Amifostine Improves the Antileukemic Therapeutic Index of Mafosfamide: Implications for Bone Marrow Purging

By Luc Douay, Chen Hu, Marie-Catherine Giarratana, Sandrine Bouchet, John Conlon, Robert L. Capizzi, and Norbert-Claude Gorin

One of the principal challenges of cancer chemotherapy is the relative inability of most anticancer drugs to distinguish between normal and neoplastic tissues. Consequently, a broad range of toxicities are experienced by patients, especially myelosuppression. Amifostine, a phosphorylated aminothiol, increases the selectivity of specific anticancer drugs for neoplastic cells by protecting normal tissues. One potential application of this protector is during bone marrow purging to selectively remove contaminating cancer cells. This study took normal or leukemic marrow from human subjects and evaluated the ability of amifostine to selectively protect normal bone marrow progenitor cells versus leukemic progenitor cells from the cytotoxic effect of mafosfamide. The dose response of mafosfamide-amifostine on leukemia colony-forming units or normal marrow progenitor cells was determined and the LD₅₀ was calculated. Amifostine pretreatment resulted in a statistically significant protection of granulocyte-macrophage colony-forming units and erythroid blast-forming units from the toxicity of mafosfamide (P = .031). Thus, amifostine protection of normal marrow progenitor cells allows a higher LD₅₀ concentration of mafosfamide to be used in ex vivo purging. In contrast, amifostine pretreatment increased the cytotoxicity of mafosfamide on the fresh human leukemia progenitor cells (P = .006). The dual effect of amifostine protection of normal marrow progenitor cells coupled with amifostine-induced sensitization of the leukemia cells increases the possible cell-kill of leukemic stem cells. With amifostine pretreatment, at the LD₅₀ concentrations of mafosfamide for marrow progenitor cells, there was an estimated 6 log increase in cell-kill of the leukemia cells. This selective cell-kill offers the potential for lowering the incidence of leukemic relapse, while preserving more normal stem cells for autologous transplantation.

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treatment (3 subjects had non-Hodgkin’s lymphoma, 2 had ALL, and 4 had ANLL).

**Treatment with amifostine and mafosfamide.** Light-density mononuclear cells in the bone marrow samples were separated by Ficoll-isopaque density gradient (d = 1.077 g/mL) centrifugation at 400g for 20 minutes. Interface cells were washed and suspended in Medium 199 (GIBCO, Egupny, France) supplemented with 20% autologous plasma and diluted to 2 × 10^6/mL. These cells were incubated with 3 mg/mL amifostine for 15 minutes at 37°C and then washed twice in cold RPMI medium with 3% autologous plasma.

After dilution to 10^6 cells/mL, the cells were incubated with agitation in freshly diluted mafosfamide (4-[(mercaptoethylsulfonate)-cyclophosphamide; ASTA Z 7654; ASTA Pharma, Bielefeld, Germany) for 30 minutes at 37°C on concentrations of 20 to 200 µg/mL in steps of 10 to 20 µg. The cells were then washed with the same medium and cultured to assess the number of viable residual bone marrow and leukemia colony-forming units (CFU-L). In all cases, a control experiment was performed using the same conditions and mafosfamide treatment without prior amifostine incubation.

**Progenitor cell studies.** The assay for leukemic progenitor cells (CFU-L) was performed according to our published technique.18 A total of 5 × 10^5 to 2 × 10^6 cells were suspended in McCoy’s 5A medium containing 0.72% methylcellulose (Fluka, Buchs, Switzerland), 5% fetal calf serum (FCS), 2.5% phytohemagglutinin (PHA), 7.5 × 10^3 mol/L α-thioglycerol, and 25 U recombinant human interleukin-2 (IL-2) for ALL and 10 ng/ml IL-3, 10 ng/ml granulocyte-macrophage colony-stimulating factor (GM-CSF), and 10 ng/ml stem cell factor (SCF) for ANLL. IL-2 (specific activity, 2.5 × 10^5 U/mg) was purchased from Genzyme (Boston, MA), GM-CSF (specific activity, 1 to 5 × 10^5 CFU/mg protein) was kindly provided by Behring (Marburg, Germany), SCF (specific activity, 10^5 U/mg) was kindly provided by Immunex (Seattle, WA). IL-3 (specific activity, 1 to 3.5 × 10^5 U/mg protein) was kindly provided by Genzyme. Granulocyte colony-stimulating factor (G-CSF) was kindly provided by Shuag Rhone Poulenc (Ukima, Japan). The mixture was overlaid onto a 0.5% agar feeder layer containing 2 X McCoy’s 5A medium and cultured to assess the number of viable residual bone marrow and leukemia colony-forming units (CFU-L). In all cases, a control experiment was performed using the same conditions and mafosfamide treatment without prior amifostine incubation.

**Progenitor cell studies.** The assay for leukemic progenitor cells (CFU-L) was performed according to our published technique.18 A total of 5 × 10^5 to 2 × 10^6 cells were suspended in McCoy’s 5A medium containing 0.72% methylcellulose (Fluka, Buchs, Switzerland), 5% fetal calf serum (FCS), 2.5% phytohemagglutinin (PHA), 7.5 × 10^3 mol/L α-thioglycerol, and 25 U recombinant human interleukin-2 (IL-2) for ALL and 10 ng/ml IL-3, 10 ng/ml granulocyte-macrophage colony-stimulating factor (GM-CSF), and 10 ng/ml stem cell factor (SCF) for ANLL. IL-2 (specific activity, 2.5 × 10^5 U/mg) was purchased from Genzyme (Boston, MA), GM-CSF (specific activity, 1 to 5 × 10^5 CFU/mg protein) was kindly provided by Behring (Marburg, Germany), SCF (specific activity, 10^5 U/mg) was kindly provided by Immunex (Seattle, WA). IL-3 (specific activity, 1 to 3.5 × 10^5 U/mg protein) was kindly provided by Genzyme. Granulocyte colony-stimulating factor (G-CSF) was kindly provided by Shuag Rhone Poulenc (Ukima, Japan). The mixture was overlaid onto a 0.5% agar feeder layer containing 2 × 10^5 irradiated leukocytes in 35 × 10 mm petri dishes and incubated in an atmosphere of 5% CO2. Colonies (>50 cells) were scored after 7 days. Identification of the leukemic origin of the colonies was performed by light microscopic examination of cells stained with May-Grünewald-Giemsa on cytopsin slides prepared from pooled harvested colonies; the cytology was compared with that of specimens before culture. The identity of the colonies before and after growth in culture were similar. These tests were performed using flow cytometric direct immunofluorescence (Becton Dickinson FAC sort; Becton Dickinson, Mountain View, CA). The following monoclonal antibodies were used: for the myelomonocytic panel, CD13 (My7), CD14 (My4), and CD15 (80H7); for the T-cell panel, CD2 (0275B), CD3 (T3), CD5 (K39), and CD7 (I-21); for the B-cell panel, CD9 (BA2), CD10 (J5), CD19 (B4), and CD20 (Bl); for HLA-DR, G-157 for CD34 (HPCA-2); and for negative control, L191 (against human epithelium).

The assay for late CFU-GM was performed in agar according to the technique of Pike and Robinson.19 The basic medium was McCoy’s 5A medium without serum (GIBCO) supplemented with 30% heat-inactivated FCS (GIBCO). Colony-stimulating activity was supplied by 10% human placental-conditioned medium (PCM). Cells were seeded at 5 × 10^5/mL of medium containing equal volumes of 0.6% agar and 2 × McCoy’s 5A medium to achieve a final serum concentration of 15%. Three 35 × 10 mm petri dishes (Becton Dickinson) were plated for each assay. The cultures were incubated for 10 days at 37°C in a humidified 5% CO2 atmosphere. The colonies were scored as PCM CFU-GM containing more than 50 cells were scored on day 10.

The assay for early CFU-GM and burst-forming units-erythroid (BFU-E) was performed according to a modification of the Eaves and Eaves technique.18 Briefly, 5 × 10^5 untreated and mafosfamide-treated bone marrow nucleated mononuclear cells (MNCs) were seeded in 35-mm petri dishes in 1-mL aliquots of Iscove’s modified Dulbecco’s medium (IMDM) containing 30% FCS (GIBCO), 10 mg/mL detoxified bovine serum albumin (Sigma, Saint-Quentin, France), and 0.92% methylcellulose (Fluka). Cultures were stimulated with two different mixtures of human recombinant growth factors: IL-3 (10 ng/mL), granulocyte growth factor (G-CSF, 10 ng/mL), granulocyte-macrophage growth factor (GM-CSF, 10 ng/mL), erythropoietin (EPO, 3 U/mL), and SCF (100 ng/mL). EPO (specific activity, 173,000 U/mg protein) was kindly provided by Boehringer Mannheim (Mannheim, Germany). The following combinations were used: IL-3 + G-CSF + GM-CSF + EPO (referred to as 4R) and IL-3 + G-CSF + GM-CSF + EPO + SCF (referred to as 5R). All growth factors were used at optimal concentrations, as determined in preliminary experiments. Dishes were incubated at 37°C in a humidified atmosphere supplemented with 5% CO2. Three dishes were plated for each individual data point per experiment. Granulocyte-macrophage colonies (CFU-GM) and erythroblastic colonies (BFU-E) were scored on day 14.

**Long-term bone marrow culture-initiating cell technique (LTC-IC).** The LTC-IC assay was performed according to a modification of the technique of Sutherland et al.19 Long-term culture medium (L(MC) was Iscove’s medium supplemented with 400 mg/mL L-glutamine (GIBCO), 1.5 U/mL heparin (Fournier, Gennevilliers, France), 10^-6 mol/L hydrocortisone hemisuccinate (Sigma), 40 mg/mL myoinositol (Sigma), 1.6 × 10^-4 mol/L monothioglycerol (Sigma), 40 U/mL penicillin, 40 mg/mL streptomycin (GIBCO), 0.5 mmol/L amphotericin (Squibb, Neufly, France), 12.5% heat-inactivated (56°C for 30 minutes) FCS (PBS organs), and 12.5% horse serum (GIBCO). LTC-IC assays were initiated by seeding an aliquot of 3 × 10^5 MNCs/0.6 cm^2 in a final volume of 200 µL LMC over a preestablished irradiated (15 Gy) human normal marrow feeder layer. At weekly intervals, half the supernatant medium and nonadherent cells were removed and replaced with fresh supplemented medium. The cultures were incubated in a humidified 5% CO2 atmosphere at 37°C for 3 days and transferred to 33°C. After a total of 6 weeks, the nonadherent cells were removed, washed, and combined with cells harvested from the adherent fraction by trypsination. Cells were then plated in methylcellulose assays in the presence of GM-CSF, G-CSF, IL-3, EPO, and SCF, as described above. The number of adherent and nonadherent progenitor cells (CFU-GM + BFU-E + colony-forming unit granulocyte, erythroid, monocyte, megakaryocyte [CFU-GEMM]) present in LTC at that time, which was diluted to 3 × 10^5 MNCs, was divided by four to estimate the absolute number of LTC-IC.19

**Statistical methods.** Probit transformation was used to model the relationship between the concentration of mafosfamide and the percentage of viable leukemic or bone marrow colonies as a linear function for each patient. The concentration of mafosfamide that produced a 95% reduction in the number of colonies (LD95) was determined from this line.20 The paired differences (± amifostine) in LD95 concentrations for each progenitor cell type were calculated and analyzed for significance using a Wilcoxon signed-rank test.

**RESULTS**

Effect of amifostine on the sensitivity of normal bone marrow progenitor cells to mafosfamide. The dose-response effect for mafosfamide ± pretreatment with amifostine was determined for each marrow specimen. After these treatments, viable late and early marrow progenitor cells were quantified using the culture techniques described above. Probit analysis of each patient’s dose-response allowed the calculation of LD95 values for individual patients. As is evi-
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Table 1. Amifostine Protection of Late Normal Marrow Progenitor Cells From LD₅₀ Concentration (μg/mL) of Mafosfamide

<table>
<thead>
<tr>
<th></th>
<th>Mafosfamide</th>
<th>Amifostine + Mafosfamide</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCM CFU-GM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>38 ± 14</td>
<td>42 ± 12</td>
<td>.058</td>
</tr>
<tr>
<td>Median (range)</td>
<td>38 (17-66)</td>
<td>39 (24-64)</td>
<td></td>
</tr>
<tr>
<td>4R BFU-E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>54 ± 13</td>
<td>66 ± 14</td>
<td>.031</td>
</tr>
<tr>
<td>Median (range)</td>
<td>50 (32-101)</td>
<td>60 (38-119)</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

The leukemia cells, and the individual patient LD₅₀ concentration for mafosfamide ± amifostine pretreatment are shown in Table 3. The immunophenotype of the fresh leukemia cells taken directly from the patients and those in the colonies were comparable. In marked contrast to the protection of normal bone marrow with amifostine, preincubation of leukemic blast cells with amifostine from 12 patients sensitized the leukemia cells to the cytotoxic effect of mafosfamide. The mean LD₅₀ ± SEM for mafosfamide alone was 33 ± 4 μg/mL versus 27 ± 3 μg/mL when preincubated with amifostine (P = .003). The intrinsic sensitivity of the various leukemic subtypes to mafosfamide reflected in the mean LD₅₀ concentration without pretreatment were 19, 34, and 44 μg/mL for ALL, ANLL, and AP-CML. Figure 1 shows the decrease in the slope of the percentage of viability for CFU-L as a function of mafosfamide concentration ± pretreatment with amifostine. These data represent the grouped data derived from individual dose-response determinations from all 12 patients.

To determine if human leukemic progenitors (CFU-L) could be eliminated by mafosfamide while normal progenitor/stem cells were protected by amifostine in the same suspension, one experiment was performed in which irradiated (15 Gy) normal bone marrow cells were admixed with 10% ANLL cells. This mixture was then treated in vitro with increasing doses of mafosfamide ± amifostine. Amifostine pretreatment sensitized the leukemia cells to the cytotoxic effects of mafosfamide. The LD₅₀ for CFU-L treated with mafosfamide alone was 45 μg/mL versus 36 μg/mL when pretreated with amifostine; the difference was statistically significant (P < .05).

DISCUSSION

Autologous bone marrow transplantation invokes dose-response concepts of cancer therapy. Using therapies for which the bone marrow is the primary dose-limiting organ, reinfusion of stored marrow after supraventral doses of chemotherapy effectively reconstitutes hematopoietic function. Contamination of harvested marrow with tumor is well-recognized and, indeed, recurrence of disease after autolo-

Table 2. Effect of Amifostine Pretreatment on LD₅₀ Concentration (μg/mL) of Mafosfamide on Early Normal Marrow Progenitor Cells

<table>
<thead>
<tr>
<th></th>
<th>Mafosfamide</th>
<th>Amifostine + Mafosfamide</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5R BFU-E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>89 ± 36</td>
<td>93 ± 41</td>
<td>NS</td>
</tr>
<tr>
<td>Median (range)</td>
<td>79 (28-136)</td>
<td>77 (37-164)</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>LTC-IC</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>89 ± 23</td>
<td>75 ± 26</td>
<td>NS</td>
</tr>
<tr>
<td>Median (range)</td>
<td>62 (29-99)</td>
<td>70 (32-117)</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: NS, not significant.
gous transplant has been linked to tumor contamination of the autotransplant. For this reason, ex vivo purging methods have been used to remove contaminating tumor cells from the transplant; however, chemical purging procedures are limited by their toxicity to normal marrow progenitor cells that are responsible for marrow engraftment. Although chemical purging is used regularly in certain centers around the world, its practice is not universally accepted. However, certain studies in patients with lymphoma or acute myeloid leukemia indicated improved survival of patients whose marrows were purged before reinfusion of the marrow. Key to the success of the purging method is selective cytotoxicity of the purging agent on tumor stem cells versus normal marrow progenitor cells. It has been estimated that, for a purging procedure to have meaningful clinical utility, it must result in at least a 6-log reduction of tumor cells in the autograft while sparing sufficient normal marrow progenitor cells to allow engraftment.

Chemical purging to eliminate leukemic cells from suspensions of normal bone marrow has been extensively studied since the initial report by Sharkis et al. Numerous preclinical and clinical studies have shown the efficacy of 4-HC and mafosfamide for in vitro purging of human bone marrow transplantation in patients with acute myeloblastic leukemia in first complete remission using mafosfamide-purged marrow resulted in 58% leukemia-free survival at 8 years. The doses that can be used are limited because these agents are also toxic to normal progenitor cells. More effective purging would be possible if normal marrow progenitor cells could be selectively protected from the cytotoxicity of the cyclophosphamide derivatives. The phosphorylated aminothiol, amifostine, has been shown to have this property. Amifostine is a prodrug that is dephosphorylated by membrane-bound alkaline phosphatase to the free thiol, WR-1065. The free thiol is then very rapidly taken up by normal cells, but very slowly, if at all, by tumor cells. The free thiol of WR-1065 binds to the active species of alkylating agents and platinum analogs, thus lessening their binding capacity to DNA.

The prominent amifostine-mediated cytoprotection from cyclophosphamide-induced bone marrow cytotoxicity prompted Shpall et al to study the possible utility of amifostine in the setting of autologous bone marrow transplantation that used purging with 4-HC. Selective cytoprotection was shown in laboratory experiments intended to mimic actual purging conditions. These experiments involved the admixture of normal human bone marrow with 10% human breast cancer cells. In this setting, whereas amifostine resulted in a 10-fold protection of CFU-GM, there was no protection of the breast cancer at concentrations of 4-HC actually used in purging protocols. Assay of residual CFU-GM after purg-
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Table 3. Sensitization of Human Leukemia Cells to Mafosfamide Cytotoxicity by Pretreatment With Amifostine

<table>
<thead>
<tr>
<th>Diagnosis (FAW immunophenotype)</th>
<th>CFU-L LD50*</th>
<th>Mafosfamide</th>
<th>Amifostine - Mafosfamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANLL (4) CD34, 13, 14, 15, 33</td>
<td>47</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>ANLL (4) CD34, 13, 33</td>
<td>41</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>ALL (2) CD34, 13, 33</td>
<td>15</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>ANLL (5) CD11, 15</td>
<td>36</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>ALL (2) CD34, 13, 33</td>
<td>35</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>ALL (2) CD10, 19, 13, 33, 34</td>
<td>10</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>ALL (2) CD10, 19, 13, 33, 34</td>
<td>14</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>AP-CML (2) CD34, 13, 14, 33, 36</td>
<td>43</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>AP-CML (2) CD33, 36</td>
<td>30</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>AP-CML (2) CD33, 15, 41</td>
<td>60</td>
<td>46</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SEM: 33 ± 4 = 27 ± 3
Median (range): 33.5 (10-60) 29 (9-46)

P value = .003

* Estimated dose (µg/mL) allowing a 5% survival of progenitor cells after treatment with mafosfamide.

ing with 4-HC ± amifostine pretreatment before autologous bone marrow transplantation in patients with breast cancer or non-Hodgkin’s lymphoma showed a 10-fold higher number of viable CFU-GM in the marrows pretreated with amifostine compared with those treated with 4-HC alone. Infusion of these marrows in the patients resulted in a significant shortening in the time to marrow engraftment and a significant decrease in the need for various supportive care measures such as antibiotic use and platelet and red blood cell transfusions for those patients whose bone marrows were pretreated with amifostine.

These data prompted us to perform the present study to determine the comparative effects of the cyclophosphamide derivative, mafosfamide, on normal bone marrow stem cells versus the malignant hematopoietic stem cells of leukemia. This is of interest because the tumor cells are derived from similar marrow progenitors as their normal counterparts after a mutational event. Several culture assays were used to investigate mafosfamide toxicity and amifostine protection of late and early marrow progenitor cells, as previously described. In brief, we had previously shown an inverse relationship between the maturational level of the progenitors, ie, late versus early cells and their degree of sensitivity for cyclophosphamide derivatives. The late progenitors assayed under PCM CFU-GM, 4R CFU-GM, and 4R BFU-E conditions were relatively more sensitive to the cytotoxic effect of mafosfamide compared with the early progenitor cells assayed under 5R CFU-GM, 5R BFU-E, and LTC-IC conditions. The apparent discordance in progenitor cell protection of the late progenitor pool and its lack of protection of the earlier pool may be explained by the observation that the latter is more resistant to mafosfamide, as evident by the higher LD50 dose. The possibility that a combination of growth factors, including the SCF, could protect progenitor cells from mafosfamide toxicity can be ruled out because LTC-ICs not grown in presence of growth factors display similar resistance.

We show here that fresh leukemic progenitor cells from patients with ANLL, ALL, and AP-CML are more sensitive to mafosfamide when pretreated with amifostine. Under the same conditions, late myeloid (PCM CFU-GM and 4R CFU-GM) and erythroid (4R BFU-E) progenitor cells from normal bone marrow were significantly protected from the toxicity of mafosfamide. It is intriguing to hypothesize that leukemic transformation of the hematopoietic progenitor is associated with some change in the metabolism or uptake of amifostine leading to enhancement of cytotoxicity rather than protection from this alkylating agent.

Valeriote and Tolen studied amifostine cytoprotection from the cytotoxicity of nitrogen mustard on colony-forming units-spleen (CFU-S) in AKR mice and the corresponding AKR leukemia. Whereas amifostine pretreatment clearly protected CFU-S, there not only was no protection of the AKR leukemia but also a paradoxical enhancement of the cytotoxic effects of nitrogen mustard on the leukemia. At a nitrogen mustard dose of 0.3 mg, amifostine pretreatment resulted in a 100-fold protection of CFU-S; in contrast, at the same dose of nitrogen mustard, there was a 100-fold enhancement of cell-kill to the AKR leukemia. Our results with human leukemia and normal progenitor stem cells were comparable to those observed in the murine model. Other laboratory studies have shown similar protection of bone marrow stem cells by amifostine while enhancing cytotoxic drug effect on the tumor. These studies involved melphalan effect on normal mouse marrow (CFU-S) versus human melanoma and phototherapy with mercocyanine on normal human marrow (CFU-GEMM) admixed with human small cell lung cancer cells.

Most of the international protocols for clinical marrow purging rely on the incubation of 10^7 MNCs/mL with a fixed dose of 50 µg/mL mafosfamide. This usually results in the destruction of ≥95% of late progenitor cells, which sometimes results in delayed engraftment or failure to engraft, especially in ANLL patients. The data we report here (Table 4) show that amifostine pretreatment of bone marrow before purging with a fixed concentration of 50 µg/mL of mafosfamide would allow a 3-log increase in leukemia cell-kill with protection of late progenitor cells. It is of major interest to observe in Fig 1 that amifostine-mediated protection of normal marrow progenitor cells becomes increasingly more evident with higher concentrations of mafosfamide coupled with a paradoxical increase in cytotoxicity to human leukemia cells. For comparative purposes, the mafosfamide ± amifostine pretreatment dose-response effect on the viability of CFU-L versus 4R CFU-GM and CFU-L versus BFU-E, respectively, is shown in Figs 1 and 2. Assuming a linear dose-response relationship for mafosfamide cytotoxicity, at the corresponding LD50 concentrations of mafosfamide ± amifostine pretreatment for 4R CFU-GM, extrapolation from these curves shows that pretreatment with amifostine reduces viability of CFU-L in excess of 6 logs from that achieved with mafosfamide alone (Fig 1). Similar effects have been noted for CFU-L versus 4R BFU-E (Fig 2). Because remission duration and possible cure are inversely related to resid-


bone marrow for autologous transplantation. Exp Hematol 101:13, 1982

of marrow purging by mafosfamide. Leukemia 5:296, 1991

greatly increase the safety and efficacy of autologous bone marrow transplantation in leukemic patients receiving mafosfamide-purged bone marrow by preserving an increased number of normal marrow progenitors while enhancing the elimination of contaminating neoplastic cells.

REFERENCES


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Table 4. Percentage of Viability of Leukemic and Normal Marrow Progenitor Cells After Bone Marrow Purging

<table>
<thead>
<tr>
<th>CFU-L</th>
<th>PCM</th>
<th>4R</th>
<th>5R</th>
<th>4R</th>
<th>5R</th>
<th>LTC-IC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>0.1</td>
<td>~10</td>
<td>~50</td>
<td>~5</td>
<td>~40</td>
<td>~70</td>
</tr>
<tr>
<td>0.00001</td>
<td>1</td>
<td>~30</td>
<td>~50</td>
<td>~30</td>
<td>~40</td>
<td>~70</td>
</tr>
</tbody>
</table>

Values are percentages.


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