



High-dose cyclophosphamide + carboplatin and interleukin-2 (IL-2) activated autologous stem cell transplantation followed by maintenance IL-2 therapy in metastatic breast carcinoma – a phase II study

HC Toh^{1,2}, SL McAfee¹, R Sackstein¹, P Multani¹, BF Cox¹, R Garcia-Carbonero², C Colby¹ and TR Spitzer¹

¹Bone Marrow Transplant Program and ²Division of Hematology-Oncology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Summary:

While high-dose chemotherapy and stem cell transplantation is associated with higher complete response rates than conventional chemotherapy in patients with metastatic breast cancer (MBC), its role in conferring a survival advantage is unproven. We report the results of a prospective phase II trial of 33 patients accrued between 1996 to 1998 with chemosensitive MBC, who received cyclophosphamide (Cy) 2000 mg/m²/day and carboplatin (Cb) 600 mg/m²/day for 3 consecutive days, followed by infusion of peripheral blood stem cells cultured in IL-2 for 24 h on day 0 as adoptive immunotherapy. Low-dose interleukin-2 (IL-2) was administered from day 0 to +4 and/or +7 to +11, +14 to +18, +21 to +25, then 5 days per month for 11 months to augment a graft-versus-tumor effect. The results of this study were compared to those of a historical control group treated with an identical high-dose Cb + Cy regimen with SCT but without IL-2 treatment. Only gastrointestinal (GI) toxicity was more frequent in the IL-2 cohort ($P = 0.0031$). At a median follow-up of 18.6 months, the median progression-free survival (PFS) is 9 months (2.4–40) and the median OS has not been reached yet. The Kaplan–Meier estimated 2 year PFS is 35%, compared with 17% in the control arm ($P = 0.73$), and the estimated 2 year OS is 78%, compared with 61% in the control arm ($P = 0.22$). Multivariate analysis showed that ER status was an independent predictor for OS and PFS, and less chemotherapy prior to HDCSCT predicted for a better PFS. These results show that augmenting HDC with IL-2 activated SCT is well-tolerated. Whether a therapeutic advantage is achievable in patients with MBC remains to be determined. *Bone Marrow Transplantation* (2000) 25, 19–24.

Keywords: high dose chemotherapy and stem cell transplantation; metastatic breast cancer; interleukin-2; graft-versus-tumor effect; adoptive immunotherapy

Immunotherapeutic strategies have shown promising anti-tumor activity in animal and human studies. Immune modulation provides a potentially non cross-resistant modality to eradicate residual disease following maximal cytoreduction with high-dose chemotherapy.¹ Interleukin-2 (IL-2)-activated autologous stem cells followed by low-dose maintenance parenteral IL-2 resulted in successful hematopoietic engraftment, reduced tumor cell contamination in the graft, increased cytotoxic effector cells that induced a graft-versus-tumor (GVT) effect and a significantly better OS in mice compared with mice that only received conventional HDCSCT.^{2,3} More recently in a human study, the incubation of hematopoietic stem cells in low doses of IL-2 was shown to generate activated killer cells with potent *in vitro* major histocompatibility complex (MHC) unrestricted anti-tumor cytotoxicity.^{4–6} This cytotoxicity compared favorably with the anti-tumor cytotoxicity of peripheral blood lymphocyte-activated killer (LAK) cells.⁷

A recent phase I study^{4,8} showed that high-dose carboplatin (Cb) and cyclophosphamide (Cy) followed by the infusion of autologous hematopoietic stem cells (HSC) activated by *in vitro* incubation with IL-2 for 24 h with subsequent low-dose parenteral IL-2 resulted in successful engraftment and acceptable toxicities in patients with stage II–IV breast cancer. In this series, IL-2-activated stem cells were shown to induce an *in vitro* cytotoxicity against breast cancer cell lines that was not seen with pre-activated stem cells. The subsequent low-dose IL-2 may have provided a continued GVT effect without the attendant toxicities associated with high-dose IL-2.⁹

We now report the results of a phase II clinical study of high-dose carboplatin with cyclophosphamide followed by the infusion of IL-2-activated peripheral blood stem cells and maintenance low-dose parenteral IL-2. Our study aimed to assess the tolerability and efficacy of this regimen in metastatic breast cancer (MBC), a uniformly fatal disease with a median survival of 2 years.

Patients and methods

Patient selection

Between April 1996 and September 1998, 33 consecutive female patients with histologically confirmed, chemosensi-

tive *de novo* MBC or MBC diagnosed following relapse after treatment with adjuvant chemotherapy for operable disease were enrolled on this study. Eligibility criteria included: age range of 18–65 years, ECOG performance status 0–2, adequate bone marrow function (absolute granulocyte count of $>1000/\mu\text{l}$, total WBC $\geq 4000/\mu\text{l}$, hemoglobin of $>10\text{ g/dl}$ and a platelet count of $\geq 10^5/\mu\text{l}$), acceptable liver function including bilirubin $\leq 2.0\text{ mg/dl}$, SGOT and SGPT <2 times the upper limit of normal range and a negative hepatitis B surface antigen and adequate renal function (serum creatinine $\leq 1.5\text{ mg/dl}$ and creatinine clearance of $>60\text{ ml/min}$). An acceptable cardiac and pulmonary status with left ventricular ejection fraction $\geq 45\%$ by radio-nuclide scan and DLCO $>60\%$ of predicted value were also required. All patients provided written informed consent. The protocol was approved by the Institutional Review Board of the Massachusetts General Hospital (MGH). All patients were treated at the MGH.

High-dose chemotherapy treatment plan

Peripheral blood stem cells (PBSC) were mobilized with cyclophosphamide 3 g/m^2 i.v. followed by recombinant myeloid growth factor (G-CSF) at a dose of $5\text{ }\mu\text{g/kg}$ subcutaneously. Leukopheresis was performed once the peripheral WBC reached $>1 \times 10^9/l$ using a Cobe Spectra Cell Separator (Cobe Laboratories, Lakewood, CO, USA) and continued daily until $>5.0 \times 10^8/\text{kg}$ mononuclear cells were collected. Peripheral blood stem cells (PBSC) were cryopreserved at -200°C using DMSO as a cryoprotectant. The conditioning regimen schedule consisted of: cyclophosphamide $2000\text{ mg/m}^2/\text{day}$ administered i.v. over 2 h on days -5 , -4 and -3 , carboplatin $600\text{ mg/m}^2/\text{day}$ administered i.v. immediately following cyclophosphamide also on days -5 , -4 and -3 ; and Mesna (sodium 2-mercaptoethane sulfonate) 15 mg/kg i.v. administered 15 min prior to and 3, 6 and 9 h following cyclophosphamide to prevent hemorrhagic cystitis. The anti-emetic regimen included dexamethasone 20 mg i.v., diphenhydramine $25\text{--}50\text{ mg}$ i.v., lorazepam 1 mg i.v., and either granisetron at 1 mg twice a day or ondansetron 8 mg every 8 h prior to start of HDC. The details of supportive care have been previously described.¹⁰

Interleukin-2 activation of PBSC and interleukin-2 therapy

On day -1 , harvested PBSC were thawed rapidly in a 37°C waterbath and then incubated in 5% CO_2 in X-VIVO serum-free medium (BioWhittaker, Walkersville, MD, USA) containing $50\text{ }\mu\text{g/ml}$ of gentamicin. A portion of the patient's PBSC was stored as backup without IL-2 activation. The PBSC to be infused were then treated with 6000 IU IL-2/ml (Chiron, Emeryville, CA, USA) at an approximate concentration of 10×10^6 cells/ml in Baxter LIFE cell culture bags. On day 0, 24 h after IL-2 activation of PBSC, the contents of the 1 liter bags were harvested on the Fenwall CS 3000 cell separator (Baxter Healthcare), and collected sterilely into receiving bags in 200 ml of normal saline.

IL-2 was administered subcutaneously at a daily dose of

$1.8 \times 10^6\text{ IU/m}^2$ in the first patient. Due to toxicity, the dose was reduced to $1.0 \times 10^6\text{ IU/m}^2/\text{day}$ in the next 10 patients, and further reduced to $5 \times 10^5\text{ IU/m}^2/\text{day}$ in the remaining 24 patients. In the first 11 patients, IL-2 was administered from transplant day 0 to day +4, days +7 to +11, +14 to +18, +21 to +25, and then for 5 days monthly for 12 months. To reduce toxicity and shorten the time to hematologic recovery, week 2 of IL-2 therapy was replaced in the remaining 24 patients with 5 days of G-CSF $5\text{ }\mu\text{g/kg}$ on days +7 to +11. All study patients received IL-2 as a twice daily divided dose. All 33 patients were instructed to administer IL-2 for 5 days each month for an additional 11 months. Pentoxifylline at an oral dose of 400 mg three times per day was administered to ameliorate IL-2-related symptoms. Patients who relapsed following HDCSCT on this study did not continue further maintenance IL-2.

Statistical analysis

Progression-free survival (PFS) was calculated from the first day of high-dose chemotherapy (HDC) to the first documented evidence of treatment failure (local or systemic relapse or treatment-related death). Death due to all causes was used as the endpoint for overall survival (OS). PFS and OS were estimated according to the Kaplan–Meier product-limit method in the IL-2-treated study patients.¹¹ An additional cohort of 29 patients with the same eligibility criteria was treated at the MGH between 1993 and 1997 in a different trial with an identical high-dose chemotherapy schedule but without IL-2. This separate cohort of patients was also analyzed for PFS and OS, and served as a historical control group. The statistical analysis of differences observed in PFS and OS between the two groups was assessed by the log-rank test.¹² To adjust for any confounding factors, and to assess the relative importance of different prognostic variables for survival, the Cox proportional hazards model was used.¹³ Estrogen receptor (ER) status, number of metastatic sites, sites of metastasis (visceral or non-visceral), previous disease-free interval (time to relapse), numbers of lines of chemotherapy and response to induction chemotherapy just prior to HDCSCT were included as covariates in the regression model.

Results

Patient characteristics

Thirty-three consecutive female patients with histologically confirmed MBC were enrolled onto this prospective phase II study (Table 1). The median age of the 33 patients was 47 years (range 28–60 years). Seven patients (21.2%) had *de novo* MBC and 26 patients (78.8%) had relapsed after surgery and/or adjuvant or neoadjuvant chemoradiotherapy for stage I to III breast cancer. The previous median disease-free interval in this subgroup was 26 months (range 2–144 months). The median time from diagnosis of MBC to HDCSCT was 8 months (range 5–116 months). Almost all patients were previously exposed to doxorubicin-containing ($n = 30$ or 91%) and/or cyclophosphamide-contain-

Table 1 Patient characteristics in the HD CbCy + IL-2-activated SCT + SC IL-2 and the HD CbCy + SCT groups

	IL-2 treatment group	Non-IL-2 treatment group
Total No. of patients	33	29
ER status		
Positive	14 (42.4%)	8 (27.6%)
Negative	13 (39.4%)	16 (55.2%)
Unknown	6 (18.2%)	5 (17.2%)
<i>De novo</i> metastatic disease	7 (21.2%)	5 (17.2%)
Median time from diagnosis to distant metastasis (months)	26 (2–144)	40 (6–84)
No. of metastatic sites		
1	20 (60.6%)	18 (62.1%)
2	9 (27.3%)	7 (24.1%)
3	3 (9.1%)	2 (6.9%)
4	1 (3%)	2 (6.9%)
Visceral metastasis	13 (39.4%)	16 (55.2%)
Non-visceral metastasis	20 (60.6%)	13 (44.8%)
Regimens of chemotherapy prior to HDCSCT		
1	8 (24.2%)	7 (24.1%)
2	20 (60.6%)	18 (62.1%)
3	3 (9.1%)	3 (10.3%)
≥4	2 (6.1%)	1 (3.4%)
Status prior to HDCSCT (response to induction chemotherapy)		
CR	13 (39.4%)	12 (41.4%)
PR	20 (60.6%)	16 (55.2%)
Unknown	0	1 (3.4%)

ing regimens ($n = 31$ or 94%) prior to HDCSCT. All 33 patients had chemosensitive disease.

Hospital course

All patients received their planned treatment at the MGH. The mean number of infused post-IL-2 incubated CD34⁺ progenitor cells/kg and nucleated cells/kg were $11.97 \pm 2.6 \times 10^6$ CD34⁺ stem cells/kg and $3.32 \pm 0.25 \times 10^8$ nucleated cells/kg respectively. One of 33 patients did not receive IL-2-activated stem cells because the stem cell bacterial culture demonstrated gram-negative rods identified as *Pseudomonas putida*, and so she received untreated PSCT. She was still included in the IL-2 treatment group for the ‘intention-to-treat’ analysis. All patients achieved successful engraftment (ANC > 500/ μ l) with a median time to engraftment of 11 days (range 8–17 days). The median engraftment in the historical control group (non-IL-2 treatment group) was 9 days, the difference between the two groups being significant ($P = 0.0001$). The median length of hospital stay in the IL-2 treatment group was 18 days (range 7–27 days). The median length in the historical control study was 15 days, the difference between the two groups also being significant ($P = 0.0001$).

An in-hospital temperature of $\geq 38^\circ\text{C}$ was detected in 28 of 33 patients with a median duration of 2 days (1–12 days) per febrile patient. Twenty-three (70%) patients required broad-spectrum antibiotics (the majority receiving ceftazidime and vancomycin) for the management of febrile neutropenia. Two of the 23 patients (UPN 189, UPN 267) yielded

positive blood cultures of *Streptococcus mitis* and coagulase-negative *Staphylococcus aureus* respectively which were successfully treated.

Toxicity

Toxicities were graded according to the Seattle transplant program criteria. There was no treatment-related mortality (Table 2). None of the patients developed organ failure or required medical intensive care management. Also, there was no evidence of hepatic veno-occlusive disease among the 33 patients. Gastrointestinal (GI) toxicity of \geq grade 2 was the most common organ-site toxicity encountered in the study group (23%). While grade 3 toxicity was absent from the historical cohort, there were two grade 3 toxicities in the study group, one skin and one GI. The grade 3 skin toxicity began on day +7 post transplant and progressed to an extensive erythematous maculopapular rash covering >50% of the body surface area, associated with fever, chills and diarrhea. Her skin biopsy was inconclusive and the skin rash recurred and was clinically diagnosed as erythema multiforme. This necessitated a 50% dose reduction of IL-2 from day +15 (dose held on day +14) and the eventual removal of further IL-2 treatment following hospital discharge. The grade 3 GI toxicity was an episode of large volume bloody diarrhea, which eventually resolved with symptomatic treatment. Only gastrointestinal (excluding liver) toxicity was significantly more frequent in the patients in the IL-2 group ($P = 0.0031$). However, 17 of these 22 patients developed grade 2 GI toxicity prior to infusion of IL-2-activated stem cells (<day +0), and only five patients developed it after day +0, with three of these five patients having received the higher doses of IL-2 amongst the first 11 treated study patients.

Within 24 h of receiving IL-2-activated stem cells, nine patients (27%) remained symptom-free, whereas 24 patients (73%) experienced transient chill and/or rigors lasting minutes that were successfully terminated with i.v. meperidine. Three patients had transient oxygen desaturation which resolved rapidly with intranasal low-dose oxygen. None of the patients developed significant (>grade 2) pulmonary toxicity during the stem cell infusion or post transplant.

Of the first 11 patients treated with the higher dose of IL-2 (1.8×10^6 IU/m² in one patient and 1.0×10^6 IU/m² in 10 patients), three patients discontinued IL-2 due to toxicity (day +1, day +7 and day +8, respectively) and were not restarted on IL-2 therapy. In the first case, as described earlier, IL-2 was discontinued due to a grade 3 skin rash. In the second case, the patient decided not to continue with IL-2 due to the presence of fever, nausea, vomiting, diarrhea and myalgias. The third patient was removed from IL-2 therapy due to a sustained temperature of $>39^\circ\text{C}$. Eight additional patients were not given the full dose of IL-2 in the second week due to the development of significant side-effects including: fever of $>39^\circ\text{C}$ with nausea, vomiting and diarrhea. In each of these cases, IL-2 was resumed on the third week at half the dose. For this reason, the next 24 patients were treated at a 50% dose reduction of IL-2 at 5.0×10^5 IU/m².

Of the subsequently treated 24 patients, only two patients

Table 2 Toxicity profiles of patients on the HDCbCy + IL-2-activated SCT and HDCbCy + SCT treatment groups

	IL-2 treatment group toxicity grade				Non-IL-2 treatment group toxicity grade			
	1	2	3	4	1	2	3	4
Skin	4 (12.1%)	0	1 (3%)	0	1 (3.4%)	0	0	0
Mucosa	2 (6.1%)	0	0	0	3 (10.1%)	1 (3.4%)	0	0
Cardiac	0	1 (3%)	0	0	1 (3.4%)	0	0	0
Lung	5 (15.2%)	1 (3%)	0	0	1 (3.4%)	0	0	0
GI	10 (30.3%)	22 (66.7%)	1 (3%)	0	18 (62.1%)	9 (31%)	0	0
Hepatic	17 (51.5%)	6 (18.2%)	0	0	16 (55.2%)	8 (27.6%)	0	0
CNS	1 (3%)	1 (3%)	0	0	1 (3.4%)	0	0	0
Renal	4 (12.1%)	1 (3%)	0	0	0	0	0	0
Bladder	0	2 (6.1%)	0	0	2 (6.9%)	1 (3.4%)	0	0

The data is represented as actual patient and percentage patient numbers. In the statistical comparison, only GI toxicity was significantly greater in the IL-2 treatment group ($P = 0.0031$) compared to the historical control group and there were no life-threatening GI toxicities in either group of treated patients.

discontinued IL-2, one for non-toxicity-related reasons, and in the second case, because of supraventricular tachycardia on day +14 post transplant. In patients who completed the full 12-month IL-2 regimen, there were no reports of significant toxicities. IL-2 treatment was also discontinued in 20 patients who relapsed.

Treatment outcome

With a median follow-up of 18.6 months (range 4.7–40 months), 20 patients have relapsed and six patients have died. The median progression-free survival (PFS) is 9 months and median OS has not yet been reached. The Kaplan–Meier (KM) estimated 2 year PFS is 35% (95% CI of 15–54%), and the 2 year OS rate is 78% (95% CI of 62–94%) (Figures 1 and 2). By univariate analysis, neither PFS nor OS were significantly different between the IL-2 group and non-IL-2 group (log rank test values of $P = 0.73$ and $P = 0.22$, respectively). The 2 year KM estimates of PFS and OS of patients on the historical control group ($n = 29$) are 17% (95% CI of 3.5–31%) and 61% (95% CI

of 43–79%), respectively at a median follow-up of 43 months (16–57.5 months).

Multivariate analysis

We performed a multivariate analysis for both PFS and OS using Cox multiple regression. When PFS was considered, the only independent significant predictors for a favourable PFS were a positive ER status (positive vs negative) (RR = 0.53, 95% CI = 0.32–0.86, $P = 0.01$) and a low number of prior chemotherapy regimens (RR = 5.2, 95% CI = 2.3–11.8, $P = 0.0001$). In the multivariate analysis for OS, ER status was again a predictor for OS (RR = 0.43, 95% CI = 0.21–0.86, $P = 0.0165$). The number of chemotherapy regimens patients received prior to HDCSCT showed a trend towards significance as a predictor for OS, the fewer regimens resulting in better OS (RR = 1.89, 95% CI = 0.93–3.84, $P = 0.007$). Treatment protocols (IL-2-treated vs non-IL-2-treated group) were not significant for PFS or OS in the multivariate analysis.

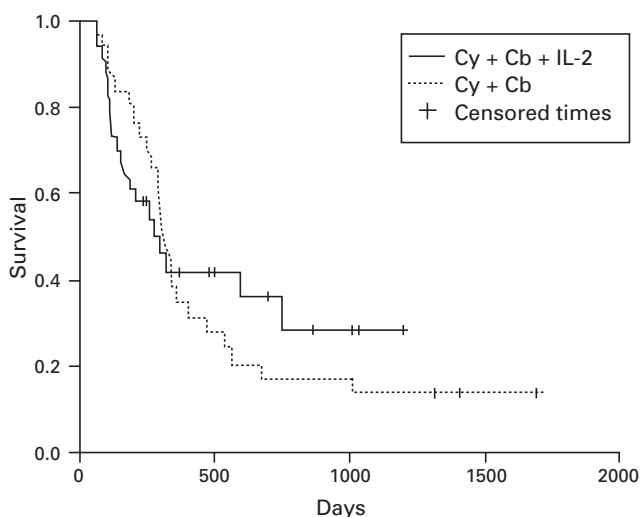


Figure 1 Kaplan–Meier estimates of PFS for patients treated with IL-2 compared with the historical control group. Log-rank P value = 0.73.

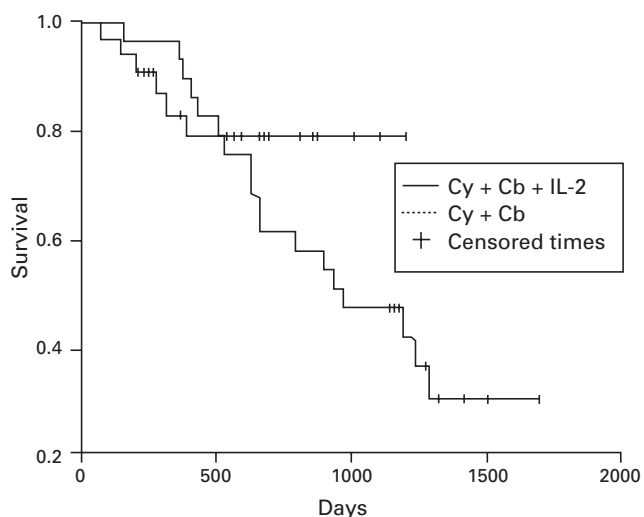


Figure 2 Kaplan–Meier estimates of OS for patients treated with IL-2 compared with the historical control group. Log-rank P value = 0.22.

Discussion

Even with HDCSCT, the median time to progression in MBC is 1 year, and the majority of MBC patients (nearly 80%) will still relapse and die of their disease.¹⁴ Furthermore, with more patients receiving adjuvant chemotherapy for resectable breast cancer, eventual relapses are characterized by poorer responses and survival rates to subsequent chemotherapy.¹⁵ Therefore, we have employed an approach combining the cytoreductive efficacy of HDC with a non cross-resistant biological modality which can further provide anti-tumor activity in MBC, especially in the situation of minimal residual disease. Instead of solely rescuing ablated marrow by SCT following HDC, the approach of activating stem cells with IL-2 converts them into an active therapeutic product with proven anti-tumor cytotoxicity. This approach aims *in vitro* to purge tumor cell contamination in the autograft and *in vivo* to eradicate residual MBC in the patient,⁴ and will hopefully contribute to improve clinical outcome in MBC patients who achieve maximal cytoreduction from HDC. In this study, low-dose maintenance parenteral IL-2 was initiated to further enhance cytotoxic activity of the adoptively transferred IL-2 activated stem cells *in vivo* (containing killer T cells and activated NK cells).⁴⁻⁶ Many human studies have shown that even at low dose ($<1 \times 10^6$ IU/m²/day), IL-2 is capable of inducing NK and antigen-activated T cell proliferation.¹⁶⁻¹⁸ Several groups have tested the use of induction and maintenance IL-2 following HDCSCT for hematologic malignancies, where increased (10-fold) tumor-lysing NK cells were generated after at least 1 month of maintenance IL-2 (0.25 or 0.5×10^6 IU/m²/day) in one study,¹⁷ and both CD8⁺ cytotoxic T cells and NK cells generated in another study of HDCSCT + maintenance IL-2 (1.6×10^6 IU/m²/day).¹⁶

Our study demonstrated successful engraftment in all patients with received IL-2-activated PBSCT with a median time to engraftment of 11 days, the same as the result reported by Areman *et al.*¹⁹ With the employment of the eventual low-dose IL-2 (5×10^5 IU/m²/day) treatment in a schedule allowing for adequate rest intervals, immediate and maximal toxicities of patients on this study were expectably very tolerable. Previous studies have shown that at such low doses of IL-2, only high affinity IL-2 receptors (and not intermediate affinity receptors) are occupied, resulting in the favorable profile of optimal cellular cytotoxicity (T and NK cells) with lesser secondary cytokine production, and hence lesser side-effects and an improved therapeutic index.²⁰ The infusion of IL-2 cultured stem cells in our cohort was marked only by transient and reversible chills and rigors (73%) easily reversed by i.v. meperidine. Capillary leak syndrome and hypotension were not significant events, and the transient oxygen desaturation during infusion witnessed in three patients was the only possible early feature of this syndrome. Moreover, four patients from the historical control group also developed features suggesting capillary leak syndrome. Febrile episodes and febrile neutropenia were not significantly increased in our study group compared with historical HDCSCT reports.²¹

The IL-2-related toxicities in our study were transient and reversible. Only GI toxicities (\geq grade 2) were more

common in the study arm. However, 77% of the grade 2 GI toxicities occurred before day +0, so that it cannot be totally attributed to IL-2 therapy. Moreover, only three patients treated with the initially higher dose of IL-2 had grade 2 GI toxicities after day +0, while seven experienced these toxicities before day +0, again absolving IL-2 as the cause of these toxicities.

With a trend towards superior survival in the IL-2-treated group, a longer follow-up time and a larger cohort of patients may be necessary to reveal a statistically significant difference between the two treatment arms. Using Cox regression, we did not find that IL-2 treatment was a statistically significant independent predictor of PFS or OS.

We have shown that this treatment strategy does not compromise stem cell viability or engraftment,¹⁹ and is associated with very acceptable immediate and long-term toxicities comparable to a purely HDCSCT regimen.⁴ A chemotherapy non cross-resistant treatment adjunct (immunotherapy) may contribute to small volume tumor eradication,^{1,6} since by the Gompertzian model, a smaller tumor load presents with increased tumor growth kinetics, so that maximizing tumor cell kill in the setting of small volume disease is critical.²²

Interleukin-2 activation of stem cells to generate effector T and NK cells which can purge the autograft *in vitro* and enhance GVT in autologous SCT *in vivo* is an attractive adoptive cellular immunotherapeutic strategy that can readily be combined with a HDCSCT regimen and potentially improve clinical outcomes in MBC patients.

References

- 1 Guillaume T, Rubenstein DB, Symann M. Immune reconstitution and immunotherapy after hematopoietic stem cell transplantation. *Blood* 1998; **92**: 1471-1490.
- 2 Charak BS, Malloy B, Agah R *et al*. Interaction of various cytokines with interleukin-2 in the generation of killer cells from human bone marrow: application in the purging of leukemia. *Leukemia Res* 1991; **9**: 801-810.
- 3 Charak BS, Brynes RK, Chogyoji M *et al*. Graft versus leukemia effect of interleukin-2 activated bone marrow: correlation with eradication of residual disease. *Transplantation* 1993; **56**: 31-37.
- 4 Meehan KR, Verma UN, Cahill R *et al*. Interleukin-2-activated hematopoietic stem cell transplantation for breast cancer: investigation of dose level with clinical correlates. *Bone Marrow Transplant* 1997; **20**: 643-651.
- 5 Charak BS, Brynes RK, Katsuda S *et al*. Induction of graft versus leukemia effect in bone marrow transplantation: dosage and time schedule dependency of interleukin-2 therapy. *Cancer Res* 1991; **51**: 2015-2020.
- 6 Charak BS, Verma UN, Mazumder A. Immunomodulation in autologous bone marrow transplantation: experimental approaches. In: Spitzer T, Mazumder A (eds). *Immunotherapy and Bone Marrow Transplantation*. Futura: Armonk, NY, 1995, pp 35-58.
- 7 Agah R, Malloy B, Kerner M *et al*. Generation and characterization of IL-2 activated bone marrow cells as potent graft versus tumor effectors in transplantation. *J Immunol* 1989; **143**: 3093-3099.
- 8 Meehan KR, Verma UN, Rajagopal C *et al*. Stem cell transplantation with chemoradiotherapy myeloablation and interleukin-2. *J Infus Chemother* 1996; **6**: 28-32.

- 9 Lotze MT, Chang AE, Seipp CA *et al*. High dose recombinant IL-2 in the treatment of patients with disseminated cancer. *JAMA* 1986; **256**: 3117–3124.
- 10 Spitzer TR, Cirenza E, McAfee S *et al*. Phase I–II trial of high-dose cyclophosphamide, carboplatin and autologous bone marrow or peripheral blood stem cell rescue. *Bone Marrow Transplant* 1995; **15**: 537–542.
- 11 Kaplan EL, Meier P. Non parametric estimation from incomplete observations. *J Am Stat Assoc* 1958; **53**: 457–481.
- 12 Peto R, Peto J. Asymmetrically efficient rank invariant test procedures. *J R Stat Soc A* 1972; **35**: 185–206.
- 13 Cox DR. Regression models and life tables. *J R Stat Soc B* 1972; **34**: 187–220.
- 14 Hortobagyi GN. Treatment of breast cancer. *New Engl J Med* 1998; **339**: 974–984.
- 15 Rahman ZU, Frye DK, Smith TL *et al*. Results and long-term follow-up for 1581 patients with metastatic breast carcinoma treated with standard dose doxorubicin-containing chemotherapy. *Cancer* 1999; **85**: 104–111.
- 16 Robinson N, Benyunes MC, Thompson JA *et al*. Interleukin-2 after autologous stem cell transplantation for hematologic malignancy – a phase I/II study. *Bone Marrow Transplant* 1997; **19**: 435–442.
- 17 Miller JS, Tessmer-Tuck J, Pierson BA *et al*. Low dose subcutaneous interleukin-2 after autologous transplantation generates sustained *in vivo* natural killer activity. *Biol Blood Marrow Transplant* 1997; **3**: 34–44.
- 18 Soiffer RJ, Murray C, Shapiro C *et al*. Expansion and manipulation of natural killer cells in patients with metastatic cancer by low-dose continuous infusion and intermittent bolus administration of interleukin 2. *Clin Cancer Res* 1996; **2**: 493–499.
- 19 Areman EM, Mazumder A, Kotula PL *et al*. Hematopoietic potential of IL-2 cultured peripheral blood stem cells from breast cancer patients. *Bone Marrow Transplant* 1999; **23**: 27–33.
- 20 Kopp WC, Holmlund JT. Cytokines and immunological monitoring. In Pinedo HM, Longo DL, Chabner BA (eds). *Cancer Chemotherapy and Biological Response Modifiers Annual 16*. Elsevier Science BV: Amsterdam, 1996, pp 189–238.
- 21 Salazar R, Sola C, Maroto P *et al*. Infectious complications in 126 patients treated with high-dose chemotherapy and autologous peripheral blood stem cell transplantation. *Bone Marrow Transplant* 1999; **23**: 27–33.
- 22 Hudis CA, Munster PN. High-dose therapy for breast cancer. *Semin Oncol* 1999; **26**: 35–47.