Feasibility of Multiple Courses of High-Dose Cyclophosphamide, Thiotepa, and Carboplatin for Breast Cancer or Germ Cell Cancer

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Purpose: **To determine the feasibility and safety of multiple, closely timed courses of high-dose cyclophosphamide, thiotepa, and carboplatin (CTC) with peripheral-blood progenitor-cell transplantation (PBPCT).**

Patients and Methods: **Forty-eight patients with advanced cancer were scheduled to undergo either two or three courses of CTC with PBPCT. All PBPCs were harvested before high-dose therapy began. Full-dose CTC courses incorporated cyclophosphamide (6,000 mg/m^2), thiotepa (480 mg/m^2), and carboplatin (1,600 mg/m^2) divided over days −6, −5, −4, and −3. Tiny CTC courses (tCTC) contained 67% of the doses of each of these agents. Second or third courses of CTC or tCTC began on day 28.**

Results: **A sufficient number of PBPC could be harvested from all but two patients. Thirty-five first full-dose courses of CTC were given, 28 second courses, and 10 third courses. Second courses could be given on time and at full dose in 80% of the patients, but there was one toxic death from venoocclusive disease (VOD). Only four of 12 patients scheduled to receive three courses of full-dose CTC could be treated at the time and dose planned. There were three toxic deaths: one of VOD, one of sepsis, and one of hemolytic uremic syndrome (HUS). Eight patients were scheduled to receive three courses of tCTC. Eight first, seven second, and six third courses were given. One of the third courses had to be delayed and one had to be reduced in dose.**

Conclusion: **A sufficient number of PBPCs for two or three transplantations can be harvested from most patients without much difficulty before high-dose therapy. Two full-dose CTC courses or three tCTC courses can be given safely and with acceptable toxicity at 5-week intervals. Organ toxicity rather than bone marrow toxicity has become dose-limiting for alkylating agents.**

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HIGH-DOSE CHEMOTHERAPY with autologous bone marrow support is increasingly used in solid tumors as a potentially curative treatment strategy for advanced disease. Although results of randomized studies are lacking, many investigators believe that this treatment modality may be beneficial in a number of rare conditions, such as relapsing germ cell cancer or neuroblastoma. It is in breast cancer that high-dose therapy has been used most frequently, but this practice is the subject of considerable controversy.[1,2] Based on retrospective analyses of bone marrow transplant registries, it has been suggested that high-dose therapy may be beneficial in a small subgroup of advanced breast cancer patients: those who have achieved a complete remission (or a very good partial remission) with standard-dose chemotherapy. Of these, approximately one fourth will survive more than 3 years without relapse after transplantation.[3-6]

It is not entirely clear whether comparable long-term survival rates could be achieved with conventional therapy in similarly highly selected patients. Even if high-dose therapy would eventually be shown to be more effective than standard-dose therapy in this situation, its efficacy is far from satisfactory. If one assumes that 20% of patients with advanced disease can achieve a complete remission with standard therapy, and 25% of these can be effectively consolidated with high-dose therapy, then only 5% of patients would experience long-term survival. One strategy to improve on these figures is to increase further the dose of chemotherapy delivered. The advent of peripheral-blood progenitor-cell transplantation (PBPCT) has allowed much faster recovery from bone marrow depression than after bone marrow transplantation, and multiple transplants are now under investigation in a number of centers.

Multiple transplantation procedures may lead to cumulative dosages of cytotoxic agents that are much higher than in previous experience, and novel patterns of toxicity are likely to emerge. Most of the agents used in high-dose therapy are associated with specific forms of extramedullary toxicity, which may include potentially lethal organ toxicities such as interstitial pneumonitis or venoocclusive disease (VOD) of the liver.[7] To prevent these serious toxicities, investigators have either substantially

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lowered the dose of the high-dose therapy courses (to nonmyeloablative levels) or have used alternating therapy in which each of two to three high-dose courses incorporates different agents. Two or more truly high-dose courses have only rarely been given in a closely timed fashion.

We have previously reported the development of a high-dose chemotherapy regimen that incorporates cyclophosphamide, thiotepa and carboplatin (CTC).[8] This regimen resembles the Solid Tumor Autologous Marrow Program (STAMP V) regimen developed by the Boston group,[9] but it is administered by short-term infusions over 4 days, rather than by continuous infusion, and it contains a double dose of carboplatin. The CTC regimen is safe and free from serious organ toxicity, and it is currently being used in a large randomized study of adjuvant high-dose therapy in high-risk breast cancer.[10] We have recently published our experience in five patients who received double courses of CTC as part of the salvage treatment of germ cell cancer.[11] Encouraged by these results, we have greatly extended our experience with double courses of CTC and have investigated triple courses at two different dose levels. This report summarizes our single-institution experience with multiple CTC courses and includes all patients ever scheduled to receive double or triple courses of CTC in the Netherlands Cancer Institute before September 1995.

PATIENTS AND METHODS

Patients

All patients had histologically verified malignant disease that could not be cured by local treatment modalities such as surgery or radiation therapy. All except three participated in clinical studies that had been approved by both the Institutional Review Committee and by the Medical Ethics Committee of the Netherlands Cancer Institute. The three other patients underwent double transplants because of peripheral neuroectodermal tumors (n = 2) or as third-line therapy for refractory choriocarcinoma (n = 1). Written informed consent was obtained from all patients according to institutional guidelines.

All protocols required adequate bone marrow function (WBC count $\geq 4.0 \times 10^9$/L and platelet count $\geq 100 \times 10^9$/L), normal liver function tests (bilirubin level < 20 μmol/L, and ALT and AST levels < 1.5 times the upper limit of normal), negative human immunodeficiency virus test, and negative test for hepatitis B antigens, negative tests for antiplatelet antibodies, and age less than 55 years. In germ cell cancer patients, renal function was considered to be acceptable when the creatine clearance was greater than 50 mL/min, but 80 mL/min was required for all other patients. Also in germ cell cancer, a World Health Organization (WHO) performance status of 2 or 3 was accepted, while it had to be 0 or 1 in all other patients. Breast cancer patients had to have estrogen receptor-negative disease or had to have failed to respond to first-line hormonal treatment. In most patients, high-dose chemotherapy with CTC was used as consolidation therapy immediately following standard-dose remission-induction therapy. Germ cell cancer patients scheduled to undergo double transplants received a mobilization course of ifosfamide, etoposide, and granulocyte colony-stimulating factor (G-CSF) (see later) followed by a single course of carboplatin (800 mg/m^2) and etoposide (1,500 mg/m^2).[12] The other patients scheduled for double CTC were mobilized with ifosfamide, etoposide, and G-CSF immediately before their first transplantation procedure. Patients scheduled to receive three courses of CTC received a mobilization course of either ifosfamide, etoposide, and G-CSF (in case of germ cell cancer, ovarian cancer, or rhabdomyosarcoma) or of fluorouracil, epirubicin, and cyclophosphamide with G-CSF (see later, in case of breast cancer).

PBPC Mobilization

One of two mobilization chemotherapy regimens was used, depending on the type of cancer to be treated. For all tumors except breast cancer, ifosfamide (4 g/m^2 on day 1) and etoposide (100 mg/m^2 on days 1, 2, and 3) were used. G-CSF (filgrastim) was started on day 4 at a dose of 10 μg/kg/d until leukocytaphereses were completed. The mobilizing properties of this regimen will be published elsewhere (Baars JW, Holtkamp MJ, Nooijen WJ, et al, submitted). For breast cancer, a regimen was used that consisted of fluorouracil (500 mg/m^2), epirubicin (120 mg/m^2), and cyclophosphamide (500 mg/m^2), all given by intravenous push on day 1.[13] Filgrastim was started on day 2.

PBPC Harvest

Irrespective of the mobilization regimen used, leukocytaphereses began when the WBC count was greater than 3.0×10^9/L and the CD34$^+$ cell count in the peripheral blood was $\geq 0.5\%$. To facilitate apheresis, all patients had 13.5-F double-lumen Hickman catheters. A continuous-flow blood-cell separator was used (Fenwal CS 3000; Baxter, Utrecht, The Netherlands). Both the number of CD34$^+$ cells and the number of granulocyte-macrophage colony-forming units (GM-CFU) were determined in the cell collections. All methods used in the stem-cell harvests have been described previously.[14]

Based on earlier findings,[14] we considered a graft size of 3.0×10^6 CD34$^+$ cells/kg body weight sufficient for sustained bone marrow recovery, and 1.0×10^6 CD34$^+$ cells/kg body weight sufficient for rapid (but possibly transient) granulocyte recovery after high-dose therapy. Using these criteria, a simple algorithm could be used to determine the distribution of the available CD34$^+$ cells over the transplants. The case for three planned transplants is listed in Table 1.

High-Dose Chemotherapy: CTC

The high-dose chemotherapy regimen CTC was administered as published previously,[8] but with two major modifications: (1) the period between the last infusion and the transplantation was reduced by 1 day, and (2) the carboplatin dose was corrected for renal function using a modified Calvert formula.[15] We have previously shown that such a modified formula adequately predicts the carboplatin area under the curve (AUC) in this patient group.[16]

Briefly, carboplatin was administered intravenously as daily 2-hour infusions on days -6, -5, -4, and -3. The total dose of carboplatin was 1,600 mg/m^2 in patients with normal renal function, but in those with creatinine clearances ≤ 110 mL/min, it was deter-

Table 1. Distribution of CD34$^+$ Cells Harvested Over Three Transplants

CD34$^+$ Cells Harvested ($\times 10^6$/kg)	Action
< 3.0	Patient must be taken off study.
$\geq 3.0 < 5.0$	Bone marrow harvest before first transplant. Divide harvested cells into 3 equal portions, 1 for each transplant. Reinfuse bone marrow together with stem cells after third course.
$\geq 5.0 < 9.0$	Reserve 3.0×10^6 CD34$^+$ cells for third transplant. Divide remainder of cells into equal halves for first and second transplant, respectively.
$\geq 9.0 < 11.0$	Reserve 3.0×10^6 CD34$^+$ cells for each of the transplants and add the remaining cells to the reinfusion after the third course.
$\geq 11.0 < 15.0$	Reserve 5×10^6/kg for third course, divide remaining cells into two equal halves for first and second transplant.
≥ 15.0	Divide into three equal portions for each transplant.

mined by the following formula: dose (mg) = 20 × (creatinine clearance + 25). Cyclophosphamide (total dose, 6,000 mg/m^2) was divided over four daily 1-hour infusions and thiotepa (total dose, 480 mg/m^2) was divided over eight twice-daily 1-hour infusions. Both agents were given on days −6, −5, −4, and −3. Mesna (total dose, 500 mg) was given six times daily for a total of 36 doses, beginning 1 hour before the first cyclophosphamide infusion. All infusions were administered through double-lumen Hickman catheters inserted in a subclavian vein. PBPCs were reinfused on day 0.

Antiemetics were used both prophylactically and as needed, and usually included dexamethasone, ondansetron, or granisetron. All patients received prophylactic antibiotics, including ciprofloxacin and amphotericin orally as selective bowel decontamination. Penicillin G (1 million units, four times daily) and amphotericin B (0.25 mg/kg/d) were given prophylactically from day 0 and were discontinued when the neutrophil count was greater than 0.5×10^9 cells/L. Patients who tested positive for anti–Herpes simplex antibodies received acyclovir prophylactically, in an oral dose of 400 mg twice daily. Irradiated platelet transfusions were administered to maintain platelet counts $\geq 10 \times 10^9$/L, and leukocyte-free irradiated RBCs were given to maintain hemoglobin levels ≥ 5.5 mmol/L. Patients were nursed in private rooms between day 0 and the end of absolute neutropenia, but no other reverse-isolation measures were used.

G-CSF (filgrastim, received as a gift from Amgen-Roche, Breda, the Netherlands) was administered as a daily subcutaneous injection of 300 μg, regardless of body weight, from day 1 until the WBC count was greater than 5.0×10^9/L.

Modified High-Dose Regimen: Tiny CTC

Eight patients were scheduled to receive three consecutive transplantations at a lower dose level. The tiny CTC (tCTC) regimen was identical to the standard CTC regimen, except that each of the cytotoxic drugs was given at a 33% lower dose. As a result, the cumulative dose of three tCTC courses equaled the cumulative dose of two CTC courses. The carboplatin dose was 1,100 mg/m^2, or was determined according to the following formula: dose (mg) = 13.3 × (creatinine clearance + 25), in patients with impaired renal function. The total doses of cyclophosphamide and thiotepa were 4,000 mg/m^2 and 320 mg/m^2, respectively. Supportive care was identical to that given for full-dose CTC courses.

Recovery Before Repeat High-Dose Chemotherapy Courses

Second and third courses of CTC or tCTC were scheduled to begin on day 28 after the previous transplantation, for an interval between first days of CTC or tCTC of 5 weeks.

The following requirements had to be fulfilled before a second or third course was administered: complete resolution of any infections and normal body temperature (consistently ≤ 37°C) for at least 5 days; WBC count $\geq 2.0 \times 10^9$/L and granulocyte count $\geq 1.0 \times 10^9$/L for at least 1 week; creatinine clearance ≥ 40 mL/min and renal function loss due to previous course not exceeding 20% of the baseline estimate; bilirubin within normal limits, and alkaline phosphatase, ALT, and AST less than two times the upper limit of normal; no severe symptomatic neuropathy or symptomatic hearing loss that could be ascribed to the previous CTC course; if one of these symptoms was present to a moderate degree, the carboplatin dose in the next CTC course had to be reduced by 25% (targeted AUC); and no significant hemorrhagic cystitis with (intermittent or continuous) macroscopic hematuria.

Dose Adaptations in Repeat High-Dose Chemotherapy Courses

Subsequent courses of CTC or tCTC were, whenever possible, given in the same dose as the previous ones. The following dose reduction rules applied. In case of renal function loss of greater than 20% over baseline creatinine clearance, the carboplatin dose in the next CTC or tCTC course was reduced to 50% (targeted AUC). The remaining creatinine clearance had to be greater than 40 mL/min; otherwise, the patient was taken off study. In case of incapacitating neuropathy (grade 3) or significant hearing loss (difficulty to conduct a one-on-one conversation), the carboplatin dose in the next CTC or tCTC course was reduced to 75% (targeted AUC). If grade IV mucositis had occurred in any CTC or tCTC course, the dose of all three agents was reduced by 25% in the next course (for carboplatin, 25% reduction in targeted AUC). If grade 4 diarrhea had occurred in combination with severe cramping and abdominal pain that required the administration of morphine (after failure of loperamide and spasmolytics), the dose of cyclohosphamide and thiotepa had to be reduced by 50% in the next tCTC course (targeted AUC for carboplatin). In case of unexpected or unexpectedly severe toxicity, the managing physicians were free to apply dose reductions that they thought were in the best interest of the patient.

RESULTS

A total of 48 patients were included in studies that entailed either two or three consecutive courses of CTC (Table 2). Five of these patients never received high-dose therapy. Two patients who had been referred for salvage treatment of unresectable partial remissions of germ cell cancer showed gradual marker normalizations and underwent surgery instead of chemotherapy. Both are currently

Table 2. Diagnoses of 48 Patients Scheduled to Receive Either Two or Three Subsequent Courses of High-Dose Therapy With PBPCT

Diagnosis	No. of Procedures Planned	
	2	3
Germ cell cancer	19	8
Breast cancer		15
Ovarian cancer		2
Choriocarcinoma	1	
PNET	2	
Rhabdomyosarcoma		1
Total	22	26

Abbreviation: PNET, peripheral neuroectodermal tumor.

alive and disease-free without further treatment. One patient with germ cell cancer and one with advanced breast cancer failed to mobilize $CD34^+$ cells and were taken off study. One patient with advanced breast cancer had progression during standard-dose chemotherapy and was taken off study. The latter three patients died of their diseases.

PBPC Mobilization and Harvests

In all but two patients, a sufficient number of $CD34^+$ cells could be harvested for two or three courses of high-dose therapy. As described earlier, the minimum requirement was 1.0×10^6 $CD34^+$ cells/kg for each high-dose therapy course plus 2.0×10^6 $CD34^+$ cells/kg for the last high-dose course. As can be seen in Table 3, the numbers harvested were variable between patients. However, in general, breast cancer patients who received the fluorouracil, epirubicin, and cyclophosphamide ($FE_{120}C$) mobilization regimen required less leukocytapheresis sessions, while the heavily pretreated patients with relapsing germ cell cancer or other solid tumors required more.

Table 3. Progenitor-Cell Harvests in 47 Patients Scheduled to Receive Multiple High-Dose Chemotherapy Courses

Variable	Mobilization Regimen			
	Ifosfamide/Etoposide (n = 33)		$FE_{120}C$ (n = 14*)	
	Median	Range	Median	Range
Second mobilization procedure required†	13		2	
No. of leukocyte-apheresis sessions	4.5	1-10	3.3	1-7
MNC harvested ($\times 10^{-6}$/kg)	1,009	446-3,054	732	385-1,455
$CD34^+$ cells harvested ($\times 10^{-6}$/kg)	10.5	0.9-21.3	17.7	9.4-50.6
CFU-GM harvested ($\times 10^{-4}$/kg)	189	0-525	255	18.5-417

Abbreviations: MNC, mononuclear cells; CFU-GM, colony-forming units granulocyte-macrophage.

*One patient did not undergo leukocytapheresis because of failure to mobilize $CD34^+$ cells.

†To obtain an adequate graft size (see text).

The chemotherapy regimens used for mobilization of PBPCs ($FE_{120}C$ for breast cancer and ifosfamide/etoposide for germ cell cancer and other solid tumors) were selected to have antitumor activity of their own, in order to avoid tumor progression during the 3 (and sometimes 6) weeks required to obtain grafts of sufficient sizes. Because of the brief duration of the mobilization phase, no formal response evaluations were possible, but simple tumor measurements or serum marker determinations could be performed in some patients. Any increase in size of a measurable or assessable tumor, or any increase in concentration of a pertinent serum tumor marker, was interpreted as a sign of progression; any decrease in size of a measurable tumor or 50% decrease in concentration of a pertinent tumor marker was interpreted as regression. Using these simple criteria, only a few patients had clear progression during the mobilization phase (Table 4).

Bone Marrow Reconstitution After Multiple Transplantations

Neutrophil recovery was rapid after first, second, and third cycles. As expected, platelet recovery usually lagged somewhat behind and depended on the number of $CD34^+$ cells reinfused. Despite the fact that the highest numbers of progenitor cells were reinfused after the last course of CTC, the times to platelet recovery and the transfusion requirements tended to be higher in subsequent courses of full-dose CTC (Table 5). This resulted in part from the fact that several cases of hemorrhagic cystitis or VOD

Table 4. Antitumor Effect of Chemotherapy Regimens Used for Progenitor-Cell Mobilization

Effect	$FE_{120}C$	Ifosfamide/Etoposide	
	Breast Cancer (n = 15)	Germ Cell Cancer (n = 27)	Other Solid Tumors (n = 6)
Not assessable	1	7	3
Regression*	9	9	2
Stable	2	6	1
Progression	3†	5‡	0

*See text for response definitions.

†Two of these patients had mixed responses; the third patient had clear progression.

‡One patient had clear progression of markers, lung metastases and pain; 3 patients had increase of markers only; 1 had questionable enlargement of a cervical lymphoma.

Table 5. Bone Marrow Reconstitution and Transfusion Requirements After Multiple Courses of CTC

Treatment	CTC Course No. 1 Median	1 Range	2 Median	2 Range	3 Median	3 Range
Two courses of full-dose CTC						
Day of neutrophil recovery*	11	9-14	11	9-12		
Days to platelet transfusion independence†	17	11-24	17	11-35		
No. of platelet transfusions‡	5	2-14	8	3-20+		
No. of blood transfusions (U)	8	5-10	10	4-21+		
Three courses of full-dose CTC						
Day of neutrophil recovery	11	10-13	10	9-19	11	9-19
Days to platelet transfusion independence	21	11-28	25	13-28	29	13-47+
No. of platelet transfusions	4	3-9	6	3-19	14	3-35
No. of blood transfusions (U)	6	4-16	8	4-17	9	3-26
Three courses of tCTC						
Day of neutrophil recovery	10	10-11	10	9-12	10	10-11
Days to platelet transfusion independence	12	11-15	15	10-19	14	9-21
No. of platelet transfusions	4	2-10	3.5	2-9	2.5	2-12
No. of blood transfusions (U)	6	4-10	6.5	5-8	7.5	3-10

*First day after transplantation (= day 0) with at least 500 neutrophils/μL.

†First day after transplantation on which platelets $\geq 20 \times 10^9$/L without platelet transfusions.

‡Five to 6 donor units per transfusion.

were observed in the second and third courses, which were associated with blood loss and rapid consumption of platelets (see later).

The tCTC courses were not clearly associated with increased times to bone marrow reconstitution after the second or third courses.

Organ Toxicity of Multiple Courses of Full-Dose CTC

Gastrointestinal and skin toxicity. A total of 73 full-dose CTC courses were given to 35 patients: 35 first, 28 second, and 10 third courses. All courses led to varying degrees and durations of nausea and vomiting, which ranged from mild nausea with occasional vomiting on the days of chemotherapy administration to refractory vomiting that required sedation. This toxicity did not tend to increase in severity in subsequent courses (data not shown). Diarrhea was common and grade 2 or higher occurred in 69%, 85%, and 70% of first, second, and third courses, respectively. Since many third courses were administered with dose reductions (see later), the frequency in third courses may be underestimated. Refractory diarrhea with severe abdominal cramps that required continuous infusion of morphine was observed in two first courses (6%) and in three second courses (11%). When observed, dose reductions were applied in the following course, which may explain its absence in third courses.

Mucositis was generally absent or mild in first courses, but its severity increased in subsequent courses. Grade 3 mucositis was observed in a single first course (3%), while grade 3 or 4 mucositis were present in seven second courses (25%) and four third courses (40%). Again, the frequency of mucositis in third courses may have been limited by dose reductions.

Rashes were frequent and occurred in 42 courses (58%). Most were mild (grade 1 or 2), and only four courses were associated with grade 3 or 4 rashes (5%). All cleared within 1 to 2 weeks and frequently left irregular skin pigmentations behind that were slow to clear. Skin pigmentation irregularities also occurred in many patients in the absence of other skin toxicity. Despite high-dose dexamethasone, one patient developed a generalized rash, fever, hypotension, and periorbital edema suggestive of severe allergy on the first day of the third CTC course. She had developed fever without the other symptoms during the first course of CTC. A similar, although less dramatic allergic reaction to a third course was seen in one patient who received a third course of tCTC (see later).

Neurotoxicity and ototoxicity. Symptomatic cisplatin neuropathy was reported by 15 germ cell cancer patients before the start of high-dose therapy. In six of these, tingling and numbness increased following the first CTC course, while it remained unchanged in the others. Three patients who had never received neurotoxic drugs reported minimal symptoms consistent with neuropathy after the first course. In all others, symptoms were absent. Thus, the frequency of subjective neurotoxicity was 26% in the first course. After second courses, six patients with preexistent neuropathy reported worsening of symptoms,

Table 6. Cumulative Hearing Loss Caused by Subsequent Full-Dose CTC Courses

Hearing Loss	One Course (n = 31)		Two Courses (n = 14)		Three Courses (n = 8)	
	Median	Range	Median	Range	Median	Range
1,000 Hz	0	−13-+10	0	−22-+13	−4	−33-+3
4,000 Hz	−1	−27-+20	−15	−42-+10	−13	−50-+2
12 kHz	−10	−37-+20	−15	−58-+12	−15	−60-+5

NOTE. Values represent the difference in auditory threshold (averaged for both ears) between measurements taken immediately before the first course and 4 weeks after first, second, or third courses. Negative values indicate hearing loss; positive values indicate improvement.

and three previously symptom-free patients had mild complaints suggestive of carboplatin neuropathy (32%). Worsening of symptoms was reported in three of 10 third courses.

Mild ototoxicity was common. Eleven of 35 patients who received the first course of CTC already had signs of ototoxicity, which usually consisted of tinnitus or mild loss of hearing acuity. Seven of these reported worsening of symptoms. Twelve of 24 previously symptom-free patients reported tinnitus (six patients) or tinnitus with some degree of hearing loss (six patients). In four of these, the symptoms were transient. First courses caused little hearing loss as documented by audiography (Table 6). Symptoms of ototoxicity were reported in 16 of 28 second courses. Half of these consisted of tinnitus only, the other eight also involved some hearing loss. In the 10 third courses, further hearing loss was reported by four patients. Only three of 10 patients remained entirely symptom-free. Despite the clear ototoxicity of CTC, no or only mild disability resulted. No hearing aids were required in any of the patients. Audiograms correlated relatively poorly with patients' complaints, but consistently showed a trend toward marked hearing loss at high frequencies that increased in subsequent courses (Table 6). The pattern and degree of hearing loss closely resembled that of standard-dose cisplatin.[17]

Cardiopulmonary toxicity. Minor signs of cardiotoxicity occurred in five of 35 first courses (14%), including transient fluid retention and dyspnea (n = 2) that responded to furosemide, pericardial friction rub (n = 1), increase in heart size (n = 1), and development of a right bundle-branch block not previously present. Both patients with fluid retention developed the same symptoms in their second course, which was again easily manageable with diuretics. A third patient had similar symptoms of mild cyclophosphamide carditis in the second course, which led to an 11% cardiac toxicity rate. Of 10 third courses, only one was associated with fluid retention and dyspnea. This patient had had the same syndrome in his first and second courses, which suggests that this type of cardiac toxicity is reversible and not cumulative.

Eight of 35 first courses, four of 28 second courses, and three of 10 third courses were complicated by pulmonary symptoms. These included pleural friction rubs, cough and dyspnea, parenchymatous densities on chest roentgenograms, and pleural effusions in various combinations. Most patients had temperature elevations and differentiation between pulmonary infection, drug hypersensitivity reactions, and direct toxicity of CTC was impossible. All pulmonary symptoms subsided with antibiotics and symptomatic therapy.

Pulmonary function tests were performed before each course of CTC and 2 to 3 months after the last course in most patients. The tests showed a gradual and apparently cumulative decrease in lung volumes and diffusion capacity (data not shown). These changes did not lead to clinical symptoms and their long-term reversibility will be the subject of future study. Details of the pulmonary function tests will be published elsewhere.

Liver and renal toxicity. Acute renal toxicity was of little importance. In some patients, a mild increase in creatinine was observed, but in only seven of 35 first courses did this lead to a greater than 20% decrease in creatinine clearance. The clearance never fell below 50 mL/min. Renal function had completely normalized in five of these seven patients before the start of course 2. Seven patients had a greater than 20% decrease in creatinine clearance after the second CTC course (25%), but this renal function decrease was of little clinical concern and completely reversible in five patients. Minor and transient decreases in creatinine clearance were observed in three third courses.

Chronic renal toxicity was absent except in two patients who developed a hemolytic uremic syndrome (HUS), 5 and 7 months after their last transplantations, respectively. Both had received three full courses of CTC without dose reductions and were in complete remissions. The first patient, a 35-year-old man with refractory germ cell cancer, died of acute circulatory arrest when undergoing plasmapheresis. At autopsy, extensive atherosclerosis was found but no tumor. The second patient, a 48-year old woman with relapsed ovarian cancer, recovered without specific therapy. She is currently alive and disease-free with mild hypertension and mild renal function impairment (creatinine clearance, 55 mL/min) as the only long-term sequelae. Details of the clinical course of these two patients and a review of the literature regarding posttransplantation HUS have been published elsewhere.[18]

Four patients developed hemorrhagic cystitis, all following the second course of CTC (14%). One of these had previously undergone radiation therapy with the bladder in the radiation field. A second one had previously received eight ifosfamide-based chemotherapy courses for a peripheral neuroectodermal tumor (PNET). The other two had no risk factors. All four required bladder irrigation. In three patients, the hemorrhagic cystitis was still active when they died of their tumors (n = 2) or of VOD (n = 1).

Elevations of liver enzymes were observed in nearly all patients. Almost invariably, ALT elevations occurred on or near the day of reinfusion and returned to normal within a few days (Table 7). Mild bilirubin elevations commonly followed (Table 7), peaked at 6 to 10 days after transplantation, and normalized a few days thereafter. These abnormalities did not worsen in subsequent courses. However, VOD occurred in two patients after course 2, and in two further patients after course 3. Two patients died, while the other two recovered without sequelae. There was no relationship between the degree of (early) ALT or bilirubin elevations and the risk of VOD.

Other toxicities. One patient with an unresectable partial remission of germ cell cancer, who had previously experienced excessive toxicity from standard-dose chemotherapy, developed a syndrome that was characterized by malaise, exhaustion, muscle weakness, nausea, and vomiting after his first CTC course. His tumor was in complete remission and further high-dose therapy was canceled. He subsequently relapsed and died of disease 6 months after his last chemotherapy administration. The exhaustion syndrome had not improved by that time.

Reversible but painful hand-foot syndromes developed in three patients following a second course of CTC and in one patient after a third course.

Toxic deaths. Four patients died as a result of toxicity of high-dose chemotherapy (Table 8). One patient, a 30-year-old woman with advanced breast cancer, developed VOD and hemorrhagic cystitis after the second CTC course. She died of liver failure on the twenty-third day after the second transplantation.

A second patient, a 32-year-old woman with advanced breast cancer, developed septicemia with *Streptococcus faecalis* and died of multiorgan failure on the seventh day after her third transplantation. A 39-year-old woman with advanced breast cancer developed VOD after her third transplantation and died of liver failure on the thirty-first day.

The fourth patient died of cardiac arrest during plasmapheresis because of a late HUS (described earlier). At autopsy, extensive atherosclerosis was found.

Table 7. Elevations of ALT and Bilirubin After Successive CTC Courses

Variable	CTC Course No. 1	CTC Course No. 2	CTC Course No. 3
ALT			
Median*	92	75	53
Range	18-263	15-2,820†	16-533†
Bilirubin‡			
Median‡	20	22	18
Range	11-52	9-143†	8-1,000†

*Median of highest value; normal < 25 U/L.
†Highest value from patient with VOD.
‡Median of highest value; normal < 15 mmol/L.

Table 8. Toxic Deaths and Major Organ Toxicity

CTC Course No.	No. of Courses	Toxic Deaths	Major Nonlethal Toxicity
1	35	0	Exhaustion syndrome (1)
2	28	VOD (1)	VOD (1)
		VOD (1)	Hemorrhagic cystitis (4)
		Sepsis (1)	HUS (1)
3	10	HUS* (1)	VOD (1)

*The cause of death was circulatory arrest in a patient with severe atherosclerosis (see text).

Feasibility of Administering Closely Timed, Full-Dose CTC Courses

Of 35 second CTC courses planned, only 28 were given. Reasons for cancellation of second courses included disappointing antitumor efficacy of CTC course 1 (five patients) or excess toxicity (two patients). The excess toxicity included one exhaustion syndrome (described earlier) and one patient with germ cell cancer who experienced severe (although largely transient) ototoxicity.

Dose reductions in the second course were applied in three patients. The dose of carboplatin was decreased 50% in one because of renal function impairment after CTC course 1; it was decreased 25% in a second course because of ototoxicity. In a third patient, cyclophosphamide and thiotepa were decreased by 50% because of severe colitis in the first course.

Second courses were scheduled to begin on day 28 after the first transplantation. Three patients actually started on days 23, 23, and 27, respectively, as they had rapidly recovered from the first course and early continuation of therapy appeared to be desirable. Treatment was delayed in three patients for ≥ 1 week, either because of infections (one patient, herpes zoster and sinusitis), impaired renal

function (one patient), or resection of a residual abdominal mass before CTC course 2 was performed (one patient, germ cell cancer).

In summary, two patients could not receive their second course because of toxicity, three patients required dose reductions, and three patients required delays. Since one patient had both a dose reduction and a delay, a total of seven patients were not able to receive CTC course 2 as planned (20%) and only two patients were not considered able to tolerate a second procedure at all (6%).

Of 28 patients who received a second course of CTC, 12 were planned to receive a third course. Two patients were not able to continue; one because of treatment-related death following CTC course, the other because of excess toxicity (nonfatal VOD).

Four of 10 third courses of CTC had to be reduced in dose. One patient did not receive cyclophosphamide at all because of hemorrhagic cystitis following course 2. One patient had a 25% carboplatin dose reduction because of severe ototoxicity after course 2. A third patient received only 50% of cyclophosphamide and thiotepa to avoid repetition of a severe colitis after the second course. A fourth patient discontinued CTC course 3 after day 1 because of a violent hypersensitivity reaction with hypotension.

Three third courses had to be delayed for $\geq$ 1 week. The reasons for delay included persistent nausea and vomiting after course 2, herpes zoster infection, and resection of a residual abdominal mass.

In summary, two courses were canceled, four reduced, and three delayed because of toxicity. One patient whose third course was delayed also had a dose reduction, so that only four of 12 courses could be given as planned (33%). Two of four patients who received their third course on time and at full dose died of toxicity (one lethal VOD and one HUS, described earlier).

Multiple Courses of tCTC

Eight patients with advanced, receptor-negative breast cancer were scheduled to receive three subsequent courses of tCTC, which contains two thirds of each active drug of a regular CTC course. Thus, the total cumulative dose of CTC at the end of treatment is equal to that after two full courses of CTC.

Six patients received all three courses, one received a single course, and one received two courses. Both patients who did not complete the planned therapy were taken off study because of lack of tumor response to tCTC. Both were thought to be able to continue in terms of tolerance. One of seven second courses was delayed for 1 week to have more time for an evaluation of the antitumor effect. One of six third courses was delayed for 1 week to allow time for recovery from prolonged nausea and anorexia.

tCTC courses were reasonably well tolerated. Nausea and vomiting were common, but generally mild. Loose stools occurred in most patients, but only one had severe refractory diarrhea that required a continuous infusion of morphine in all three cycles. Third cycles were associated with grade 4 diarrhea in two other patients who had had only grade 2 diarrhea in earlier courses, and grade 3 diarrhea was present in the third course of a patient who had no diarrhea at all in earlier courses. Some accumulation of bowel toxicity may thus occur.

Mucositis was absent or mild, and never exceeded grade 2. Three patients developed minor rashes that were of no clinical consequence. However, one patient had a strong allergic reaction tCTC in her third course that included fever, erythema, and facial edema. The high-dose chemotherapy course was discontinued early and she recovered uneventfully.

Neurotoxicity and ototoxicity were absent or subtle. One patient reported mild tingling sensations in her feet after the third course. Another patient with preexistent audiographic abnormalities showed increased hearing loss at frequencies greater than 2,000 Hz. All six patients who completed the three courses denied subjective hearing loss.

Liver or renal toxicity was not seen, and cardiopulmonary toxicity was absent. In this small series of patients, triple tCTC appears to be both feasible and well tolerated.

DISCUSSION

We have previously reported that high-dose chemotherapy with the trialkylator regimen CTC is safe and reasonably well tolerated when administered as a single course in conjunction with a bone marrow or PBPCT.[10] This is confirmed by the 35 first courses described here. Severe irreversible organ toxicity was not observed, and there were no treatment-related deaths. Double procedures are also feasible, but VOD or hemorrhagic cystitis may be encountered occasionally. One of 28 patients who underwent a second transplant died (of liver toxicity), which resulted in a treatment-related mortality rate of 4%. At present, there is no way to avoid the occurrence of VOD and it is likely that this type of toxicity may continue to limit the repeated administration of very high doses of alkylating agents. Nevertheless, double CTC courses could be given safely, at the planned time and at full dose in the large majority of patients (Table 8). Other investigators have reported the feasibility of double trans-

plantations using related regimens.[19] Preliminary evidence suggests that double CTC courses may be effective in the salvage treatment of relapsing germ cell cancer.[11] A prospective phase II study that attempts to reproduce these results in a multicenter setting is currently in progress in the Netherlands.

However, three of ten patients who received a third course of CTC died of toxicity: one of sepsis, one of a late HUS (similar to that described after cyclophosphamide and total-body irradiation,[20] and one of VOD. This severe toxicity occurred despite the fact that most third courses were not given at full dose or were delayed to allow additional time for recovery. In fact, only four of 12 patients scheduled to receive a third course were treated at the planned time and at full dose. Two of these eventually died of toxicity. Although the number of patients is small, it is reasonable to conclude that three courses of full-dose CTC are too toxic for most patients. The efficacy of triple CTC to eradicate residual cancer cells cannot be determined from this small and heterogeneous series of patients. Only one patient, who had an early relapse of ovarian cancer after surgery and standard first-line chemotherapy, is currently alive and disease-free, 2 years after completion of therapy.

The reason that the triple CTC regimen was not tolerated was clearly not the dose-intensity (which is usually defined as the amount of chemotherapy delivered per unit time), but must have been either the total cumulative dose of the agents or the total treatment time. To investigate this further, we entered eight patients onto a part of the study that studied a triple transplantation regimen with a lowered chemotherapy dose, called tCTC. Three courses of tCTC deliver precisely the same cumulative drug dose as two courses of standard CTC. Six patients completed the three cycles of treatment without severe toxicity. The other two patients were taken off study because their tumors did not appear to respond sufficiently to the chemotherapy and their physicians felt that long-term disease-free survival could not be achieved by continuing high-dose therapy. In terms of toxicity, both patients would have been able to continue protocol treatment as planned. Individual courses of tCTC were clearly better tolerated than full-dose CTC. Thus, the excess toxicity of full-dose CTC is caused by the high cumulative doses of the three alkylating agents.

Clearly, bone marrow suppression no longer limits the dose of alkylating agents. The hematopoietic reconstitution after first and second courses was similar. After a full-dose third CTC course, both granulocytopenia and thrombocytopenia took a few days longer to recover. Since the number of $CD34^+$ cells reinfused after the third course was higher rather than lower than after earlier courses, this could result from damage to the bone marrow stroma. Delayed bone marrow recovery was not observed after three tCTC courses.

The mobilizing and harvesting of sufficient PBPCs presented few problems and was successful in all but two patients. The median number of leukocytapheresis sessions to harvest a sufficiently large number of cells for three transplantations was three (range, one to 10), and only 17 of 48 patients (35%) required a second mobilizing chemotherapy course. In none of the patients did a bone marrow transplantation have to be added to the last progenitor cell reinfusion to ensure sustained bone marrow recovery. It is possible that selection of another mobilizing regimen could lead to even better progenitor cell yields, but the importance of using chemotherapy that is appropriate for the tumor type to be treated must be balanced against that consideration.

In tumor types that can be cured by cytotoxic drugs at conventional doses, chemotherapy is invariably divided over multiple courses. One rationale for this is derived from the log-cell kill hypothesis reported by Skipper et al.[21,22] When the number of tumors cells is large and a course of chemotherapy can only kill a fixed proportion of the tumor stem cells, repeated courses of chemotherapy will be required. This intuitive concept is supported by laboratory models that show repeated doses of chemotherapy cause more cytotoxicity than single doses, even when the total amount of drug administered is the same.[23] Clearly, the courses should be timed as closely as tolerable to limit outgrowth of tumor cells in the treatment-free intervals.[24] Based on the Goldie-Coltman hypothesis,[25,26] alternating non–cross-resistant therapy has been advocated and this strategy has been applied in several feasibility studies of high-dose therapy. For instance, the Boston group has studied a regimen for breast cancer in which high-dose melphalan is followed by CTC.[27] Gianni et al[28] reported a randomized single-institution study that successfully used alternating high-dose therapy as part of first-line therapy in poor-prognosis non-Hodgkin's lymphoma. However, in general, alternating non–cross-resistant chemotherapy has not convincingly fulfilled its promise in standard-dose regimens as recently reviewed by Goldie.[29] Modern computer models of cell kill by chemotherapy tend to favor repeated courses of the same drug(s) and switching to an alternative regimen after a number of cycles.[30,31] Considerations of this kind provide a rationale for a series of three CTC courses that may follow a series of standard-dosed chemotherapy courses.

At present, the optimal number of high-dose therapy courses is unknown, but treatment duration is likely to be as important for cure as dose-intensity or peak plasma drug levels.[5] In addition, the precise shape of the dose-effect curves that relate the duration of clinical remissions to the delivered dose of the drug combination[32] are unknown for different tumors and for different drugs. It is possible that the dose-effect curve for CTC becomes shallow beyond a certain dose, and that a full-dose CTC course is not proportionally more effective than a tCTC course in a given tumor. In this case, three tCTC courses could eradicate a significantly greater proportion of tumor cells than two full-dose CTC courses. Such a mechanism could be of particular importance in solid tumors with relatively low growth fractions, such as breast cancer. Macroscopic tumors usually contain a large proportion of noncycling cells that are relatively resistant to chemotherapy.[33] A first course of high-dose therapy may increase that proportion by recruiting cells into the cell cycle,[34] rendering the tumor more sensitive to the next course. Used in this way, CTC or tCTC could also aid in the induction of the complete remission that appears to be required before consolidation by high-dose therapy can lead to prolonged disease-free survival.[4,34] The ability of three courses of tCTC to achieve durable remissions in advanced breast cancer and other solid tumors remains to evaluated.

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