Increasing 1-β-D-Arabinofuranosylcytosine Efficacy by Scheduled Dosing Intervals Based on Direct Measurements of Bone Marrow Cell Kinetics[1]

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ABSTRACT

The therapeutic efficacy of cell cycle phase-specific drugs can be improved by repeated administrations, the dosing interval being related to the cell cycle time of the susceptible normal host tissue. Kinetic measurements of bone marrow cell proliferation, with bromodeoxyuridine labeling and flow cytometry analysis, were used to determine the optimal dosing intervals of 1-β-D-arabinofuranosylcytosine for minimizing bone marrow cell damage in mice. The results showed that cells surviving a single dose 1-β-D-arabinofuranosylcytosine treatment remained temporarily blocked at the G_1-S boundary, and upon release from the block the cells crossed through S phase in a nearly synchronized way. The optimal spacing of repeated treatments, evaluated by measurements of the drug-induced transit times through the different cell cycle phases, equaled the bone marrow cell cycle time following treatment. Repeated 1-β-D-arabinofuranosylcytosine injections according to this protocol markedly diminished drug toxicity in C3H mice, as compared to protocols of other time intervals. A therapeutic schedule based on these measurements was highly effective in lymphoma-bearing mice: the designed protocol of dosing intervals significantly delayed tumor growth whereas other intervals were highly toxic.

INTRODUCTION

Earlier attempts to harness cell cycle kinetics for improving drug selectivity suggested that the efficacy of cancer chemotherapeutic agents can be improved by manipulating dosing intervals (1–3). Repeated administrations of a cell cycle phase-specific drug at appropriate time intervals can enhance drug toxicity against the tumor. However, this approach, based on the kinetic properties of the tumor cell alone, was impeded by two main obstacles: (*a*) the large distribution of intermitotic intervals in malignant cells; and (*b*) the difficulty in obtaining the necessary data from human cancer cells in patients, particularly in the case of solid tumors (4). In addition, it was soon recognized that considering the proliferative properties of the tumor while neglecting those of the susceptible normal cells is bound to result in significant adverse reactions (5, 6). These were the main reasons why the kinetic approach to cancer chemotherapy was altogether abandoned, and to date protocols are still largely determined by an intuitive "trial and error" method. However, Skipper (7) notes that "trial and error" dose manipulations are apt to result in no improvement and that a quantitative approach to chemotherapy is highly essential.

Recently, it has been shown mathematically how drug selectivity can be increased if one considers the resonance effect that can be created by a drug dosing with a frequency equal to the cell replication frequency. Thus, increased survival of the sensitive host cells is achieved by a periodic dosing of cell cycle phase-specific drugs, the dosing intervals being equal to the mean intermitotic time in the susceptible host tissue (Z method). Under this regimen the resonance effect is prevented in the cancer cells if the heterogeneity in their cell cycle time is larger or if they differ from the host cells in mean cell cycle time (8, 9). The theory can be intuitively understood if one bears in mind that most of the host cells that were in their drug-resistant phases when the first dose was applied will survive also the following doses, provided the dosing intervals will allow them to be repeatedly exposed to the drug while being in their resistant phases. Thus, differences between normal and cancer cells in mean or variance in the cell cycle time can be exploited for increasing treatment selectivity and for justifying the focus on the kinetics of normal cells alone.

Preliminary experiments in lymphoma-bearing mice treated by repeated pulse dosing of ara-C[3] supported the prediction of the Z-method (10). Dosing intervals, which are related to BM kinetics, were associated with a significant reduction of myelotoxicity and increased survival. In fact, the high ara-C dosage reached in those studies led to toxic death of mice on one hand or strongly enhanced mouse survival and recovery on the other hand, depending on drug scheduling.

However, a major consideration is the effect of a multiple-dosings treatment on the cell cycle parameters of the host sensitive cells, such as the induction of blocks in certain phases. The present study evaluates the effects of repeated drug treatment by a myelotoxic drug [ara-C (11)] on BM cell kinetics and demonstrates an applicable method designed to directly measure *in vivo* the kinetics of the drug sensitive population. The method, based on BrdUrd incorporation followed by flow cytometry detection (12, 13), enabled the evaluation of drug effects and the definition of optimal scheduling parameters for drug treatment.

MATERIALS AND METHODS

Mice and *in Vivo* Treatments

Female C3H/HeJ mice 6–10 weeks old were treated with i.p. injections of 0.3 ml of PBS containing ara-C (Sigma) at doses of 1, 2, or 5 mg/mouse (50, 100, or 250 mg/kg). BrdUrd labeling was performed by injecting 1 mg BrdUrd (50 mg/kg, from Sigma) into the peritoneal cavity of the mice. Immediately (10 min) or after the appropriate time interval as indicated, mice were sacrificed and BM was flushed from the femurs using a saline solution. The cells were suspended in saline and fixed in 70% ethanol.

The studies on the antitumor effect of ara-C schedules were performed using a C3H/Hej originated 38C-13B lymphoma (14). Mice were inoculated i.p. with 5×10^4 lymphoma cells and ara-C treatment was initiated 3 days later.

Procedures involving laboratory animals, animal care, and use were conducted under institutional guidelines that are in compliance with national and international laws and policies (15, 16).

Flow Cytometry

Each sample of ethanol-fixed BM cell suspension was centrifuged and incubated with 3 N HCl for 20 min to obtain partially denatured DNA. After a washing with 0.1 M $Na_2B_4O_7$ to neutralize the acid, the cells were resuspended with 50 μl 0.5% Tween 20 (Merck) in PBS. Following this step, 50 μl bovine

[3] The abbreviations used are: ara-C, 1-β-D-arabinofuranosylcytosine (cytarabine); BM, bone marrow; BrdUrd, bromodeoxyuridine; PC, pulse-chase; IC, immediate BM collection; RL, repeated labeling; FSC, forward scatter; L, lymphocytes; E, erythroid cells; M, myeloid cells; B, blast cells.

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serum albumin (0.5% in PBS) and 20 μl fluoresceinated anti-BrdUrd (Becton-Dickinson, Mountain View, CA) were added and the mixture was incubated for 30 min at room temperature. After incubation with antibody, the cells were centrifuged and resuspended in 1 μg/ml propidium iodide to counterstain the DNA content; and at least 1 h later the cells were analyzed by flow cytometry with laser excitation at 488 nm. Green fluorescein fluorescence was detected in the 515–555 nm wavelength band and red propidium iodide fluorescence was detected at above 630 nm. The flow cytometer (FACScan; Becton Dickinson) was equipped with a pulse processing unit and data analysis was performed with the LYSYS software (Becton Dickinson) gating out aggregated cells.

BrdUrd Protocols

IC. In experiments where BM collection immediately follows a single BrdUrd injection, we evaluated the percentage of cells in G_0-G_1 (BrdUrd– cells with G_1 DNA content), in G_2-M (BrdUrd– cells with DNA content twice the value of G_1 cells) and in S (BrdUrd+ cells). The percentage of BrdUrd+ cells represents the fraction of cells which incorporate BrdUrd (defined as the BrdUrd labeling index). Quiescent or dying S-phase cells were detected as BrdUrd– cells with DNA content between G_1 and G_2 values.

PC. A detailed analysis of cell cycle transit time has been performed by experiments in which mice were given a BrdUrd injection and BM cells were collected at varying intervals later on. In this way BrdUrd+ cells, which were in S phase at the time of labeling, had time to flow out and were detected successively in G_2-M, in G_1, etc., in the following cycle. The time course enabled us to follow the duration of the different cell cycle phases, If, for example, S transit time is 6 h and G_2-M transit time is 3 h, a cell entering S phase at the time of labeling, $t = 0$ h, will be detected as a BrdUrd+ cell in middle S at $t = 3$ h, as a BrdU+ cell in G_2 at $t = 6$ h and as two BrdUrd+ cells in G_1 at $t = 9$ h. Hence the increase of the percentage of BrdUrd+ cells by a PC experiment reflects the amplification, by subsequent mitotic division(s), of the cells being in S phase at the time of labeling.

RL. In RL experiments mice were repeatedly labeled with BrdUrd at intervals (4 h) not larger than the S-phase transit time, in order to label all cells entering S phase. Percentage of BrdUrd+ cells here comprises cells labeled in S phase by the first BrdUrd injection at $t = 0$ h (as in PC experiments) but also those that were in G_2-M and G_0-G_1 at $t = 0$ h but are in S phase at $t = 4$ h, 8 h, etc.

RESULTS

BM Proliferating Pool

A typical biparametric analysis of DNA *versus* FSC of fixed normal BM cells is shown in Fig. 1. Two subpopulations of nucleated cells are distinguishable on the basis of volume related FSC parameter (17); the ratio between the two subpopulations is maintained following fixation and staining, although a shift in signal intensity has been observed. Region A delimits a well defined subpopulation (25 to 35% of all nucleated cells in C3H mice), identified as lymphocyte and erythroid (L+E) cells that are distinguishable from the larger myeloid and blast (M+B) cells in region B. Region A includes some larger cells that are in S and G_2-M, which on the basis of FSC alone would belong to the range of G_0-G_1 M+B cells in region B, but by using the biparametric histogram, where the information on DNA contents is added to the FSC, the L+E and M+B are well defined. Unnucleated mature erythrocytes are excluded from the measurement. An injection of BrdUrd a few min before BM collection allows the contemporaneous data analysis of L+E and M+B subpopulations in S phase (Fig. 2). S-phase cells that are BrdUrd+ (above the dashed line) are clearly distinguishable from G_1 and G_2-M BrdUrd– cells (below the dashed line). We found that 23.8 ± 2.5% L+E cells and 17.7 ± 2.9% M+B cells were in S phase, as the mean ± SD of 15 control mice used throughout the experiments presented in this study. These results, as well as the percentage of L+E cells within the total nucleated cells, are in good agreement with Lord *et al.* (18), using different methods. Lord

et al. also showed that lymphocytes and erythroid cells (polychromatophilic and orthochromatic normoblasts) are present in a near 1:1 ratio and proliferating cells are predominantly erythroid; proliferating cells in the M+B group are mostly late myeloid cells, but the proportion of early progenitors is expected to increase following a severe challenge (19).

Effect of a Single ara-C Dose on Cell Cycle Progression

Mice were given injections of BrdUrd at different time points following a single dose of 5 mg ara-C. Results of these experiments, performed by three distinct BrdUrd protocols (IC, PC, and RL), showed the following effects of ara-C: complete arrest of DNA synthesis and cell death in S phase; arrest and unscheduled DNA synthesis in G_1; and synchronous progression through the cell cycle of cells released from the G_1 arrest. Loss of synchrony, due to recruitment of cells from the G_0 compartment, occurs upon entering the subsequent cell cycle.

DNA Synthesis Arrest and Cell Death. The cytograms of BrdUrd *versus* DNA (IC protocol) for the total BM population are shown in Fig. 3, with L+E and M+B cells both exhibiting the same kinetic behavior. The panel at $t = 0$ h, for untreated mice showed a significant BrdUrd+ population. In contrast, no BrdUrd+ cells were observed in mice 30 min after ara-C, indicating an arrest of DNA synthesis. Cells that were hit by the drug during their S phase were detected as BrdUrd– at $t = 30$ min and progressively disappeared from the cytogram due to cell death (evidenced by the decreasing density of BrdUrd– dots between the G_1 and G_2 areas at $t = 14$ h and 18 h; Fig. 3).

G_1 Block and Unscheduled DNA Synthesis. A population of dim BrdUrd+ cells of DNA content typical to G_1 cells appeared 4 h after treatment with 5 mg ara-C (Fig. 4). However, although under this dose the number of BrdUrd+ increased over time, there was no movement into S phase at $t = 6$ h (Fig. 4). This suggests that BrdUrd incorporation was due to unscheduled DNA synthesis preceding a release from the G_1 block.

Synchronization and Loss of Synchrony. Cells that were blocked in G_1 went through early S phase at $t = 7$ h, reaching middle S phase at $t = 10$ h in a nearly synchronized manner (Fig. 3). The kinetics of these cells has been followed by a pulse-chase experiment, labeling the cells at $t = 8$ h after 5 mg ara-C treatment (Fig. 5), while in early S phase. The cohort of BrdUrd+ cells flows through S phase reaching late S phase at $t = 12$ h, with a few cells already in G_1 (after division). At $t = 14$ h most of the labeled cells are detected in G_2 and G_1 (Fig. 5). However a minority of cells was still in late S phase and a few cells
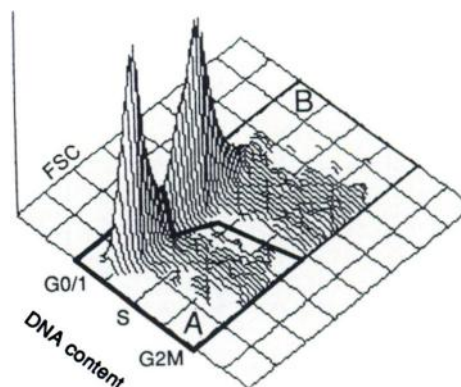

Fig. 1. Murine bone marrow nucleated cells analyzed by flow cytometry. In the biparametric flow cytometry histogram DNA and FSC (*horizontal axes*) are plotted *versus* number of cells (*vertical axis*). The two boxes delimit lymphocytes and erythroid cells (*A*) and myeloid and blast cells (*B*).

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Fig. 2. Murine bone marrow nucleated cells labeled with BrdUrd (*BrdU*) *in vivo* and collected immediately (IC protocol). Cells belonging to the L+E group (*A*) and M+B group (*B*) are separated during the computer analysis of data and the dot-plots (BrdUrd *versus* DNA content) showing the proliferating activity are presented separately. Each *dot* represents the couple of values of BrdUrd and DNA of a single cell. G_1, G_2, and S boxes delimit cells detected in the respective cell cycle phases. G_0 cells are detected in the G_1 box and mitotic cells in the G_2 box. G_0-G_1 and G_2-M cells are BrdUrd– because they exhibit very low BrdUrd content (below the *dashed line*) and are distinct by the DNA content, G_2-M cells exhibiting nearly twice the value of G_0-G_1 cells. S-phase cells are BrdUrd+ (above the *dashed line*) and have DNA content ranging from G_1 to G_2, which allows a rough distinction of early S (near G_1 DNA content), middle S, and late S (near G_2-M DNA content) cells.
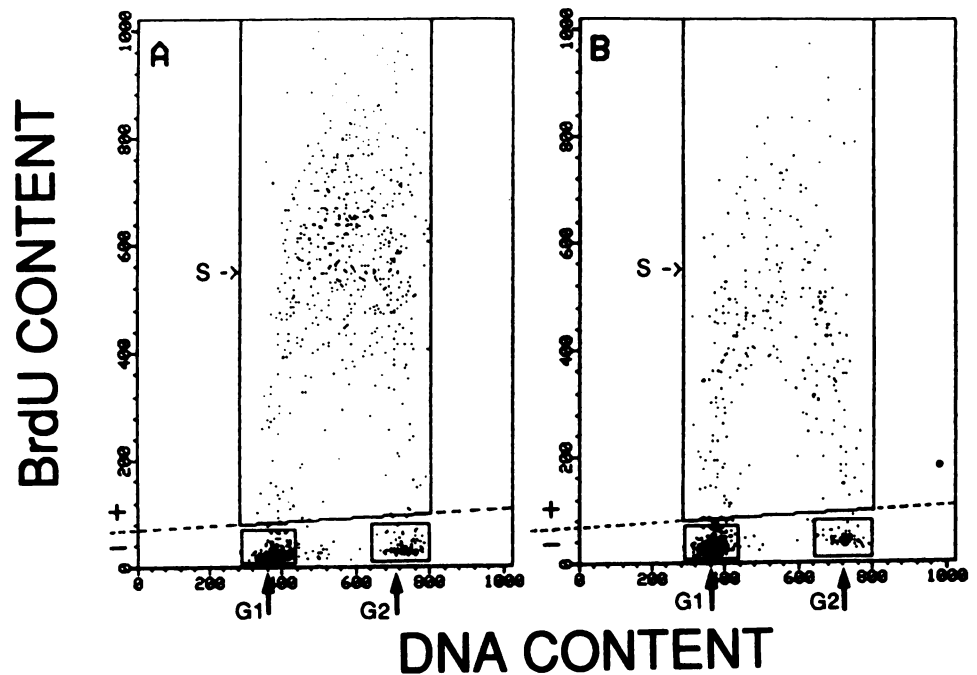
were already in the early S phase of a second cycle (detected as BrdUrd+ also in the IC experiment of Fig. 3, *14h*). At $t = 17$ h, more cells were detected in S phase (early and middle S phase) of the second cycle and at $t = 19$ h and later labeled cells were detected in all cell cycle phases, so that the synchronization wave was no longer detectable. Fig. 5, *19 h* to *28 h*, also indicates that a fraction of BrdUrd+ cells remained in G_0-G_1 after division, but this occurs in a way similar to that in untreated mice (not shown), where the cells leaving cycle are balanced by cells recruited into the proliferative pool.
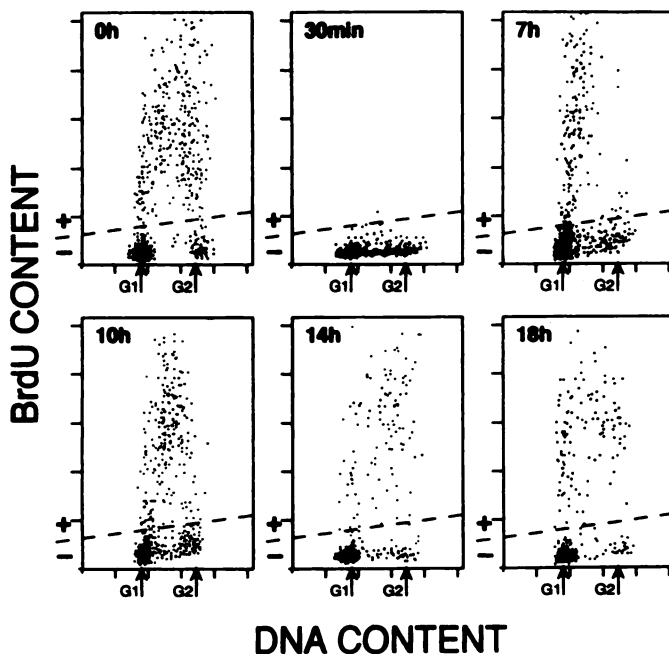

Fig. 3. BrdUrd (*BrdU*) labeling at different time points (IC protocol) after ara-C treatment (5 mg/mouse). No BrdUrd+ cells are detected at $t = 30$ min, while all BrdUrd+ cells are at the beginning of S phase at 7 h (near G_1 DNA content) and in the middle of S phase at 10 h.

Cell Recruitment. Information about cell recruitment following ara-C treatment was obtained by the RL experiment where mice were given injections of BrdUrd at $t = 10$, 14, 18, and 22 h, in parallel to the PC experiment. No difference in the percentage of labeled cells in PC and RL was observed at $t = 14$ h (Table 1), suggesting that only cells overcoming the G_1 block at $t = 7$ h were present in the proliferating pool up to that time. Later on, a significant difference was detected, in both L+E and M+B groups, between RL and PC, signifying that new cells were entering S phase, in addition to the cells overcoming the G_1 block at $t = 7$ h and their descendants. Because these cells appeared only at $t > 14$ h, this phenomenon can be interpreted probably as resumption of the recruitment from G_0 into proliferation, which was inhibited before that time, or less probably to resumption of proliferation from a long (>14 h) block. We believe that recruitment contributed to the progressive loss of synchrony of the proliferating cells.

Effect of Different Drug Doses

The recovery from the G_1 block following treatments with 1 or 2 mg ara-C was generally similar to that after 5 mg (Fig. 4). However, the block after 1 mg was slightly shorter; cells which survived 1 mg ara-C were out of the G_1 block in early S at $t = 6$ h and reached the resistant G_2-M/G_1 phases at 12–14 h. It is interesting to note that following 1 mg ara-C, some S-phase cells, particularly those of late S, resume cycling, although with lower levels of BrdUrd incorporation. These cells demonstrate incomplete damage at late S, suggesting that early or middle S cells are more susceptible to ara-C than late S (Fig. 4).

Effect of Dosing Intervals on BM Toxicity

The above results showed that ara-C was toxic to S-phase cells, causing an arrest in G_1 for about 4 to 6 h, following which cells crossed S phase in a nearly synchronized manner. This scenario suggests that an optimal window for a second ara-C dose, designed to preserve the BM proliferating pool, would be at 14 to 16 h (for 5 mg) and at 12 to 14 h (for 1 mg) after the first ara-C dose, since at that time most of the surviving cells will be in the less susceptible phases (G_1 and G_2-M). In contrast, a time interval of 7 to 11 h is expected to
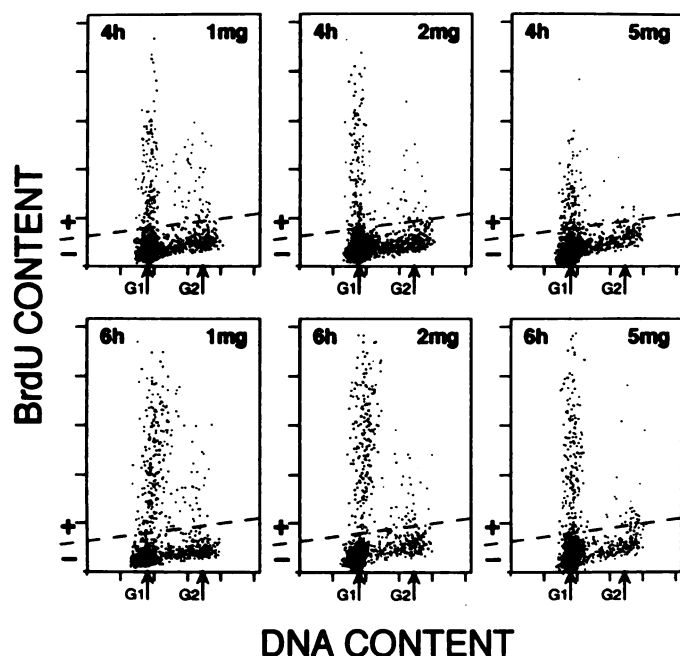
Fig. 4. BrdUrd (*BrdU*) labeling at *t* = 4 h and *t* = 6 h (IC protocol) after 1, 2, and 5 mg ara-C treatment. Cells emerging from the block are detected as BrdUrd+ cells at the indicated time points (above the *dashed line*).
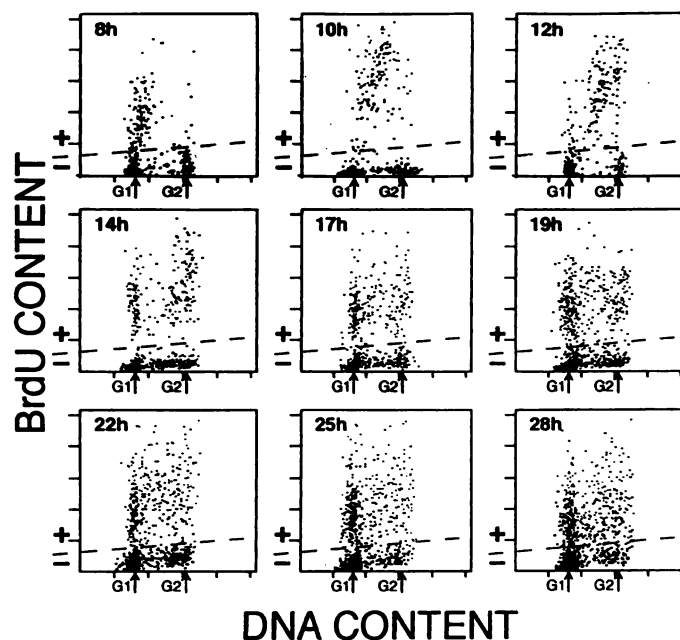

Fig. 5. Pulse-chase experiment. S-phase cells were labeled with BrdUrd (*BrdU*) 8 h following 5 mg ara-C treatment (*upper left*). The cohort of BrdUrd-labeled cells was followed by collecting bone marrow cells at the indicated time intervals.

impose maximal toxicity on the BM, since at that time surviving cells will be in the susceptible S phase.

However, if subsequent drug administrations had had different effects on the host cell cycle then the optimal dosing interval should have been calculated and adjusted following each administration. Luckily this is not the case as we showed that the kinetic behavior of BM cells following 2 or 3 ara-C dosings remained similar to that observed after a single dosing (Fig. 6), and that cell cycle kinetics was not affected by the duration of the dosing interval (not shown). Interestingly, a high degree of synchrony was observed already fol-

lowing the first 5-mg dose, whereas two 1-mg ara-C doses were required to obtain a similar effect, as indicated by the sharpness of the synchronization wave on the DNA axis (Fig. 6).

Subsequently the proportion of proliferating cells that survived different ara-C protocols was evaluated. The test actually measured the ability of cells of the proliferating pool to recycle, by the fraction of cells resuming from the block (BrdUrd+ cells 8 to 10 h after the last ara-C treatment). We focused on the percentage of actively proliferating surviving cells since during a certain period after treatment the total number of detected cells is not considerably altered, because (*a*) about 70% of the cells are nonproliferating and are not immediately susceptible to ara-C and (*b*) dead cells, still not degraded at *t* = 10 h, are not excluded from the measure, as indicated by the amount of S-phase cells unable to incorporate BrdUrd. In this experiment, mice were given 2 or 3 ara-C injections (5 mg) at 7-, 10-, and 14-h intervals, and the proportion of BrdUrd+ was determined at 8 to 10 h after the last injection. As is shown in Fig. 7, 4.2% L+E cells and 10.6% M+B cells

Table 1 *Percentage of BrdUrd labeled BM cells following a single 5-mg ara-C injection*

Single labeling PC: BrdUrd injected at *t* = 10 h after ara-C injection. RL: BrdUrd injected every 4 h starting at *t* = 10 h after ara-C injection. The difference between RL and PC values is due to cells entering S phase after *t* = 10 h, besides the cells initially labeled and their descendants. For each time point, means ± SD of the measurements in BM of 3 mice are presented.

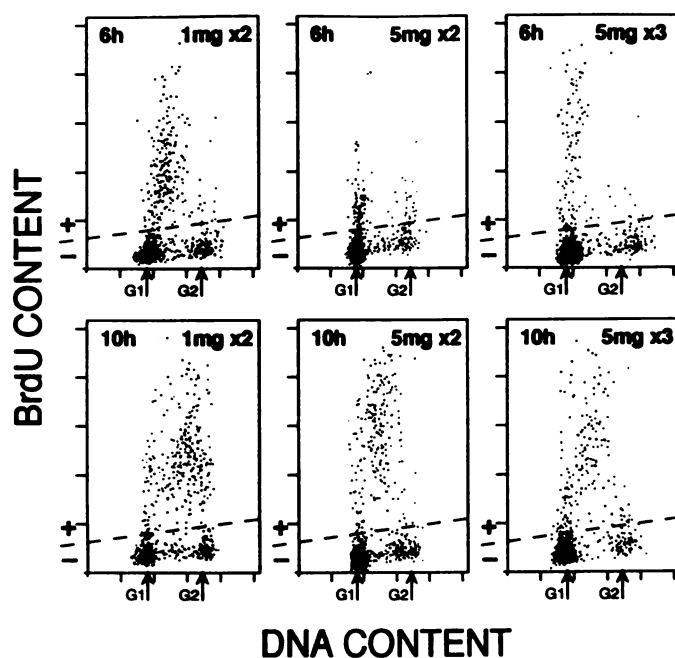| Time from ara-C injection	L+E cells		M+B cells	
	PC	RL	PC	RL
t = 10 h	4.2 ± 1.9		9.9 ± 1.6	
t = 14 h	3.9 ± 0.6	4.0 ± 0.4	11.2 ± 0.6	11.4 ± 1.9
New S-phase cells 10–14 h	0.1		0.2	
t = 18 h	4.9 ± 0.8	6.8 ± 0.8	15.6 ± 1.2	17.9 ± 0.7
New S-phase cells 10–18	1.9		2.3	
New S-phase cells 14–18	1.8		2.1	
t = 22 h	8.4 ± 0.6	11.4 ± 1.9	20.9 ± 1.8	26.3 ± 2.6
New S-phase cells 10–22 h	3.0		5.4	
New S-phase cells 18–22 h	1.1		3.1	

Fig. 6. BrdUrd (*BrdU*) labeling at *t* = 6 h and *t* = 10 h (IC protocol) after the last ara-C treatment in three examples of repeated doses scheduling. Mice received either 2 treatments of 1 mg ara-C (*left*) or 2 treatments of 5 mg ara-C (*middle*) or 3 treatments of 5 mg ara-C (*right*). Spacing between treatments was 14 h.

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were detected in the "surviving" group following the first dose. A second dose given 7 or 10 h later resulted in a considerable depletion (to around 1.0% L+E and 3.8% M+B cells) of BrdUrd+ labeled cells. However, a time interval of 14 h was significantly less damaging to the cells, since 2.7% L+E and 8% M+B were viable proliferating cells. In this group, a third dose given after a second 14-h interval did not increase cell damage in the L+E group and caused only a small subsequent loss of the cells in the M+B group. In both L+E and M+B subsets, survival of cells after these 3 ara-C doses was significantly higher than after only 2 ara-C doses in the 7- and 10-h regimens. This experiment shows that the second and third ara-C doses, given at 14-h intervals did not induce severe damage to the proliferating BM cells, In contrast, a second dose at the 7- and 10-h intervals caused a considerable decrease in the percentage of proliferating cells.

Effect of Dosing Interval on Tumor Elimination *in Vivo*

A close correlation between BM damage and mouse survival was observed. As shown in Fig. 8, 4 of 5 mice died as a result of treatment with 4 ara-C doses given at 10-h intervals. Reducing the number of doses to 3 did not decrease mortality. On the other hand, increasing time intervals to 14 h dramatically improved survival since 5 of 5 mice survived 3 ara-C doses given at 14-h intervals; 4 doses caused the death of only 1 of 5 mice.

The next step was to examine the above scheme on tumor-bearing mice. In this case, both 4 × 14 h and 4 × 16 h protocols were tested and compared to a treatment in which 4 drug doses were applied at "at random" (unequal) time intervals. The "random" protocol included time intervals longer than 16 h and shorter than 14 h to reach the same overall duration of treatment as in the 14-h schedule. Schedules with

Fig. 9. Effect of different time regimens of repeated treatments on the survival of tumor-bearing mice. Mice were inoculated with 50,000 38C-13B lymphoma cells (5 times the lethal dose) and 3 days later mice were given 4 injections of 5 mg ara-C at 14-h, 16-h, or unequal intervals (*16h–17.5h–8.5h*). Control mice were given 4 PBS injections. Survival was followed for 30 days.

two intervals in the 8- to 10-h range were yet known to be lethal. Here mice were inoculated with 38C-13B lymphoma cells and treatment was initiated 3 days later (Fig. 9). Results were indisputable as the 4 × 14 h was not only nontoxic but it also delayed tumor development, nearly doubling survival time as compared to the control. The 16-h protocol was similar to the 14-h (except for the death of 1 of 4 mice) defining the width of the BM-preserving time window. In contrast, the control untreated mice died within 14 days due to tumor development whereas the "random" treatment was highly toxic causing 100% deaths within 7 days.

DISCUSSION

Most anticancer drugs are cell cycle phase specific, hitting cells in particular stages of their cell cycle. Cell kinetics could therefore be exploited for manipulating drug effect. The importance of cell kinetics is usually acknowledged by clinicians (20) but its practical clinical application has been rare (21). Most schedules are assessed and optimized in an empirical manner, leaving aside kinetic considerations. The approach evaluated in this study is new in exploiting the drug resonance effect on cell population dynamics, enabling one to focus on the kinetics of the host tissues alone. In addition, we tested the possibility of obtaining the appropriate data by flow cytometric BrdUrd techniques.

In order to examine the theoretical method we first evaluated the effect of a single and multiple dosing of a phase-specific agent, such as ara-C, on the kinetic properties of the limiting normal target cells. Using these data we predicted the optimal dosing interval for ara-C and tested the prediction in murine trials, keeping in mind that the methodology must be applicable in the clinic.

The study was performed with the prototype phase-specific anticancer drug ara-C, which is known to be cytotoxic during S phase (22, 23). Myelotoxicity is the limiting toxicity for ara-C as it is for other anticancer drugs (24). Thus, setting up a method for decreasing toxicity to the hematopoietic system by using cell kinetics data is not limited to ara-C alone.

The cytokinetic study in the hematopoietic system is conveniently performed at the level of the proliferative pool of the BM, *i.e.*, cells in the intermediate stages of differentiation. We expect any drug effect on earlier differentiative stages, *e.g.*, on stem cells, to be detected in the proliferative pool. Moreover, the proliferative pool itself is probably the first target of phase-specific myelotoxic drugs and may regulate the response of the hematopoietic system to challenges that
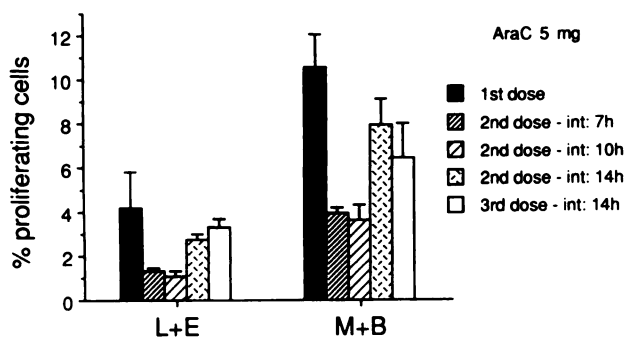

Fig. 7. BM cytotoxicity induced by different time regimens of repeated treatments with 5 mg ara-C (at 7 h, 10 h and 14 h spacing). A BrdUrd injection was given 8 h–10 h after the last treatment and percent BrdUrd+ cells was determined.
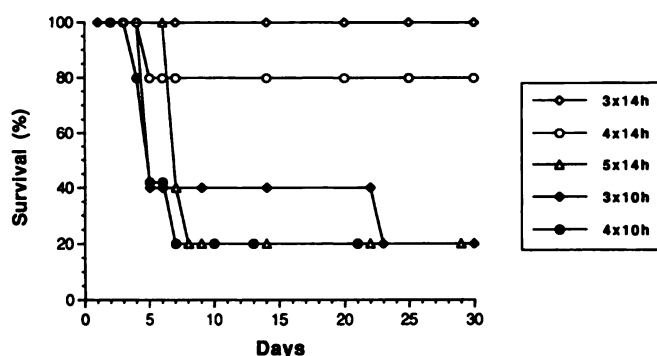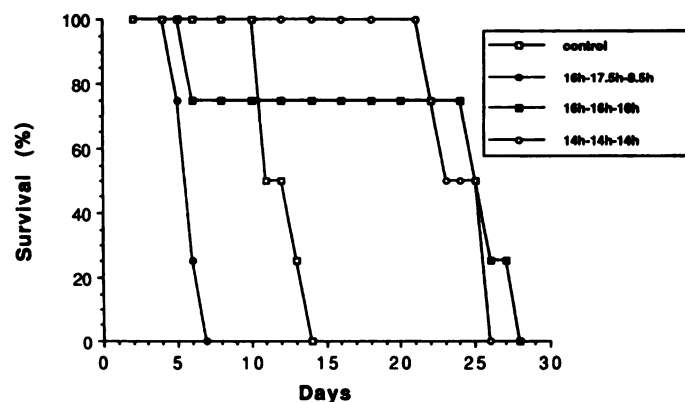

Fig. 8. Effect of different time regimens of repeated treatments on mouse survival. Mice were given 3, 4, or 5 injections of 5 mg ara-C at 10- or 14-h intervals as indicated. Survival was followed for 30 days. Surviving mice had the maximum weight reduction at day 6, but they resume the original weight at day 12. Later on they continue to increase in weight and were sacrificed 3 months from the start of ara-C treatment.

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do not involve severe BM depletion requiring the recruitment of early progenitor cells (19). The present study shows that the kinetic measurement at the level of the BM proliferating pool is a strong indicator of the toxicity induced by ara-C and allows the design of bone marrow-preserving regimens. However, more experiments, e.g., those focusing on the kinetics of the small stem cells population or on that of the intestinal epithelium, etc., may be warranted for further refinement of the method. Note that optimal treatment for reducing toxicity to more than one host tissue is now studied theoretically.[4]

In the first part of the work we studied the kinetic effects of a single dose of ara-C on the proliferative pool, leading to the determination of the optimal intervals between treatments. In the second part, the treatment schedule was tested evaluating the effects of successive drug administrations on bone marrow kinetics, on toxicity and on antitumor activity.

Results showed that within 30 min following ara-C treatment, DNA synthesis in the BM was inhibited and S-phase cells successively died. DNA synthesis was restored 7 h following a 5-mg ara-C dosing (5 h following a 1 mg dosing), due to emergence of surviving cells from a block at the G_1-S boundary. A lower number of surviving cells was found in the group of erythroid proliferating cells, as compared to myeloid and blast cells, indicating higher toxicity of single dose ara-C to the former group. Surviving proliferating cells underwent a delayed transit through S phase, so that most of them were found in G_2-M-G_1 phases of the cell cycle 14 to 16 h after treatment, irrespective of the lineage group.

The above results remained unchanged by additional administrations of ara-C. This result suggests that the effect of ara-C on BM cells is not a dynamic process, so that kinetic measurements following a single dose administration suffice for its evaluation. We thus concluded that the kinetic properties of the proliferative hemopoietic cells do not change in a short duration therapy. Our results led us to choose the 14-h interval as a putative optimal interval for repeated treatments. At $t = 14$ h, i.e., the sum of the duration of the block and the S-phase transit time, cells that recovered from the first treatment were in the ara-C less sensitive phases of the cell cycle. Shorter or longer time intervals are expected to have less favorable effects since recovered cells in this case would be in the ara-C-sensitive phase. Moreover, schedules based on interval time shorter than the duration of the block will mimic, from the kinetic point of view, a continuous infusion, and the "kinetic selectivity" at the basis of the Z-method could not be exploited.

The data presented here show that the 14-h protocol exerted low toxicity to the BM, whereas the 10-h protocol was extremely toxic. Using a tumor model in C3H mice, a high antitumor activity was demonstrated by four administrations of ara-C with 14- or 16-h spacing, whereas a random protocol was toxic. These results demonstrate the feasibility of controlling BM toxicity by time scheduling of the drug, based on the Z-method and using flow cytometry data.

From the clinician's point of view, the present work suggests that high doses of ara-C can be given with expected limited toxicity in a protocol of repeated doses at regular intervals, on the basis of the kinetics of BM cells. As for the practical problem of determining the optimal interval in a clinical environment, some suggestions emerge from the present work. First, the observation that BM cells surviving an ara-C dosing cross S-phase in a semisynchronous wave is favorable for measuring BM cell kinetics in patients. In such a case, pulse-chase

measurements, requiring BrdUrd injections in patients are not essential in order to follow progression of surviving cells during the first cycle. A BM aspirate, at two particular time points after ara-C treatment, followed by in vitro BrdUrd labeling and a rapid flow cytometry analysis should enable to estimate the optimal interval. Moreover, because the kinetic characteristics are reproducible by subsequent dosings, this estimation can be done following the first or an independent ara-C treatment.

It is still not known whether a wide interpatient variation exists in BM cell kinetics following ara-C treatment. Reproducibility in interpatient response will provide a characteristic value of an optimal time interval and will eliminate the necessity for individual kinetic measurements.

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Increasing 1-β-d-Arabinofuranosylcytosine Efficacy by Scheduled Dosing Intervals Based on Direct Measurements of Bone Marrow Cell Kinetics

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