High-Dose Infusional Doxorubicin and Cyclophosphamide: A Feasibility Study of Tandem High-Dose Chemotherapy Cycles without Stem Cell Support

Robert J. Morgan, Jr., James H. Doroshow, Kalyanasundaram Venkataraman, Karen Chang, James Raschko, George Somlo, Lucille Leong, Merry Tetef, Stephen Shibata, Victor Hamasaki, Kim Margolin, Stephen Forman, Steven Akman, Paul Coluzzi, Chul Ahn, Lawrence Weiss, Uday Gadgil, and Jonathan Harrison


ABSTRACT

The purpose of this study was to determine the maximally tolerated dose of doxorubicin administered during two cycles of intensive chemotherapy with cyclophosphamide and doxorubicin without stem cell support in patients with advanced cancer and to assess the cumulative cardiac toxicity of the regimen by noninvasive radionuclide imaging and by pre- and postchemotherapy endomyocardial biopsies. Thirty-eight patients (thirty-six with high risk or metastatic breast cancer) were treated in a dose-escalation trial using a fixed dose of i.v. cyclophosphamide (4.2 g/m²) administered over 2 h on day 5 and escalating doses of doxorubicin (50–175 mg/m²) given as a 96-h continuous i.v. infusion on days 1–4, using Filgrastim (granulocyte colony-stimulating factor) for hematological support beginning on day 6. All patients underwent pretreatment, and 28 patients underwent postchemotherapy endomyocardial biopsies. Twenty-nine of 38 patients received two cycles of treatment (median number of days between cycles, 44; range, 34–62). Twenty-one patients had previously had doxorubicin, and 20 had previously had granulocyte colony-stimulating factor. Twenty-one patients had previously had doxorubicin at a dose of 150 mg/m² without stem cell support. Twenty-nine of 38 patients received two cycles of treatment (median number of days between cycles, 44; range, 34–62). Twenty-one patients had previously had doxorubicin at a dose of 150 mg/m² without stem cell support. Twenty-nine of 38 patients received two cycles of treatment (median number of days between cycles, 44; range, 34–62). Twenty-one patients had previously had doxorubicin at a dose of 150 mg/m² without stem cell support.

The recommended Phase II dose is doxorubicin 150 mg/m² administered as a 96-h infusion on days 1–4, with cyclophosphamide 4.2 g/m² on day 5 and G-CSF 5 μg/kg/day started on day 6 and administered until the total WBC is above 10,000/μl for three consecutive days.

INTRODUCTION

Dose intensity plays an important role in the efficacy of chemotherapy for breast cancer and other tumors (1, 2). Steep dose-response relationships for both alkylating agents and anthracyclines have been demonstrated in the laboratory and confirmed in clinical trials. Experiments using incremental increases in exposure of experimental tumors to cyclophosphamide in vivo demonstrate a linear log relationship between dose and tumor cell kill; a 2-fold difference in dose increases cell kill...
by three to greater than 10-fold (2). Buckner et al. (3), Collins et al. (4), and Mullins et al. (5) have performed separate trials administering repeated courses of single-agent cyclophosphamide 120 mg/kg without stem cell support in patients with a variety of solid tumors and found that two to six cycles are feasible with acceptable hematological and cardiac toxicity. Similarly, clinical trials designed to assess the effect of dose intensity in combination regimens have consistently demonstrated improved complete and partial response rates for therapeutic regimens incorporating increased dose levels (6, 7).

Clinical dose-response data exist for doxorubicin in the treatment of breast cancer where an overall response rate of 85% (38% complete responses) was demonstrated for metastatic disease when the anthracycline dose was escalated 2–3-fold, double that expected with conventional doses (8). Wheeler et al. (9) used high-dose doxorubicin (120 mg/m² every 3 weeks) to treat cancers normally considered to be minimally responsive to this agent, including non-small cell lung cancer, melanoma, and soft-tissue sarcoma, and obtained an overall response rate of 46%.

Despite these results, it is commonly assumed that the cumulative cardiac toxicity of doxorubicin precludes its routine use in high-dose combination chemotherapy regimens, regardless of the fact that this drug is the single most active agent in the treatment of breast cancer. However, definitive evaluation of the risk of congestive heart failure in patients treated with anthracyclines is possible by the use of endomyocardial biopsy (10). This technique has been shown to be safe and to have significant predictive power. Billingham and Bristow (10) reported that of more than 5000 biopsies performed at Stanford University Medical Center, there were no deaths and <0.3% morbidity. Thus, we were encouraged to examine the feasibility, safety, and efficacy of the combination of high-dose cyclophosphamide and high-dose doxorubicin without peripheral blood progenitor cell support.

We report here the results of a dose-escalation trial in which two cycles of the combination of cyclophosphamide (4.2 g/m²) and doxorubicin (50–175 mg/m²) were administered using G-CSF3 alone and in which a prospective assessment of cumulative myocardial toxicity was performed using pre- and posttreatment endomyocardial biopsies.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Dosage escalation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dox dose/cycle</td>
<td>No. of patients treated</td>
</tr>
<tr>
<td>50</td>
<td>3</td>
</tr>
<tr>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td>125</td>
<td>8</td>
</tr>
<tr>
<td>150</td>
<td>15</td>
</tr>
<tr>
<td>175</td>
<td>7</td>
</tr>
</tbody>
</table>

* D0x, doxorubicin; dose in mg/m².

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3 The abbreviations used are: G-CSF, granulocyte colony-stimulating factor; MUGA, multiple gated radionuclide cardiac wall motion study; TPN, total parenteral nutrition.

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PATIENTS AND METHODS

**Patient Selection.** Between August, 1991, and June, 1994, 38 patients were entered in this Phase I trial. Eligible patients included: those with high risk stage 2 or 3 breast cancer following standard adjuvant chemotherapy; metastatic breast cancer patients demonstrating an objective response to standard chemotherapy; sarcoma patients following complete surgical resection of high-grade primary tumor; and individuals with other histologies who were considered for treatment on an individual basis. Patients were required to be ≥60 years old and have a Karnofsky performance status of ≥70%. Laboratory parameters included 24-h creatinine clearance of ≥50 ml/min, total WBCs ≥4000/µl with a normal differential count, platelet count ≥150,000/µl, hemoglobin ≥10 g/dl, serum bilirubin ≤1.5 mg/dl, and aspartate aminotransferase and alanine aminotransferase within twice the upper limit of normal. Patients may have received no more than two previous regimens of chemotherapy, which must have been completed at least 4 weeks prior to beginning treatment on this protocol. Patients who had previously received radiotherapy to >20% of marrow-bearing bone or who had received doxorubicin >150 mg/m² were ineligible. Left ventricular ejection fraction by radionuclide scan (MUGA) was required to be ≥52%. All patients were required to undergo pre- and posttreatment endomyocardial biopsies; a score of less than grade 1 [endomyocardial biopsy pathological grading scale (11)] was necessary on the baseline examination for study entry. All patients gave their voluntary informed consent and signed a consent document reviewed and...
Phosphamide 4.2 g/m² was administered as a 2-h iv. infusion. Four h after the completion of the doxorubicin infusion, cyclophosphamide was escalated in doses (see Table 1 for dose escalation schema) according to actual body weight. Doxorubicin was administered as a 96-h course of dose-intensive treatment. All doses were calculated according to body surface area. Sodium 2-mercaptoethanesulfonate (MESNA; 1.7 g/m²) was administered prior to the beginning of the cyclophosphamide infusion and repeated every 3 h for a total of eight doses. I.v. hydration was administered during the cyclophosphamide infusion using normal saline at 250 ml/h and was continued for 24 h, with diuretics given as needed to maintain fluid balance. G-CSF (Filgrastim; Amgen, Thousand Oaks, CA) 5 µg/kg/day was administered once daily, i.v., beginning 24 h following the cyclophosphamide infusion, until the total WBCs reached 10,000/µl for 3 consecutive days. Patients remaining granulocytopenic (absolute granulocyte count, <500/µl) for >10 days received a dosage escalation of G-CSF to 10 µg/kg/day. Supportive care for all patients included protective isolation in a room with HEPA-filtered positive pressure ventilation until the granulocyte count had recovered to >500/µl. All patients received transfusions to maintain the platelet count >20,000/µl and hemoglobin >8 g/dl. Oral nonabsorbable antibiotics included vancomycin 250 mg and neomycin 1 g three times per day for 3 days and then daily until granulocyte recovery; nystatin vaginal tablets, USP 100,000 units, were used intravaginally twice daily. Oral care included peroxide mouthwashes and oral nystatin or amphotericin B solution six times daily. All blood products were irradiated, and appropriate antibiotics, analgesics, and parenteral nutrition were administered as necessary.

Patients remained hospitalized until they had recovered from the acute side effects of the therapy. Planned date of readmission for the second cycle of therapy was day 35 from the first day of G-CSF administration, although a delay of up to 14 days was allowed for full recovery of toxicity from the first cycle of chemotherapy. Patients were required to meet preprotocol eligibility requirements prior to the administration of cycle 2 of protocol therapy. The second cycle of chemotherapy was administered exactly as outlined for cycle 1. Toxicities were graded according to the Common Toxicity Criteria; hematological toxicity and mucositis were not considered dose-limiting. Accrual at each dose level continued until at least three patients received two cycles of chemotherapy with no grade 3 or 4 toxicities and, additionally, had been evaluated with posttreatment endomyocardial biopsies that demonstrated a cardiac toxicity score of less than grade 1 (11). Maximal tolerated dose was established by the observation of one grade 4 toxicity at any dose level or two grade 3 toxicities in a cohort of six patients. Response criteria were recorded using standard criteria (15).

**Statistical Methods.** Repeated measures analysis was performed to examine whether there were significant associations between changes in measured ejection fraction determined by MUGA scan and age, prior doxorubicin dose, total lifetime doxorubicin dose, and doxorubicin received on this study (16). Linear trend analysis was performed to determine whether there was a significant linear decrease in ejection fraction over time (17). The number of days of TPN, days with platelets below 100,000/i.l for 3 consecutive days. Patients remaining granulocytopenic (absolute granulocyte count, <500/µl) for >10 days received a dosage escalation of G-CSF to 10 µg/kg/day. Supportive care for all patients included protective isolation in a room with HEPA-filtered positive pressure ventilation until the granulocyte count had recovered to >500/µl. All patients received transfusions to maintain the platelet count >20,000/µl and hemoglobin >8 g/dl. Oral nonabsorbable antibiotics included vancomycin 250 mg and neomycin 1 g three times per day for 3 days and then daily until granulocyte recovery; nystatin vaginal tablets, USP 100,000 units, were used intravaginally twice daily. Oral care included peroxide mouthwashes and oral nystatin or amphotericin B solution six times daily. All blood products were irradiated, and appropriate antibiotics, analgesics, and parenteral nutrition were administered as necessary.

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**Pretreatment Evaluation.** All patients underwent a complete history and physical examination including documentation of weight, Karnofsky performance status, presence of measurable or evaluable disease, a complete blood count with platelet count and differential, 18-channel blood chemistry analysis, chest X-ray, radionuclide bone scan, and computed tomographic scans of the abdomen and pelvis. Patients with measurable disease were required to repeat scans for analysis of measurable disease after the two planned cycles of high-dose therapy. Cardiac evaluation included a two-dimensional and M-mode echocardiogram and MUGA scan performed prior to the initial endomyocardial biopsy. MUGA scanning was repeated prior to and following the second cycle of chemotherapy. Patients were followed monthly for 3 months and then as per the routine of the referring physician. Endomyocardial biopsies were performed prior to the first cycle and following the second cycle of high-dose chemotherapy by the Department of Cardiology at the City of Hope using a percutaneous transfemoral approach. An average of two to three biopsy samples was taken from the intraventricular septum (12, 13). Tissue was processed for light and electron microscopy by the Department of Pathology at the City of Hope National Medical Center as described previously (14).

**Treatment Plan.** Chemotherapy consisted of two cycles of dose-intensive treatment. All doses were calculated according to actual body weight. Doxorubicin was administered as a 96-h continuous i.v. infusion through a central venous catheter with escalating doses (see Table 1 for dose escalation schema) beginning at 50 mg/m²/cycle (total dose, 100 mg/m²). Twenty-four h after the completion of the doxorubicin infusion, cyclophosphamide 4.2 g/m², was administered as a 2-h i.v. infusion. Sodium 2-mercaptoethanesulfonate (MESNA; 1.7 g/m²) was administered prior to the beginning of the cyclophosphamide infusion and repeated every 3 h for a total of eight doses. I.v. hydration was administered during the cyclophosphamide infusion using normal saline at 250 ml/h and was continued for 24 h, with diuretics given as needed to maintain fluid balance. G-CSF (Filgrastim; Amgen, Thousand Oaks, CA) 5 µg/kg/day was administered once daily, i.v., beginning 24 h following the cyclophosphamide infusion, until the total WBCs reached 10,000/µl for 3 consecutive days. Patients remaining granulocytopenic (absolute granulocyte count, <500/µl) for >10 days received a dosage escalation of G-CSF to 10 µg/kg/day. Supportive care for all patients included protective isolation in a room with HEPA-filtered positive pressure ventilation until the granulocyte count had recovered to >500/µl. All patients received transfusions to maintain the platelet count >20,000/µl and hemoglobin >8 g/dl. Oral nonabsorbable antibiotics included vancomycin 250 mg and neomycin 1 g three times per day for 3 days and then daily until granulocyte recovery; nystatin vaginal tablets, USP 100,000 units, were used intravaginally twice daily. Oral care included peroxide mouthwashes and oral nystatin or amphotericin B solution six times daily. All blood products were irradiated, and appropriate antibiotics, analgesics, and parenteral nutrition were administered as necessary.

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**Fig. 1** From left to right, the first three closed circles represent pre-, post-course 1, and post-course 2 ejection fractions measured by a MUGA scan of patients receiving two complete courses of therapy who had pre- and posttreatment endomyocardial biopsies. The second group of three closed circles are summaries of results of all patients. There is a significant linear decrease over time within the normal range (see text).
RESULTS

Patient Characteristics. Patient characteristics are summarized in Table 2. All 38 patients entered in this study were female. Median age was 45 years (range, 23–60), and median Karnofsky performance status was 100% (range, 70%–100%). Thirty-six patients had breast cancer, 1 patient had primary angiosarcoma of the breast and received adjuvant treatment on this protocol, and 1 patient had metastatic pancreatic islet cell cancer that had demonstrated a partial response to prior chemotherapy. Of the patients with breast cancer, 12 had high-risk stage 2 disease (11 with ≥10 involved lymph nodes, one patient with 8 involved nodes), 11 were stage 3A or 3B, 12 patients had responding metastatic disease, and 1 patient had newly relapsed cancer with bone metastases. Twenty-one patients had received prior radiotherapy to the left chest wall.

Treatment Delivered. The number of treatment cycles delivered at each dose level is summarized in Table 1. Twenty-nine of the 38 patients enrolled on this study received two planned courses of chemotherapy. Nine patients did not receive the second cycle of chemotherapy for the following reasons: five developed decreased cardiac ejection fractions observed on MUGA scanning postcycle 1 (one patient developed seronegative hepatitis in addition to the decreased ejection fraction), one patient’s disease progressed following cycle one, one patient refused cycle 2, one patient developed a persistent Clostridium difficile enterotoxin necessitating cancellation of the planned second cycle of therapy, and one patient died during the first cycle of therapy due to sepsis. Of the 29 patients receiving two courses of therapy, the median number of days from the initial day of chemotherapy of cycle one until the beginning of chemotherapy in cycle 2 was 44 days (range, 34–62).

Summary of Cardiac Evaluation by Radionuclide Scan (MUGA). A summary of MUGA scan data are shown in Fig. 1 and Tables 3 and 4. Eight of nine patients who received one course of therapy and 28 of 29 patients who received two courses of chemotherapy had pre- and posttreatment radionuclide scans. All patients had normal scans prior to treatment (ejection fraction, >52%). Six of nine patients receiving only one course of chemotherapy had asymptomatic decreases in cardiac ejection fraction to ≤52% (range of ejection fraction postchemotherapy, 43–52% Table 3). Four of these patients had received prior doxorubicin, and the other two had received prior left chest irradiation. Two of these patients had a normal (grade 0) endomyocardial biopsy score following the first cycle of chemotherapy despite a decrease in ejection fraction by MUGA, two patients had posttreatment biopsy scores of grade 1, and the other two patients did not undergo posttreatment biopsies.

Twenty-eight of 29 patients who received two courses of high-dose chemotherapy had pre- and posttreatment MUGA scans (a summary of the cardiac evaluations of those patients who had abnormal results is found in Table 4). The six patients with abnormal posttreatment ejection fractions (≤52%) after two cycles of treatment included one patient treated with two cycles of doxorubicin 100 mg/m²/cycle, two patients with 125 mg/m²/cycle, one patient with 150 mg/m²/cycle, and two patients with 175 mg/m²/cycle. Both patients treated at the highest dose level had received prior doxorubicin, and one had additionally received prior left chest irradiation. The median ejection fraction of those patients with ≤52% ejection fractions after two cycles of chemotherapy was 47.5% (range, 39–51%). Only one of these six patients, who had received prior doxorubicin and left chest irradiation (treated with doxorubicin 175 mg/m²/cycle), suffered symptoms of congestive heart failure; these symptoms were of minor severity and were easily controlled with medication.

Follow-up MUGA scans of patients receiving one cycle of therapy performed from 1 to 10 months after doxorubicin treatment revealed improvement to normal ejection fraction values from 48, 43, and 48% to 61, 62, and 55%, respectively, in three patients, stability in three other patients with follow-up scans, and one patient with further decrease in ejection fraction from 43 to 34% who remained asymptomatic (see Table 3). The patients with grade 1 posttreatment biopsy scores included those with posttreatment ejection fractions of 61 and 34% at 6 and 8 months, respectively. Patients receiving two cycles of therapy had similar findings; however, no patient in this group had worsening of the ejection fraction (Table 4).

Repeated measures analysis revealed that there was no
Three patients at dose level 125 mg/m² had abnormal posttreatment endomyocardial biopsies: two of which were normal (grade 0); and pretreatment, between-treatment, and posttreatment biopsy scores were abnormal at the two lower dose levels had corresponding MUGA scan results within the normal range. The two patients at the highest dose level with scores of 1 on the posttreatment biopsy had corresponding posttreatment ejection fractions of 43 and 45%, respectively, as measured by MUGA scan. One of these patients had symptoms of mild congestive heart failure, which appeared approximately 4 weeks after hospital discharge. Only one of the other six patients with pathologically abnormal biopsies had an abnormal ejection fraction as measured by MUGA scanning, and none had symptomatic congestive heart failure. Of the five patients who received only one cycle of therapy and were not retreated due to decreases in ejection fraction measured by MUGA scanning, four consented to posttreatment biopsies: two of which were normal (grade 0); and two with biopsy scores of grade 1.

One patient treated with doxorubicin 100 mg/m²/course developed supraventricular tachycardia following hematological recovery and hospital discharge after the first cycle of therapy. Because these symptoms were well controlled on medication, a second cycle of therapy was administered. The supraventricular tachycardia recurred during the second cycle of treatment, requiring intensive care unit monitoring. The patient recovered without sequelae. Pre- and posttreatment biopsy results were grade 0; and pretreatment, between-treatment, and posttreatment MUGA scans revealed ejection fractions of 67. 67, and 66%, respectively.

Hence, one patient enrolled on this study developed mild symptomatic congestive heart failure. This patient had a fall in cardiac ejection fraction as measured by MUGA scanning from 65% pretreatment to 55% after one cycle of treatment to 45% following two cycles of therapy. Pathological correlation revealed grade 1 myofibrillar changes (isolated myocytes affected by a distended sarcotubular system and/or early myofibrillar loss). No other patient developed clinical symptoms of cardiac insufficiency irrespective of noninvasive or invasive cardiac evaluation results.

**Hematological Recovery.** Duration of granulocytopenia and thrombocytopenia by dosage level of doxorubicin is summarized in Table 5. The median number of days of granulocy-
Table 5 Hematological recovery and transfusions

<table>
<thead>
<tr>
<th>Dox dose&lt;sup&gt;a&lt;/sup&gt; mg/m&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Days of AGC &lt;500/μl Median (range)</th>
<th>Days of Plt &lt;20,000/μl Median (range)</th>
<th>RBC transfusions Median (range)</th>
<th>Plt transfusions Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>4 (4–6)</td>
<td>0 (0–2)</td>
<td>2 (0–2)</td>
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<td>100</td>
<td>7 (3–9)</td>
<td>0 (0–10)</td>
<td>4 (1–6)</td>
<td>1 (0–18)</td>
</tr>
<tr>
<td>125</td>
<td>9 (8–13)</td>
<td>2 (1–9)</td>
<td>4 (1–6)</td>
<td>3 (1–8)</td>
</tr>
<tr>
<td>150</td>
<td>9 (5–13)</td>
<td>2 (0–5)</td>
<td>4 (2–6)</td>
<td>4 (2–8)</td>
</tr>
<tr>
<td>175</td>
<td>8 (6–13)</td>
<td>2 (0–4)</td>
<td>4.5 (3–5)</td>
<td>4 (1–6)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Dox, doxorubicin; AGC, absolute granulocyte count; Plt, platelet.

Table 6 Hospital stay and days on TPN

<table>
<thead>
<tr>
<th>Dox dose mg/m&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Hospital stay (days) Median (range)</th>
<th>Days on TPN Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>19 (18–21)</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td>100</td>
<td>20 (16–30)</td>
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<td>125</td>
<td>24 (21–26)</td>
<td>12 (0–16)</td>
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<td>150</td>
<td>24 (19–36)</td>
<td>15 (0–24)</td>
</tr>
<tr>
<td>175</td>
<td>23 (21–34)</td>
<td>17 (10–22)</td>
</tr>
</tbody>
</table>

Hospital stay and days on TPN

The number of days of hospitalization is also summarized in Table 6. Again, the median number of days of hospitalization rose from 19 to 23 between 50 mg/m<sup>2</sup> and 125 mg/m<sup>2</sup> doxorubicin but remained stable as the doxorubicin dose was increased further.

Fever and Infection. Fever \( \geq 38^\circ \text{C} \) (grade 2) developed in 26 of 38 patients (72%) during cycle one of chemotherapy and in 18 of 28 patients (64%) during cycle two. Confirmed positive blood cultures associated with episodes of fever are summarized in Table 7. Gram-positive bacteremias included: *Staphylococcus* (coagulase negative) isolated in 12 instances of fever, and *Staphylococcus aureus*, *Bacillus cereus*, and *Streptococcus Group D* isolated in one or two instances. Three patients had confirmed *Streptococcus viridans* infections; this included the single patient who died from respiratory failure caused by the adult respiratory distress syndrome associated with sepsis. Gram-negative infections included: *Escherichia coli* in three patients, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pseudomonas paraumbilicus*, and *Enterococcus sp.* in one or two instances. No patient developed positive fungal blood cultures. Six patients had positive oral or pharyngeal cultures for *Herpes simplex*, and six patients had positive stool cultures or assays for *C. difficile* and/or toxin.

Other Toxicities. The nonhematological toxicities observed are consistent with those experienced in the setting of dose-intensive chemotherapy and are summarized in Table 7. Episodes of mucositis consisting of pain with ulceration requiring narcotic analgesics for less than 2 weeks duration were very common at all dose levels. At the maximally tolerated dose of doxorubicin, 150 mg/m<sup>2</sup>, 10 of 15 patients experienced these symptoms during cycle one, and 9 of 11 patients during cycle 2. Other significant toxicities included emesis and diarrhea in smaller numbers of patients. There was no significant difference in nonhematological toxicity between cycles one and two of chemotherapy at any dosage level.

One patient treated at the highest doxorubicin dose level sustained a grand mal seizure associated with orthostatic hypotension, 4 days following chemotherapy. The patient was treated symptomatically and recovered without sequelae; however, this event was considered a dose-limiting toxicity and established the maximally tolerated dose.

One patient treated on the third dose level was found to have pancytopenia 54 months following treatment in this study. Further evaluation revealed a myelodysplastic syndrome that evolved into acute leukemia. She had previously undergone left modified radical mastectomy and right segmentectomy for two...
separate breast cancers diagnosed 2 years apart and had undergone adjuvant chemotherapy using cyclophosphamide/methotrexate/5-fluorouracil following the first mastectomy, and cyclophosphamide/doxorubicin (total prior doxorubicin dose, 150 mg/m²) prior to treatment on this study, followed by right-sided regional irradiation.

**Therapeutic Responses in Breast Cancer Patients.** Thirty-six patients with breast cancer were enrolled in this study. Twenty-three patients were classified as having high-risk stage 2 or stage 3 breast cancer, of whom 21 were without evidence of disease both before and after intensive chemotherapy with doxorubicin and cyclophosphamide (6 of these patients received only one cycle of high-dose chemotherapy). One patient in this category progressed immediately following the second cycle of therapy. One patient with locally advanced disease achieved a partial response following induction and high-dose consolidation chemotherapy and subsequently was rendered surgically free of disease.

Twelve patients had stage 4 breast cancer objectively responsive to induction chemotherapy. Three of these patients achieved a complete response prior to consolidation therapy on this study and remained without evidence of disease at the completion of therapy on this protocol. One patient with a preprotocol partial response converted to an objective complete response; five patients (all with bone metastases only) continued in partial response following two cycles of high-dose chemotherapy; one patient with a pre-high-dose therapy partial response is too early in her course of treatment to judge response; and one patient progressed following one cycle of chemotherapy. One patient who achieved a partial response to induction chemotherapy died in the study during the first cycle of treatment of complications related to sepsis.

In addition to the patients noted above, one patient was treated immediately following her diagnosis of new bone metastases and had stable disease following treatment.

**Survival Data for Breast Cancer Patients.** The progression-free and overall survival of the high-risk and metastatic breast cancer patients, estimated using the product-limit method of Kaplan and Meier and calculated from the first day of therapy, are shown in Table 8. High-risk patients with stage 2 or 3 disease have had a median progression-free survival of 35.3 months, overall survival duration has not reached the median. Patients with metastatic disease have had a median progression-free survival of 18.6 months with an overall median survival of 22.5 months.

TABLE 7  No. of patients with other toxicities

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Cycle no.</th>
<th>50</th>
<th>100</th>
<th>125</th>
<th>150</th>
<th>175</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emesis (no. of episodes of grade 2 toxicity)*</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2/3</td>
<td>3/5</td>
<td>6/8</td>
<td>10/15</td>
<td>6/7</td>
</tr>
<tr>
<td>Mucositis (requiring narcotic analgesics &lt;2 weeks)/No.</td>
<td>1</td>
<td>2/3</td>
<td>3/5</td>
<td>6/8</td>
<td>10/15</td>
<td>6/7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1/3</td>
<td>3/5</td>
<td>5/6</td>
<td>9/11</td>
<td>3/4</td>
</tr>
<tr>
<td>Diarrhea (grade 2)/No. of stool cultures (+) for C. difficile</td>
<td>1</td>
<td>2/0</td>
<td>3/1</td>
<td>2/1</td>
<td>0/0</td>
<td>1/0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3/1</td>
<td>1/2</td>
<td>0/1</td>
<td>1/0</td>
<td>1/0</td>
</tr>
<tr>
<td>Fever &gt;38°C/No. of (+) blood cultures</td>
<td>1</td>
<td>1</td>
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* Common toxicity scale.

DISCUSSION

Dose intensity of chemotherapeutic agents (defined as the amount of drug delivered per unit time; Ref. 18) and total administered dose (2) have been shown to be critical features of patient outcome after cancer treatment (18). Although it is clear that increasing dose intensity results in increased objective response rates in breast cancer (8, 9), the shape and slope of the dose-response curve remain controversial. (19) Tannock et al. (7) reported a survival benefit for standard dose chemotherapy with cyclophosphamide, methotrexate, and 5-fluorouracil compared to lower doses of the same drugs when administered as adjuvant therapy in breast cancer. Wood et al. (20), in a randomized three-arm study, recently showed that low-dose chemotherapy for breast cancer in the adjuvant setting is inferior to standard dose therapy; however, one arm of this Cancer and Leukemia Group B study, consisting of moderately increased doses of chemotherapy, did not improve disease-free survival compared to the control. The National Surgical Adjuvant Breast and Bowel Project reported recently that modest increases in total dose or dose intensity of cyclophosphamide, in a doxorubicin-containing adjuvant combination chemotherapy regimen, did not appear to affect relapse-free survival compared to standard doses of the same agents (21). In contrast to these studies, however, Peters et al. (22) and others have shown a major effect on disease-free survival for breast cancer patients with responding metastatic or high-risk disease using very high-dose chemotherapy and stem cell rescue. Thus, it appears that major increases in dose intensity may be necessary before improvements in disease-free or overall survival can be achieved for patients with breast cancer.

Doxorubicin and related anthracyclines have played a central role in cancer chemotherapy for the past 20 years because of their activity in a variety of hematological and solid tumors. Their primary limitation has been dose-dependent cardiac toxicity, which can result in a congestive cardiomyopathy due to myocyte loss. Prediction of the onset of functional myocardial toxicity by noninvasive testing has been useful in population studies but limited in individual patients (23, 24). However, definitive evaluation of the risk of development of congestive heart failure in patients treated with anthracyclines is possible by endomyocardial biopsy (10) with documentation of typical myocardial muscle fiber damage, including vacuolization and myofibrillar loss seen on ultrastructural examination. The extent of these changes is predictive of the development of clinical symptoms of heart failure. In our study, five patients received
only one of two planned cycles of intensive chemotherapy due to a decreased ejection fraction observed on the MUGA scan following the first cycle of treatment. Two of these patients had endomyocardial biopsy scores of zero, and two patients had biopsy scores of one on the posttreatment pathological evaluation. Eight additional patients had abnormal endomyocardial biopsy scores following two cycles of dose-intensive doxorubicin. In only three of these patients was the MUGA scan result below the lower limit of normal, and one of these patients was shown to normalize her ejection fraction on a scan obtained 5 months later. These observations of minimal clinical cardiotoxicity are consistent with the findings of Green and colleagues (25), who observed decreases in ejection fractions in 8 of 15 patients treated with three cycles of dose-intensive epirubicin and cyclophosphamide but no episodes of congestive heart failure. Thus, these data strongly suggest that prediction of latent cardiac damage by radionuclide scanning in individual patients may be unreliable. The endomyocardial biopsies indicate that patients may be safely treated with dose-intensive doxorubicin therapy without fear of unsuspected severe myofibrillar damage due to anthracyclines.

Cyclophosphamide has also been reported to cause cardiac toxicity (26) in the high-dose setting. Changes seen in autopsy series, including myocardial hemorrhage and evidence of fibrinous pericarditis, however, differ from the classic morphological picture of anthracycline cardiac toxicity (27).

This study has shown that patients treated with two cycles of dose-intensive therapy using these two chemotherapeutic agents tolerate such treatment well, with minimal clinical cardiac symptomatology and no pathological evidence of cyclophosphamide toxicity. The low-grade morphological evidence of anthracycline toxicity noted on biopsy specimens at the higher dose levels in this study is consistent with the observations of Billingham et al. (10, 11), who have described evidence of myocardial cell degeneration in virtually all patients treated with cumulative doxorubicin doses of ≥ 240 mg/m² with the degree of toxicity noted related to cumulative doxorubicin dose. Increased levels of toxicity in this study were noted in patients previously treated with left chest wall irradiation. The low level of morphological cardiac injury observed was due, at least in part, to the delivery of doxorubicin by 96-h continuous infusion, which has been shown to allow higher cumulative doses of anthracyclines with decreased morphological evidence of cardiac toxicity (28).

Statistical analysis of sequential ejection fraction measurements by MUGA scanning revealed a trend toward decreased cardiac ejection fraction with increasing total lifetime dose of doxorubicin in this study. Follow-up scans revealed that this trend was at least partially reversible, with normalization of the ejection fraction in some patients after several months, and stability of ejection fraction in other patients with follow-up measurements. Despite prior doxorubicin treatment, left chest irradiation, or any abnormal MUGA scan measurements or biopsy findings, only one patient developed symptoms of mild congestive heart failure, which was easily controlled with minimal medications, indicating that a 2-3-fold dose intensification with this cyclophosphamide/doxorubicin regimen over dosages considered standard is clinically feasible and allows a dose-intensive program in breast cancer patients using the most active agents in this disease, providing that the prior anthracycline dose is limited and the high-dose doxorubicin is administered as a 96-h infusion. This includes patients who may display involvement of the bone marrow with metastases, in whom contamination of stem cells otherwise used as a part of supportive care would be a concern.

Efforts to administer dose-intensive treatment require aggressive blood product and antibiotic support. Morbidity and mortality are directly related to the length of myelosuppression induced by the administered chemotherapy. Hematological supportive therapy often includes hematopoietic stem cell rescue, using cells obtained from either the bone marrow or peripheral blood. These procedures are expensive, require significant time for preparation prior to the administration of dose intensive therapy, and have the additional potential risk of reinfusion of contaminating breast cancer cells (29). More recent attempts to increase dose intensity with decreased toxicity and cost have focused on the use of recombinant human growth factors to stimulate bone marrow recovery (30, 31). This present trial is one of the first to combine high-dose cyclophosphamide and high-dose doxorubicin without stem cell support; it demonstrates durations of neutropenia and thrombocytopenia comparable to those reported with regimens that include both stem cell reinfusion and growth factor (32, 33). All treatment in this Phase I study was administered as inpatient to ensure patient safety; therefore, the duration of hospitalization reported in this study appears to be prolonged. It has been determined, however, that chemotherapy on this schedule can be safely administered as outpatient, and hospital days on an ongoing Phase 2 study are expected to be at least 10 days shorter than those reported here.

One patient on this study experienced a grand mal seizure, which following neurology consultation and institutional review board review was considered the dose-limiting toxicity. On a previous single-cycle, stem cell-supported, doxorubicin-based protocol at our institution, dose-limiting mucositis was experienced at a level concordant with the observed dose-limiting toxicity in this study (33).

We investigated the feasibility of administering this two-drug, dose-intensive chemotherapy regimen in patients with solid tumors and determined that treatment with two cycles of high-dose cyclophosphamide 4.2 g/m² and doxorubicin 150 mg/m²/cycle using G-CSF for hematological support is feasible in patients who have previously received ≤ 150 mg/m² of anthracycline. This regimen is safe and has demonstrable therapeutic activity when administered in a medical center skilled in the delivery and management of the complications of high-dose chemo-
therapy. Furthermore, the myocardial toxicity of the high-dose combination is tolerable within the above dosage range, thereby allowing a 2-3-fold dose escalation of doxorubicin. We are presently conducting a Phase 2 trial to determine the long-term efficacy of two cycles of consolidation treatment with this regimen in patients with high-risk or responsive metastatic breast cancer.

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High-dose infusional doxorubicin and cyclophosphamide: a feasibility study of tandem high-dose chemotherapy cycles without stem cell support.

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