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Original Paper

Tandem High-dose Therapy with Ifosfamide, Epirubicin, Carboplatin and Peripheral Blood Stem Cell Support is an Effective Adjuvant Treatment for High-risk Primary Breast Cancer

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We evaluated the therapeutic efficacy and toxicity of a tandem high-dose therapy with peripheral blood stem cell (PBSC) support in 40 patients with high-risk, primary breast cancer (stage II–III) and involvement of ten or more positive axillary lymph nodes. Their median age was 44 years (range 23–56). Two cycles of cytotoxic chemotherapy with ifosfamide (10000 mg/m^2) and epirubicin (100 mg/m^2) were administered. Granulocyte colony-stimulating factor (G-CSF) was given to hasten neutrophil reconstitution and to mobilise PBSC during marrow recovery. Leukaphereses were performed following the first and/or second cycle. Tandem high-dose therapy consisted of two cycles with ifosfamide (15 or 12 g/m^2) and epirubicin (150 mg/m^2), while carboplatin (900 mg/m^2) was added for the last 24 patients included. Using an immunocytochemical method, two of 11 patients had cytokeratin-positive tumour cells in three leukapheresis products that were collected following the first G-CSF-supported cycle with ifosfamide and epirubicin, whereas only two harvests obtained following the second cycle in 26 patients contained cytokeratin-positive tumour cells. The number of CD34+ cells/kg re-infused following both high-dose cycles was similar ($4.20 \pm 0.29 \times 10^6$, first cycle and $5.25 \pm 0.63 \times 10^6$, second cycle), and no notable difference was noted in the speed of haematological reconstitution. An absolute neutrophil count (ANC) of 0.5×10^9/l was reached after a median time of 13 days, while an unsupported platelet count of 20.0×10^9/l was achieved after a median time of 8 (first cycle) and 9 (second cycle) days post-transplantation. Patients autografted with more than 7.5×10^6 CD34+ cells/kg had platelet counts above 20×10^9/l within less than 10 days. 6 patients relapsed between 7 and 11 months (median 8 months) post-transplantation. 37 patients are alive and in remission with a median follow-up time of 11 months (range 1–38). This translates into a probability of disease-free survival (DFS) of 77% (95% CI 32–95%) at 38 months. The probability of overall survival is 85%, since 3 patients with local relapse achieved a second complete remission following surgery and involved-field radiotherapy. In conclusion, a sequential high-dose therapy including ifosfamide, epirubicin, carboplatin and PBSC support is well tolerated and effective in patients with high-risk primary breast cancer. Involved-field irradiation should be performed post-transplantation to reduce the risk of local relapse.

Key words: tandem high-dose therapy, primary breast cancer, peripheral blood stem cell support

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INTRODUCTION

SEVERAL STUDIES have shown that adjuvant chemotherapy is effective in patients with stage II/III breast cancer and invol-

vement of 10 or more axillary lymph nodes [1–3]. Despite the improvements achieved with conventional cytotoxic chemotherapy, a significant proportion of these patients is still at risk of treatment failure. Considering that breast cancer is generally a chemosensitive tumour, an increase in dose intensity appears to be particularly beneficial at a time when tumour burden is low and before drug resistance develops [4, 5]. Peters and associates [6, 7] first reported on 85 patients with poor-prognosis primary breast cancer who received four cycles of cyclophosphamide, doxorubicin and 5-fluorouracil. Unpurged autologous bone marrow was used for supporting high-dose therapy with cyclophosphamide, cisplatin and carmustine. The result was remarkable, as the probability of disease-free survival (DFS) at 2.5 years was 72% compared with 38% and 52% in historical control patients. Instead of using a single high-dose conditioning therapy which is almost myeloablative, dose intensification can also be obtained by administering two or more cycles of a dose-escalated therapy [8–10]. This approach can help to reduce treatment-related toxicity so that dose-escalated regimens can be introduced in the adjuvant setting. Administration of more than one cycle of high-dose therapy can also be facilitated if a sufficient number of peripheral blood stem cells (PBSC) can be mobilised in the majority of patients following cytokine-supported chemotherapy [11].

Regardless of the number of high-dose cycles to be administered, the choice of drugs plays an important role. Alkylating agents, such as cyclophosphamide or ifosfamide, are effective at conventional dose and suitable for dose escalation. Anthracyclines are valuable despite their cumulative cardiotoxicity, as they have been very effective in several single-agent studies [12]. In our study, epirubicin was preferred, because it is less cardiotoxic than doxorubicin when used in equivalent doses [13]. Carboplatin is a prime candidate for high-dose protocols in solid tumours, as its dose-limiting myelotoxic side-effects can be circumvented by haematopoietic stem cell grafting [14]. In this report, data on the therapeutic efficacy and toxicity of a tandem high-dose regimen including ifosfamide, epirubicin and carboplatin will be presented for 40 patients with high-risk primary breast cancer.

PATIENTS AND METHODS

Patients

Between September 1992 and March 1996, 40 female patients with primary breast cancer were enrolled onto this study. They had a median age of 44 years (range 23–56 years). The evaluation of patients included clinical examination, a full blood count, liver and kidney function tests, mammography, chest X-ray, abdominal ultrasound examination, chest and abdominal CT scan and scintigraphic bone scan. According to the TNM classification [15], 28 patients had stage IIA/B and 12 patients stage IIIA/B. 30 patients underwent mastectomy, while 10 had breast-conserving surgery. The oestrogen and progesterone receptor status, S-phase and DNA index were assessed. The patient characteristics including the number of axillary lymph nodes involved and hormone receptor status are further detailed in Table 1. The time interval from initial diagnosis to the start of cytotoxic chemotherapy varied between 2 and 8 weeks (median 4 weeks).

Table 1. Patient characteristics

No. of patients	40
Age (years)	44 (23–56)*
Surgical treatment	
Mastectomy	30
Segmentectomy	10
Stage	
IIA	17
B	11
IIIA	10
B	2
No. of positive axillary lymph nodes	
10–15	24
16–20	8
>20	8
Hormone receptor status	
ER+ /PgR+	18
ER+ /PgR	5
ER-/PgR+	1
ER- /PgR	16
Time from initial diagnosis to entry into the study (weeks)	4 (2–8)

ER, oestrogen receptor; PgR, progesterone receptor.
*Median and range.

The study was conducted under the guidelines of the Joint Ethical Committee of the University of Heidelberg. Each patient gave her informed consent to participate in this study. The cut-off date of this report was 31 March 1996.

Cytotoxic chemotherapy

Cytotoxic chemotherapy consisted of two cycles of ifosfamide (Holoxan®, ASTA Medica, Frankfurt, Germany), 5000 mg/m^2, as a 24 h continuous i.v. infusion, on days 1 and 2 followed by epirubicin (Farmorubicin®, Pharmacia GmbH, Erlangen, Germany), 100 mg/m^2, as a 4 h i.v. infusion, on day 2. The first 7 patients included received ifosfamide (5000 mg/m^2) only on day 1. Mesna (Uromitexan®, ASTA Medica, Frankfurt, Germany) was administered at the same dose as ifosfamide 5000 mg/m^2, on days 1 and 2, as a 24 h continuous i.v. infusion.

Both cycles were supported with R-metHuG-CSF, 300 μg/day, s.c. (Filgrastim, AMGEN Inc., Thousand Oaks, California, U.S.A.). The growth factor was not only given to shorten the period of neutropenia, but also to increase the number of circulating progenitor cells during marrow recovery. PBSC collection began when a distinct population of CD34+ cells was measurable in the peripheral blood. The leukaphereses were performed using a Fenwal CS 3000 (Baxter Deutschland GmbH, Munich, Germany) and/or Spectra (COBE Laboratories, Lakewood, California, U.S.A.). Between 10 and 20 litres were processed at flow rates of 70–150 ml/min. The leukaphereses were well tolerated, and no procedure-related complications were observed.

The cytotoxic therapy was continued with two cycles of PBSC-supported high-dose ifosfamide (15000 mg/m^2) and epirubicin (150 mg/m^2). 6 patients received a total dose of 15000 mg/m^2 ifosfamide on 3 consecutive days. In these patients, mesna (5000 mg/m^2/day) was administered as a 24 h continuous i.v. infusion, on days 1 to 3. After the completion of ifosfamide administration, the patients received the last dose of mesna 2500 mg/m^2 as a 12 h con-

tinuous i.v. infusion on day 4. Later, the schedule was changed to a 5 day course with a total dose of 12000 mg/m^2 ifosfamide in order to reduce renal toxicity. At the same time carboplatin (Carboplat®, ASTA Medica, Frankfurt, Germany) was added at a total dose of 900 mg/m^2 to increase the anti-neoplastic efficacy of the regimen. Mesna was administered at the same dose as ifosfamide, on days 1 to 5, followed by an additional administration of 50% of the daily dose on day 6. The hydration regimen consisted of 1000 ml NaCl 0.9% combined with 80 mval potassium chloride and 100 mval sodium bicarbonate given over 24 h. No cytokines were given following re-infusion of PBSC.

The patients received prophylactic antimicrobial therapy with ciprofloxazine (1000 mg/day) and fluconazole (400 mg/day). Empirical antibiotic therapy was administered for fever of greater than 38.5°C. A platelet count above 20×10^9/l was maintained by platelet transfusions, and packed red cells were given when the haemoglobin was less that 8.0 g/dl.

Following high-dose therapy, two-thirds of the patients received hormonal therapy with goserelin (Zoladex®, Zeneca GmbH, Plankstadt, Germany) at a dose of 3.6 mg per day, once a month, or tamoxifen (Nolvadex®, Zeneca GmbH, Plankstadt, Germany) at a daily dose of 30 mg.

Immunofluorescence staining and flow cytometry

For immunofluorescence analysis, 1×10^6 mononuclear cells (MNC) of the leukapheresis product or 20–50 µl of whole blood (EDTA) were incubated for 30 min at 4°C with the phycoerythrin (PE)- or fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody (MAb) CD34 (HPCA-2) and CD45 (HLe-1) FITC, CD33 (My9) PE and CD38 (Leu-17) PE. With the exception of CD33 (Coulter Clone, Hialeah, Florida, U.S.A.), all MAbs were obtained from Becton-Dickinson (Heidelberg, Germany). Isotype-identical antibodies served as controls: IgG1, IgG2a (FITC/PE-conjugated, Becton-Dickinson, Heidelberg, Germany). The cells were analysed with a Becton-Dickinson FACScan as previously described [11].

Clonogenic assay for haematopoietic progenitor cells

The concentration of haematopoietic progenitor cells in peripheral blood was assessed using a semisolid clonogenic culture assay (Stem Cell Technologies Inc., Vancouver, Canada), as previously described [11].

Immunocytochemistry

The mononuclear cells from bone marrow samples and leukapheresis products were isolated by density centrifugation (Lymphoprep TM, Nycomed, Oslo, Norway). The cells of the interphase were washed twice in phosphate-buffered saline (PBS) and adjusted to a concentration of 1×10^6 cells/ml. Using a cytocentrifuge (Cytospin 2, Shandon, Cheshire, U.K.), 1×10^5 cells were transferred on to glass slides. Following air drying for 12–24 h, the slides were stored at −70°C. Immunostaining was performed using the monoclonal antibody CK2 (IgG1; Boehringer, Mannheim, Germany). This antibody recognises the intracellular cytokeratin component No. 18 which represents an intermediate filament. The antigen is expressed on normal and malignant epithelial cells. No expression has been found on cells of mesenchymal origin. For the staining procedure, the antibody was diluted to a concentration of 2.5 µg/ml. Corresponding dilutions of a mouse-myeloma protein served as IgG1 isotype control (MOPC21; Sigma, Deisenhofen, Germany). The antibody reaction was developed with an alkaline-phosphatase anti-alkaline-phosphatase method, using a polyvalent rabbit anti-mouse immunoglobulin antiserum including preformed complexes of alkaline-phosphatase and monoclonal anti-alkaline-phosphatase antibodies. Endogenous phosphatase was inhibited by pre-incubation with levamisole. Five slides, comprising an average of 5×10^5 cells, were examined per specimen. One additional slide served as an IgG1 isotype control. Cells containing cytokeratin No. 18 are detectable by their red staining. The method is associated with low background reaction without the need for counterstaining. Cytospin preparations were scored positive, if at least one stained cell could be detected.

Statistical analysis

The clinical and laboratory data of the patients were analysed according to standard statistical methods using a commercially available computer program (Statworks, Cricket Software, Philadelphia, Pennsylvania, U.S.A.). The results are given as mean ± standard error of the mean (SEM) or as median and range. Statistical significance between differences of grouped data was determined using the Students *t*-test for paired samples. A significance level of $P < 0.05$ was chosen.

To compare the proportions of patients with leukapheresis products containing cytokeratin positive tumour cells after the first and second cycle, an exact χ^2 test was used.

The correlation between the number of CD34+ cells reinfused and the speed of haematological recovery to an absolute neutrophil count $\geqslant 0.5 \times 10^9$/l and platelets $\geqslant 20 \times 10^9$/l was assessed by a regression analysis. A linear mixed effects model was used to account for the dependency between repeated measurements for the same patient.

The estimate of the survival curves was computed using the Kaplan–Meier method. Two-sided 95% confidence intervals for the survival curves were computed on the cumulative-hazard scale. A modified Peto limit was used to modify the lower confidence limit which agrees with the standard limits at each event time, but is based on the effective number at risk between event times. Numerical and graphical computations were done using the statistical software package S-PLUS, Version 3.3 (Statistical Sciences, 1995) on the Sun SPARCstation.

RESULTS

G-CSF-supported cytotoxic therapy with ifosfamide and epirubicin

The conventional cytotoxic chemotherapy with ifosfamide and epirubicin was well tolerated without significant non-haematological toxicity. At the beginning, PBSC were collected during G-CSF-enhanced recovery following the first and second cycles. An interim analysis showed that three of 23 leukapheresis products, which were obtained in 2 of 11 patients following the first cycle, contained cytokeratin positive tumour cells. In contrast, 25 leukapheresis products collected following the second cycle were free of cytokeratin-positive cells. Bone marrow biopsies of these 2 patients were rated negative by histological examination, whereas the immunocytochemical assessment was positive. For the last 29 patients enrolled, the decision was made to harvest PBSC after the second cycle. A total of 62 leukapheresis products

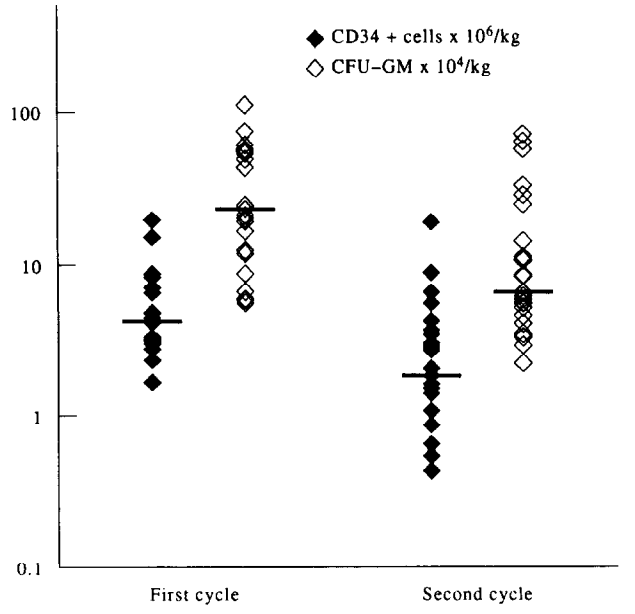

Figure 1. Yield of CD34+ cells and colony-forming units granulocyte-macrophage (CFU-GM) following the first and second cycle of G-CSF-supported cytotoxic chemotherapy with epirubicin and ifosfamide. The intra-individual comparison includes 10 patients with a total of 23 and 25 products obtained after the first and second cycle, respectively. The median number of CD34+ cells harvested following the first cycle was 2.2-fold more compared with harvests obtained following the second cycle (3.8×10^6/kg versus 1.7×10^6/kg, $P < 0.05$).

obtained from 26 patients were evaluable and only two products contained cytokeratin positive tumour cells from 2 patients with stage IIa.

Although the difference observed in the small cohort of patients studied was not statistically significant, the likelihood of harvesting malignant cells may be reduced when PBSC are collected after the second cycle. The median number of CD34+ cells harvested was 2.2-fold less than following the first cycle (1.7×10^6/kg versus 3.8×10^6/kg, $P < 0.05$), as shown in Figure 1. In addition, the CD34+ cells collected following the first cycle comprised of a greater proportion of more primitive progenitor cells. This could be concluded from an intra-individual assessment of 28 leukapheresis products of 7 patients. 14 products were harvested after the first and second cycles, respectively. The proportion of more primitive CD34+ cells, as defined by the lack of CD38 co-expression, was 7.5-fold more after the first cycle when compared with the second cycle ($1.5\% \pm 0.6\%$ versus $0.2\% \pm 0.1\%$, $P < 0.05$). Similarly, the proportion of CD34 + /CD33+ cells, indicating lineage-commitment, was 1.5-fold smaller following the first cycle ($54\% \pm 5.1\%$) in comparison with the second cycle ($80.9 \pm 6.1\%$). The difference was statistically significant, as reflected by a P value of 0.005 (Figure 2).

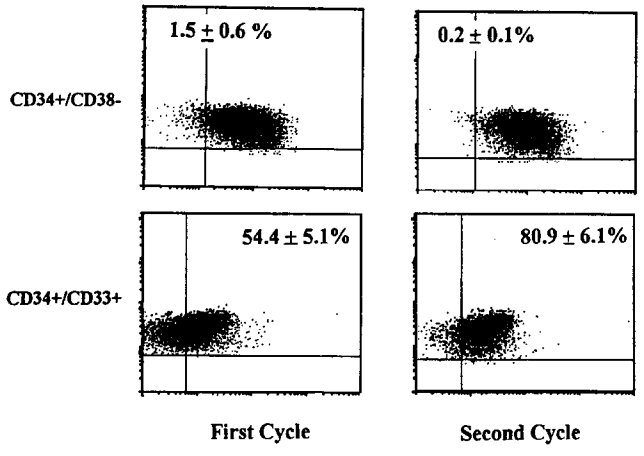

Figure 2. Dual-colour immunofluorescence analysis of CD34+ cells from harvests obtained following the first and second cycle of G-CSF-supported cytotoxic chemotherapy. The overlay plots which are based on 14 paired samples show that a 7.5-fold greater proportion of CD34 + /CD38-negative cells was harvested following the first cycle, while at the same time the proportion of lineage-committed CD34 + / CD33+ progenitor cells was 1.5-fold smaller. The values represent the mean and standard error of the mean (±SEM), $P = 0.005$.

High-dose therapy with PBSC support

There were 78 PBSC-supported cycles evaluable for toxicity, since 2 patients withdrew after the first cycle of high-dose therapy. One patient declined the second cycle because of severe anxiety, while the other patient was taken off study following prolonged enterocolitis of unknown actiology. The median time elapsed between the last cycle of ifosfamide plus epirubicin and the first cycle of PBSC-supported high-dose therapy was 6 weeks (range 4–16), while the time interval between the two high-dose therapies varied between 5 and 9 weeks (median 7 weeks). The variation occurred because of patients' requests for postponing treatment and not because treatment-related toxicity necessitated a delay.

A median of two leukapheresis products (range 1–6) were used for supporting both cycles of high-dose therapy.

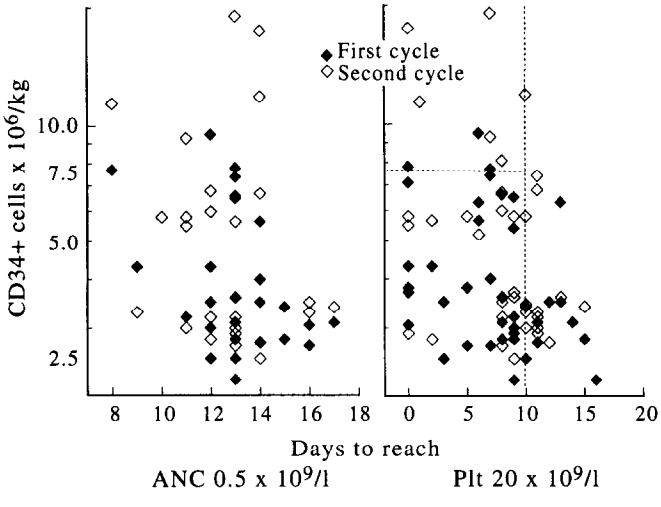

Figure 3. Haematological reconstitution following the first (◆) and second (◇) cycle of high-dose chemotherapy. The evaluation was based on a total of 40 patients who underwent 78 PBSC-supported courses of high-dose chemotherapy. No difference was observed in the speed of haematological reconstitution following both cycles: recovery of neutrophils to 0.5×10^9/l took a median time of 13 days, while an unsupported platelet count of 20×10^9/l was reached after a median time of 8 and 9 days, respectively. The platelet reconstitution shows a dose–response relationship between the number of CD34+ cells re-infused and the speed of recovery ($P < 0.05$). Patients autografted with more than 7.5×10^6 CD34+ cells/kg achieved platelet counts of 20×10^9/l within 10 days.

Harvests were chosen on the basis of their CD34+ cell content, aiming at a minimum number of 2.5×10^6/kg CD34+ cells for each transplantation. A total of 60 high-dose cycles (77%) could be supported with a single leukapheresis product. The number of CD34+ cells/kg re-infused after the second cycle was similar to that transplanted following the first cycle ($5.52 \pm 0.63 \times 10^6$ versus $4.20 \pm 0.29 \times 10^6$), and no notable difference was noted in the speed of haematological reconstitution. An absolute neutrophil count of 0.5×10^9/l was reached after a median time of 13 days, while an unsupported platelet count of 20×10^9/l was achieved after a median time of 8 and 9 days following the first and second cycle, respectively. The effect of the number of CD34+ cells autografted on the speed of platelet recovery was statistically significant ($P < 0.05$). As shown in Figure 3, 10 cycles of high-dose therapy were administered to patients with platelet nadirs above 20×10^9/l (i.e. 0 days to reach 20×10^9/l), and these received ifosfamide (15000 mg/m^2) and epirubicin (150 mg/m^2) without carboplatin. Following the addition of 900 mg/m^2 carboplatin, the regimen was severely myelosuppressive with nadirs for the WBC between 0.05 and 0.15×10^9/l, and all patients required platelet transfusion (median 2, range 1–10). Patients autografted with more than 7.5×10^6 CD34+ cells/kg had platelet counts above 20×10^9/l within less than 10 days.

There was no toxic death in our study, and non-haematological toxicity predominantly consisted of mucositis and renal toxicity. Following the 3-day course of 15000 mg/m^2 ifosfamide, 8 patients developed renal tubular acidosis which required substitution of sodium bicarbonate. At the same time, individual peak levels of serum creatinine reached values of 1.5–3.6 mg/dl. Except for 2 patients with persistent impairment, renal function recovered after a median time of 21 days following the last cycle of high-dose therapy. As a result of the toxicity encountered in these patients, the dose of ifosfamide was reduced to 12000 mg/m^2 and delivered over a period of 5 days instead of 3 days. Following this dose adjustment, no further renal toxicity was noted.

Fever of greater than 38.5°C was observed in 33 and 31 patients after the first and second cycle, respectively (Table 2). There were 5 cases of septicaemia after the second cycle, whereas fungal infections were not observed. Central nervous system- or neurotoxicity, which were found to be dose-limiting by other investigators, were not observed [14, 16, 17]. Left ventricular ejection fraction, as measured by two-dimensional-echocardiography, did not change during the treatment period arguing against a significant therapy-related cardiotoxicity. There was no difference in the tolerability of the two high-dose cycles. The second cycle was tolerated as well as the first cycle, which is best reflected in the duration of hospitalisation. The median time in hospital after the first and second cycle was 14 days (range 10–47) and 14 days (range 7–20), respectively. The median time required to complete the entire protocol was 23 weeks (range 18–26).

Table 2. Non-haematological toxicity following two cycles of PBSC-supported high-dose chemotherapy

	First cycle	Second cycle
No. of patients with fever >38.5°C	33	31
FUO	33	26
Septicaemia	0	5
Days of fever >38.5°C	2(0–9)*	1.5 (0–6)
Days of i.v. antibiotics	8 (0–14)	6 (0–16)
No. of transfusions per patient		
Red blood cells	2 (0–10)	2 (0–5)
Platelets	2 (0–10)	2 (0–8)

*Median and range.
i.v., intravenous; FUO, fever of unknown origin.

Response

6 patients relapsed between 7 and 11 months postgrafting (median 8 months) which included 4 patients with local recurrence. Three of the 4 obtained a second remission following surgery and involved-field irradiation of the chest

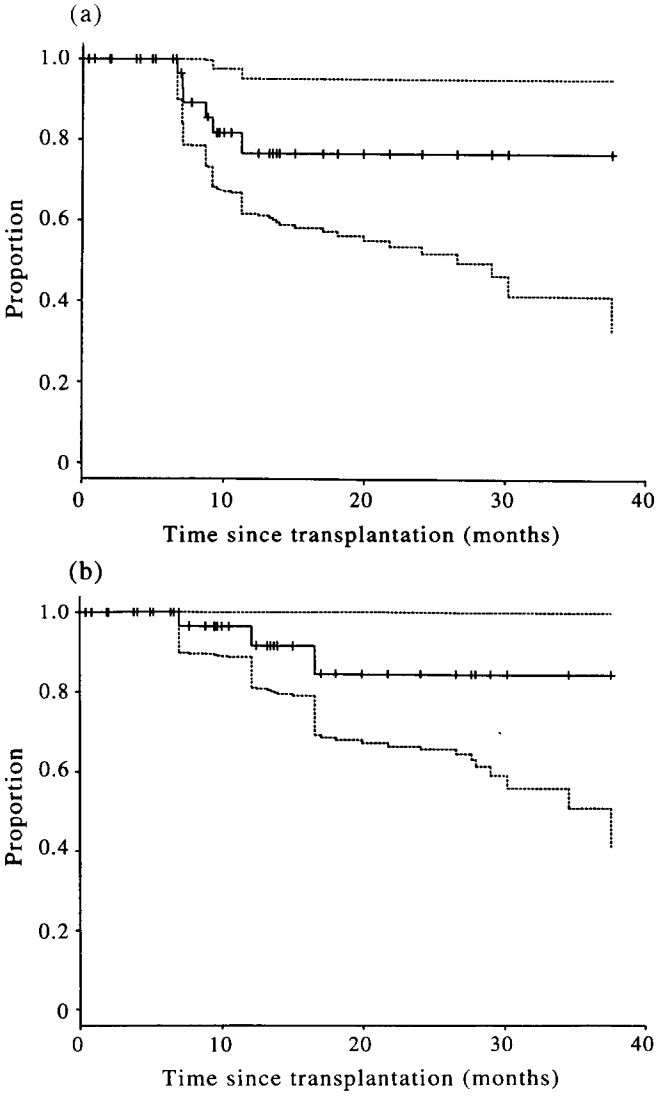

Figure 4. Probability of (a) disease-free survival (DFS) and (b) overall survival in 40 patients with primary breast cancer. With a median follow-up time of 11 months, the probability of overall survival (85% with a 95% confidence interval of 41–100%) at 38 months post-transplantation was greater than the probability of DFS (77% with a 95% confidence interval of 32–95%), since 3 patients with local relapse entered a second remission following surgery and involved-field radiotherapy. 95% CI shown with broken lines.

wall. The other 3 patients in relapse died of tumour progression 7, 12 and 17 months post-transplantation, with a median follow-up time of 12 months. This translates into a probability of disease-free survival of 77% at 38 months with a 95% confidence interval of 32–95% (Figure 4). Since 3 patients with local relapse entered a second remission, the probability of overall survival is 85%, with a 95% confidence interval of 41–100%.

Out of 78 autografts, 68 autografts from 37 patients could be evaluated for contamination with cytokeratin-positive tumour cells. Of 68 high-dose cycles, 64 were supported using leukapheresis products without detectable cytokeratin-positive cells. One patient with systemic relapse received an autograft containing cytokeratin-positive cells following the first cycle of high-dose therapy. The other 2 patients who received autografts harbouring cytokeratin-positive cells following high-dose cycles are disease free at a follow-up time of 8 and 28 months post-transplantation.

DISCUSSION

In a single-centre study, efficacy and toxicity of a sequential PBSC-supported high-dose therapy were evaluated in 40 patients with primary (stage II/III) breast cancer and involvement of 10 or more axillary lymph nodes. Blood-derived autografts were obtained during G-CSF-enhanced recovery after the first and/or second cycle of cytotoxic chemotherapy with ifosfamide and epirubicin. The drugs were chosen because they are effective in the treatment of primary and metastatic breast cancer [18, 19]. The combination was well tolerated without severe toxicity. An increase in dose intensity could be achieved by administering two cycles of high-dose therapy with PBSC support. The toxicity observed was tolerable which is a prerequisite for acceptance of any dose-escalated regimen in the adjuvant setting. In our study, there were no toxic deaths, whereas single high-dose regimens may be associated with mortality rates of up to 12% [6]. Because of severe renal toxicity that was encountered at the beginning, the total dose of ifosfamide was reduced to 12000 mg/m^2 and delivered over a period of 5 days [20]. At the same time, carboplatin was added to enhance the antineoplastic efficacy of the regimen [14]. Having made these changes, no further renal toxicity was noted. One patient was taken off study following the first high-dose cycle due to prolonged gastrointestinal toxicity, while another patient declined the second cycle due to severe anxiety. Leukapheresis products for autografting were chosen according to their content of CD34 + cells. As the autografts contained a mean number of $4.84 \pm 0.35 \times 10^6$ CD34+ cells/kg, the number re-infused was 1.9-fold greater than the threshold quantity of 2.5×10^6/kg. This amount of progenitor cells provides rapid and sustained haematological reconstitution even following myeloablative high-dose conditioning therapy [11]. The median time needed to reach an ANC of 0.5×10^9/l and an unsupported platelet count of 20×10^9/l following both cycles was similar, suggesting that marrow recovery primarily resulted from the haematopoietic progenitor cells transplanted and not from autochthonous regeneration. The speed of reconstitution was also similar to that observed in patients with haematological malignancies who received high-dose regimens with total body irradiation [21]. This finding argues against a particular regimen-related effect on engraftment and haematological reconstitution. In contrast to previous reports, we observed a statistically significant dose–response relationship between the number of CD34+ cells re-infused and the speed of platelet recovery. Patients who received more than 7.5×10^6 CD34+ cells/kg had platelet counts of 20×10^9/l within less than 10 days.

The data on the therapeutic efficacy are encouraging in comparison with results obtained with standard therapy [22, 23]. Bonadonna and associates [23] found that sequential administration of cyclophosphamide, methotrexate, 5-fluorouracil (CMF) and doxorubicin is currently the most effective conventional therapy in patients with high-risk primary breast cancer, resulting in a probability of relapse-free survival of 29% at 10 years. At 38 months, the longest time of follow-up in our patient group, the probability of disease-free survival was 77% compared with 50% in Bonadonna's cohort. Caution is warranted in the interpretation of our results, since the number of patients included is relatively small and the follow-up time relatively short with a 95% confidence interval of 32–95%.

It is worth noting that Bonadonna observed two toxic deaths due to doxorubicin-induced congestive heart failure demonstrating that standard cytotoxic therapy may also be associated with significant toxicity.

6 of our patients relapsed between 7 and 11 months post-grafting including 4 patients who developed local relapse at the chest wall. Two of them initially had breast-conserving surgery, but more importantly, all patients in relapse declined local radiotherapy following PBSC-supported high-dose therapy. These data are in line with the results of Peters and associates [7] stressing the importance of involved-field irradiation even after high-dose chemotherapy. 3 patients entered into a second remission following surgery and local radiotherapy.

The issue of whether contaminating tumour cells in the autograft may contribute to relapse can be specifically addressed in 3 patients. 2 of them are disease-free after a follow-up time of 8 and 28 months, respectively, whereas the third patient relapsed 7 months post-transplantation. It is interesting that her second cycle was supported with a single harvest which was free of tumour cells. 5 patients in relapse had autografts without detectable cytokeratin-positive cells. However, considering the detection limit of the immunocytochemical method, one cannot exclude the possibility that re-infused tumour cells have contributed to recurrence of disease [24]. If selection of CD34+ cells is envisaged for purging, leukapheresis products collected after the first cycle may be preferred, as they contain a greater number of CD34+ cells in comparison with harvests obtained after the second cycle. Furthermore, the increased number of CD34+ cells observed following the first cycle was accompanied by a greater proportion of more primitive progenitor cells.

In summary, the data presented demonstrate that G-CSF administered following an effective cytotoxic chemotherapy mobilises a sufficient number of PBSC. This is a prerequisite when sequential high-dose therapies are envisaged in patients with high-risk breast cancer. A longer follow-up is needed to ascertain the therapeutic advantage in comparison with patients who had received conventional cytotoxic chemotherapy. As the risk of local relapse is apparently

increased, future trials should include involved-field radiotherapy.

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