatment of laboratory-induced tumours with periodically timed chemotherapy that took into account cell-cycle kinetics. Other experiments carried out by Dibrov *et al*. (1985) showed survival of murine hematopoietic stem cells as a function of drug delivery period that had a peak near a period of 12 hr, which is within the experimental range of the cell-cycle time for hematopoietic stem cells in the strain of mouse they were using. Though the results are not entirely conclusive (there were no error bars plotted with these values and the difference between minimum and maximum survival is not strikingly large) a theoretical modeling explanation for the efficacy of this treatment was offered (Dibrov *et al*., 1985) that has been recently amplified (Dibrov, 1998).

Other experiments in mice also suggest that there may be optimal periods of chemotherapy administration. Agur and co-workers (Agur *et al*., 1988) inoculated mice with lymphoma cells to produce tumours and found that the 7 hr protocol matching the presumptive normal cell-cycle duration and 10 hr protocol showed significantly higher survival than random drug administration and an 8 hr protocol. While the advantage of the 7 hr protocol may lie in the fact that it is tuned to the internal cell parameters, this does not explain why the 10 hr protocol also shows high survival rates. A 12 hr protocol would, in theory, yield the lowest survival rates because it shields the cancer cells with this resonance, but unfortunately a 12 hr protocol was not attempted in these experiments. Other experiments along these lines (Ubezio *et al*., 1994) have given similar results. Agur and her co-workers have introduced a simple heuristic finite difference model equation to describe a cell population (cells in mitosis) undergoing periodic chemotherapy (Agur *et al*., 1988). They predicted that beneficial effects could be obtained if the drug administration is done at a resonant period equal to the average cell-cycle time of the normal cells. This resonance strategy works if the malignant cell population has different cell-cycle times, or greater variation in the cell-cycle times, than the normal cell population. Other modeling studies by her group (Cojocaru & Agur, 1992; Mehr & Agur, 1992) have reached the same conclusion.

Swierniak *et al*. (1996) introduce a model of phase-specific chemotherapy based on ordinary differential equations where cells flow through the G_1–M compartments, and show analytically that *periodic* chemotherapy protocols may be optimal solutions under certain circumstances but that this is by no means a universal conclusion.

Webb (1992) has presented an age-structured model and an interesting feature of this model is that an age-specific division rate is modeled using a displaced gamma distribution. The presence of long tails in this distribution mimics the effect of the resting G_0 state, where cells can stay a long time before reentering the cell cycle. Using this model, it is shown that the sharpness of the resonance can be controlled by varying the width of the gamma distribution. Dibrov *et al*. (1985) introduced an integral equation model describing the flux out of the mitotic phase. As in the age-structured model of Webb, a long tail in the gamma distribution describing the age at division also mimics the existence of a resting cell population.

Based on this previous work, three points are noteworthy and have motivated the work presented here:

Unphysiological parameters. The cumulative factor R used by Dibrov (1998), Dibrov *et al.* (1985), Agur *et al.* (1991, 1988, 1992) and Swierniak *et al.* (1996) includes apoptosis, differentiation and division and does not distinguish between these different events. None of the studies discussed above make an effort to estimate realistic physiological parameters aside from cell-cycle length and cell cycle variance in untreated tissue.

Existence of G_0. All of the above models omit explicit consideration of the resting G_0 state. Agur *et al.* (1991, 1988, 1992), Swierniak *et al.* (1996) and Webb (1990, 1992a, b) do not discuss G_0 while Dibrov *et al.* (1985) discuss why the G_0 state is excluded from their model. Dibrov *et al.* argue that both normal and malignant cells are continuously dividing (Dibrov *et al.*, 1985). The rationale behind this is the assumption that: (Dibrov *et al.*, 1985, p. 2) "…in an untreated organism most hematopoietic stem cells were resting, while all of the detectable tumour cells appeared to be proliferating…". However, in AML, it has been observed that $\sim$20% of the normal bone-marrow cells (Peters *et al.*, 1986) and $\sim$10% of the leukemic bone-marrow cells (Vidriales *et al.*, 1995) appear to be proliferating prior to treatment.

Dibrov goes on to argue how the normal cells also become continuously dividing (Dibrov *et al.*, 1985, pp. 5, 6): "Under multiple treatment by cytotoxic drug, the limiting tissue is continuously damaged, and consequently stem cells become continuously cycling soon after the beginning of therapy." When chemotherapy is applied, more cells are recruited from G_0 and more cells skip this phase resulting in a shorter average time spent here. This effect is due to *feedback* in the system.

Feedback. When chemotherapy is applied, the tissue corrects for the resulting cell loss by recruiting more cells from the resting, G_0-pool. In this way, G_0 acts as a buffer. This was clearly stated in Dibrov *et al.* (1985). A further assumption, clearly stated in Dibrov *et al.* (1985) and implicitly assumed in Agur *et al.* (1991, 1988, 1992), Swierniak *et al.* (1996) and Webb (1990, 1992a, b), is that the internal cell-cycle parameters do not change during chemotherapy (Dibrov *et al.*, 1985, p. 5): "It is assumed further that the cell-cycle parameters are independent of the time and of the population size". The argument for this assumption is as follows (Dibrov *et al.*, 1985, p. 6): "The use of the *above* assumption for tumour population is of little importance, since it will be shown that the optimal schedules obtained depend primarily on the cell-cycle parameters of normal cells and do not depend on the parameters of tumour cells as long as their mean generation time differs considerably from that of normal cells (as it does for most natural tumours)".

Unfortunately, the assumption that all cells are proliferating voilates the assumption about stable parameter values, and in many tumours these two assumptions *are not* fulfilled at the same time. When cells are being recruited from G_0 at a higher rate, the cells spend less time there on average and G_0 becomes less important. Therefore, the cell-cycle parameters are not stable during the course of chemotherapy. This is the nature of feedback. In the untreated case, the total average intermitotic interval (including time spent in G_0) differs considerably between the normal and the leukemic bone-marrow cells, see also Appendix A. But since these differences stem mainly from time spent in G_0 (Baserga, 1981) and G_1 (Alison & Sarraf, 1997), the differences vanish when both cell populations become continuously dividing during chemotherapy treatment.

3. Cell Kinetic G_0 Model

In Section 1.1, the underlying kinetics of cell replication were described. These features are captured in a simple model with a few, but physiologically meaningful, parameters (Burns & Tannock, 1970; Hearn *et al.*, 1998; Mackey, 1978, 1981; Smith & Martin, 1973) illustrated in Fig. 1. The development of this model, which has been used to explain a number of diseases in the hematopoietic system, is presented as well as an analysis of the stability of the model steady states. The effects of chemotherapy are considered in Section 4.

The number of cells in the proliferative phase, P, is made up of cells in the S, G_2, and M phases of the cell cycle. The number of cells in the non-proliferative phase, N, is made up of cells in

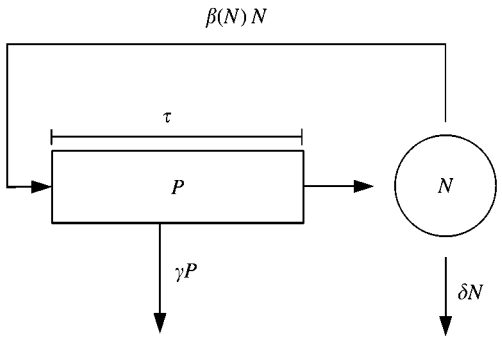

FIG. 1. Schematic diagram of the G_0 phase cell-cycle model used in this study. This model was first analysed mathematically by Burns & Tannock (1970) and later reintroduced by Smith & Martin (1973) in an examination of the statistics of cell division.

G_0 and G_1.* The cellular dynamics in the two compartments are described by the following pair of differential delay equations (Haurie *et al.*, 1998; Mackey, 1978, 1996):

$$\dot{N} = -\delta N - \beta(N)N + 2e^{-\gamma\tau}\beta(N_\tau)N_\tau, \quad (1)$$

$$\dot{P} = -\gamma P + \beta(N)N - e^{-\gamma\tau}\beta(N_\tau)N_\tau, \quad (2)$$

where we assume that β is a monotone decreasing function of N and has the explicit form of a Hill function

$$\beta(N) = \beta_0 \frac{\theta^n}{\theta^n + N^n}. \quad (3)$$

The symbols in eqns (1)–(3) have the following interpretation: N is the number of cells in non-proliferative phase $(G_0 + G_1)$, $N_\tau = N(t-\tau)$, P the number of cycling proliferating cells $(S + G_2 + M)$, γ the rate of cell loss from proliferative phase, δ the rate of cell loss from non-proliferative phase, τ the time spent in the proliferative phase, β the feedback function, rate of recruitment from non-proliferative phase, β_0 the maximum recruitment rate, and θ, n the control shape of the feedback function.

In this paper, we study the growth of normal and leukemic cell populations using this model. It is generally believed that normal and malignant cell populations have different cell-cycle times (Baserga, 1981) and thus they will be described by different parameters in our model. In particular, in untreated leukemic cells the apoptotic rate is significantly smaller than in normal cells (Macnamara *et al.*, 1999; Okita *et al.*, 2000; Ong *et al.*, 2000; Parker *et al.*, 2000), and the time spent in the non-proliferative phase is longer relative to normal cells in the bone marrow. It is a central assumption of this study that leukemic cells have a lower basal rate of apoptosis than normal cells.

We solve the model eqns (1) and (2) with the initial steady-state conditions $N(t \leqslant 0) = N^*$ and $P(t \leqslant 0) = P^*$. This requires that we estimate the entire parameter set for both leukemic and normal cell populations, including the initial conditions N^* and P^*. To do this, we have used values from the experimental and clinical literature as discussed in Appendix A. The resulting parameter values are presented in Table 1.

TABLE 1

Estimated parameters for normal and malignant bone-marrow cell population, determined using the techniques and data of Appendices A.2 and A.3, respectively

Symbol	Normal BM	Malignant BM
t_S	14.3 hr 0.60 day	17.4 hr 0.73 day
τ	20.0 hr 0.83 day	21.2 hr 0.88 day
γ_0	$0.015\ \text{hr}^{-1}$ $0.36\ \text{day}^{-1}$	$0\ \text{hr}^{-1}$ $0\ \text{day}^{-1}$
β_0	$0.14\ \text{hr}^{-1}$ $3.5\ \text{day}^{-1}$	$0.14\ \text{hr}^{-1}$ $3.5\ \text{day}^{-1}$
δ	$0.0068\ \text{hr}^{-1}$ $0.16\ \text{day}^{-1}$	$0.0047\ \text{hr}^{-1}$ $0.11\ \text{day}^{-1}$
N^*	2.87×10^8 cells kg^{-1}	3.25×10^8 cells kg^{-1}
P^*	0.70×10^8 cells kg^{-1}	0.32×10^8 cells kg^{-1}
θ	1.38×10^8 cells kg^{-1}	1.06×10^8 cells kg^{-1}
n	3	3

*Many chemotherapeutic drugs kill cells during DNA synthesis (S phase) or mitosis (M phase). Going into synthesis, the only important information is the age distribution of the cells because certain ages are not represented. There is no additional information in the transition time between G_0 and G_1. For these reasons, in the model we have combined the G_0 and G_1 phases so the time spent in the non-proliferative phases, T_N, includes time spent in both G_0 and G_1. In what follows, when we refer to G_0, we refer to G_0 and G_1.

The steady states of eqns (1) and (2) consist of the trivial steady state

$$(N^*, P^*) = (0, 0) \tag{4}$$

and the non-trivial steady state

$$(N^*, P^*) = \left(\theta^n \sqrt{\frac{\beta_0(2e^{-\gamma_0\tau} - 1) - \delta}{\delta}}, \times \frac{\delta N^*}{\gamma_0}\left[\frac{1 - e^{-\gamma_0\tau}}{2e^{-\gamma_0\tau} - 1}\right]\right). \tag{5}$$

The non-trivial steady state exists if

$$0 < \gamma_0\tau < \ln\left(\frac{2}{1 + \delta/\beta_0}\right). \tag{6}$$

With the notation and approximation,

$$\beta(N)N = \alpha(N) \simeq \alpha(N^*) + (N - N^*)\alpha'(N^*),$$

we can linearize eqn (1) in the neighborhood of a steady state. Defining the deviation in the non-proliferative population from the steady state by $Z(t) = N(t) - N^*$ it is easy to show that

$$\frac{dZ}{dt} = -AZ + BZ_\tau, \tag{7}$$

where

$$A = \delta + \alpha'(N^*), \tag{8}$$

$$B = 2e^{-\gamma_0\tau}\alpha'(N^*). \tag{9}$$

The stability of $P(t)$ follows the stability of $N(t)$. The *ansatz* $Z(t) = Z_0 e^{\lambda t}$ yields the eigenvalue equation

$$\lambda = -A + Be^{-\lambda\tau}. \tag{10}$$

According to Hayes' (1950) criteria, the eigenvalues will have negative real part and the steady state will thus be stable if

$$\left|\frac{A}{B}\right| > 1 \tag{11}$$

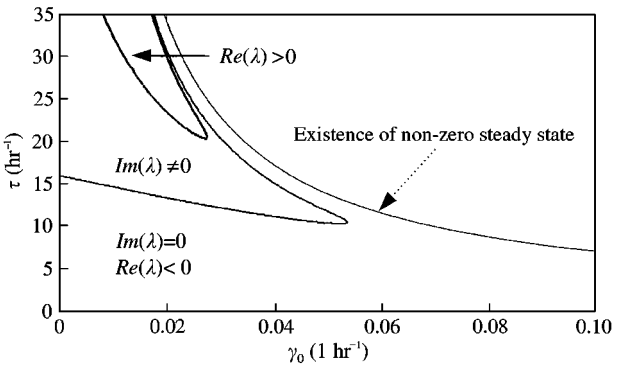

FIG. 2. This diagram shows the parameter space which is separated into two regions by the line labeled "Existence of non-zero steady state": These two regions are the upper right region, where only the zero steady state exists and is stable, and the lower left region, where the zero and non-zero steady states coexist. The big "tongue" coming from the upper left corner indicates the area where $\mathrm{Im}(\lambda) \neq 0$, e.g. where oscillatory behavior around the non-zero steady state is predicted from the linear analysis and the smaller tongue is the area in parameter space where the non-zero steady state is unstable and the system shows periodic solutions. The diagram corresponds to the normal bone-marrow value of $\delta = 0.16\ \mathrm{day}^{-1}$ and only small differences are obtained by using the leukemic bone-marrow value of $\delta = 0.11\ \mathrm{day}^{-1}$ (see Table 1). The trivial steady-state eigenvalue is always real.

or

$$\left|\frac{A}{B}\right| \leqslant 1 \quad \text{and} \quad \tau < \frac{\arccos(-A/B)}{\sqrt{B^2 - A^2}}. \tag{12}$$

These stability criteria are illustrated in the (γ_0, τ) parameter space in Fig. 2. The parameter estimates in Table 1 suggest that $\tau \simeq 20$ hr for both populations while $\gamma_0 \simeq 0\ \mathrm{hr}^{-1}$ and $0.149\ \mathrm{hr}^{-1}$ for the untreated leukemic and normal cell populations, respectively. Thus, the untreated populations are both stable, non-oscillatory fixed points far from the non-zero steady-state boundary in terms of the apoptotic loss rate γ_0. It should be noted that the malignant population is further from this boundary and a higher γ_c must be applied in order to drive the system to extinction (a stable zero steady state).

4. Chemotherapy

The chemotherapeutic drugs clinically used in remission induction therapy of AML are Ara-C

and the anthracyclines idarubicin, doxorubicin and daunorubicin. They are all *S*-phase specific, but may have an effect in other phases too, e.g. G_1/S block (Alison & Sarraf, 1997). For simplicity, we first assume that all proliferating cells are affected by the drug, but later we will show the effect of phase-specific drugs. In our model, the cells in the proliferative phase die due to an increased apoptotic rate $\gamma = \gamma_0 + \gamma_c(t)$. The parameter $\gamma_c(t)$ is the loss rate due to chemotherapy and is periodic with period $\mathscr{P}$. In Appendix B, we estimate the value of γ_c for a number of clinical schedules/doses. Here, we derive equations for the system under the influence of phase-non-specific chemotherapy and describe our simulations.

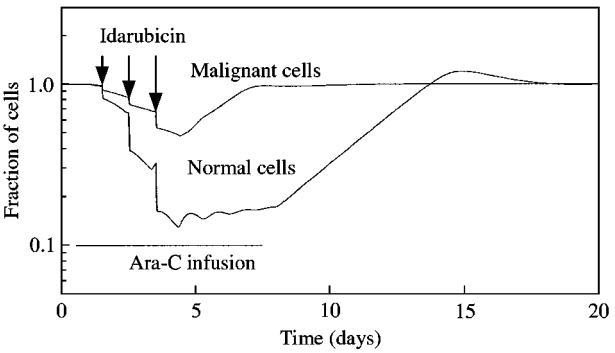

FIG. 3. Modeling results of AML remission induction schedule of Ara-C infusion for 7 days (days 1–8) together with IV bolus injection of Idarubicin on day 1, 2 and 3. The ratio of number of cells to the number of cells before treatment for normal and malignant cells is plotted vs. day number. When replacing Idarubicin with one of the other anthracyclines, similar results are obtained.

4.1. EQUATIONS WITH CHEMOTHERAPY

To derive a system of model equations that includes the effects of non-phase-specific chemotherapy, note that the number of cells recruited from the non-proliferative phase and back into the cell cycle will, until division, obey

$$\dot{N} = -\{\gamma_0 + \gamma_c(t)\}N(t). \tag{13}$$

Between $t-\tau$ and t the solution to this equation is given by

$$\int_{N(t-\tau)}^{N(t)} \frac{\mathrm{d}N}{N} = -\int_{t-\tau}^{t} \{\gamma_0 + \gamma_c(t')\}\,\mathrm{d}t', \tag{14}$$

$$N(t) = N(t-\tau)\exp\left(-\gamma_0\tau - \int_{t-\tau}^{t} \gamma_c(t')\,\mathrm{d}t'\right). \tag{15}$$

The integral of γ_c represents the fact that all proliferative cells are affected by the history of γ_c from time $t-\tau$ to time t.

Thus, when chemotherapy acts non-specifically throughout the cell cycle (duration τ) the system of governing equations is given by

$$\dot{N} = -\delta N - \beta(N)N + 2\exp\left(-\gamma_0\tau - \int_{t-\tau}^{t} \gamma_c(t')\,\mathrm{d}t'\right)\beta(N_\tau)N_\tau, \tag{16}$$

$$\dot{P} = -\gamma(t)P + \beta(N)N - \exp\left(-\gamma_0\tau - \int_{t-\tau}^{t} \gamma_c(t')\,\mathrm{d}t'\right)\beta(N_\tau)N_\tau. \tag{17}$$

4.2. CONVENTIONAL CHEMOTHERAPY SCHEDULES

Using parameters for normal and leukemic bone marrow, eqns (16) and (17) have been integrated numerically using software written in C++ with a fourth-order Runge–Kutta integration routine and a fixed time-step $\mathrm{d}t = 0.1$ hr. In Fig. 3, we present a typical simulation for a conventional schedule of Ara-C plus idarubicin discussed in Section 1.2.

Unfortunately, the simulated tumour was not killed by this protocol, but neither was the normal tissue. For the estimated parameters, the regeneration time for the tumour is shorter than for the host cells, and chemotherapy schedules are very unlikely to succeed in eradicating the tumour. This failure in the simulation results is due to two reasons, both related to the differences in the appoptotic rates of normal and leukemic cells: (i) when chemotherapy is applied, it is more devastating to the normal cells than to the leukemic cells; and (ii) leukemic cells regrow faster than their normal counterparts.

5. Resonance in the Cell Kinetic G_0 Model

Here, we study the periodic administration of chemotherapy (at a period $\mathscr{P}$) in which γ alternates between the natural loss rate, γ_0, and an elevated loss rate, $\gamma_0 + \gamma_c$, for a period of time equal to Δ. In what follows, we keep the average γ_c over one period independent of the period by varying the duration Δ so that the ratio $r = \Delta/\mathscr{P}$ is fixed. Previous work (Agur *et al.*, 1988; Dibrov, 1998; Dibrov *et al.*, 1985; Webb, 1992a) has suggested that resonant chemotherapy administration periods occur near integer multiples of the cell-cycle time. We, therefore, expect any resonance in our model to occur near the average inter-mitotic interval, which is the duration of the cell-cycle time plus the average time spent in G_0.

When chemotherapy is applied periodically with period $\mathscr{P}$, the cell number will oscillate with that period either around a constant mean value or around a decreasing mean

$$N(t) \simeq \tilde{N}(t, \mathscr{P})\mathrm{e}^{\alpha t}, \tag{18}$$

where $\tilde{N}(t, \mathscr{P})$ is periodic with period $\mathscr{P}$, $\tilde{N}(t + \mathscr{P}) = \tilde{N}(t)$. In what follows, α is calculated using

$$\alpha = \frac{\ln(N_{t_2}) - \ln(N_{t_1})}{t_2 - t_1} \tag{19}$$

for sufficiently large t_1 and t_2. The exponent α satisfies $\alpha \leqslant 0$ and will be referred to as *rate of growth* or simply *growth rate*. A zero growth rate corresponds to no effect of chemotherapy and a negative growth rate corresponds to a situation where cell numbers are being depleted. The transient period is of the order of a few multiples of $\max[\tau, \mathscr{P}]$.

In Fig. 4, we show the growth rate as a function of chemotherapy administration period $\mathscr{P}$ for both normal and malignant cells. The growth rates of *both* populations show clear peaks at $\mathscr{P} \simeq 45$, 90 and 135 hr, indicating resonance and the existence of higher harmonic responses. This is in spite of the fact that the two populations have different untreated inter-mitotic intervals.

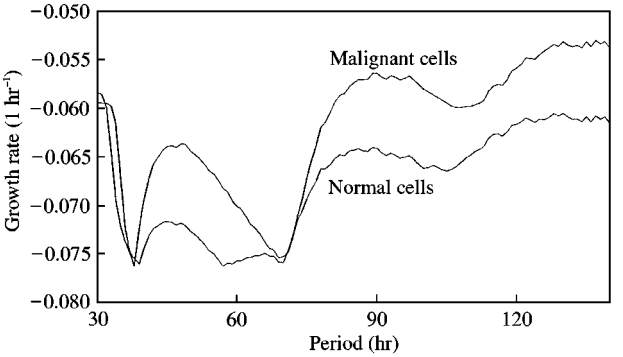

FIG. 4. The model predicted growth rate vs. administration period for normal and malignant cells. In this simulation $\gamma_c = 1.0\,\mathrm{hr}^{-1}$ and $\Delta = 0.4 \times \mathscr{P}$. There are peaks at $\mathscr{P} \simeq 45$, 90 and 135 hr, indicating resonant behavior. Note that the resonances occur at the same periods for both populations and that the malignant population always decays more slowly.

Because the peaks occur at the same periods, it is difficult to spare only the normal cells and it is not beneficial to administer chemotherapy at these resonant periods. Note also that the malignant population always decays more slowly than the normal population, even at resonance. This is due to the fact that the malignant population has a lower apoptosis rate from the proliferative phase than the normal population.

5.1. ROLE OF FEEDBACK

The feedback in the system controls the rate of recruitment out of G_0 into the proliferative phase, and affects the average inter-mitotic interval, the variance in inter-mitotic interval and resistance to chemotherapy. The average inter-mitotic interval consists of two parts: the time spent in the proliferative phase (τ) and the time spent in the non-proliferative phase $\langle T_N \rangle$. Cells are recruited from the non-proliferative phase back into the cell cycle at a rate $\beta(N)$ and move on to further differentiation and maturation at a rate δ. The distribution of time spent in the non-proliferative (G_0) phase is of the form

$$f(a) = k\mathrm{e}^{-ka}, \tag{20}$$

where a is the chronological age of the cell and $k = \delta + \beta(N)$ is the total loss rate out of the non-proliferative phase. The mean and the

variance of inter-mitotic intervals, T_{IM}, are given by

$$\langle T_{IM} \rangle = \tau + \frac{1}{k}, \tag{21}$$

$$\sigma^2 = \frac{1}{k^2}, \tag{22}$$

while the coefficient of variation (CV, standard deviation relative to the mean) is given by

$$CV = \frac{\sigma}{\langle T_{IM} \rangle} = \frac{1}{k \langle T_{IM} \rangle}. \tag{23}$$

With our estimated β_0 value, after only a few pulses of chemotherapy, the system will be at cell number levels such that $\beta(N) \simeq \beta_0$. In this case, the cells are rapidly recruited out of the non-proliferative phase, supporting the assumption (Dibrov *et al.*, 1985) of a short transit time through the non-proliferative phase. This explains why both Dibrov's model and our model display resonance. Also, when $\beta(N) \simeq \beta_0$, the mean inter-mitotic interval for both malignant and normal cells is around 27 hr and the coefficient of variation is $\simeq 25\%$ for both normal and malignant populations, making it impossible to shield only normal cells from chemotherapy effects. This explains why the resonance occurs at the *same* periods in both populations. In the presence of feedback, the cell number N will decrease over a course of chemotherapy, increasing the recruitment rate $\beta(N)$ and both the mean and variance of T_{IM} will decrease. If, however, we assume that the recruitment rate back into the active proliferating cycle is constant and equal to the estimated untreated steady-state value, $\beta(N) = \beta(N^*)$, the average inter-mitotic interval does not change as the cells are being depleted by chemotherapy.

In Fig. 5, the growth rate is plotted for three different values of β_0 which give rise to three different average inter-mitotic intervals and variation. Note that the absence of feedback wipes out the resonance and no differences with different administration periods are observed. It is strikingly clear that, in spite of the lack of resonance, the system will decay more slowly than it would at resonant period. This is because of a resistance

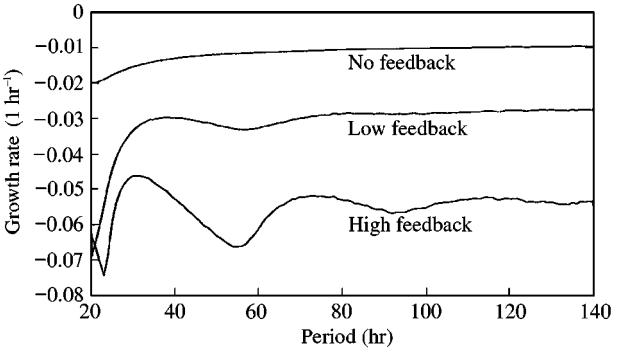

FIG. 5. Model predicted growth rates for normal bone-marrow cells vs. chemotherapy administration period for $\gamma_c = 1.0\ \mathrm{hr}^{-1}$. Top curve: $\beta_0 = \beta^*$. Middle curve: $\beta_0 = 5\beta^*$. Bottom curve: $\beta_0 = 10\beta^*$. The duration of the chemotherapy on-phase is $\Delta = 0.4 \times$ period. The simulations are all carried out for *S*-phase drugs (see later).

to chemotherapy caused by a low proportion of proliferating cells.

Most chemotherapeutic agents affect cells in one or more of the phases of the proliferative cycle. Only drugs of this type are considered here. It is, therefore, not possible to target the large number of resting cells, and in order to kill these they must be recruited back into the cycle. A strong feedback allows the system to respond to low cell numbers quickly as opposed to a weaker feedback or no feedback at all. This effect is emphasized by the large number of cells initially in the resting phase (80–90%, Peters *et al.*, 1986; Vidriales *et al.*, 1995). If the feedback is strong, most of the cells will be back in the cycle even after a few chemotherapeutic drug pulses to make up for the apoptotic-induced loss. A weak or non-existent feedback slows the replenishment and thus the resultant suicide happens more slowly for all periods. This is shown in Fig. 5 and explains why, in spite of the lack of resonance, a system with no feedback decays more slowly than a system with large feedback.

Dibrov *et al.* (1998) suggest that the resonance phenomena can only be observed when the coefficient of variation in inter-mitotic intervals is less than 50% and only if there is a stable distribution of inter-mitotic intervals and stable average inter-mitotic interval. Estimating the coefficient of variation using eqn (23), we find that it is 70% when $\beta_0 = \beta^*$ (no feedback), 39% when $\beta_0 = 5\beta^*$ (low feedback) and 25% when $\beta_0 = 10\beta^*$ (high

feedback). In Fig. 5, we can clearly see resonance when the coefficient of variation is less than 50%. This is in spite of the fact that our model has neither a constant inter-mitotic interval distribution nor mean.

The two main differences between our considerations and the previous results discussed in Section 2 are that our cell kinetic model explicitly includes both a resting G_0 state as well as feedback. Feedback controls the rate of recruitment out of G_0 into the proliferative phase. As we have seen, this has three main effects:

- *Average inter-mitotic interval.* The distribution of inter-mitotic intervals is not constant under feedback, and the average inter-mitotic interval for a given cell population can change during chemotherapy. This capacity for adjustment is an important feature of the G_0 state.
- *Variance in inter-mitotic interval.* The strength of the resonance is controlled by the variance in the distribution of inter-mitotic intervals. A low feedback tends to increase the time spent in the resting G_0 phase, and thus also increases the variance in inter-mitotic intervals.
- *Resistance to chemotherapy.* A cell population is more resistant to chemotherapy when fewer cells are in the proliferative compartment. A low feedback tends to make the population more resistant to chemotherapy because cells are not immediately recruited back into the proliferative phase from G_0.

Previous models (Agur *et al.*, 1988; Dibrov, 1998; Dibrov *et al.*, 1985; Webb, 1992a) of periodic applications of chemotherapy have all concluded that the normal cell population will decay more slowly than the malignant cells when chemotherapy is administered at the resonant period of the normal cells as long as the cell-cycle times of the two populations differ. This is based on the assumption that one can estimate the average cell-cycle time based on information about the *untreated* tissue. In untreated tissues, the average inter-mitotic intervals differ between normal and malignant cells but, when chemotherapy is applied, the inter-mitotic intervals may change. In our model, it is observed that the resonant periods of the normal and malignant populations can approach each other for parameters estimated for normal marrow and AML.

5.2. DRUG SPECIFICITY

The external parameters in our model consist of the chemotherapy administration schedule: duration of susceptible phase (S, M or cycle-non-specific), duration of drug effectiveness, Δ, and the intensity of chemotherapy γ_c. When the integrations of eqns (16) and (17) for phase-specific chemotherapy are carried out, the differences between cycle-non-specific, S-phase-specific and M-phase-specific drugs can be uncovered.

By analogy with our previous considerations, the equations describing the system under the influence of an S-phase-specific chemotherapeutic agent are given by

$$\dot{N} = -\delta N - \beta(N)N + 2\exp\left(-\gamma_0\tau - \int_{t-\tau}^{t-\tau+T_S}\gamma_c(t')\,dt'\right)\beta(N_\tau)N_\tau, \tag{24}$$

$$\dot{S} = -(\gamma_0 + \gamma_c(t))P + \beta(N)N - \exp\left(-\gamma_0 T_S - \int_{t-T_S}^{t}\gamma_c(t')\,dt'\right)\beta(N_{T_S})N_{T_S}, \tag{25}$$

$$\dot{M} = -\gamma_0 M + \exp\left(-\gamma_0 T_S - \int_{t-T_S}^{t}\gamma_c(t')\,dt'\right)\beta(N_{T_S})N_{T_S} - \exp\left(-\gamma_0\tau - \int_{t-\tau}^{t-\tau+T_S}\gamma_c(t')\,dt'\right)\beta(N_\tau)N_\tau. \tag{26}$$

Here, N is the number of non-proliferative cells, S is the number of cells in the DNA synthesis phase and M is the number of cells in mitosis. The main difference between the original model and this version is that the integral over γ_c is carried out from $t-\tau$ to $t-\tau+T_S$ instead of $t-\tau$ to t. Now, we can only kill *true* S-phase cells.

A number of drugs are anti-mitotic and affect only cells in mitosis or entering mitosis. Examples

are Vincristine and Vinblastine (Calabresi & Schein, 1993; Foon & Casciato, 1995). For such cases, the system equations are only slightly different from those for *S*-phase-specific drugs, and are given by

$$\dot{N} = -\delta N - \beta(N)N + 2\exp\left(-\gamma_0\tau - \int_{t-\tau+T_S}^{t} \gamma_c(t')\,dt'\right)\beta(N_\tau)N_\tau, \tag{27}$$

$$\dot{S} = -\gamma_0 P + \beta(N)N - \exp(-\gamma_0 T_S)\beta(N_{T_S})N_{T_S} \tag{28}$$

$$\dot{M} = -(\gamma_0 + \gamma_c)M + \exp(-\gamma_0 T_S)\beta(N_{T_S})N_{T_S} - \exp\left(-\gamma_0\tau - \int_{t-\tau+T_S}^{t} \gamma_c(t')\,dt'\right)\beta(N_\tau)N_\tau. \tag{29}$$

Figure 6 shows a typical example of the resonance observed for these three different drug mechanisms. *S*-phase-specific resonance is more pronounced than cycle-non-specific resonance, and *M*-phase-specific resonance is even sharper than the *S*-phase resonance. This is true for both normal and malignant cells, where the growth rates for the malignant cells are elevated relative to normal. In general, the shorter the susceptible phase, the sharper the resonance. This is also a feature of other models that predict resonance (Agur *et al.*, 1998; Webb, 1992a).

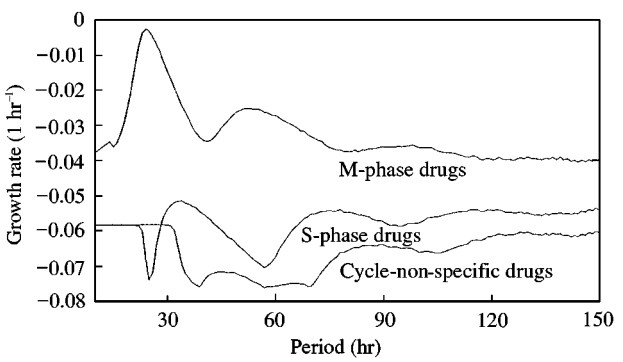

FIG. 6. The growth rate vs. administration period predicted from the modeling for cycle-non-specific, *S*-phase- and *M*-phase-specific drugs. The chemotherapy duration is $\Delta = 0.4 \times$ period and γ_c is $1.0\,\mathrm{hr}^{-1}$. The simulations were carried out for normal cell parameters.

5.3. INTENSIVE CHEMOTHERAPY

For intensive drug therapy, γ_c becomes large in both magnitude and duration of effectiveness. When this is the case and there is a long susceptible phase, a new form of resonance can occur. In this case, the resonance does not occur at the inter-mitotic interval but is shifted toward longer periods as in Fig. 6.

In the previous work described in Section 2, the drug pulse was short compared to the inter-mitotic interval. In this case, heterogeneity in cycle phases means that the same few depleted populations are *killed* at every administration, while most of the cells are shielded because they are non-susceptible.

Under intensive drug therapy, the opposite situation holds. The drug kills off all populations in the cycle except the few populations that are *shielded* because their susceptible phase exactly fits the off-phase of chemotherapy during each period.

Periods of drug administration near the intermitotic interval cannot generate resonance if the susceptible phase is longer than the off-phase. In this case, the resonance moves towards longer periods, where the off-phase is long enough for a single or a few lineages/populations to be shielded. Because the on-phase fraction is constant, periods to the right of the shifted resonance show poorer survival until a population can be shielded *twice* each period. This leads to a new resonance peak. A practical consequence of this shift may be that the resonance periods will depend on the drug dose and be harder to predict.

5.4. OPTIMAL CHEMOTHERAPY SCHEDULING

It is now established that resonance can occur in the G_0 model of the cell cycle. The next question is whether this resonance can be used to predict an optimal chemotherapy schedule. Although previous studies, as discussed in Section 2, have all concluded that these resonances can be used to predict optimal chemotherapy schedules, *we conclude that such an optimal schedule cannot be found within our model.* This is because our model is constrained by the physiological parameters

estimated in Appendix A and by a physiologically realistic drug effectiveness, while previous studies failed to include these constraints.

From our estimation of the physiological parameters, malignant cells seem to have a significantly lower apoptosis rate from the proliferative phase than do normal cells. This has two important consequences:

1. The minimum chemotherapeutic apoptotic rate such that the cell number will decay to the zero steady state can be found by rearranging eqn (6):

$$\gamma_c < \frac{1}{\tau} \ln\left(\frac{2}{1+\delta/\beta_0}\right) - \gamma_0.$$

   With the parameters we have estimated, this yields $\gamma_c \simeq 0.017\,\mathrm{hr}^{-1}$ and $0.030\,\mathrm{hr}^{-1}$ for the normal and malignant cells, respectively, and thus the malignant cells require a minimum chemotherapeutic kill rate almost twice as high as the normal cell kill rate to cross the boundary where the zero steady state becomes stable.
2. A lower apoptotic rate also means that the malignant cells regrow faster after depletion. This is because the eigenvalue of the steady state is proportional to the distance to the boundary where the zero steady state becomes stable.

Previous studies (Agur *et al.*, 1988, 1992; Dibrov *et al.*, 1985; Dibrov, 1998; Webb, 1990, 1992a, b) have ignored the role of feedback and have assumed a stable distribution of cell-cycle parameters. While it is true that the average inter-mitotic intervals of the normal and malignant populations in the *untreated* organism differ, the important cell parameter under chemotherapy is the average time spent in the proliferative phase (τ). This is because our modeling predicts that the average time spent in G_0 tends to zero during chemotherapy. Our parameter analysis (Appendix A) suggests that the average time spent in the proliferative phase for the two populations are similar $\tau_m \simeq \tau_n$. Agur (1988, 1992) discusses this point, and suggests that one can still benefit from resonance as long as the variance in the malignant cell population is greater than in the normal cell population.

In our model, the larger variance in inter-mitotic interval would come from a lower malignant maximal feedback $(\beta_0)_m < (\beta_0)_n$. This is illustrated in Fig. 7 in which two feedback curves are shown. Before treatment, the normal and malignant populations are situated at $n(0)$ and $m(0)$, respectively. With our current parameter estimates, both will follow the upper feedback curves during treatment. Here, the inter-mitotic intervals and variances will approach each other as treatment proceeds and no benefit can be obtained from resonance. However if, as in Dibrov *et al.* (1985), we assume that the malignant cell population has a longer inter-mitotic interval (lower feedback), the malignant cell population follows the lower curve in Fig. 7. This has several effects:

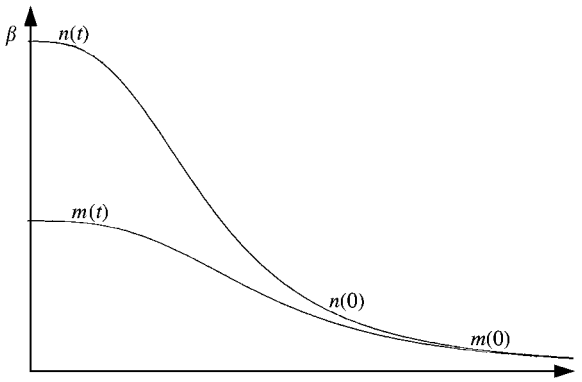

FIG. 7. The steady-state recruitment rate for normal $n(0)$ and malignant $m(0)$ cells and feedback curves for strong feedback $n(t)$ and weak feedback $m(t)$ resulting in higher coefficient of variation for the malignant cells, but slower decay.

- The inter-mitotic intervals change: $\langle T_{IM}\rangle_n \neq \langle T_{IM}\rangle_m$. The resonant periods no longer coincide and the schedule designed to maximally shield the normal cell population may not maximally kill the malignant cell population.
- The variance in inter-mitotic interval for the malignant cell population increases and the resonance in the malignant cell population is less pronounced.
- The recruitment of malignant cells from G_0 happens more slowly while the normal cells try and make up for the loss with a faster recruitment. More normal cells are sent back into the cycle to try and replenish the population. The normal cell population is thus more susceptible to chemotherapy and will experience an increased cell kill.

It is this last effect that has been ignored in previous studies. In these studies, the model starts

with two untreated populations with feedback of $m(t)$ and $n(t)$, respectively. This ignores the fact that, with lower feedback in the malignant population, it takes longer to reach the maximum feedback rate $m(t)$ than to reach $n(t)$ and the initially large malignant cell population in G_0 (Peters *et al*., 1986; Vidriales *et al*., 1995) will be depleted more slowly.

6. Summary

Based on this study, we have seen that the system can show resonance for periods slightly shorter than the mean inter-mitotic interval if the coefficient of variation is less than around 50% as in Dibrov *et al*. (1985). This condition will hold when a large number of non-proliferative cells are being recruited back into the cell cycle after a few drug pulses and the average time spent in the non-proliferative phase becomes very short.

In our model, leukemic bone marrow cells have a longer inter-mitotic interval (and variance) than normal bone marrow cells when *untreated*, but both tend to the same value as chemotherapy proceeds. However, resonance alone does *not* guarantee a cure for the patient, especially if regrowth is faster in tumour cells than in the limiting tissue.

We varied the duration of the susceptible phase in order to mimic the effects of cycle-non-specific, *S*-phase-specific and *M*-phase-specific drugs and observed that differences in drug phase effects strongly affect the resonance properties. A shorter susceptible phase gives a sharper resonance, which agrees with the results of Webb (1992b).

When the drug dose is large, resonance can be observed until the on-phase becomes longer than the inter-mitotic interval, and the shielding effect is lost. Just before this happens, resonance can be seen for periods longer than the average inter-mitotic interval. When the off-phase exceeds the duration of susceptible phase, these long resonant periods can be observed for *S*-phase drugs and cycle-non-specific drugs. Based on our results, we conclude that in order to successfully take advantage of resonance in periodic chemotherapy treatment of cancer, one must be very careful when predicting optimal administration periods based on cell-cycle times alone. It is important to note that the average inter-mitotic interval and its variance may change over the course of chemotherapy, the speed of recruitment of cells out of G_0 may favor one population, the depletion and regrowth rate may differ between the two cell populations and the resonance period itself may be difficult to predict especially under intense drug administration (which is actually required in order to observe resonance at all).

It is now interesting to ask who would benefit from these considerations. To successfully take advantage of the resonance effect, both cell populations need to have high feedback in order to recruit the cells into the susceptible proliferative phase together with parameters such that either:

- The inter-mitotic intervals at low cell numbers of the two populations differ (Agur *et al*., 1988; Dibrov, 1998; Dibrov *et al*., 1985; Webb, 1992a). In this case, resonance at low cell numbers occurs for different periods so the normal population can be shielded; or
- There is a larger variance in the malignant cell inter-mitotic interval T_{IM} (Agur *et al*., 1988). In this case, even though the inter-mitotic intervals may be the same, the malignant cells do not benefit from the resonance condition and only the normal cell population is shielded by the resonance.

The average inter-mitotic interval and the variance in inter-mitotic interval are primarily controlled by two parameters: the strength of feedback β_0 (which controls $\langle T_N \rangle$), and the average time spent in the proliferative phase τ. The effect of changing these two parameters are shown in Fig. 8 where all simulations were carried out for normal cell parameters.

We have shown that the resonance is eliminated by changing the strength of the feedback, and therefore also the average time spent in the resting phase, as discussed previously. Assuming a longer proliferative phase, together with an unchanged duration of susceptible phase, the resonance becomes more pronounced. Both the resonance and the higher harmonics are quite pronounced allowing this cell type, with a longer inter-mitotic interval and smaller variation, to respond more effectively to resonant periodic chemotherapy scheduling. This is in disagreement with results of previous work (Agur *et al*., 1988, 1992; Dibrov *et al*., 1985; Dibrov, 1998; Webb,

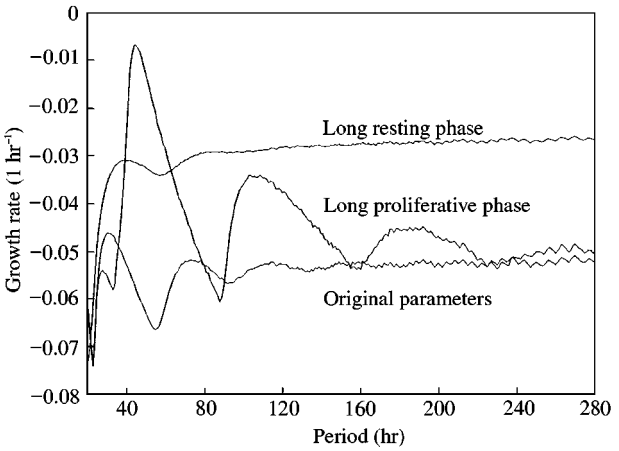

FIG. 8. The growth rate vs. administration period predicted from our modeling for different cell populations with different inter-mitotic intervals. Here, $\langle T_{IM} \rangle = 27$ hr and $CV = 25\%$. In the long proliferative phase system, $\tau \to 2\tau$, and has a sharper resonance than the reference population because of a larger $\langle T_{IM} \rangle = 47$ hr and a smaller $CV = 15\%$. In the lower feedback system, $\beta_0 \to 0.5\beta_0$. The mean and variance for this population are 33 hr and 39%, respectively. The chemotherapy duration is $\Delta = 0.4 \times$ Period and the simulations are all carried out for S-phase drugs.

1990, 1992a, b), where malignant cells are assumed *not* to benefit from resonant periodic scheduling because of their *longer* cell-cycle time.

Three major facets of this study demand further investigation, and are under active investigation. The first of these is related to the lack of adequate parameter estimation for the hematopoietic stem cell compartments of normal humans and patients as alluded to in Appendix A. The second has to do with the fact that the application of chemotherapeutic drugs can cause cell arrest within the cell cycle and then a subsequent synchronization.

The final one is related to our neglect of the communication between normal and malignant cell populations through the exchange of cytokines. Though it is difficult to forsee the consequence of the inclusion of this effect in the model developed here, there are clues from the work of Łoskot *et al.* (1991). Using a model for two types of communicating cells, they demonstrated that there existed a region of parameter values that guaranteed the stable coexistence of coupled cellular populations. The analysis also showed that increases in the coupling between populations could ultimately lead to the destruction of one population and the persistence of the other.

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APPENDIX A

Parameter Estimation

This appendix uses published data to estimate the parameters for simulations. In spite of the fact that a great deal of *qualitative* information is currently being rapidly accumulated on the hematopoietic stem cells, there is little *quantitative* data on which one can base the type of parameter estimation that this study requires. Arguably the best estimates for stem cell parameters are those of Abkowitz *et al.* (1996, 2000, 1995) in the cat and mouse, and Mackey (2000) in the mouse. However, there is little experimental data on human hematopoietic stem cells in either normal individuals or leukemic patients on which to base similar estimates.

The hematopoietic stem cells are not the only proliferating cells in the bone marrow. In fact, they are vastly outnumbered by the differentiated progeny of these stem cells that are committed to the production of red blood cells, platelets, and white blood cells. However, in terms of tissue growth, we have considered these populations as a single source (Baserga, 1981) because of the paucity of data allowing us to distinguish between them. Our parameters are evaluated under this assumption.

In the following, the fraction of cells in G_0/G_1 phase is given by A and the labeling index (the fraction of cells in DNA synthesis) is represented by B.

A.1. STEADY-STATE EQUATIONS

At steady state, the fraction of cells in proliferative phase is given by

$$1 - A = \frac{P^*}{P^* + N^*} = \frac{(\beta^*/\gamma_0)(1 - e^{-\gamma_0 \tau})}{1 + (\beta^*/\gamma_0)(1 - e^{-\gamma_0 \tau})}. \tag{A.1}$$

Similarly, the fraction of cells in S phase is given by

$$B = \frac{P_S^*}{P^* + N^*} = \frac{(\beta^*/\gamma_0)(1 - e^{-\gamma_0 t_S})}{1 + (\beta^*/\gamma_0)(1 - e^{-\gamma_0 \tau})}. \tag{A.2}$$

The ratio of the cells in S phase (B) to all proliferative cells ($1 - A$) yields

$$\tau = -\frac{1}{\gamma_0} \ln\left[1 - \frac{1 - A}{B}(1 - e^{-\gamma_0 t_S})\right]. \tag{A.3}$$

The steady-state recruitment rate (β^*) is derived from

$$A = \frac{N^*}{P^* + N^*} = \frac{1}{1 + (\beta^*/\gamma_0)(1 - e^{-\gamma_0 \tau})} \Rightarrow$$

$$\beta^* = \frac{1 - A}{A} \frac{\gamma_0}{1 - e^{-\gamma_0 \tau}} = \frac{B}{A} \frac{\gamma_0}{1 - e^{-\gamma_0 \tau_S}}. \tag{A.4}$$

Knowing β^*, δ can be derived:

$$\delta = \beta^*(2e^{-\gamma_0 \tau} - 1) = \frac{B}{A} \frac{\gamma_0}{1 - e^{-\gamma_0 t_S}} \times \left[1 - 2\frac{1 - A}{B}(1 - e^{-\gamma_0 t_S})\right]. \tag{A.5}$$

A.2. NORMAL BONE MARROW

The following data are from the literature: $t_S = 0.60$ day (Aglietta *et al.*, 1989), $\delta = 0.164\ \text{days}^{-1}$ (Mackey, 1978), $A = 80.4\%$ (Peters *et al.*, 1986), and $B = 14.6$ (Peters *et al.*, 1986). Using eqns (32)–(34) together with these data we calculate $\gamma_0 = 0.35\ \text{day}^{-1}$, $\beta^* = 0.35\ \text{day}^{-1}$, $\tau = 0.83$ day, $t_{G_2/M} = \tau - t_S = 0.24$ day, and $\langle t_{G_0/G_1} \rangle = 1/(\delta + \beta^*) = 1.4$ day. With these estimates of γ_0 and τ, approximately 81% of the cells survive through proliferative phase. Under the assumptions (Mackey, 1978) that $N_{\text{total}} = 5 \times 10^{10}$ cells/70 kg, 50% of the bone marrow cells are normal, β^* is one-tenth of maximum value and $n = 3$ the normal bone-marrow parameters in Table 1 were derived.

A.3. MALIGNANT BONE MARROW

Translocation of the *bcl*-2 gene (seen in virus-induced AML) to a transcriptionally active site may be expected to increase *bcl*-2 expression and protect cells, which under normal circumstances would undergo apoptic cell death (Alison & Sarraf, 1997). Expression of the *bcl*-2 oncoprotein inhibits programmed cell death and has been shown to predict poor survival, since these forms of cancer will often be resistant to chemotherapy which induces apoptosis. In the following it will be assumed that leukemic cells do respond to chemotherapy.

Using the following data from the literature: $\gamma_0 = 0$, $t_S = 0.73$ day (Preisler *et al.*, 1995), $A = 91\%$ (Vidriales *et al.*, 1995), and $B = 7.4\%$ (Vidriales *et al.*, 1995), we find that $\beta^* = 0.0047\ \text{hr}^{-1} = 0.11\ \text{day}^{-1}$, $\delta = \beta^*$, $\tau = 21.2$ hr, $t_{G_2/M} = \tau - t_S = 3.8$ hr, $\langle t_{G_0/G_1} \rangle = 1/(\delta + \beta^*) = 4.5$ days, $N^* = 3.25 \times 10^8\ \text{cells kg}^{-1}$, and $P^* = 0.32 \times 10^8\ \text{cells kg}^{-1}$.

Estimating the maximum recruitment rate (β_0) is not easy. An argument for the form of $\beta(N)$ is given in Mackey (1978), and in the following it is assumed that the leukemic cells resemble those of their host tissues in maximum recruitment capacity (β_0), but the untreated recruitment levels (β^*) can differ. An expression for the doubling time at depleted levels is also given in Mackey (1978). For the choice of β_0, this doubling time equals 53 hr for normal cells and 29 hr for malignant cells. Unfortunately, these numbers have not been verified experimentally but they may be reasonable since the malignant cell doubling time is shorter than the normal cell doubling time. When the maximal rate of reentry from non-proliferative phase into the cell cycle, β_0, is assumed the same for normal and malignant cells we obtain $\theta = 1.06 \times 10^8\ \text{cells kg}^{-1}$. The malignant bone-marrow parameter values are summarized in Table 1.

APPENDIX B

Estimation of Apoptotic Rate

To estimate the rate of apoptosis due to chemotherapy, we assume that γ_c can be fitted to exponentials, i.e. the drug binds and induces apoptosis in a constant fraction of cells per unit time and that γ_c is the same for normal and AML cells.

B.1. ARA-C

Kufe & Spriggs (1985) show dose–response curves of human leukemic myoloblasts after exposure to Ara-C for 1, 3, 6 and 24 hr. They also show the survival of cells in thymidine suicide experiments as a measure of cells in *S* phase during the exposure periods. The survival fraction was converted into a fraction of surviving *S*-phase cells by subtracting the fraction of cells that survived the thymidine suicide experiment. This fraction was plotted vs. the duration of exposure for each concentration.

In Chabner & Longo (1996), figures of Ara-C plasma concentrations under different administration schedules are given. A dose of $3\ \text{g m}^{-2}$ over 2 hr results in plasma concentration that falls off close to exponentially, i.e. $c(t) \simeq k_1 e^{-\alpha t}$, where c is the concentration of Ara-C. For the two hour injections, effective drug is assumed to be in the system only in the initial phase, and the killing rates are the slopes fit through the first data points up to $t = 6$ hr. The four slopes obtained are $\gamma_c = 0.09\ \text{hr}^{-1}$ (10^{-7} M), $\gamma_c = 0.34\ \text{hr}^{-1}$ (10^{-6} M), $\gamma_c = 0.74\ \text{hr}^{-1}$ (10^{-5} M) and $\gamma_c = 2.0\ \text{hr}^{-1}$ (10^{-4} M).

In plotting the four killing rates, γ_c vs. Ara-C concentration on a double logarithmic scale, one finds the $\gamma_c(c) \simeq k_2 c^{1/2}$.

Using this relation together with $c(t)$, the killing rate of the 2 hr injection is given by: $\gamma_c(t) \simeq a e^{-\beta t}$ with $a = 2\ \text{hr}^{-1}$ and $\beta = 0.4\ \text{hr}^{-1}$.

TABLE B.1
Induced apoptosis rate for a number of chemotherapeutic drugs under certain schedules

Dose	γ_c	Duration	Concentration	Reference
$100\,\mathrm{mg\,m^{-2}\,hr^{-1}}$ Ara-C	$0.015\,\mathrm{hr^{-1}}$	cont	Chabner & Longo (1996)	Kufe & Spriggs (1985)
$3\,\mathrm{g\,m^{-2}}$ Ara-C over 2 hr	$2e^{-0.4t}\,\mathrm{hr^{-1}}$	—	Chabner & Longo (1996)	Kufe & Spriggs (1985)
$12\,\mathrm{mg\,m^{-2}}$ idarubicin	$1.6\,\mathrm{hr^{-1}}$	1 hr	Tidefelt *et al.* (1994)	Sundman-Engberg *et al.* (1998)
$60\,\mathrm{mg\,m^{-2}}$ daunorubicin	$1.2\,\mathrm{hr^{-1}}$	1 hr	Sundman-Engberg *et al.* (1990)	Sundman-Engberg *et al.* (1998)
$30\,\mathrm{mg\,m^{-2}}$ doxorubicin	$0.4\,\mathrm{hr^{-1}}$	3 hr	Sundman-Engberg *et al.* (1990)	Sundman-Engberg *et al.* (1998)

A dose of $100\,\mathrm{mg\,m^{-2}\,hr^{-1}}$ results in a constant plasma concentration $\simeq 2\times10^{-6}$ M (Chabner & Longo, 1996). Between 6 and 24 hr for $c = 10^{-7}$ M Ara-C, the fraction of surviving cells falls from 50 to 32%. For $c = 10^{-6}$ M, the fraction falls from 19 to 18% and for $c = 10^{-5}$ M the fall is from 4 to 3%. The slopes of these three curves are: $\gamma_c\ (10^{-7}) = 0.025\,\mathrm{hr^{-1}}$, $\gamma_c\ (10^{-6}) = 0.003\,\mathrm{hr^{-1}}$, $\gamma_c\ (10^{-5}) = 0.016\,\mathrm{hr^{-1}}$.

Since there is no obvious pattern in these data, we simply use the average value, $\gamma_c = 0.015\,\mathrm{hr^{-1}}$ (Table B.1).

B.2. IDARUBICIN

Sundman-Engberg *et al.* (1998) show the survival fraction after incubating normal bone-marrow cells with concentrations of 0.05 and 0.2 μM idarubicin for 1 and 3 hr. A concentration of 0.05 μM shows no further effect after 1 hr. During this first hour, the apoptotic killing rate is $\gamma_c \simeq -\ln(0.27\times14.6/19.6) = 1.6\,\mathrm{hr^{-1}}$, where 0.27 is the fraction of surviving S phase cells, 14.6 is the percentage of cells in S phase and 19.6 is the percentage of all cycling cells for normal bone marrow (Peters *et al.*, 1986).

Tidefelt *et al.* (1994) measure the plasma concentration of idarubicin in leukemic peripheral blood cells after administration of $10\,\mathrm{mg\,m^{-2}}$ as a 10 min or a 1 hr infusion. Within 1 hr, the concentration is in the range $0.025 < c < 0.2$ μM and we, therefore, assume that the γ_c value estimated at $c = 0.05$ μM will be in the range of acceptance.

B.3. DAUNORUBICIN

The concentration of daunorubicin in vivo after a 10 min infusion of $60\,\mathrm{mg\,m^{-2}}$ results in intra-cellular concentration in leukemia cells that resemble 1 hr incubation of 0.2 μM daunorubicin *in vitro* (Sundman-Engberg *et al.*, 1990).

The cytotoxicity of 0.2 μM daunorubicin is estimated according to survival fractions after 1 and 3 hr of incubation (Sundman-Engberg *et al.*, 1998) of normal bone marrow cells. We find $\gamma_c \simeq 1.2\,\mathrm{hr^{-1}}$ for 1 hr.

B.4. DOXORUBICIN/ADRIAMYCIN

As in the case of daunorubicin, the concentration of doxorubicin *in vivo* after a 10 min infusion of $60\,\mathrm{mg\,m^{-2}}$ resembles a 3 hr *in vitro* infusion of 0.2 μM (Sundman-Engberg *et al.*, 1998).

This concentration will result in a $\gamma_c \simeq 0.6\,\mathrm{hr^{-1}}$. Since we are interested in γ_c after infusion of only $30\,\mathrm{mg\,m^{-2}}$, we take $\gamma_c = 0.4\,\mathrm{hr^{-1}}$, keeping in mind that the product $\gamma_c\Delta$ for the three anthracycline protocols probably should be of the same order: $\gamma_c\Delta$ (idarubicin) = 1.6, $\gamma_c\Delta$ (daunorubicin) = 1.2, and $\gamma_c\Delta$ (doxorubicin) = 1.2 when $\gamma_c = 0.4\,\mathrm{hr^{-1}}$.

The rationale for keeping $\gamma_c\Delta$ constant is based on the idea that the integrated effect of these three anthracyclines must be the same, since they are used interchangeably in combination chemotherapy with Ara-C.