Amifostine (WR-2721) Shortens the Engraftment Period of 4-Hydroperoxycyclophosphamide-Purged Bone Marrow in Breast Cancer Patients Receiving High-Dose Chemotherapy With Autologous Bone Marrow Support


4-Hydroperoxycyclophosphamide (4-HC), a commonly used marrow-purging agent, is active against many tumors, but is also toxic to normal marrow progenitors. Amifostine (WR-2721) is a sulfhydryl compound with chemoprotectant activity. Preclinical studies using suspensions of bone marrow and breast cancer cells demonstrated that ex vivo treatment with amifostine followed by 4-HC resulted in protection of marrow progenitors, with no compromise in the antitumor effect of 4-HC. This fact stimulated the development of a clinical trial. Bone marrow was harvested from 15 poor-prognosis breast cancer patients and randomly assigned to ex vivo treatment with amifostine followed by 4-HC (amifostine + 4-HC), or treatment with 4-HC alone. High-dose chemotherapy was then administered followed by infusion of the purged autologous bone marrow support (ABMS). Leukocyte engraftment, defined as a white blood cell count $\geq 1 \times 10^9/L$, was achieved in an average of 26 days for patients whose marrow was purged with amifostine + 4-HC versus 36 days for patients whose marrow was purged with 4-HC alone ($P = .032$). The average number of platelet transfusions (12 v 29; $P = .017$) and days of antibiotic therapy (28 v 40; $P = .012$) were significantly less for patients whose marrow was exposed to amifostine + 4-HC, compared with 4-HC alone. Unpurged backup marrow fractions were infused into three patients whose marrow was purged with 4-HC alone, because of inadequate marrow recovery. None of the patients who received amifostine + 4-HC-purged marrow required a backup marrow fraction. Complete remissions were achieved in 83% of patients with measurable disease, with no difference between the two cohorts. Forty-three percent of patients remained alive and progression-free at a mean of 13 months posttransplant. There was no significant difference in the rate or pattern of relapse for patients whose marrow was purged with amifostine + 4-HC compared with those whose marrow was purged with 4-HC alone. Ex vivo treatment of marrow with amifostine significantly shortens the time to marrow recovery, thereby reducing the risk of myelosuppressive complications in breast cancer patients receiving high-dose chemotherapy and 4-HC-purged ABMS. Since supportive care requirements are also significantly decreased, amifostine may reduce the cost of such therapy.

HIGH-DOSE CHEMOTHERAPY with autologous bone marrow support (ABMS) is effective treatment for selected patients with high-risk breast cancer.1,2 Since breast cancer commonly involves the bone marrow,3,4 a potential concern with this approach is the reinfusion of clonogenic tumor into patients in addition to normal marrow. Several recent studies using ABMS suggest that purging the marrow ex vivo may reduce residual tumor, which in turn may have a positive impact on disease-free survival.5-7

4-Hydroperoxycyclophosphamide (4-HC), a synthetic analog of cyclophosphamide's active metabolite, is widely used to purge the marrow of patients who receive ABMS.8,9 However, in addition to killing tumor cells, 4-HC is toxic to normal marrow as measured by reduced colony-forming units granulocyte-macrophage (CFU-GM). Clinical studies have shown that in patients receiving 4-HC-purged ABMS, the lower the CFU-GM recovery following ex vivo treatment, the longer the time to marrow reconstitution.9,10 Delayed hematopoietic recovery increases the risk of myelosuppressive complications, as well as the cost of marrow-supported therapy. Thus, a method that might reduce 4-HC toxicity to normal marrow without compromising the antitumor effect was investigated.

Amifostine is a phosphorylated sulfhydryl compound that is converted by alkaline phosphatase to its active form WR-1065.11 This drug arose from a classified nuclear warfare project sponsored by the United States Army, in which more than 4,500 chemicals were screened as potential radiation protectors; because of its superior activity, amifostine was selected for development.12 Amifostine demonstrated a unique ability to selectively protect a broad range of normal tissues, including bone marrow, but not neoplastic tissues, from damage produced by radiotherapy and alkylating agent chemotherapy.13-17

Preclinical studies described in this report demonstrated that ex vivo treatment of normal marrow with amifostine, which was then washed out before incubation with 4-HC, resulted in a 100-fold higher recovery of CFU-GM compared with that achieved following treatment with 4-HC alone, with no compromise in the antitumor effect.18 We postulated that the 100-fold increase in CFU-GM produced by adding amifostine to 4-HC would translate into a reduction in time to marrow engraftment in patients receiving 4-HC-purged ABMS, and thus the clinical trial described here was designed and executed.

MATERIALS AND METHODS

Preclinical studies. Suspensions of human CAMA breast cancer cells19 and a 10-fold excess of human bone marrow from normal...
donors were prepared, using $2 \times 10^7$ marrow mononuclear cells/mL, which were isolated using a ficoll-diatrizoate density gradient (Lymphocyte Separation Medium; Organon Teknika, Durham, NC). The suspensions were divided into three treatment groups, each of which was incubated with 60, 80, 100, and 120 $\mu$g/mL of 4-HC, respectively, as follows.

Treatment 1 consisted of incubation with the designated concentrations of 4-HC (Nova Pharmaceutical, Baltimore, MD) alone for 30 minutes in a 37°C water bath (4-HC alone).

Treatment 2 consisted of incubation with 3.0 mg/mL amifostine (US Bioscience, West Conshohocken, PA) for 15 minutes in a 37°C water bath followed immediately by washing twice with phosphate-buffered saline (PBS), then incubation with 4-HC as described for treatment 1 (amifostine-washed + 4-HC).

Treatment 3 consisted of incubation with amifostine as described for treatment 2, followed immediately by incubation with 4-HC as described in treatment 1, without washing to remove the amifostine (amifostine-no wash + 4-HC). Following 4-HC treatment, the cells were washed twice with PBS, and evaluated after 14 days in culture for breast cancer cell depletion in a limiting-dilution clonogenic assay, or for CFU-GM recovery in a short-term methylcellulose-based tissue culture assay, as previously described. For tumor depletion, the untreated control suspensions were evaluated for the presence of breast cancer cells, and the clonogenic breast cancer cell growth calculated as described by Johnson and Brown. The treated suspensions were similarly evaluated. The depletion of breast cancer was then calculated as the difference in clonogenic growth between the control and the treated suspensions. For progenitor cell recovery, the specimens purged at increasing 4-HC concentrations (with and without amifostine) were scored for CFU-GM content. The recovery was then reported as a percent of the control CFU-GM recovery, the latter depicted as 100%. The marrow cells used in the breast cancer clonogenic assays were irradiated with 2,500 cGy at 926 cGy/min. The tumor cells used in the progenitor cell assays were irradiated similarly.

In a separate series of experiments, the suspensions of marrow plus 10% CAMA breast cancer cells were treated with Amifostine-no wash + 4-HC and amifostine-washed + 4-HC. The concentration of 4-HC used was 80 $\mu$g/mL. The treated suspensions were centrifuged at 6,000 rpm for 5 minutes and a cell pellet formed. The supernatant was then assayed for WR-1065 using a mercury/gold electrochemical detection liquid chromatography method.

Clinical study: trial design and chemotherapy administration. Fourteen breast cancer patients with hormone-insensitive stage IV, and one patient with stage III disease involving 13 axillary lymph nodes, entered the study.

<table>
<thead>
<tr>
<th>Adjuvant Therapy</th>
<th>WR + 4-HC (Cohort A; N = 8)</th>
<th>4-HC Alone (Cohort B; N = 7)</th>
<th>Total (N = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMF</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>CAF</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>None</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

Dominant disease

| Stage III        | 0                           | 1                           | 1              |
| Stage IV NED     | 0                           | 1                           | 1              |
| Bone scan        | 4                           | 1                           | 5              |
| Soft tissue/node | 2                           | 4                           | 6              |
| Liver            | 1                           | 0                           | 1              |
| Lung             | 1                           | 0                           | 1              |
| Positive marrow Bx | 2                          | 1                           | 3              |

Fourteen breast cancer patients with hormone-insensitive stage IV, and one patient with stage III disease involving 13 axillary lymph nodes, entered the study.

Abbreviations: WR, amifostine; C, cyclophosphamide; M, methotrexate; F, 5-fluorouracil; A, Adriamycin; NED, no evidence of disease; Bx, biopsy.

All patients received a single donor pheresed platelet transfusion for a platelet count less than $20 \times 10^9/L$, and a red blood cell transfusion for a hematocrit less than 35% until granulocyte engraftment was documented, at which time a single donor pheresed platelet transfusion was administered for a platelet count less than $10 \times 10^9/L$, and a red blood cell transfusion administered for a hematocrit less than 28%. The primary end point of the trial was time to leukocyte engraftment, defined as a white blood cell count $\geq 1.0 \times 10^9$ cells/L. A secondary end point of platelet transfusion independence was defined as time to an unsupported platelet count less than $10 \times 10^9/L$. If on day +36 the leukocyte count was less than $0.3 \times 10^9/L$, or on day +50 the platelet count was less than $10 \times 10^9/L$ and transfusion-dependent, with a corresponding bone marrow biopsy showing less than 5% cellularity, then the protocol specified that the untreated back-up marrow be reinfused.

Informal consent was obtained from all patients and normal marrow donors. Both the clinical study and the protocol for obtaining research specimens were approved by the University of Colorado Institutional Review Board. Toxicities were scored using the Southwest Oncology Group criteria. Response criteria were those of the International Union Against Cancer and were clinically defined.

Bone marrow processing. Bone marrow was harvested from patients under general anesthesia and a buffy-coat fraction was prepared using a COBE 2991 Blood Cell Processor (Cobe Laboratories, Lakewood, CO). A backup marrow fraction containing $0.5 \times 10^8$ nucleated cells/kg was removed and cryopreserved without purging. A mononuclear cell fraction was obtained from the remainder of the buffy coat, using a Ficoll-diatrizoate density gradient on the COBE Processor, as previously described. The marrow then was randomly allocated to cohort A, ex vivo treatment with amifostine followed by washing and then incubation with 4-HC, or cohort B, purging with 4-HC alone. The randomization procedure was performed by investigators from US Bioscience, while the marrow harvest was in progress. The purging procedures were performed as follows.

For cohort A (amifostine + 4-HC), the washed mononuclear cell fraction was incubated for 15 minutes in a 37°C water bath with 3.0 mg/mL WR, 20% autologous plasma, and 200 mL of tissue culture (TC)-199, in a concentration of $2 \times 10^7$ cells/mL. The cells were washed twice with RPMI-1640 on the COBE Processor, and then incubated with 80 $\mu$g/mL of 4-HC, 20% autologous plasma, and 200 mL of TC-199 for 30 minutes in a 37°C water bath.
For cohort B (4-HC alone), the washed mononuclear cell fraction was incubated immediately with 4-HC as described for cohort A, with no exposure to amifostine.

After 4-HC incubation, the treated cells from patients in both cohorts were washed twice on the COBE Processor with RPMI-1640 containing 3% autologous plasma, and cryopreserved with 10% dimethyl sulfoxide, 20% autologous plasma, and 70% TC-199 using a rate-controlled freezing method. The cells were stored at −196°C.

Immunohistochemical evaluation of breast cancer cells. Ten cyto-
tosin preparations were evaluated for cells bearing breast cancer antigens, using a sensitive, quantitative, immunohistochemical staining technique. The number of antigen-positive cells present in both the prepurge buffy-coat and postpurge mononuclear cell marrow fractions were enumerated, as previously described. The method used a panel of four monoclonal antibodies that react strongly with breast cancer cell surface antigens, but not with normal marrow cells. Antigen-positive cells were identified by both immunohistochemical staining and morphologic criteria. The latter included large nuclei, high nuclear to cytoplasmic ratio, abnormal chromatin clumping, and frequent clustering.

Statistical methods. Cohorts A and B were compared with respect to time to leukocyte engraftment, platelet transfusion independence, and supportive care required, as well as complete response and event-free survival rates. Supportive care measures evaluated included the number of days of antibiotic administration, as well as the number of red blood cell and platelet transfusions received. The data were compared nonparametrically using the paired Wilcoxon sum test; two-sided tests of significance are reported. All patients who received the entire chemotherapy regimen were included in all analyses. Time to engraftment was also analyzed using the Kaplan-Meier method, with the times of death for the two patients who died before engraftment treated as censored times of engraftment.

RESULTS

Preclinical studies. The clonogenic assay results summarized in Fig 1 represent data from three replicate experiments and show the log of CAMA breast cancer cell elimination obtained with the three different treatments: (1) 4-HC alone, (2) amifostine-washed + 4-HC, and (3) amifostine-no wash + 4-HC. With 60 to 120 μg/mL of 4-HC, amifostine-washed + 4-HC depleted greater than 1 log of more of the breast cancer cells than amifostine-no wash + 4-HC. There was no significant difference in the breast cancer cell depletion achieved with 4-HC alone compared with Amifostine-washed + 4-HC (P = .903).

The tissue culture assay results represent data from four replicate experiments, and are summarized in Fig 2. At every concentration tested, amifostine-washed + 4-HC produced 1 to 2 logs more CFU-GM recovery than treatment with 4-HC alone (P = .013). With 80 μg/mL of 4-HC, which was the concentration used clinically, amifostine-washed + 4-HC produced 35% recovery of CFU-GM, compared with 3% recovery produced with 4-HC alone (P = .026).

Substantial concentrations of WR-1065 were produced in the medium very rapidly, reaching a maximal concentration of 2.8 mmol/L within 15 minutes after the addition of the parent drug. By 30 minutes, the WR-1065 was decreased to 1.8 mmol/L. Washing out the amifostine before 4-HC incubation produced a greater than 10-fold reduction in the WR-1065 concentration of the tissue culture medium.

Marrow processing. Table 2 lists marrow processing data by cohort. There was no significant difference in the number of cells collected, processed, or purged in patients from cohort A (amifostine + 4-HC) when compared with those in cohort B (4-HC alone).

Toxicity. Three of 15 patients developed significant toxicities. One patient developed congestive heart failure on the third day of chemotherapy administration, requiring intubation, and never received the carbustine. The congestive heart failure resolved rapidly and her marrow recovered 10 days following infusion, but because she did not receive the entire chemotherapy regimen, she was not assessable for time to engraftment. Fourteen patients completed the entire chemo-
AMIFOSTINE PROTECTS MARROW

Therapy program. Two of 14 (one from each cohort) developed early fatal treatment-related multiorgan failure before marrow recovery, on days 12 and 15, respectively.

Engraftment. In the assessable patients, the mean time to leukocyte engraftment was 26 days for cohort A (amifostine + 4-HC), and 36 days for cohort B (4-HC alone) (P = .042) (Fig 3). The mean time to platelet transfusion independence was 37 days for cohort A (amifostine + 4-HC) and 45 days for cohort B (4-HC alone) (P = .046). Supportive care data listed in Table 3 demonstrated that the mean number of platelet transfusions was 12 for cohort A (amifostine + 4-HC), and 29 for cohort B (4-HC alone) (P = .017). The mean number of days antibiotics were administered was 28 for cohort A (amifostine + 4-HC), and 40 for cohort B (4-HC alone) (P = .012). Two patients in cohort B (4-HC alone) received their unpurged backup marrow fraction and systemic growth factor support beginning on day +36, because of inadequate leukocyte recovery. Both patients subsequently achieved leukocyte engraftment on day +47 and +51, respectively (11 and 12 days, respectively, following infusion of backup marrow). A third patient, also from cohort B (4-HC alone), received her backup marrow fraction 1 month after discharge from the hospital, because of persistent thrombocytopenia and bone marrow biopsies showing an inadequate number of megakaryocytes. By day +80, she was platelet transfusion–independent. None of the patients in cohort A (amifostine + 4-HC) received backup marrow fractions or growth factor support.

Marrow progenitor cell recovery. The mean CFU-GM recovery following the purge procedure was 6.3% of the pretreatment control for patients from cohort A (amifostine + 4-HC) and 0.57% of control for patients from cohort B (4-HC alone) (P = .32). A median of 19.0 × 10⁶ CFU-GM/KG and 5.6 × 10⁶ CFU-GM/KG were infused into the patients from cohort A (amifostine + 4-HC) and B (4-HC alone), respectively (P = .19). There appears to be a strong association between the CFU-GM content in the 4-HC–purged marrow graft and time to marrow reconstitution (P = −.07), but more data are needed to confirm this. There was no correlation between the total number, nor the total viable number of marrow cells harvested, purged, frozen, or reinfused and the time to marrow recovery.

Tumor detection. Immunohistochemical evaluation detected breast cancer antigen in the prepurge buffy-coat marrow specimens of three patients at the time of harvest. This included two patients from cohort A (amifostine + 4-HC) and one from cohort B (4-HC alone). Quantitative evaluation showed a log reduction in breast cancer antigen in all three postpurge mononuclear cell specimens of 0.6 and 0.9 logs, respectively, for cohort A patients, and 1.4 logs for the patient in cohort B.

Therapeutic results. Complete remissions were achieved in 83% of patients with measurable disease, with no difference between the two cohorts. Forty-three percent of patients

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**Table 2. Marrow Processing Data**

<table>
<thead>
<tr>
<th></th>
<th>WR + 4-HC</th>
<th>4-HC Alone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cohort A:</td>
<td>Cohort B:</td>
</tr>
<tr>
<td>Cells/kg (×10⁶)</td>
<td>N = 8</td>
<td>N = 7</td>
</tr>
<tr>
<td>Harvested</td>
<td>5.7</td>
<td>5.9</td>
</tr>
<tr>
<td>(3.9-7.9)</td>
<td>(4.2-6.8)</td>
<td></td>
</tr>
<tr>
<td>Buffy-coat</td>
<td>3.8</td>
<td>3.7</td>
</tr>
<tr>
<td>(2.4-6.0)</td>
<td>(2.5-5.2)</td>
<td></td>
</tr>
<tr>
<td>MNCs prepurge</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>(0.3-0.9)</td>
<td>(0.1-1.3)</td>
<td></td>
</tr>
<tr>
<td>MNCs postpurge</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>(0.2-0.7)</td>
<td>(0.1-0.9)</td>
<td></td>
</tr>
<tr>
<td>CFU-GM/kg</td>
<td>19</td>
<td>6</td>
</tr>
<tr>
<td>(2-29)</td>
<td>(1.6-1)</td>
<td></td>
</tr>
</tbody>
</table>

There was no significant difference in the number of cells collected, processed, or purged in patients from cohort A (WR + 4-HC) when compared with those in cohort B (4-HC alone). Values are mean (range).

Abbreviation: MNCs, mononuclear cells.

**Table 3. Engraftment and Supportive Care Data**

<table>
<thead>
<tr>
<th></th>
<th>WR + 4-HC</th>
<th>4-HC Alone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cohort A:</td>
<td>Cohort B:</td>
</tr>
<tr>
<td>No. of d to WBC &gt; 1,000/µL</td>
<td>26</td>
<td>36</td>
</tr>
<tr>
<td>(21-31)</td>
<td>(26-51)</td>
<td></td>
</tr>
<tr>
<td>No. of d to PLAT INDEPENDENT</td>
<td>37</td>
<td>46</td>
</tr>
<tr>
<td>(22-41)</td>
<td>(30-67)</td>
<td></td>
</tr>
<tr>
<td>No. of PLAT transfusions</td>
<td>12</td>
<td>29</td>
</tr>
<tr>
<td>(3-28)</td>
<td>(10-40)</td>
<td></td>
</tr>
<tr>
<td>No. of RBC transfusions</td>
<td>18</td>
<td>26</td>
</tr>
<tr>
<td>(4-50)</td>
<td>(14-60)</td>
<td></td>
</tr>
<tr>
<td>No. of d with antibiotic RX</td>
<td>28</td>
<td>40</td>
</tr>
<tr>
<td>(16-32)</td>
<td>(14-54)</td>
<td></td>
</tr>
</tbody>
</table>

Time to engraftment, number of platelet transfusions, and number of days of antibiotic administration were significantly less for cohort A when compared with cohort B. Values are mean (range).

Abbreviations: PLAT, platelet; INDEP, independence; WBC, white blood cell; RBC, red blood cell; RX, therapy.
(three patients each from cohorts A and B) remained alive and progression-free at a mean of 13 months (range, 6-24 months) following treatment. There was no significant difference in the rate or pattern of relapse for patients in cohort A compared with those in cohort B. Patients relapsed in sites of previous bulk disease.

**DISCUSSION**

The preclinical studies described here demonstrated that purging the bone marrow with amifostine followed immediately by 4-HC preserved the highest frequency of CFU-GM of the three treatments evaluated. However, without washing to remove the amifostine, there was also protection of breast cancer cells from 4-HC-mediated killing. If the amifostine was washed out before addition of 4-HC, there was no significant protection of breast cancer cell killing, and yet the CFU-GM recovery was significantly higher than that documented following treatment with 4-HC alone, at all 4-HC concentrations evaluated. These results stimulated the design of the randomized clinical trial consisting of marrow purging with 4-HC either preceded by an amifostine incubation for 15 minutes followed by washing, versus treatment with 4-HC alone. The choice of 80 µg/mL as the concentration of 4-HC used in this trial was based on our previously published phase I purging study, where it was determined to be the maximal dose that produced an acceptable length of marrow aplasia in a similar patient population.

The current randomized clinical study results are consistent with our preclinical findings demonstrating amifostine’s protection of CFU-GM. Preincubating patient marrow with amifostine produced leukocyte engraftment in an average of 26 days, which is significantly shorter than the 36 days documented for patients whose marrows were treated with 4-HC alone ($P = 0.042$). The average time to leukocyte engraftment of 36 days in the control arm of this trial is longer than the 28 days reported in our previous phase I trial for a cohort of eight breast cancer patients whose marrow was purged with 80 µg/mL of 4-HC. While differences in patient selection may have produced this disparity, the randomized design used in the current trial, and the large differences produced by pretreatment with amifostine, suggest that the effect of amifostine is not likely the result of differences in patient selection.

The mechanisms by which amifostine selectively protects normal but not neoplastic tissue from chemotherapy-induced toxicity have been studied extensively. Amifostine, the phosphorilated prodrug, has a limited protective effect. When administered, it is rapidly cleared from the plasma and taken up into normal tissues, with a half-life of less than 10 minutes. Intracellularly amifostine is converted to the free thiol, WR-1065, which has been demonstrated to be the active species. It has been shown that within 5 minutes after administration, the concentration of WR-1065 in normal tissues is as much as 100-fold greater than in malignant tissues, and that a difference of 1 log or greater is maintained for at least 90 minutes. Our preclinical pharmacology studies demonstrated that the concentration of WR-1065 was maximal within 15 minutes following addition of parent drug, which is consistent with previously published data. A variety of mechanisms by which WR-1065 is selectively concentrated in normal versus malignant tissues have been proposed. Normal tissues, specifically at the capillary level, have a higher activity of alkaline phosphatase, which can convert amifostine to the dephosphorylated WR-1065 for rapid local uptake into normal tissues. The activity of alkaline phosphatase in tumor-associated capillaries is appreciably less. Additionally, a facilitated transport system for WR-1065 has been demonstrated in normal, but not neoplastic tissues. Once inside the marrow cell, WR-1065 probably exerts its protective effect by binding directly to the reactive intermediates produced by the spontaneous decomposition of 4-HC, thus detoxifying the drug. Finally, WR-1065 is a potent scavenger of free radicals. Thus, WR-1065 provides a form of temporary augmentation of protection to the normal marrow, which supplements the intrinsic protection provided by intracellular glutathione.

Using a sensitive immunohistochemical technique, 0.6 to 1.4 logs of breast cancer antigen-positive cells were eliminated from the buffy-coat marrow fraction of the three patients in whom these cells were initially detected. This depletion is likely due to the reduction in cellular concentration produced by density gradient separation, rather than to an immediate 4-HC effect. The tumor assay does not distinguish viable from intact but nonviable cells. Clearly, longer follow-up and larger patient numbers are required to assess the significance of residual cancer in the marrow of breast cancer patients who receive ABMS.

In conclusion, amifostine significantly shortens the time to marrow recovery and reduces the supportive care requirements of breast cancer patients receiving high-dose chemotherapy and ABMS with 4-HC–purged marrow. Since the supportive care measures for infection and bleeding are major contributors to the cost of marrow transplantation, it is likely that amifostine will reduce the cost of therapy, in addition to reducing the risk of myelosuppressive complications, for patients receiving 4-HC–purged ABMS.

**ACKNOWLEDGMENT**

We gratefully acknowledge the support of the nurses and residents who helped care for these patients in the University of Colorado Bone Marrow Transplant Unit.

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Amifostine (WR-2721) shortens the engraftment period of 4-hydroperoxycyclophosphamide-purged bone marrow in breast cancer patients receiving high-dose chemotherapy with autologous bone marrow support

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