Cancer Chemother Pharmacol (1999) 44 (Suppl): S13–S17

ORIGINAL ARTICLE

S. Hohaus · S. Martin · A. Schneeweiß · M.T. Voso
R.F. Schlenk · D. Wallwiener · G. Bastert · R. Haas

Adjuvant high-dose therapy with peripheral blood stem cell support for patients with high-risk breast cancer

Abstract We report on the efficacy and toxicity of a sequential high-dose therapy with peripheral blood stem cell (PBSC) support in 107 patients with high-risk stage II/III breast cancer. There were 90 patients with more than 9 tumour-positive axillary lymph nodes. An induction therapy of two cycles of ifosfamide (total dose, 7,500 mg/m^2) and epirubicin (120 mg/m^2) was given, and PBSC were harvested during granulocyte colony-stimulating factor (G-CSF)-supported leukocyte recovery following the second cycle. The PBSC-supported high-dose chemotherapy consisted of two cycles of ifosfamide (total dose 12,000 mg/m^2), carboplatin (900 mg/m^2) and epirubicin (180 mg/m^2). Patients were autografted with a median number of 4.1×10^6 CD34+ cells/kg (range 1.9–26.5×10^6), resulting in haematological reconstitution within approximately 2 weeks following high-dose therapy. The toxicity was moderate in general, and there was no treatment-related toxic death. Twenty-nine patients (27.1% of all patients) relapsed between 3 and 46 months following the last cycle of high-dose therapy (median 15 months). The probability of disease-free and overall survival at 3 years was 56% and 83%, respectively. A multivariate analysis showed that patients with stage II disease had a significantly better probability of disease-free survival (71%) in comparison with patients with stage III disease (30%). The probability of disease-free survival was also significantly better for patients with oestrogen receptor-positive tumours (62%) compared with patients with receptor-negative ones (40%). In conclusion, sequential high-dose chemotherapy with PBSC support can be safely administered to patients with high-risk stage II/III breast cancer. Further intensification of the therapy including the addition of non-cross-resistant drugs or immunological approaches may be envisaged for patients with stage III disease and hormone receptor-negative tumours.

Key words Breast cancer · High-dose chemotherapy · Peripheral blood stem cell transplantation · Prognostic indicators · Tumour cells

Work presented at the Satellite Symposium "New Options for Palliative Treatment of Breast Cancer", 29 September 1998, at the First European Breast Cancer Conference, Florence, Italy

S. Hohaus (✉) · S. Martin · M.T. Voso · R.F. Schlenk · R. Haas
Department of Internal Medicine V,
University of Heidelberg,
Hospitalstr. 3,
D-69115 Heidelberg, Germany
Tel.: +49-6221-562781; Fax: +49-6221-565813

A. Schneeweiß · G. Bastert
Department of Gynaecology,
University of Heidelberg, Germany

M.T. Voso · R. Haas
Clinical Cooperation Unit Molecular Haematology and Oncology,
German Cancer Research Centre, Germany

D. Wallwiener
Department of Gynaecology,
University of Tübingen, Germany

Introduction

Following conventional cytotoxic chemotherapy, long-term disease-free survival is only achieved in 15–30% of the patients with primary breast cancer and tumour involvement of more than 9 axillary lymph nodes [1, 2]. As a consequence, high-dose therapy has been envisaged to improve the treatment results for this particular group of patients [3–8]. For instance, in patients with primary breast cancer and tumour involvement of more than 9 axillary lymph nodes, Gianni et al. [6] observed a probability of disease-free survival of 57% after 5 years for the 67 patients treated with high-dose therapy, compared with 41% for those who had received cytotoxic chemotherapy at conventional doses.

Instead of a single cycle of PBSC-supported high-dose therapy, 2 or more cycles can be administered to achieve a dose intensification [5, 9–12]. Here, we report on 107 patients with high-risk stage II/III breast cancer, including 90 patients with more than 9 tumour-positive axillary lymph nodes who were treated with a sequential

high-dose therapy supported by PBSC. Particular emphasis was put on the evaluation of prognostic factors such as stage of the disease, number of involved axillary lymph nodes and the hormone receptor status.

Patients and methods

Patients

Between July 1993 and July 1998, 107 female patients with primary breast cancer were enrolled onto this study and completed high-dose therapy by September 30, 1998. The common denominator for including them into our study was the presence of high-risk factors. The majority of patients (90 of 107 patients) had tumour involvement of more than 9 axillary lymph nodes, while 17 patients had a median of 7 lymph nodes with additional risk factors such as hormone receptor-negative tumour, young age, high proliferation index, or tumour cells in the bone marrow. Patient characteristics are given in Table 1. 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 the study.

Cytotoxic therapy

Cytotoxic chemotherapy consisted of 2 cycles of ifosfamide (2,500 mg/m^2 i.v. infusion on 3 days) and epirubicin (40 mg/m^2 i.v. infusion on 3 days) (Fig. 1). Mesna was given at the same dose as ifosfamide on 4 days. Both cycles were supported with R-metHuG-CSF (filgrastim, 300 μg/day, s.c., Neupogen, Amgen Inc., Thousand Oaks, Calif., USA).

PBSC collection began when a distinct population of CD34+ cells was measurable in the peripheral blood. The leukaphereses were performed using a Fenwal CS3000 (Baxter Deutschland GmbH, Munich, Germany) or a Spectra (Cobe Laboratories, Lakewood, Calif., USA). Between 10 l and 20 l were processed at flow rates between 70 and 150 ml/min. The yield of CD34+ haematopoietic stem cells was assessed by immunostaining and flow cytometry as previously described [13, 14].

The cytotoxic therapy was continued with 2 cycles of PBSC-supported high-dose ifosfamide (total dose 12,000 mg/m^2), epirubicin (180 mg/m^2) and carboplatin (900 mg/m^2) (Fig. 1). The dose of all drugs was delivered over a period of 5 days. Ifosfamide was given as 24-h continuous i.v. infusion, while epirubicin and carboplatin were administered over 4 and 2 h, respectively. Mesna was given at the same dose as ifosfamide, on days 1–5, followed by an additional administration of 50% of the dose on day 6.

PBSCs were reinfused 1 day after the end of cytotoxic chemotherapy, and no cytokines were given following transplantation. The patients received prophylactic antimicrobial therapy with ciprofloxacin (1,000 mg/day) and fluconazole (400 mg/day). Empirical antibiotic therapy was administered for fever ≥38.5 °C. Platelet counts above 20×10^9/l were maintained by platelet transfusions, and packed red cells were given when the hemoglobin was less than 8.0 g/dl.

Pre-menopausal women received anti-hormonal therapy with goserelin at a dose of 3.6 mg administered subcutaneously once per

Table 1 Patient characteristics (*n.a.* not available, *ER* oestrogen receptor, *PR* progesterone receptor)

No. of patients	107
Age, years	
Median	45
Range	23–63
Menopausal status	
Pre-menopausal	74
Post-menopausal	33
Type of surgical procedure	
Mastectomy	35
Breast-conserving surgery	72
Stage	
II A	25
II B	38
III A	33
III B	11
No. of axillary involved lymph nodes	
<10	17
10–19	58
>19	32
Receptor status	
ER-positive	59
ER-negative	44
n.a.	4
PR-positive	61
PR-negative	42
n.a.	4

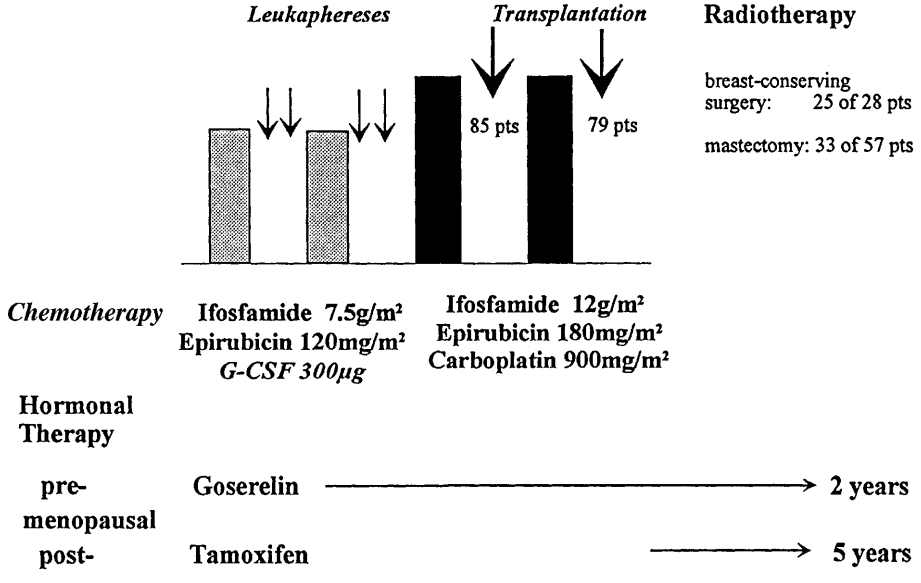

Fig. 1 Treatment plan (*pts* patients, *G-CSF* granulocyte colony-stimulating factor)

month. Post-menopausal women received tamoxifen per os at a daily dose of 30 mg, which was commenced 6 weeks following PBSC-supported high-dose therapy.

Thirty-two of 35 patients who underwent breast-conserving surgery received local-regional irradiation of the breast and draining lymph nodes at a dose of 50 Gy, with a boost of 10 Gy to the tumour bed. For patients undergoing modified radical mastectomy, there was no general recommendation for radiotherapy of the chest wall at the beginning. From August 1996 onwards, irradiation of the chest wall and draining lymph nodes was included for all patients. As a result, 43 of 72 mastectomized patients underwent local radiotherapy.

Statistical analysis

Survival curves were estimated using the Kaplan-Meier product limit method. Differences between the survival curves were compared using the log-rank test. Multivariate analysis was performed using the Cox regression model with stepwise analysis (P values for entry = 0.15 and for removal = 0.05). The following risk factors were first examined in a univariate analysis using the log-rank test: age, menopausal status, type of surgical procedure, stage (II A or B versus III A or B), number of tumour-positive axillary lymph nodes (group 1: <10, group 2: 10–19, group 3: >19), proportion of tumour-involved axillary nodes (group 1: $<50\%$, group 2: 50–85%, group 3: $>85\%$), histological grading (G2 versus G3), oestrogen receptor (ER) status, progesterone receptor (PR) status, radiation therapy. In a second step, variables which were significant in the univariate analysis were included in a multivariate analysis as stated above. Statistical computations were performed using the software program Statistica.

Results and discussion

High-dose therapy with PBSC support

One-hundred-and-seven patients with high-risk stage II/III breast cancer were included in the study to receive PBSC-supported sequential high-dose therapy. The treatment was completed as scheduled in 99 patients, while 8 patients were withdrawn following the first cycle of high-dose therapy. The reasons for discontinuation were severe enterocolitis in 2 patients, cardiac toxicity with T-wave changes indicative for ischaemic heart disease in 1 patient, CNS toxicity in 2 patients, subjective decrease in hearing in 1 patient, and hepatitis B in 1 patient. There was 1 patient who declined the second cycle because of severe anxiety. The time interval between the 2 cycles of high-dose therapy varied between 4 and 15 weeks (median 7 weeks). In 1 patient with alloreactive antibodies against platelets, the second cycle was postponed until appropriate histocompatibility leucocyte antigen (HLA)-cross-match negative donors were found for platelet donations.

High-dose therapy was supported with a median number of 4.1×10^6 CD34+ cells/kg (range 1.9–26.5×10^6), which were mainly collected following the second cycle of G-CSF-supported induction therapy. Considering a threshold number of 2.5×10^6 CD34+ cells/kg as an adequate amount for sustained engraftment, the sequential high-dose therapy could be supported with portions of a single leukapheresis (LP) product in 45% of the patients, while the percentage of patients requiring between 3 and 6 LP products was only 14%.

The time needed for haematological reconstitution following both cycles of high-dose therapy was not different. A neutrophil count of 0.5×10^9/l was observed after a median time of 13 days (range 8–18 days), and an unsupported platelet count of 20×10^9/l was reached after 9 days (range 0–20). The non-haematological toxicity was generally moderate. In particular, there was no day-100 treatment-related mortality. Thirty per cent of the patients required total parenteral nutrition owing to severe mucositis, while the median number of days with fever of ≥38.5 °C following both cycles was 2 days. As a result, the number of days of i.v. antibiotic therapy amounted to a median of 7 days. Considering the relatively moderate toxicity, the high-dose therapy will be administered in an outpatient setting for selected patients.

Therapeutic outcome

Twenty-nine patients (27.1% of all patients) relapsed between 3 and 46 months following the last cycle of high-dose therapy (median 15 months) (Table 2). Three of 32 patients who did not receive irradiation had local-regional relapse. In contrast, among 75 patients who underwent local-regional radiotherapy, there was only 1 patient with local-regional relapse, which was accompanied by metastases to the lung. There was 1 patient with local-regional relapse outside the irradiation field. Patients with locally advanced breast cancer are apparently at risk for local relapse and benefit from involved-field radiotherapy which is associated with moderate toxicity [15, 16]. Two patients developed tumours in the contralateral breast 5 and 7 months post-transplantation, respectively. As a result of surgery and radiotherapy, 5 of the 6 patients with local relapse or tumours at the contralateral site have been disease-free for between 1.5 and 44 months. One patient with hepatitis-C died of liver failure owing due to a hepatocellular carcinoma 21 months post-transplantation.

The corresponding Kaplan-Meier estimate for disease-free survival at 3 years following the last cycle of high-dose therapy was 56%, while the probability of overall survival was 83% (Fig. 2A). The data are similar to those reported by other groups and suggest that high-dose therapy may be superior to cytotoxic chemotherapy

Table 2 Sites of relapse (*CNS* central nervous system)

Local-regional	4
Contralateral breast	2
Soft tissue	2
Bone	5
Lung	2
Liver	5
CNS	3
Multiple	6

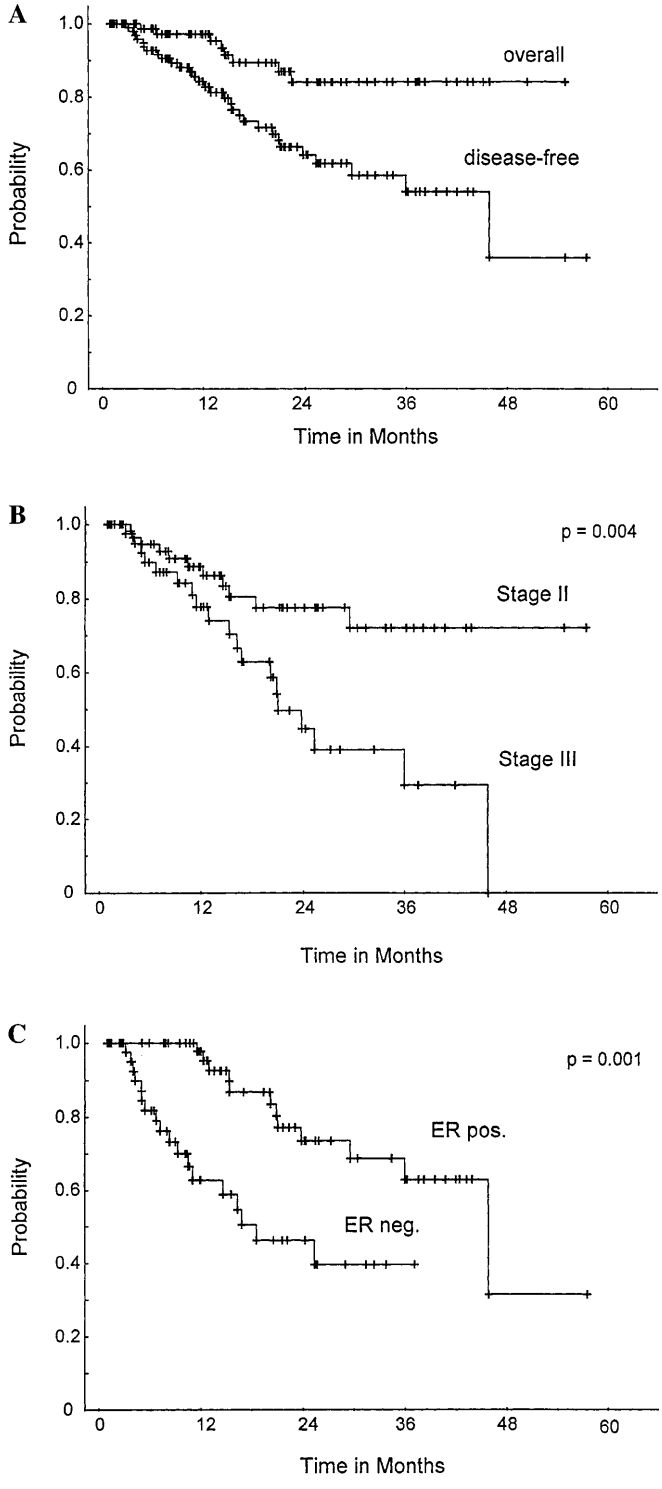

Fig. 2 **A** Overall survival and disease-free survival following the last cycle of adjuvant peripheral blood stem cell (PBSC)-supported high-dose chemotherapy in 107 patients with high-risk stage II/III breast cancer. **B** Disease-free survival after the last cycle of adjuvant PBSC-supported high-dose chemotherapy according to stage of disease and **C** oestrogen receptor (ER) status

at conventional doses [6]. Still, a significant proportion of patients treated with high-dose therapy is at risk for relapse and eventually dies of progressive disease. We therefore looked for prognostic factors associated with an increased risk of relapse.

Prognostic factors

By univariate analysis, the risk of relapse for patients with stage II disease was significantly smaller in comparison with patients with stage III disease ($P = 0.004$). Similarly, the risk of relapse was also significantly reduced for patients with ER-positive tumours ($P = 0.001$) when compared with patients with negative tumours. According to the multivariate analysis, the stage of disease and the ER-status were independent prognostic factors. As a consequence, patients with stage II disease had a Kaplan-Meier estimate of disease-free survival at 3 years of 71% in comparison with 30% for patients with stage III disease (Fig. 2B). In the same line, the probability of disease-free survival was significantly better for patients with ER-positive tumours in comparison with those with receptor-negative ones (62% vs 40%) (Fig. 2C).

These findings are in line with data of Somlo et al. showing that patients with stage III B disease had a greater likelihood of relapse compared with patients with stage II disease [7]. Similar to our data, the lack of hormone receptor expression in that study was also associated with a bad prognosis. Since the presence of ERs is also associated with response to anti-hormonal treatment, the better disease-free survival observed in patients with ER-positive tumours may also reflect the efficacy of the anti-hormonal treatment that we administered post-transplantation. Gianni et al. observed a better disease-free survival in patients presenting with 10–20 tumour-positive axillary lymph nodes in comparison with patients with more than 20 lymph nodes, a finding which we could not confirm in our patient group [6]. However, the proportion of tumour-involved lymph nodes proved to be of significance. The probability of disease-free survival at 3 years was 74% for patients with less than 50% tumour-involved lymph nodes in comparison with 40% for patients with more than 85% tumour-positive nodes.

In conclusion, patients with stage III disease and with ER-negative tumours have a poor prognosis despite sequential high-dose therapy. Further intensification of the therapy by administering additional cycles of high-dose therapy with non-cross-resistant cytotoxic drugs is feasible, as we have shown for patients with metastatic disease [17]. Immunological treatment modalities including the use of antibodies against antigens expressed on the surface of breast cancer cells such as HER-2/NEU breast mucins or the epithelial glycoprotein 17-1A might also be envisaged in addition to the high-dose therapy.

Acknowledgements We thank the nursing staff of the Department of Internal Medicine V for their outstanding care of the patients. We are grateful to Petra Schmidt, Margit Pförsich, Miriam Weis,

Magdalena Volk, Kirsten Flentje and Evi Holdermann for excellent technical assistance. We thank Ulla Scheidler and Brigitte Saur for expert secretarial help.

References

1. Valagussa P, Bonadonna G, Veronesi U (1978) Patterns of relapse and survival following radical mastectomy: analysis of 716 consecutive patients. Cancer 41: 1170–1178
2. Fisher E, Sass R, Fisher B (1984) Pathologic findings from the National Surgical Adjuvant Project for Breast Cancers (protocol no. 4). Cancer 53: 712–723
3. Peters WP, Ross M, Vredenburgh JJ, Meisenberg B, Marks LB, Winer E, Kurtzberg J, Bast RC Jr, Jones R, Shpall EJ, Wu K, Rosner G, Gilbert C, Mathias B, Consighio D, Pehosw, Henderson IC, Norton L, Weiss R, Budman D, Huid D (1993) High-dose chemotherapy and autologous bone marrow support as consolidation after standard-dose adjuvant therapy for high-risk primary breast cancer. J Clin Oncol 11: 1132–1143
4. Wall E van der, Mooijen WJ, Baars JW, Holtkamp MJ, Schornagel JH, Richel DJ, Rutgers EJ, Slaper-Cortenbach IC, Schoot CE van der, Rodenhuis S (1995) High-dose carboplatin, thiotepa and cyclophosphamide (CTC) with peripheral blood stem cell support in the adjuvant therapy of high-risk breast cancer: a practical approach. Br J Cancer 71: 857–862
5. Haas R, Schmid H, Hahn U, Hohaus S, Goldschmidt H, Murea S, Kaufmann M, Wannenmacher M, Wallwiener D, Bastert G, Hunstein W (1997) 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. Eur J Cancer 33: 372–378
6. Gianni AM, Siena S, Bregni SM, Di Nicola M, Orefice S, Cusumano F, Salvadori B, Luini A, Greco M, Zucali R, Rilke F, Zambetti M, Valagussa P, Bonadonna G (1997) Efficacy, toxicity, and applicability of high-dose sequential chemotherapy as adjuvant treatment in operable breast cancer with 10 or more involved axillary nodes: five-year results. J Clin Oncol 15: 2312–2321
7. Somlo G, Doroshow JH, Forman SJ, Odom-Maryon T, Lee J, Chow W, Hamasaki V, Leong L, Morgan R Jr, Margolin K, Raschko J, Shibata S, Tetef M, Yen Y, Simpson J, Molina A (1997) High-dose chemotherapy and stem-cell rescue in the treatment of high-risk breast cancer: prognostic indicators of progression-free and overall survival. J Clin Oncol 15: 2882–2893
8. Antman KH, Rowlings PA, Vaughan WP, Pelz CJ, Fay JW, Fields KK, Freytes CO, Gale RP, Hillner BE, Holland HK, Kennedy MJ, Klein JP, Lazarus HM, McCarthy PL Jr, Saez R, Spitzer G, Stadtmauer EA, Williams SF, Wolff S, Sobocinski KA, Armitage JO, Horowitz MM (1997) High-dose chemotherapy with autologous hematopoietic stem-cell support for breast cancer in North America. J Clin Oncol 15: 1870–1879
9. Dunphy FR, Spitzer G, Buzdar AU, Hortobagyi GN, Horwitz LJ, Yau JC, Spinolo JA, Jagannath S, Holmes F, Wallerstein RO, Bohannan PA, Dicke KA (1990) Treatment of estrogen receptor negative or hormonally refractory breast cancer with double high-dose chemotherapy for intensification and bone marrow support. J Clin Oncol 8: 1207–1216
10. Ayash LJ, Elias A, Wheeler C, Reich E, Schwartz G, Mazanet R, Tepler I, Warren D, Lynch C, Gonin R, Schnipper L, Frei E, Antman K (1994) Double dose-intensive chemotherapy with autologous marrow and peripheral blood progenitor cell support for metastatic breast cancer: a feasibility study. J Clin Oncol 12: 37–44
11. Broun ER, Sridhara R, Sledge GW, Loesch D, Kneebone PH, Hanna M, Hromas R, Cornetta K, Einhorn LH (1995) Tandem autotransplantation for the treatment of metastatic breast cancer. J Clin Oncol 13: 2050–2055
12. Bitran JD, Samuels B, Klein L, Hanauer S, Johnson L, Martinec J, Harris E, Kempler J, White W (1996) Tandem high-dose chemotherapy supported by hematopoietic progenitor cells yield prolonged survival in stage IV breast cancer. Bone Marrow Transplant 17: 157–162
13. Hohaus S, Goldschmidt H, Ehrhardt R, Haas R (1993) Successful autografting following myeloablative conditioning therapy with blood stem cells mobilized by chemotherapy plus rhG-CSF. Exp Hematol 21: 508–514
14. Haas R, Möhle R, Frühauf S, Goldschmidt H, Witt B, Flentje M, Wannenmacher M, Hunstein W (1994) Patient characteristics associated with successful mobilizing and autografting of peripheral blood progenitor cells in malignant lymphoma. Blood 83: 3787–3794
15. Marks C, Halperin E, Prosnitz L, Ross M, Vredenburgh JJ, Rosner GL, Peters W (1992) Post-mastectomy radiotherapy following adjuvant chemotherapy and autologous bone marrow transplantation for breast cancer patients with greater than or equal to 10 positive axillary lymph nodes. Cancer and Leukemia Group B. Int J Radiat Oncol Biol Phys 23: 1021–1026
16. Wall E van der, Schaake-Koning CC, Zandwijk N van, Baars JW, Schornagel J, Richel DJ, Rutgers EJ, Borger JH, Beijnen JH, Rodenhuis S (1996) The toxicity of radiotherapy following high-dose chemotherapy with peripheral blood stem cell support in high-risk breast cancer: a preliminary analysis. Eur J Cancer 32A: 1490–1497
17. Hohaus S, Wallwiener D, Martin S, Voso MT, Huober J, Fersis N, Bastert G, Haas R (1998) Efficacy and toxicity of sequential high-dose therapy with peripheral blood stem cell support in patients with high-risk breast cancer. Semin Oncol 25: 7–11