

A Theoretical Analysis of Interval Drug Dosing for Cell-Cycle-Phase-Specific Drugs

LUTZY COJOCARU

AND

ZVIA AGUR*

*Department of Applied Mathematics and Computer Science,
The Weizmann Institute of Science, Rehovot, Israel*

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ABSTRACT

A formal method is provided for predicting the effect on treatment efficacy of cell-cycle-phase-specific drugs, such as the AIDS drug zidovudine (AZT) or the cancer drug cytosine arabinoside (ara-C). Our analysis shows that the elimination of somatic cells or viruses depends not only on the drug's pharmacokinetic and pharmacodynamic properties, but also on the duration of the dosing interval per se and on the life-cycle parameters, that is, the duration of the drug-susceptible life phase, the duration of the whole life cycle, and the proliferation rate. The results support those of simplified models in showing that drug toxicity to the host may be minimized when the dosing interval is an integer multiple of the average cycle time of the host susceptible cells. This prediction has been verified in mice treated with AZT or ara-C.

1. INTRODUCTION

Many drugs used in the treatment of cancer and viral infections impair genome synthesis while having no effect on cells or viruses that are not in the DNA synthesis phase. The effect of such drugs on proliferating normal host cells can bring about bone marrow depression and other toxic effects that often result in the patient's death. Reduction to about half the original dose is usually recommended for controlling drug toxicity, whereas high doses are often necessary to achieve responsiveness [9, 15, 19]. These

* Present address: Department of Biology, Imperial College of Science Technology and Medicine, London, England.

contradicting requirements have led to a practice of tedious trial-and-error dosage manipulations in the treatment of patients. The aim of the present work is to provide a formal method for predicting treatment efficacy. This method may aid in limiting the clinical trials to a reasonable minimum.

In previous studies simple models of cell population dynamics in regimes of cell-cycle-phase-specific drugs have been investigated. Analysis of these models suggests that the variability in the cycle duration of cancer cells or the HIV virus can be employed for designing drug regimens with reduced host toxicity. More specifically, it was suggested that toxic cell-cycle-phase-specific drugs should be used so that the dosing interval is an integer multiple of the average cycle time of the susceptible host cells [1, 3, 4]. As the average cell-cycle duration in human bone marrow is about 24 h, it was suggested that AZT should be administered in a *single* high daily dose (the *Z-method*) [2].

In the above models, specificity and precision were traded off for tractability and generality, the major simplification being a dichotomous (“all or none”) function for the drug effect. However, for rendering our theoretical results useful to clinicians it is necessary to verify their robustness under realistic assumptions about the drug’s pharmacokinetics and pharmacodynamics and about the age density distribution of the cell population. This task is taken up in the present work.

2. THE BASIC MODEL

2.1. BIOLOGICAL ASSUMPTIONS

Based on the time of DNA synthesis, the cell division cycle can be divided into four distinct phases: G_1 (before DNA synthesis), S (DNA synthesis), G_2 (after DNA synthesis), and M (cell division). The life cycle of the HIV virus can be divided into several phases that include attachment to cell surface, reverse transcription, integration within cellular DNA, and the production and release of new HIV particles [13]. However, for analyzing drug effect on the persistence of somatic and neoplastic cells or viruses, the different life-cycle phases of the cells, or those of the virus, can be grouped into two essential phases: a phase in which the cell or virus is susceptible to the drug, and a phase in which the cell or virus is immune to the drug. The important differences between the somatic and the viral, or neoplastic, life cycles lie in the distribution of the durations of these two phases.

In our model we consider the dynamics of cells or viruses with the above life-cycle properties. For brevity we refer in this section to both the cell and the virus as the *cell*. An important simplifying assumption is that each population is homogeneous with respect to drug susceptibility. Thus we do not take account of the possibility that drug resistance may emerge or that

some cells can be sheltered from drug effect. Previous theoretical results suggest that the existence of a drug-immune cell reservoir, such as G_0 cancer cells or latent virus particles, may require a prolonged treatment period but not a change in protocol (Agur and Norel, unpublished).

Our model considers a cell having age a at the initial moment of drug treatment, that is, at $t = 0$; by *age* we denote the cell's position in the life cycle, that is, the time since its birth. We denote the duration of the phase in which the cell is resistant to the drug (e.g., the $G_1 + G_2 + M$ phases) by ρ and the duration of the susceptible phase (e.g., the S phase in somatic cells or the reverse transcription phase in the HIV-1 virus) by ξ , so that the cell-cycle length is $\tau = \xi + \rho$. In this section all these parameters are taken as constant.

Using the above assumption we can formulate cell susceptibility to the drug treatment by the simple function

$$\chi(a) = \begin{cases} 1, & a \in \text{susceptible phase,} \\ 0, & a \in \text{resistant phase.} \end{cases} \quad (1)$$

Under the assumption that τ is constant, $\chi(a)$ is a periodic function; that is,

$$\chi(a + n\tau) = \chi(a), \quad n = 1, 2, \dots \quad (2)$$

2.2. PROBABILITY OF CELL SURVIVAL

Looking at an arbitrary cell whose age is a at the moment of treatment initiation and defining $p(t, a)$ as the probability that the lineage descending from this cell will survive up to the moment t , we obtain

$$p(t + \Delta t, a) = [1 - g(t, a) \cdot \Delta t] p(t, a), \quad (3)$$

where by $g(t, a)$ we define the effect of the toxic drug at time t . This effect is the product of the drug's killing efficacy and the availability of susceptible cells at time t , as follows:

$$g(t, a) = K(t) \cdot \chi(t + a). \quad (4)$$

To account for a saturation effect of the drug, the killing efficacy, $K(t)$, at time t may be taken as a function of the current drug concentration, $C(t)$, as follows:

$$K(t) = 1 - e^{-k_1 C(t)}, \quad k_1 \text{ constant} > 0. \quad (5)$$

Assuming a conventional first-order kinetics of drug elimination, the rate of loss of the drug from the body is given by

$$\frac{dC}{dt} = -kC, \quad C(0) = C_0, \quad (6)$$

where k is the decay coefficient.

If every drug dose is fully eliminated prior to the consecutive dosing, and the same initial concentration C_0 is given cyclically, then $K(t)$ is a periodic function, so that, for l being the dosing interval, we obtain

$$K(t + nl) = K(t), \quad n = 1, 2, \dots \quad (7)$$

When $\Delta t \rightarrow 0$, we have

$$\frac{\partial p(t, a)}{\partial t} = -g(t, a)p(t, a). \quad (8)$$

Since we consider here the case of a single cell lineage, the initial condition is

$$p(0, a) = 1, \quad (9)$$

so that

$$p(t, a) = \exp\left[-\int_0^t K(s)\chi(s+a) ds\right]. \quad (10)$$

2.3. THE DYNAMICS OF AN ASYNCHRONOUS CELL POPULATION

The analysis is now extended to allow for the dynamics of an asynchronous cell population. At treatment initiation ($t = 0$), the number of cells is N_0 , and their age density distribution is $f(a)$, $0 \leq a < \tau$. We wish to investigate the dynamics of this cell population by evaluating its size after the first, second, etc. dosing. Denoting by $N_{a,\varepsilon}(0)$ the number of cells whose age lies in the interval $(a, a + \varepsilon)$ at $t = 0$, we obtain

$$N_{a,\varepsilon}(0) = N_0 \int_a^{a+\varepsilon} f(s) ds \approx N_0 f(a)\varepsilon, \quad (11)$$

where ε is small.

After the first dosing the number of cells in the cohort whose age lies in the interval $(a, a + \varepsilon)$ is

$$N_{a,\varepsilon}(l) = \alpha^{l/\tau} [N_0 f(a) \varepsilon p(l, a)]. \quad (12)$$

The total number of cells after the first dosing is

$$N(l) = \alpha^{l/\tau} N_0 \int_0^\tau f(a) p(l, a) da, \quad (13)$$

and after m successive dosings it is

$$N(ml) = \alpha^{ml/\tau} N_0 \int_0^\tau f(a) p(ml, a) da. \quad (14)$$

Let us denote by A_i the event

$$A_i = \{ \text{a lineage initiated by a cell of age } a \text{ survives the } i\text{th dose} \}. \quad (15)$$

Assuming no overlap between any two consecutive doses, the events A_i are independent, so, using (10), we can write

$$\begin{aligned} p(ml, a) &= p(A_1 \cap A_2 \cap \cdots \cap A_m) = p(A_1) p(A_2) \cdots p(A_m) \\ &= \exp \left\{ - \sum_{i=0}^{m-1} \int_{il}^{(i+1)l} K(s \bmod l) \chi[(s+a) \bmod \tau] ds \right\} \\ &= \exp \left\{ - \int_0^l K(s) \sum_{i=0}^{m-1} \chi[(s+a+il) \bmod \tau] ds \right\}, \quad (16) \end{aligned}$$

where we define " $x \bmod y$ " (x, y positive real numbers) as the positive real number z such that $x = ny + z$ holds, n being the largest natural number that satisfies $ny \leq x$.

Substituting (16) into (14), we obtain the number of cells after m doses:

$$\begin{aligned} N(ml) &= \alpha^{ml/\tau} N_0 \int_0^\tau f(a) \\ &\quad \times \exp \left\{ - \int_0^l K(s) \sum_{i=0}^{m-1} \chi[(s+a+il) \bmod \tau] ds \right\} da. \quad (17) \end{aligned}$$

Below, we analyze the effect of long term treatment, that is, $m \rightarrow \infty$. For computing $\lim_{m \rightarrow \infty} N(ml)$, it is convenient to use the notation

$$\frac{1}{m} \sum_{i=0}^{m-1} \chi[(s + a + il) \bmod \tau] = A_m(s, a). \quad (18)$$

In this way (17) becomes

$$N(ml) = N_0 \int_0^\tau f(a) \left[\frac{\alpha^{l/\tau}}{\exp \left[\int_0^l K(s) A_m(s, a) ds \right]} \right]^m da. \quad (19)$$

As (18) is related to the dynamical aspect of ergodicity, it is natural to use some results in ergodicity to evaluate $\lim_{m \rightarrow \infty} N(ml)$. More precisely, we consider a cell of age a and look at the cell or the cell lineage states at each new dosing, that is, $\{a, (a + l) \bmod \tau, (a + 2l) \bmod \tau, \dots, [a + (m - 1)l] \bmod \tau\}$. Thus (18) measures the number of times the cell lineage has entered the S phase during m dosings.

The analysis that follows is carried out for two types of protocols: (1) protocols with an irrational relation between the dosing interval l and cell-cycle time τ , and (2) protocols with a rational relation between l and τ .

Irrational l/τ . In this case the transformation

$$t \rightarrow (t + l) \bmod \tau \quad (20)$$

is measure-preserving and ergodic, and from ergodic theory [7, 8] we obtain

$$\lim_{m \rightarrow \infty} A_m(s, a) = \frac{\xi}{\tau} \text{ a.e.} \quad (21)$$

Substituting (21) into (19) we obtain the *necessary* and *sufficient* condition for exponential decay of $N(ml)$:

$$\frac{\alpha^{l/\tau}}{\exp \left[\left(\xi / \tau \right) \int_0^l K(s) ds \right]} < 1 \text{ a.e.} \quad (22)$$

From here,

$$\ln \alpha < \frac{\xi}{l} \int_0^l K(s) ds, \quad (23)$$

which yields the condition for treatment efficacy.

Rational l/τ . If l/τ is rational, then

$$\chi[(s + a + il) \bmod \tau] \text{ is periodic with period } T = p\tau = ql, \quad (24)$$

p, q coprime integers.

A straightforward computation shows that in this case

$$\lim_{m \rightarrow \infty} N(ml) = \lim_{m \rightarrow \infty} N_0 \int_0^\tau f(a) \left[\frac{\alpha^{l/\tau}}{\exp \left[\int_0^l K(s) A_q(s, a) ds \right]} \right]^m da. \quad (25)$$

A *necessary* and *sufficient* condition for $N(ml) \rightarrow 0$ would therefore be

$$\frac{\alpha^{l/\tau}}{\exp \left[\int_0^l K(s) A_q(s, a) ds \right]} < 1 \text{ a.e.}, \quad (26)$$

which is equivalent to the condition

$$\ln \alpha < \frac{\tau}{l} \int_0^l K(s) A_q(s, a) ds \text{ a.e.} \quad (27)$$

Remark. From a practical point of view the distinction between rational and irrational l/τ makes no sense. Therefore our model is consistent if we capture this phenomenon mathematically as well, that is, if we show that (27) is consistent with (23). If l/τ is an irrational number and p_n/q_n is a sequence of rational numbers converging to l/τ , we have to show that

$$A_{q_n} \rightarrow \xi/\tau. \quad (28)$$

If (28) is true, then the inequalities (27) and (23) are identical, thus proving that the model is consistent. To show that (28) is true, we note that

$$\text{when } \lim_{n \rightarrow \infty} \frac{p_n}{q_n} = \frac{l}{\tau}, \text{ then } q_n \rightarrow \infty.$$

This is due to the fact that l/τ is irrational while p_n/q_n is rational, so that (28) follows in view of (21).

3. DISCUSSION

A mathematical model of cell population dynamics in regimes of cell-cycle-phase-specific drugs is studied in this work. The model allows for the pharmacokinetics and pharmacodynamics of the drug, as well as for the age density distribution, which represents the level of synchronicity in the timing of cell division. For analytic tractability, a constant cycle duration is assumed, but, as is shown below, in the numeric simulations of the model this assumption can be replaced by a more realistic distribution of this parameter. In the latter case the function χ in (17) is no longer periodic, so the summation should be performed explicitly and the integration limit should refer to τ at $t = 0$.

Our analysis shows that for eliminating a given cell population, condition (23) has to be satisfied if the relation between τ and l is irrational, and condition (27) if the relation between τ and l is rational. As real-life measurements yield a rational relation between the biological and pharmacological periodicities, we focus on condition (27) and show how it can be employed in a straightforward manner for increasing drug selectivity. To illustrate this we represent time by a circle of perimeter τ and denote the first q successive drug dosings by points on the circle, at times $t_0, t_0 + l, \dots, t_0 + (q-1)l$ (Figure 1). After q doses, because of the periodicity (24), we return to the initial point on the circle. When this happens, the interval between two neighboring such points is τ/q . Denoting the episode in which each dose is effective by δ , the drug-free interval becomes $\tau/q - \delta$. This interval is a crucial parameter in the system; for eliminating a given population we must make sure that it is short enough that no cells can complete their drug-susceptible life phase. More precisely, a *necessary* condition for population elimination is

$$\frac{\tau}{q} - \delta < \xi \leftrightarrow \frac{\tau}{q} - \xi < \delta. \quad (29)$$

In general, relation (29) can be satisfied for relatively large q 's and violated for relatively small q 's. This property leads to a selective treatment by means of which we aim to eliminate the neoplastic cells or the virus while minimizing damage to the host cells. The above analysis suggests that when ξ , τ , and δ are given both for the host cells and the neoplastic cells, or the virus, one can choose l (thus q will follow) such that (29) is satisfied for the population we wish to eliminate and violated for the host cells. Moreover, it is clear from (29) that toxicity will be minimal, for a given

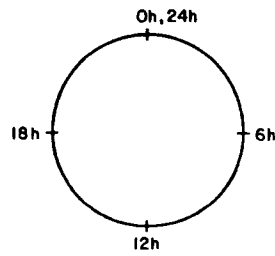


Fig. 1-a

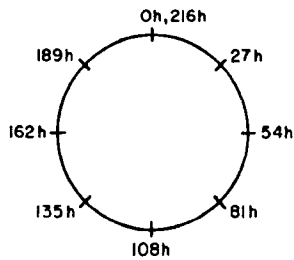


Fig. 1-b

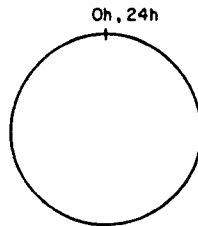


Fig. 1-c

FIG. 1. Time is represented by a circle of perimeter $\tau = 24$ h, and the marked points represent the first q consecutive times at which the drug is applied. (a) Dosing interval is 6 h. In this case, the drug-free interval is always less than 6 h, so bone marrow cells whose susceptible life phase (S phase) is longer than 6 h are progressively eliminated. (b) Dosing interval is 27 h. Here again, in spite of the relatively long dosing interval, all bone marrow lines are expected to be affected by the drug, as the ratio between the dosing interval and the cell-cycle time is $27/24 = 9/8 = p/q$. This means that in the long run the treatment "hits" at eight different timings over the 24-h cycle, leaving no rescued cell lines that can complete the S phase during the drug-free interval. (c) Dosing interval is 24 h. Here only a small fraction of the bone marrow population is expected to be eliminated by the drug; most cells will be in their susceptible life phase during the drug-free interval.

population and a given drug, if l is an integer multiple of τ , so that $q = 1$ and $\delta < \tau - \xi$. Note that condition (29) is insufficient for estimating population size because it uses only "temporal" parameters (ξ, τ, δ), leaving aside the "physical" effect of the drug on the susceptible cells (K). For incorporating the latter effect, one should use condition (27).

Our analysis is based on the assumption of a continuous age density distribution $f(a)$, but this distribution does not appear explicitly in the conditions for population elimination. For this reason the above conclusions are applicable to any continuous cell age density distribution, indicating the robustness of our model. Note, however, that $f(a)$ does appear in the formula for the number of cells at any given time (17).

What are the practical implications of our analysis for the treatment of AIDS patients with AZT? We are interested in checking if different interval AZT dosings can exert different *relative* toxicity to HIV-1 and host bone marrow cells, as a function of their different life cycles. To this end we compute the size of populations subjected to interval drug dosing, according to (17). Two populations are considered: one with a small variation in cycle duration, τ , representing the normal host cells, and one with a large variation in cycle duration, representing the virus (see below). The analysis presented above implies that dosing intervals similar to the average cycle duration of the normal cells will be more selective than other dosing intervals. This possibility is explored below.

In the results presented in Figures 2 and 3, we compare a protocol involving a single daily AZT application with the currently used protocol in which the same daily dose is divided into four dosings [10]. We assume that bone marrow cells have a constant or a normally distributed cycle duration, whose average is 24 h [12], and a drug-susceptible phase (S phase) whose duration is in the range of 9–11 h [18]. The virus cycle duration is a random variable in the range 10–50 h [11], and its drug-susceptible phase (reverse transcription) is assumed to be in the range of 1–3 h [17].

Simulation results suggest that a relatively fast viral elimination may occur under a 6-h dosing interval. However, this protocol is expected to exert extremely high bone marrow toxicity (Figure 2). A dosing interval of 24 h is somewhat slower in virus elimination; its great advantage lies in its capacity to preserve the bone marrow cell pool (Figure 3). Results in this figure also suggest that a larger bone marrow toxicity will be exerted if the variation in cycle duration of bone marrow cells is relatively large. Still, for a given distribution of cell-cycle duration, a single daily dosing is expected to be significantly less toxic than four daily dosings, using the same total dose. These conclusions remain unaltered for other mean transit times of the viral distribution as long as the fraction of time spent in the viral drug-susceptible life phase remains large enough (Figures 2 and 3). However, if the distribution of viral cycle time is very large (e.g., 5–100 h), no protocol with drug-free intervals can effectively eliminate the virus (results not shown).

The present analysis supports our previous prediction in showing that host toxicity may be minimized, while pathogen elimination may not necessarily be hampered, when the dosing interval is an integer multiple of

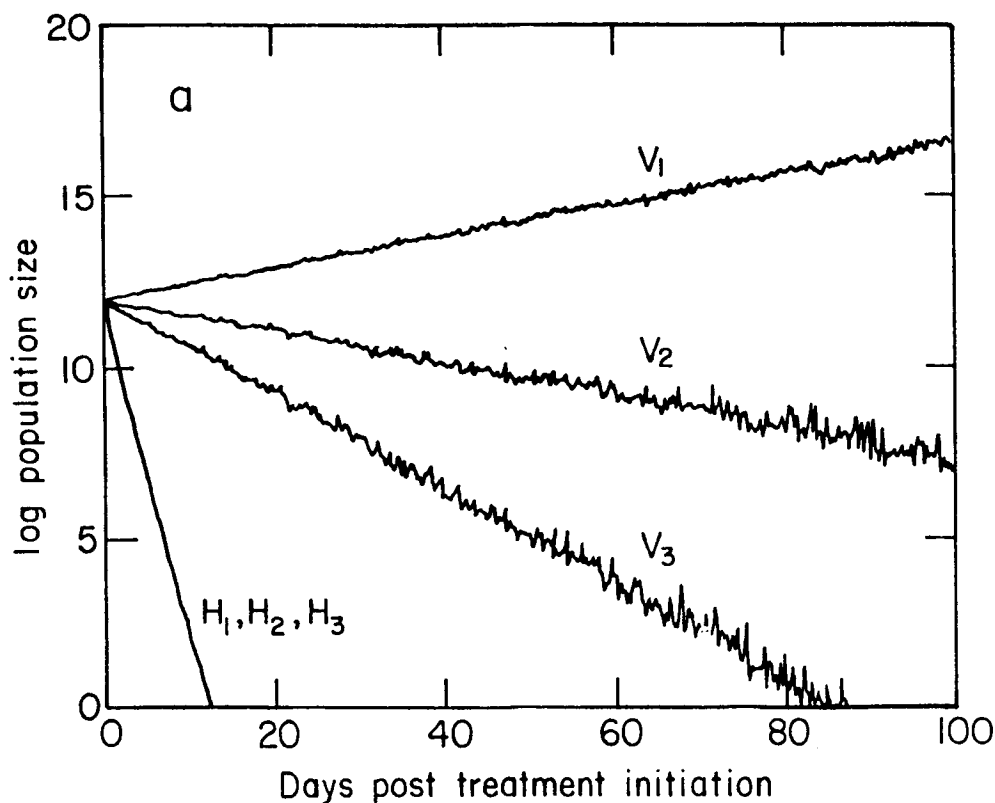


FIG. 2. Effect of 100 days of drug treatment, using a dose of 370 mg every 6 h. The numbers of bone marrow cells and of three types of viruses differing in the duration of reverse transcriptase activity are calculated according to (17). Host average cell-cycle time is 24 h, with standard deviation 0.0 (H1), 0.2 (H2), 0.5 (H3). Duration of susceptible life phase (*S* phase) is 10 h. Virus cycle duration is taken as a uniformly distributed random variable in the range 10–50 h, and the drug-susceptible life phase (reverse transcriptase) is 1 h (v_1), 2 h (v_2), 3 h (v_3); half-life of the drug 3 h, initial killing 38% ($k_1 = 0.0013$).

the average susceptible host cell-cycle time. This prediction has been verified *in vitro* for a leukemic cell line treated with ara-C [4], and *in vivo* in mice treated with AZT or ara-C. The latter experiments show that when the dosing interval is similar to the average bone marrow cell-cycle time, the drug is significantly less toxic to the host than with other dosing intervals [5, 6].

Putative effects of factors such as the circadian rhythm [14] or the drug effect on cell-cycle synchronization and on shortening cycle duration [16] can be incorporated into our model. However, further laboratory experiments are warranted for checking how significant such effects are. Moreover, as noted above, the condition for population elimination is independent of the specific age density distribution as long as this distribution is continuous. For this reason, we do not expect circadian rhythms to have a meaningful effect on the prospects of population elimination.

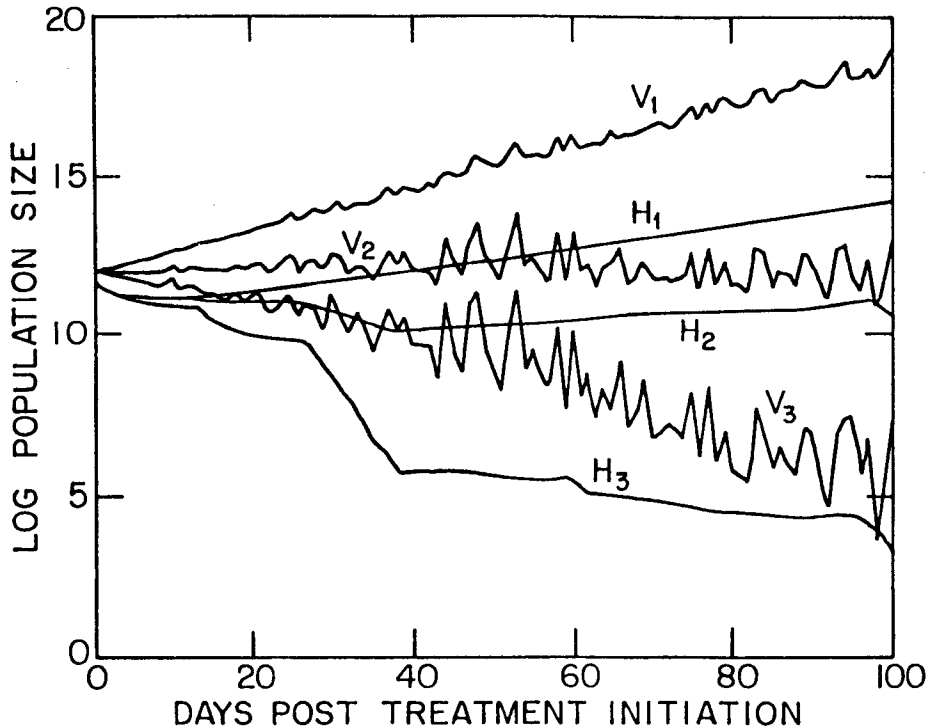


FIG. 3. Effect of 100 days of drug treatment, using a dose of 1500 mg every 24 h. The number of bone marrow cells and three types of viruses differing in the duration of reverse transcriptase activity are calculated according to (17). Host average cell-cycle time is 24 h, with standard deviation 0.0 (H1), 0.2 (H2), 0.5 (H3). Duration of susceptible life phase (*S* phase) is 10 h. Virus cycle duration is a uniformly distributed random variable in the range 10–50 h; virus-susceptible life phase (reverse transcriptase) is 1 h (v_1), 2 h (v_2), 3 h (v_3); half-life of the drug 3 h, initial killing 85% ($k_1 = 0.0013$).

The general conclusion of this work is that treatment efficacy is highly dependent on the susceptible host cell cycle or the viral cycle parameters, and that cell and pathogen dynamics should be considered when designing treatment regimens. We hope that this work provides tools for predicting the efficacy of specific drug regimens.

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