Cytotoxic Chemotherapy Regimens That Increase Dose per Cycle (Dose Intensity) by Extending Daily Dosing from 5 Consecutive Days to 28 Consecutive Days and Beyond

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ABSTRACT
Dose intensity, defined as dose administered per unit time, has emerged as a potentially important measurement of anticancer drug exposure and determinant of efficacy. There are several strategies for increasing dose intensity, one being a protracted daily dosing strategy without major dose reduction for toxicity. This strategy involves continued therapy during periods of recovery from reversible toxicity, and it inherently challenges our understanding that renewing tissues cannot repopulate (recover) in the continued presence of cytotoxic drug. We have tested this idea directly in a murine preclinical trial. Specifically, we have tested whether acutely myelotoxic doses of gemcitabine (i.p. injection, 6.0 mg/m$^2$/day), acetyldinaline [CI-994; GOE 5549; PD 123 654; 4-acetylamino-N-(2-amino phenyl)-benzamide, 150 mg/m$^2$/day p.o.], and/or melphalan (i.p. injection, 7.2 mg/m$^2$/day) can be tolerated for 28 consecutive days and whether suppressed bone marrow function recovers despite this protracted daily therapy. The three drugs all caused acute neutropenia and suppression of medullary hematopoiesis. Damage to progenitor populations exposed to acetyldinaline and gemcitabine was not as severe as that caused by melphalan, in which case absolute neutrophil count, mature progenitors (colony-forming unit granulocyte/macrophage), and immature progenitors (colony-forming unit-S) progressively declined to severely depressed levels. Marrow recovery was observed during continued daily treatment with acetyldinaline and gemcitabine but not melphalan, and marrow function completely recovered after finishing the 28-day course. Pharmacology studies proved that protracted therapy causes little, if any, change in cellular drug tolerance or systemic exposure.

INTRODUCTION
Dose intensity, defined as the ratio of delivered dose rate to planned dose rate (1), was renamed “relative dose intensity” to expand its meaning to include comparison of delivered dose rate to any arbitrarily selected standard regimen (2). Dose intensity sometimes exerts an influence on response rate, independent of total dose (3). Hryniuk and Bush (2) and Hryniuk (4) reported steeply linear relationships between dose intensity and response rate after reanalyzing published studies of cyclophosphamide, methotrexate, and 5-FU-based therapy in stage IV breast cancer and of single agent studies in a variety of solid tumors. However, both retrospective (5) and prospective (6) studies of dose intensity in lung cancer failed to find a relationship between response rate and dose intensity. Thus, the contribution of dose intensity to clinical outcome is under debate (2, 7, 8).

Some drugs, e.g., antimetabolites, require substantial reductions in daily dose to be tolerable on protracted daily regimens, and the dose reduction is large enough to decrease the dose intensity in prolonged infusional regimens compared with bolus administration. It is particularly noticeable when comparing every 21 days to multiday regimens of antimetabolites. A recent review of 27 oncology drugs (9) confirmed this prediction for 7 of 8 antimetabolites, including fluorouridine, and many alkylating agents and natural products. However, dose intensity for a small number of drugs that do not share mechanism or structure (5-FU, thiopeta, and teniposide) could be increased by twofold or more by using protracted treatment regimens instead of intermittent daily regimens. We analyzed the literature in a similar way, confirming these conclusions and identifying 10 more drugs that show this same phenomenon (Table 1). Although not mechanistically related, some can be administered on an every day for 28 days every 4 weeks regimen at a daily dose that is ≥54% of the daily MTD$^3$ on an every day for 5 days every 4 weeks regimen, resulting in at least a threefold increase in dose intensity (drug X in Table 1). Others show a threefold increase in dose intensity because the daily dose needed no more than a 30% reduction to extend dosing from every day for 5 days to every day for 21 days (drug Y in Table 1). There are, of
Acetyldinaline (CI-994) is a clear example of this type of drug regimen (Table 1). The daily MTD in the mouse is 240 mg/m². A reduction in daily dose compared with the every day for 5 days regimen (10) is possible to give some myelotoxic anticancer agents daily for long periods without causing myeloablation, rather than assessing daily efficacy (2, 4). The investigational substituted benzamide known as acetyldinaline (CI-994) is a clear example of this type of drug (10). In vitro it demonstrated activity against several tumor cell lines, with greater cytotoxicity against solid tumors relative to both leukemia and normal fibroblast cell lines (11). Reversible neutropenia was found to be dose limiting in rat and dog (12). Cures rates of 40% were found in rats bearing myelocytic leukemia. In rats acetyldinaline was shown to have a sparing effect on CFU-S in the same animals (10). In the mouse, protracted daily acetyldinaline treatment requires only a 25% reduction in daily dose compared with the every day for 5 days regimen (Table 1). The daily MTD in the mouse is 240 mg/m² for 5 consecutive days; however, acetyldinaline is well tolerated for >50 consecutive days when the daily dose is reduced 20% to 180 mg/m² (11, 13). At a dose of 150 mg/m²/day, a pharmacokinetic study showed systemic drug exposure declined just 15% over 14 days. (13). Therefore, this drug is a useful tool to begin identifying the pharmacological and biological characteristics that the small number of apparently unrelated drugs in Table 1 may share.

Given the fact that marrow toxicity often limits dosing, we studied the effect of protracted daily oral acetyldinaline on a spectrum of hematopoietic elements in murine marrow from the neutrophil and megakaryocytic lineages. Gemcitabine is an antimitabolite that, like 5-FU, belongs to the drug family in Table 1. Its efficacy improves with increasing consecutive days of therapy in murine models, despite more severe myelosuppression (14–16), so it might be highly effective on a protracted daily regimen if its suppressive effects on hematopoiesis resemble those of acetyldinaline. In preliminary toxicology studies, we found that reducing the daily dose of gemcitabine from the MTD of an every day for 5 days regimen by only 20%, from 7.5 to 6.0 mg/m², made an every day for 28 days schedule tolerable. This small 20% dose reduction combined with the substantial increase in time of treatment increases dose intensity by 3.5-fold (Table 1). In the study reported here, we have directly compared the hematotoxicity of acetyldinaline and gemcitabine. As a control for a stem cell-damaging agent, melphalan was tested at 7.2 mg/m²/day, which is its daily × 5 MTD reduced by 20%, expecting that it will be toxic both to late and early progenitor populations and probably myeloablative when used in this regimen. Thus, the goal of our study was to determine why it was possible to give some myelotoxic anticancer agents daily for long periods without causing myeloablation, rather than assessing efficacy.

### MATERIALS AND METHODS

**Mice.** Breeding pairs, originally obtained from The Jackson Laboratory (Bar Harbor, ME), were bred at the Center for Investigations of Energy, Environment and Technology (CIEMAT) Animal Facility, and the litters were allowed food and tap water ad libitum. They were housed on chips that were changed twice a week. Twelve- to 14-week-old female (C57BL/6 × DBA/2)F₁ mice were used in these experiments if they had achieved a pretreatment weight of 20–25 g.

**Drug Preparation.** Gemzar (Eli Lilly, Indianapolis, IN; acquired from the Washington Wholesale Drug Exchange, Savage, MD) was solubilized to 0.18 mg/ml in USP saline according to the manufacturer and stored in the dark at 4°C in Falcon 2099 cell culture tubes for no more than 10 days. Acetyldinaline (Parke-Davis, Morris Plains, NJ) was prepared in Falcon 2099 cell culture tubes for no more than 10 days.
Daily Chemotherapy to Increase Dose Intensity

Drug Treatment. Randomized groups of mice were placed on one of the following drug regimens and treated for 28 consecutive days: 150 mg/m^2/day of acetyldinaline via oral gavage, 6.0 mg/m^2/day of gemcitabine via i.p. injection, or 7.2 mg/ml BSA (Boehringer Ingelheim), mixed with 30% semisolid agarose, supplemented with 10% diluent provided by the manufacturer (0.2 g sodium citrate, 6.0 ml propylene glycol, 0.52 ml 96% ethanol, and water) in Falcon 2099 tubes. Within 10 min, this solution was additionally diluted in sterile USP saline to 0.18 mg/ml and injected immediately.

Study Design. At different time points during treatment (4 h, 24 h, day 4, day 10, day 14, day 28), four mice were removed from the trial for each drug group, and their bone marrow was assayed for the effect to the progenitors. Peripheral blood was obtained from the same mice to measure the ANCs. The total number of nucleated cells was determined by microscopic examination in a Neubauer chamber, and viability was determined after staining with trypan blue.

Assays of CFU-GM and CFU-Meg. In a total of 3.3 ml, 5 x 10^5 cells/ml murine mononuclear cells in IMDM were mixed with 30% semisolid agarose, supplemented with 10 mg/ml BSA (Boehringer Ingelheim), 3 µg/ml transferrin (Boehringer Ingelheim), 25 µg/ml soy bean lipids (Boehringer Ingelheim), 45 µg/ml linoleic acid (Sigma), 7.8 µg/ml cholesterol (Sigma), 10^{-4} M triglycerol (Fluka), 110 µg/ml sodium pyruvate (Sigma), 0.2 mM L-glutamine, and WeHi-conditioned medium (20% final volume). One ml of suspension was pipetted into triplicate 35-mm culture dishes (Nunc, Roskilde, Denmark). CFU-GM colonies of ≥50 cells were counted microscopically after incubation at 37°C in a fully humidified atmosphere of 5% CO_2 in air for 7 days. CFU-Meg colonies containing three or more cells were visualized by acetycholinesterase staining.

RESULTS

General Toxicity. Animals treated with chronic daily oral acetyldinaline showed no signs of toxicity. There was no significant weight loss in any of these animals. Animals treated with chronic daily i.p. gemcitabine, like the acetyldinaline-treated animals, showed no signs of toxicity. In contrast, animals treated daily with melphalan injections showed signs of hair loss and lethargy by the 28th day of treatment, and some lethality was evident by day 21 of treatment.

Peripheral Blood Cell Counts. Melphalan-treated animals exhibited severe, progressive neutropenia, characterized by declining ANC throughout the 28 days of treatment (Fig. 1A). ANC decreased from 778 to 76 neutrophils/µl from day 1 to day 28 (8.6% of baseline). In the animals surviving treatment, ANC recovered 1 week after the termination of treatment, reaching 230 neutrophils/µl (26% of baseline). This recovery indicated that the marrow still contained some capacity for renewal despite the intensive melphalan regimen. The progressive decline in ANC with protracted daily therapy is typical of what is expected from a myelosuppressive alkylating agent.

The initial effect of CI994 on ANC was more severe and faster in onset than that of melphalan (Fig. 1B). Neutrophil counts reached nadir on day 4 of acetyldinaline treatment, at 140 neutrophils/µl (11% of baseline). However, unlike during melphalan treatment, the ANC showed evidence of recovery by day 10 and reached 54% of baseline by day 14. As in prior studies, the ANC stabilized at this level for the duration of the treatment. One week after the end of treatment, ANC had fully recovered, proving the capability of the treated marrow to repopulate.

Gemcitabine-treated animals exhibited a neutropenia intermediate between that in CI994- and melphalan-treated animals (Fig. 1C). The ANC nadir was reached on day 10 at 190/µl (16% of baseline). This effect was intermediate in onset and severity between that of acetyldinaline and melphalan. Similar to CI994-treated animals, the ANC in Gemzar-treated animals began to recover while continuing treatment. The ANC recovered to 60% of baseline, where it stabilized for the remainder of
One week after completing therapy, neutrophil counts had shown a more dramatic recovery than in acetyldinaline- or melphalan-treated animals. The initial nadir in ANC that occurred over days 4–10 was similar across the three treatment groups, but melphalan was unique in causing a progressive decline in ANC after day 10. The ANC in the vehicle-treated group varied from 400 to 1200/\(\text{mL}\) throughout the treatment period (Fig. 1). Therefore, the severe decreases in ANC associated with drug treatments were attributed to drug effects and not to the frequent handling and injection of the animals.

All three of the drugs caused a similar effect on platelet counts through day 14 of therapy (Fig. 2). However, after 28 days of treatment, the melphalan-treated animals exhibited a large decrease in platelet counts to 18% of baseline. Acetyldinaline and gemcitabine treatment decreased platelet counts by 57 and 53%, respectively, compared with fluctuations in the vehicle-treated animals in which handling and repeated injections caused counts to drop by 51% on day 28.

**GM Progenitors.** The CFU-GM progenitor is a committed, relatively differentiated precursor that forms colonies of neutrophils and macrophages *in vitro*. The CFU-GM content of bone marrow was monitored to evaluate whether the response of this compartment to CI994 and gemcitabine therapy, during which partial recovery of ANC was evident during chronic therapy, differed from the response to melphalan.

In the melphalan-treated group (Fig. 3A), the CFU-GM compartment showed a progressive and severe decline through-

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**Fig. 1** ANCs. Female BDF mice were treated daily with melphalan, acetyldinaline, gemcitabine, or vehicle control for 28 days and allowed 7 days to recover. At several time points throughout the trial, 100 \(\mu\text{l}\) of peripheral blood were obtained from the lateral tail vein of four mice from each treatment group for peripheral blood analysis. The counts were obtained from individual animals and the results averaged. The figure represents the average of two individual trials. *Shaded regions*, duration of treatment.

**Fig. 2** Absolute platelet counts. Platelet counts were determined during the same analysis of the ANCs, from 100 \(\mu\text{l}\) of peripheral blood obtained from the lateral tail vein of the four animals from each group. The data shown are the result of two individual trials.
out the duration of treatment that paralleled and preceded the similar progressive decline in ANC. The number of CFU-GM/femur decreased from 26,500 on day 1 to 300 on the last day of treatment (0.7% of baseline). In animals that had survived 28 days of melphalan treatment, CFU-GM content recovered 10-fold within 1 week of completing treatment, reaching nearly 3,000 CFU-GM/femur (9.5% of baseline).

In acetyldinaline-treated animals, the CFU-GM compartment was not severely affected by the treatment (Fig. 3B). The steep decline seen with melphalan treatment was not seen in the CFU-GM compartment in these animals. Femoral content of CFU-GM was reduced 50% by the 10th day of acetyldinaline treatment, yet remained at 50% for the duration of therapy. Recovery of CFU-GM content was complete within 7 days of the end of treatment (110% of baseline).

The CFU-GM compartment in the gemcitabine-treated animals was not as severely affected as it had been by melphalan (Fig. 3C). Femoral CFU-GM content reached nadir on day 4, at 40% of baseline. This nadir level correlated with the nadir level in ANC. The progressive decline in CFU-GM content observed previously with melphalan was not apparent in the gemcitabine-treated animals. After the day-4 nadir, the CFU-GM content recovered slightly, to 52% of baseline by day 28. One week after discontinuing gemcitabine, the CFU-GM compartment had recovered completely, similar to the postacetyldinaline period. Therefore, the effects of gemcitabine on the CFU-GM compartment were qualitatively similar in all respects to that of CI994 but markedly different from that of melphalan.

The levels of CFU-GMs in vehicle-treated animals remained stable throughout the trial except for a decrease 24 h into treatment. This dip may have been the result of mobilization of progenitors in response to stress or injected volume. Femoral CFU-GM content was 20,000–40,000 and averaged 36,000 in the vehicle arm (Fig. 3D).

Melphalan, acetyldinaline, and gemcitabine treatment caused a significant decrease in CFU-Meg content on day 4 of treatment to 56, 25, and 27% of baseline, respectively (Fig. 4). From this time point, the melphalan-treated animals showed a progressive decline in CFU-Meg content to 2.5% of baseline on day 28. This decline preceded the decline in platelet counts. After the termination of treatment, CFU-Meg content increased to 21% of baseline in survivors. In contrast to melphalan, protracted daily acetyldinaline and gemcitabine treatment did not affect CFU-Meg after day 4. Instead, CFU-Meg content began to recover and eventually stabilized at 50% and ~90% of baseline in acetyldinaline- and gemcitabine-treated animals, respectively. Thus, daily gemcitabine and acetyldinaline caused similar but less severe effects on the megakaryocyte than on the neutrophil progenitors.

**CFU-S (12 days) Compartment.** To examine whether the differential effect on CFU-GM was attributable to differential effects on repopulation, the murine bone marrow was assayed for a more primitive progenitor called CFU-S (Fig. 5). In melphalan-treated animals (Fig. 5A), there was a severe, progressive decline in the femoral CFU-S content that was more severe than that in the neutrophil or CFU-GM compartments. CFU-S numbers reached nadir on day 14, falling from 2400/femur on day 1 to 48/femur on day 14 (1.3% of baseline). In the animals that survived 28 days of melphalan treatment, recovery could not be detected in the CFU-S compartment 7 days after completion of therapy. The lack of recovery from day 28 to day 35 was different from the repopulation in the CFU-GM and neutrophil compartments, indicating severe damage to the stem cell compartment that repopulates CFU-S as it in turn repopulates the CFU-GM compartment.

In contrast, acetyldinaline did not affect the CFU-S compartment until day 28, the last day of treatment (Fig. 5B). On day 28, the CFU-S content had fallen to 32% of baseline. This decrease is slightly greater than that in the CFU-GM compartment. Within 7 days of completing therapy, the CFU-S compartment had recovered to 90% of baseline.

Gemcitabine caused a decline in CFU-S content to 40% of baseline, beginning on day 10 of treatment (Fig. 5C). However, the CFU-S content stabilized and was at 30% of baseline at the end of the 28 days of treatment. Seven days after terminating therapy, there was significant recovery of this population to 81%
of baseline. The nadir values for CFU-S in acetyldinaline- and gemcitabine-treated animals were similar, but suppression by gemcitabine was distinguished by an earlier onset.

In vehicle-treated animals, the stable range for CFU-S content was 2700–4700/femur (Fig. 5D), except for the same 24-h time point when CFU-GM decreased (see Fig. 3D). Comparing to this range, it can be concluded that acetyldinaline decreases CFU-S on days 1 and 28 but not between, gemcitabine causes a prolonged period of mild CFU-S suppression, and melphalan causes a progressive, severe decline in CFU-S.

Drug-induced Resistance in Hematopoietic Cells. These results showed that the marrow content of relatively mature and immature progenitor populations was initially reduced but then stabilized at adequate levels or recovered partially despite 28-consecutive-day therapy with acetyldinaline or gemcitabine but not melphalan. This result could be attributable either to a compensatory hyperplastic response that generates extra cells to offset the proportion lost to drug toxicity or to an adaptive response of the progenitor populations to drug exposure that increases drug tolerance. To test the latter hypothesis, we investigated whether the ability of mice to tolerate acetyldinaline for 28 consecutive days was attributable to the development of an acquired drug tolerance in bone marrow hematopoietic cells. We propose that if there were acquired tolerance to CI994 during treatment, then CFU-GM isolated from mice treated for 28 days would show a higher drug tolerance than CFU-GM from animals receiving only one day of treatment when exposed to drug in vitro. However, CFU-GM in femoral bone marrow isolated from mice treated with CI994 for 1, 14, and 28 days showed identical drug tolerance under in vitro conditions (Fig. 6). Both the IC_{50} and the more predictive IC_{90} (18) were similar for all of the animals tested at all of the

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**Fig. 4** CFU-Megakaryocytes/Femur. The CFU-Megs counts were scored from the same dishes as were the CFU-GM (see Fig. 2). The cultures were visualized by acetylcholinesterase staining, and colonies of three or more megakaryocytes were counted as colonies. The data are the average of three plates per drug group from one trial.

**Fig. 5** CFU-S/Femur. Pooled bone marrow aspirates from four animals from each of the four treated groups were injected i.v. into lethally irradiated male recipients as 5 × 10^5 cells/mouse in 0.2-ml injections. Spleens were removed from seven recipients per treatment group. The results shown are the average of two individual trials.
treatment times. When coupled with published pharmacokinetic studies (13), these results prove that the stabilization and recovery of peripheral blood cell counts and hematopoietic progenitors during protracted daily therapy must be attributable to some reason other than the development of cellular drug tolerance or enhanced drug elimination.

**DISCUSSION**

Several clinical strategies have been proposed to increase dose intensity: prophylactic antibiotic coverage and/or stem cell support during episodes of myelosuppression, increased frequency of dosing even to three times daily, individualized dosing based on the individual patient’s drug tolerance, cytokine support to accelerate recovery from toxicity and reduce the interval between cycles, drug combinations or alternating drug regimens with complementary toxicity profiles, and taking advantage of circadian rhythms in drug tolerance. High-dose chemotherapy maximizes acute drug dose but not cumulative drug dose. Theoretical considerations suggest that repeated exposure to moderate doses will be more effective against tumors with a low growth fraction and a long cycle time than treatment with a single high dose.

The success or failure of these strategies is critically dependent on drug selection. Given the dissimilarity of the drugs in Table 1, the question arises how to choose the drugs that will most likely perform well on protracted daily regimens. In the present study, we tested the hypothesis that differential sensitivity of neutrophil and megakaryocyte progenitor populations to myelosuppressive agents relates to whether a drug is well tolerated on a protracted regimen.

All three of the drugs caused neutropenia, which was most severe in melphalan-treated animals. Animals treated with acetyldinaline or gemcitabine also exhibited acute neutropenia. However, in contrast to melphalan, neutrophil counts began to recover before the end of treatment and completely recovered after drug therapy. The CFU-GM compartments of animals treated with acetyldinaline or gemcitabine showed slightly different kinetics in the onset of drug toxicity or levels of toxicity. With either drug, a 50% reduction was observed in the CFU-GM compartment. This reduction did not decrease additionally with continued treatment and completely recovered on termination of the treatment. The toxicity seen in the CFU-GM compartment of these animals was not as severe as that seen with melphalan treatment and did not decline progressively. Likewise, the effect on the CFU-S compartment was not as severe with either acetyldinaline or gemcitabine treatment as it was with melphalan treatment. Both drugs reduced CFU-S levels by 70% by the last day of treatment, with recovery by 1 week after the end of treatment. The rapid recovery suggests no permanent damage to this compartment.

However, there was one distinct difference between acetyldinaline and gemcitabine. CFU-S levels in acetyldinaline-treated mice were maintained at control levels for at least 14 days and had not declined until day 28, but CFU-S fell early during gemcitabine therapy (Fig. 5). These findings suggest that maintaining CFU-GM levels during protracted gemcitabine therapy requires significant input from CFU-S that is not readily repopulated by hematopoietic stem cells. Taken together, these findings suggest that the toxicity of gemcitabine to stem cells is subtle. Such a mild, adverse effect of gemcitabine on stem cells cannot be proven here but might be detected directly using the competitive repopulating assay (18).

To reach this conclusion, it is important to show that physiological changes that reduce drug exposure levels at a fixed dose by increasing drug clearance do not contribute to marrow recovery. A previous pharmacokinetic investigation in mice found that systemic exposure to acetyldinaline at the daily dose used in our study did not change substantially over 14 days of therapy (13). The current study excluded the possibility of induced cellular resistance (Fig. 6). Taken together, these findings suggest that partial recovery and stabilization of ANC and platelet counts in mice on protracted therapy is attributable to an adaptive biological response in hematopoietic marrow.

Understanding this particular strategy to achieve increased dose intensity seems to us to have clinical promise and be worthy of additional study because it achieves a several-fold increase in dose intensity without increased costs or medical interventions while being very well tolerated. The data discussed herein suggest that it is possible to administer a myelosuppressive anticancer agent on a daily treatment regimen during toxic episodes without fear of marrow failure. The results with daily melphalan show that not all myelosuppressive agents are tolerated on this regimen.

Early results from a Phase I dose escalation trial of acetyldinaline in patients with solid tumor confirm that daily acetyldinaline therapy can continue through grade II-III myelosuppression well past 28 days (20). Patients tolerated daily dosing with acetyldinaline, and three patients completed 7–20+ months of daily oral therapy with CI-994. The hematology profiles of these patients showed that the initial days of treatment caused a
decline in ANC and platelet count to a nadir of 950–1,200/mm$^3$ and 100,000–150,000/mm$^3$, respectively. However, daily therapy was continued despite the initial onset of grade 2 hematological toxicity, and during this continued therapy, both ANC and platelet counts gradually recovered to a level that justified dose escalation in some patients. Progressive decline in bone marrow function to grade 4 toxicity was never encountered, and most patients showed stabilization of marrow function but went off trial because of disease progression. These results suggest that human and murine marrow share similar biological responses to CI-994 therapy.

Whether or not gemcitabine is a candidate drug for protracted daily therapy remains to be determined. It will be important to understand why gemcitabine and acetyldinilamine produced different effects on the CFU-S compartment. It is conceivable that this difference indicates mild gemcitabine damage to the stem cell compartment. If there is a differential effect seen between these drugs on the stem cell compartment, then this criterion may be useful in identifying agents that will be successful on an extended therapy regimen. If the stem cell sparing effect is the major determinant in success or failure of a particular drug in protracted regimens, then it might be possible to assess this potential with in vitro systems and to be able to compare the animal model and human directly in the same assay to predict clinical safety (19).

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REFERENCES
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