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

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 = .008). 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.

© 1995 by The American Society of Hematology.

Because bone marrow stem cells are the major target for many cytotoxic drugs, the field of bone marrow transplantation after high-dose chemotherapy has emerged as a recognized curative approach for certain malignancies. As an extension of the high-dose chemotherapy concept, ex vivo purging of bone marrow with the cyclophosphamide derivative, 4-hydroperoxycyclophosphamide (4-HC), has been used to remove tumor cells that contaminate the autograft and may limit its therapeutic utility. This approach has been widely applied in the treatment of patients with advanced malignancies, with reportedly superior results in patients with lymphoma and acute myeloid leukemia, although the procedure has generated some controversy.

Because both leukemic and normal bone marrow progenitor cells show similar in vitro sensitivity to the cyclophosphamide derivative, mafosfamide, a major limitation of the purging procedure is the cytotoxicity to normal bone marrow progenitor cells. Quantification of the proportion of residual viable granulocyte-macrophage colony-forming units (CFU-GM) remaining in the marrow suspension after the purging procedure has been used to assess toxicity to normal marrow progenitor cells. An inverse relationship between the percentage of viable CFU-GM and the time to engraftment and a decreased need for supportive care measures such as platelet and red blood cell transfusions and use of marrow colony-stimulating factors.

In the present study, we compared the effect of amifostine on the cytotoxicity of mafosfamide on fresh human leukemia cells versus normal bone marrow progenitor cells taken directly from patients. In this setting, the central question was whether amifostine would have a selective cytoprotective effect on the normal marrow progenitor cells compared with the closely related leukemia stem cells that represent the mutated progeny of hematopoietic progenitor cells.

Patients and Methods

Bone marrow samples. Leukemia cells were obtained from diagnostic bone marrow aspirates of 12 patients (6 with acute non-lymphoblastic leukemia [ANLL], 3 with acute lymphoblastic leukemia [ALL], and 3 with acute myelocytic leukemia [AP-CML]). Normal bone marrow cells were aspirated from the iliac crests of 14 individuals, i.e., 5 normal donors for autologous transplant and 9 patients in complete hematologic remission after treatment.
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 400 g for 20 minutes. Interface cells were washed and suspended in Medium 199 (GIBCO, Eggenburg, France) supplemented with 20% autologous plasma and diluted to 2 x 10^5/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 10^5 cells/mL, the cells were incubated with agitation in freshly diluted mafosfamide (4-mercaptoethylsulphonate-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 viability 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. A total of 5 x 10^5 to 2 x 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 (Phila), 7.5 x 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 x 10^9 U/mg) was purchased from Genzyme (Boston, MA). GM-CSF (specific activity, 1 to 5 x 10^9 CFU/mg protein) was kindly provided by Behring (Marburg, Germany). SCF (specific activity, 10^9 U/mg) was kindly provided by Immunex (Seattle, WA). IL-3 (specific activity, 5 x 10^9 U/mg protein) was kindly provided by Genzyme. Granulocyte colony-stimulating factor (G-CSF) was kindly provided by Shugu Phone Poulenc (UKima, Japan). The mixture was overlaid onto a 0.5% agar feeder layer containing 2 x 10^5 irradiated leukocytes in 35 x 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-Grunwald-Giemsa on cytospin slides prepared from pooled harvested colonies; of direct immunofluorescence (Becton Dickinson FAC sort; Becton bodies were used: for the myelomonocytic panel, CD13 (My7). The assay for late CFU-GM was performed according to a modification of the Eaves and Eaves technique. Briefly, 5 x 10^5 untreated and mafosfamide-treated (5 μg/mL) mononuclear cells (MNCs) were plated in 35 x 10 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 colony-stimulating factor (G-CSF, 5 ng/mL), granulocyte-macrophage colony-stimulating factor (GM-CSF, 7.5 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. Long-term culture medium (LMC) 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 x 10^-4 mol/L monothioglycerol (Sigma), 40 U/mL penicillin, 40 mg/mL streptomycin (GIBCO), 0.5 mg/mL amphotericin B (Sigma, Neuilly, France), 12.5% heat-inactivated (56°C for 30 minutes) FCS (PBS organics), and 12.5% horse serum (GIBCO). LTC-IC assays were initiated by seeding an aliquot of 3 x 10^5 MNCs/0.6 cm² 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 trypsinization. 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-IC at that time, which was diluted to 3 x 10^5 MNCs, was divided by four to estimate the absolute number of LTC-IC.

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 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...
AMIFOSTINE AND ANTILEUKEMIC INDEX OF MAfosfAMIDE

Table 1. Amifostine Protection of Late Normal Marrow Progenitor Cells From LD$_{50}$ Concentration (µg/mL) of Mafosfamide

<table>
<thead>
<tr>
<th>Mafosfamide</th>
<th>Amifostine + Mafosfamide</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCM CFU-GM</td>
<td>38 ± 14</td>
<td>42 ± 12</td>
</tr>
<tr>
<td>Median (range)</td>
<td>38 (17-66)</td>
<td>39 (24-64)</td>
</tr>
<tr>
<td>4R CFU-GM</td>
<td>54 ± 13</td>
<td>68 ± 14</td>
</tr>
<tr>
<td>Median (range)</td>
<td>50 (32-101)</td>
<td>60 (38-119)</td>
</tr>
<tr>
<td>4R BFU-E</td>
<td>48 ± 14</td>
<td>61 ± 11</td>
</tr>
<tr>
<td>Median (range)</td>
<td>40 (11-91)</td>
<td>57 (28-93)</td>
</tr>
</tbody>
</table>

The leukemia cells, and the individual patient LD$_{50}$ 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 LD$_{50}$ ± 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 LD$_{50}$ 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 LD$_{50}$ 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 supraventricular doses of chemotherapy effectively reconstitutes hematopoietic function. Contamination of harvested marrow with tumor is well-recognized and, indeed, recrudesces disease after autologous bone marrow transplantation as in medium containing five recombinant growth factors (5R CFU-GM and 5R BFU-E) and LTC-IC were increasingly resistant to mafosfamide, as reflected by the higher LD$_{50}$ concentrations for mafosfamide alone. Given the relative resistance of these earlier progenitor cells to mafosfamide, pretreatment with amifostine did not significantly alter the LD$_{50}$ concentration (Table 2).

**Table 2. Effect of Amifostine Pretreatment on LD$_{50}$ Concentration (µg/mL) of Mafosfamide on Early Normal Marrow Progenitor Cells**

<table>
<thead>
<tr>
<th>Mafosfamide</th>
<th>Amifostine + Mafosfamide</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5R CFU-GM</td>
<td>89 ± 36</td>
<td>93 ± 41</td>
</tr>
<tr>
<td>Median (range)</td>
<td>79 (28-136)</td>
<td>77 (37-164)</td>
</tr>
<tr>
<td>5R BFU-E</td>
<td>69 ± 23</td>
<td>75 ± 26</td>
</tr>
<tr>
<td>Median (range)</td>
<td>62 (29-99)</td>
<td>70 (32-117)</td>
</tr>
<tr>
<td>LTC-IC</td>
<td>136 ± 56</td>
<td>139 ± 50</td>
</tr>
<tr>
<td>Median (range)</td>
<td>118 (73-206)</td>
<td>132 (82-216)</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 meth
ods 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 mar
row. 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 sus
pensions 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 mafosfa
mide-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 mar
row progenitor cells could be selectively protected from the
cytotoxicity of the cyclophosphamide derivatives. The phos
phorylated aminothiol, amifostine, has been shown to have this property. Amifostine is a prodruk that is dephosphory
lated 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 amifos
tine 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 admix
ture 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

![Fig 1. Probit plot of dose response for mafosfam ide ± pretreatment with amifostine on late normal myeloid progenitor cells (4R CFU-GM; n = 5) versus leukemic progenitor cells (CFU-L; n = 12). (-- ) 4R CFU-GM, mafosfamide alone; (---) 4R CFU-GM, mafosfamide with amifostine; (---) CFU-L, mafosfamide alone; (--) CFU-L, mafosfamide with amifostine. Viability (as a percentage of control) was determined after drug treatment using colony growth factors as described in the Materials and Methods. 4R, mixtures of 4 recombinant growth factors. The horizontal lines associate the LDM concentration of mafosfamide ± amifostin e for 4R-CFU GM with the corresponding viability of L-CFU treated with mafosf amide ± amifostine. ](/www.bloodjournal.org/content/10.1182/blood-2009-01-175763/Fig1.png)

![Fig 2. Probit plot of dose response for mafosfam ide ± pretreatment with amifostine on normal erythroid progenitor cells (4R BFU-E; n = 5) versus leukemic stem cells (CFU-L; n = 12); (---) 4R-BFU-E, mafosfamide alone; (--) 4R BFU-E, mafosfamide with amifostine; (---) CFU-L, mafosfamide alone; (--) CFU-L, mafosfamide with amifostine. The horizontal lines associate the LDM concentration of mafosfamide ± amifostine for 4R BFU-E with the corresponding viability of L-CFU treated with mafosf amide ± amifostine. ](/www.bloodjournal.org/content/10.1182/blood-2009-01-175763/Fig2.png)
AMIFOSTINE AND ANTELEUKEMIC INDEX OF MAfosfAMIDE

Table 3. Sensitization of Human Leukemia Cells to Mafosfamide
Cytoxicity by Pretreatment With Amifostine

<table>
<thead>
<tr>
<th>Diagnosis (FAB)</th>
<th>Immunophenotype</th>
<th>Mafosfamide</th>
<th>Amifostine ( \cdot ) Mafosfamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANLL (4)</td>
<td>CD34, 13, 14, 15, 33</td>
<td>47</td>
<td>39</td>
</tr>
<tr>
<td>ANLL (4)</td>
<td>CD34, 13, 33</td>
<td>41</td>
<td>37</td>
</tr>
<tr>
<td>ANLL (2)</td>
<td>CD34, 33</td>
<td>31</td>
<td>17</td>
</tr>
<tr>
<td>ANLL (4)</td>
<td>CD34, 13, 14, 15, 33</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>ANLL (5)</td>
<td>CD11, 15</td>
<td>36</td>
<td>34</td>
</tr>
<tr>
<td>ANLL (2)</td>
<td>CD34, 13, 33</td>
<td>35</td>
<td>32</td>
</tr>
<tr>
<td>ALL (1)</td>
<td>CD6, 7, 13, 33, 34</td>
<td>32</td>
<td>25</td>
</tr>
<tr>
<td>ALL (2)</td>
<td>CD10, 19, 13, 33, 34</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>ALL (2)</td>
<td>CD10, 19, 13, 33, 34</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>AP-CML (2)</td>
<td>CD34, 13, 14, 33, 36</td>
<td>43</td>
<td>26</td>
</tr>
<tr>
<td>AP-CML (2)</td>
<td>CD33, 36</td>
<td>30</td>
<td>31</td>
</tr>
<tr>
<td>AP-CML (2)</td>
<td>CD33, 15, 41</td>
<td>60</td>
<td>46</td>
</tr>
</tbody>
</table>

Mean ± SEM

<table>
<thead>
<tr>
<th>Median (range)</th>
<th>CFU-L LD50*</th>
</tr>
</thead>
<tbody>
<tr>
<td>33.5 (10-60)</td>
<td>29 (19-46)</td>
</tr>
</tbody>
</table>

\( P \) value .003

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

ing with 4-HC \( \pm \) amifostine pretreatment before autologous bone marrow transplantation in patients with breast cancer12 or non-Hodgkin’s lymphoma5 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.10 In brief, we had previously shown an inverse relationship between the maturational level of the progenitors, i.e., 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.30

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 Tolen10 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 melanoma31 and phototherapy with mercouriancine on normal human marrow (CFU-GEMM) admixed with human small cell lung cancer cells.14

Most of the international protocols for clinical marrow purging rely on the incubation of \( 10^7 \) MNCs/mL with a fixed dose of 50 \( \mu g/mL \) mafosfamide.27 This usually results in the destruction of \( \approx 95% \) of late progenitor cells,26,30 which sometimes results in delayed engraftment or failure to engraft, especially in ANLL patients.26,32 The data we report here (Table 4) show that amifostine pretreatment of bone marrow before purging with a fixed concentration of 50 \( \mu g/mL \) 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 \( \pm \) 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 \( \pm \) 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-
Table 4. Percentage of Viability of Leukemic and Normal Marrow Progenitor Cells After Bone Marrow Purging

<table>
<thead>
<tr>
<th>CFU-GM</th>
<th>BFU-E</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFU-L</td>
<td>PCM</td>
</tr>
<tr>
<td>-----------</td>
<td>---------</td>
</tr>
<tr>
<td>No amifostine protection</td>
<td>0.01</td>
</tr>
<tr>
<td>Amifostine protection</td>
<td>0.00001</td>
</tr>
</tbody>
</table>

Values are percentages.

REFERENCES


4. Appelbaum FR: Purging of normal marrow progenitor cells while enhancing the cellular levels of glutathione in certain cell lines, an effect known to enhance the cytotoxic effect of alkylating agents. J Natl Cancer Inst 84:548, 1992


15. Shpall EJ, Stemmer SM, Hami L, Bearman SI, Myers SE, Tafts S, Shaw L, Caprioli R, Schein P: Amifostine (WR-2721) shortens the engraftment time of 4-hydroperoxycyclophosphamide (4- HC) purged bone marrow in lymphoma patients receiving high dose chemotherapy (HDC) with autologous bone marrow support (ABMS). Blood 80:70a, 1992 (abstr, suppl 1)


33. Issels RD, Nagele A: Promotion of cystine uptake, increase of glutathione biosynthesis, and modulation of glutathione status by S-2-(3-Aminopropylamino)ethyl Phosphorothioic acid (WR-2721) in chinese hamster cells. Cancer Res 40;2082, 1990

Amifostine improves the antileukemic therapeutic index of mafosfamide: implications for bone marrow purging

L Douay, C Hu, MC Giarratana, S Bouchet, J Conlon, RL Capizzi and NC Gorin