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Clinical trial

A multicenter randomized clinical trial evaluating interleukin-2 activated hematopoietic stem cell transplantation and post-transplant IL-2 for high risk breast cancer patients

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Summary

Purpose. This Phase III randomized multicenter trial compared progression-free (PFS) and overall survival (OS) for autologous peripheral blood stem cell (aPBSC) transplantation with or without immunotherapy in high-risk breast cancer patients.

Methods. Eligible patients had American Joint Committee on Cancer (AJCC) 5th Edition Stage II/IIIA with ≥ 4 axillary nodes, Stage IIIB, or chemotherapy-sensitive or stable Stage IV disease. Following treatment with cyclophosphamide, thiotepa and carboplatin (STAMP V), patients were randomized to aPBSC transplant with or without immunotherapy. Patients on immunotherapy received cells that were incubated in interleukin-2 (IL-2) for 24 h followed by parenteral IL-2 for 5 days then 2 days of rest for 4 weeks.

Results. Fifty-nine patients were treated (35 Stage II/IIIA; 13 Stage IIIB; 11 Stage IV), 30 patients were randomized to immunotherapy and 29 patients to no immunotherapy. Neutrophils engrafted a median of 10 days post-transplant in both groups. The median times to platelet engraftment were 9 and 10 days after transplant in the no-immunotherapy and immunotherapy groups, respectively ($p = 0.03$). There was no statistical evidence ($p = 0.61$) of a difference in progression-free and surviving (PFS) at 3 years for patients receiving immunotherapy (53%) compared with no immunotherapy (48%). There was some evidence of superiority in overall survival (OS) at 3 years for patients receiving immunotherapy (83%) compared with no immunotherapy (69%), but the difference between survival curves was not statistically significant ($p = 0.08$). Also, there was some evidence that patients developing acute graft versus host disease (aGVHD) had superior PFS ($p = 0.02$) but not OS ($p = 0.19$) than patients not developing aGVHD. Toxicities were transient and similar between groups, with no treatment-related deaths.

Conclusions. This phase III study of high-risk breast cancer patients randomized to immunotherapy or no immunotherapy demonstrated that a well-tolerated immunotherapy regimen added to aPBSC transplant did not improve PFS, but there was some improvement in OS, but not by an amount that was statistically significant ($p = 0.08$).

Introduction

The role of high dose chemotherapy with peripheral blood stem cell (PBSC) transplantation for the treatment of high-risk breast cancer remains controversial [1–8]. Although *in vitro* data have demonstrated enhanced cytotoxicity with increasing doses of chemotherapy, escalation of dose alone is unlikely to eliminate all malignant cells [9]. Recent clinical trials question the role of high dose chemotherapy and bone marrow transplantation and support an urgent need to develop innovative therapies [10]. Immunotherapy following myelosuppressive chemotherapy provides an attractive modality of non-cross-resistant tumor cell killing in patients with minimal residual disease.

We previously generated activated effector cells *in vitro* with 24 h of incubation of PBSC with interleukin-2 (IL-2). In high-risk breast cancer patients, a clinical syndrome suggestive of cutaneous autologous graft versus host disease (aGVHD) associated with IL-2 activated autologous PBSC (aPBSC) transplantation followed by 4 weeks of low dose IL-2 was observed [11–13].

Because of encouraging preclinical and clinical Phase I and II trial results, a multicenter phase III randomized clinical trial was designed to compare this immunotherapy regimen to a standard transplant regimen [14], involving cyclophosphamide, thiotepa and carboplatin (STAMP V). The primary endpoint was progression-free survival (PFS) and the major objectives were to compare the PFS and overall survival (OS) between the two treatment arms. Secondary objectives included comparison of engraftment and toxicities.

Methods

Patient population, eligibility criteria and staging evaluation

Women between the ages of 17 and 70 years with AJCC 5th Edition Stage II (T1 or T2 with ≥4 axillary lymph nodes involved with disease), Stage III (T3 with involved lymph nodes or T4) or chemotherapy-sensitive or stable Stage IV disease, were eligible. Patients had to have adequate bone marrow, liver and renal function, no history of debilitating cardiac or pulmonary disease, and a Karnofsky performance status of ≥80%. A left ventricular ejection fraction of ≥50% measured by radionuclide scan and pulmonary function tests demonstrating a forced expiratory volume (FEV_1) of $>70\%$ and a diffusion capacity $>60\%$ were needed. No radiographic evidence of malignant central nervous system involvement could exist. Negative serologies for Hepatitis B surface antigen, HIV, and HTLV I, and histological negative bone marrow aspirate and biopsy were required. The protocol received institutional review board approval and all patients signed informed consent.

The initial staging evaluation included a complete physical examination, chest radiograph, bone scan, computer tomographs of the head, thorax, abdomen, and pelvis, electrocardiogram, and audiogram. Laboratory evaluation included a complete blood count with differential and platelet count, coagulation profile, and renal, liver, and thyroid function tests. Patients with measurable disease had baseline tumor measurements by physical examination and/or radiographic studies.

Peripheral blood stem cell (PBSC) collection and harvest

Prior to priming chemotherapy, a central venous catheter was placed. Paclitaxel (300 mg/m^2) was administered as an intravenous infusion over 24 h to mobilize PBSC (Figure 1). Recombinant human granulocyte colony stimulating factor (rhG-CSF) (5 μg/kg) was administered subcutaneously 48 h after priming chemotherapy administration and continued daily until apheresis completion. Complete blood counts were obtained every other day until the start of leukapheresis.

PBSC collection began 24 h after the white blood count (WBC) returned to 1×10^9/l. Cells were collected using a Cobe Spectra Cell Separator (Cobe Laboratories, Lakewood, Colorado) with Anticoagulant Citrate Dextrose–formula A (ACD-A) (Baxter Healthcare, Deerfield, IL). Three to four whole blood volumes (10–20 l) were processed for each collection. Leukapheresis continued until 3×10^6 $CD34^+$ cells/kg and 7.5×10^8 mononuclear cells/kg of body weight were obtained. Clonogenic progenitor cell assays were performed for each collection. Concentrated cell suspensions were cryopreserved in medium consisting of 6% Pentastarch (McGaw, Irvine, CA), 5% DMSO (Cryoserv, Research Industries, Salt Lake City, UT), 4% human serum albumin (American Red Cross, Washington, DC), and either sterile endotoxin- tested bovine pancreatic DNase (Sigma Chemicals, St. Louis, MO) or recombinant human DNase (Pulmozyme, Genentech, San Francisco, CA). Cells were frozen in Cryocyte bags (Baxter Healthcare) and stored in liquid nitrogen.

Interleukin-2 activation of PBSC

IL-2 activation of PBSC has been described previous [15] Briefly, harvested cells were thawed rapidly in a 37 °C water bath and incubated for 24 h. Incubation was in 5% CO_2 at 37 °C in the serum-free medium X-VIVO 10 (BioWhittaker, Walkersville, MD) containing gentamicin (50 ug/ml), L-glutamine, heparin (50 units/ml) (Elkins-Simm, Inc., Cherry Hill, NJ) and IL-2 (6000 IU/ml) (Chiron, Emeryville, CA). Samples were obtained from the thawed cell suspension prior to and following IL-2 activation and analyzed for cell count, viability, Gram stain, immunophenotyping, cytotoxicity assays and sterility. Approximately one third of the PBSC were stored for backup without IL-2 culturing.

Treatment plan

Cyclophosphamide (1500 mg/m^2), carboplatin (200 mg/m^2) and Thiotepa (125 mg/m^2) each were administered intravenously over 2 h for 4 days on days −6 to −3. MESNA (sodium 2-mercaptoethane sulfonate) and hydration were given as prophylaxis for hemorrhagic cystitis. The anti-emetic regimen included ondansetron, lorazepam, and diphenhydramine.

On day 0, the PBSC were infused over 30–90 min at the patient's bedside with cardiovascular monitoring. Patients randomized to no immunotherapy received no further treatment. Patients randomized to immunotherapy received subcutaneous IL-2 (Chiron Therapeutics, California) the same day and continued

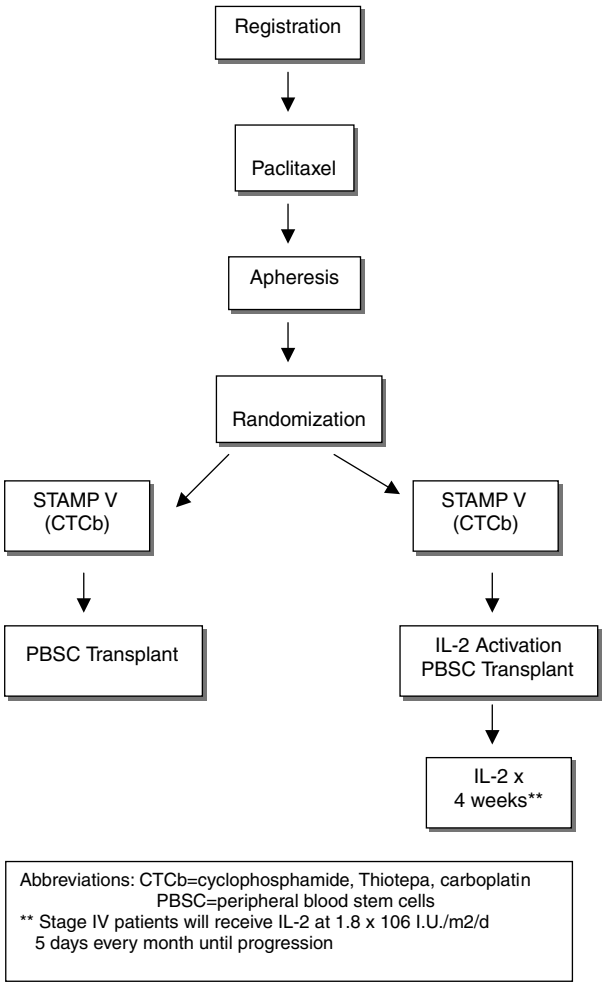

Figure 1. Trial Schema. 30 Patients were randomized to immunotherapy and 29 patients to no immunotherapy. All randomized patients received transplant.

for 4 weeks [11] IL-2 began at 6×10^5 IU/m^2/d, based on previous clinical trials [11,13]. A week of therapy was 5 days of treatment and 2 days of rest. Engraftment was defined as an absolute neutrophil count of $\geq 500/mm^3$ for 3 days and a platelet count of $\geq 20 \times 10^9$/l for 3 days (untransfused).

Toxicities were monitored daily and graded according to CALGB Toxicity Criteria as follows: Grade 0 (no toxicity), Grade 1 (mild), Grades 2 and 3 (moderate), Grade 4 (severe) and Grade 5 (toxic death). IL-2 was held if severe non-hematologic toxicities occurred during therapy. If the toxicities returned to Grade 1 or resolved within 48 h, IL-2 was resumed. If signs and symptoms of the non-hematologic toxicities returned after resolution or failed to improve in 72 h, the IL-2 was discontinued but the patient remained on study.

Evaluation of skin for GVHD

Since prior clinical trials using this immunotherapy regimen demonstrated signs consistent with skin aGVHD, each patient received a daily skin exam by a bone marrow transplant physician during their in-hospital stay. Individuals randomized to immunotherapy treatment at Georgetown University Medical Center (GUMC) additionally were to receive a 4-mm skin punch biopsy prior to initiation of week 3 therapy. All biopsy specimens were graded for the presence or absence of 4 histologic criteria using a scale developed by Horn [16] including basal cell vacuolization, exocytosis, dyskeratotic cells and dermal lymphatic infiltration by a dermatopathologist blinded to the treatment the patient was receiving. Skin biopsy specimens demonstrating ≥3 criteria were considered compatible with aGVHD.

Supportive care

Patients were treated in HEPA-filtered rooms. Routine supportive care was started at the time of admission and included Norfloxacin for gastrointestinal bacterial decontamination, Fluconazole, and Acyclovir (both continued until day 30). With the development of a temperature ≥38.5 °C, broad-spectrum antibiotics were initiated and Norfloxacin was discontinued. Amphotericin B was administered empirically when patients remained febrile after 48–72 h on broad-spectrum antibiotic therapy. All patients received daily rhG-CSF (5 μg/kg/day) beginning on day 5 after transplantation until the absolute neutrophil count (ANC) reached 5.0×10^9/l for 2 days.

In-hospital clinical monitoring

Patients underwent daily physical examination and blood work, including a complete blood count with differential and platelet count, electrolytes, and liver and renal function tests. Packed red blood cells and platelets were administered if the hemoglobin level fell below 8.5 g/dl, platelets dropped below 20×10^9/l, or for symptomatic anemia or bleeding.

Clinical monitoring after discharge

Patients were evaluated weekly in the outpatient clinic for 1 month before returning to their referring oncologist. Evaluation at each clinic visit included a history and physical examination, a complete blood count with differential and platelet count, electrolytes, liver and renal function tests. Response was evaluated 100 days after transplantation by physical examination, bone scan and CT scans of the thorax, abdomen and pelvis. Re-evaluation was performed every 6 months for the first 2 years and yearly thereafter. Patients with inflammatory breast cancer or 4 or more axillary lymph nodes involved with metastatic carcinoma received irradiation of the ipsilateral chest wall, supraclavicular, and axillary areas beginning 3 months post-transplantation (total dose 5040 cGy chest wall). Patients with estrogen receptor (ER) positive tumors started on Tamoxifen (20 mg twice a day) after completion of chest wall irradiation.

Statistical considerations

Design of the study

This randomized, phase III clinical trial was stratified by disease stage (II–IIIA, IIIB, and IV) and conducted at multiple institutions. The trial was designed to determine whether patients receiving additional immunotherapy with subcutaneous IL-2 cells (immunotherapy group) would demonstrate improved PFS at 3 years compared with patients receiving combined therapy and transplant with PBSCs alone (no-immunotherapy group). Originally, 195 patients were to be accrued and followed for 3 years to detect an odds ratio (OR) for PFS of 2.0 or more over all strata with statistical power of 80% at a 5% significance level (two-sided test). In stratum Stage II–IIIA, an OR of 2.0 would mean an improvement in 3-year PFS from 60% for the no-immunotherapy group to 75% for the immunotherapy group, and there would be similar amounts of improvement in PFS in the other stages. Secondary endpoints included OS, hematopoietic reconstitution, and toxicity.

Conduct of the study

Between December 1997 and December 2000, 59 patients were accrued by Georgetown University Medical Center (GUMC) ($n = 45$), University of Massachusetts Memorial Hospital ($n = 8$) and Holy Cross Hospital ($n = 6$). Patients were stratified by stage of disease and randomized equally to the two treatment arms. A biostatistician prepared the randomization list that was concealed from other study personnel. At completion of mobilization treatment, the research nurse provided the patient's disease stage to the biostatistician for randomization. Study personnel and patients were unblinded to treatment after randomization since different procedures were required for immunotherapy treatment. The dermatopathologist was blinded to treatment arm (immunotherapy versus not) for aGVHD ascertainment.

In accord with NCI requirements for phase III clinical trials, an independent Data Monitoring Committee (DMC) was organized, that included one biostatistician and two clinicians. The DMC met in January 1999 and December 2000 and recommended termination of the study following the latter meeting. The major reason for termination was a marked drop in patient accrual following publications questioning the role of transplantation for breast cancer [1,6,17].

Analysis of the study

PFS, OS, and post-progression survival (PPS) curves were calculated using the Kaplan–Meier [18] method. PFS was defined as the time from randomization to progression, death, or date of last follow-up, whichever came first. Overall survival was the time between randomization and date of death or last contact. Patients surviving progression-free were censored at the date of last contact for PFS. PPS, the time between progression and date of death or last follow-up, included only patients who progressed. Survival curves for all patients were compared by the generalized Wilcoxon test [19] since hazard rates were not proportional between groups. Survival curves were also calculated for subsets of patients by stage and by aGVHD without adjustment of p-values since interpretation of outcomes was based upon patients from all stages combined. The combination of treatment and aGVHD effects on PFS and OS was explored with Cox's proportional hazards regression [20]. Standard χ^2 tests were used to compare the distributions of patients and incidence rates (e.g. toxicity) between groups. All statistical tests were two-sided with a 0.05 significance level. Analyses were performed using the 'intention-to-treat' principle. The Haybittle–Peto approach to the analysis of accumulating data was planned with interim tests to be performed at a 0.001 significance level, assuring that the final test would be nearly at the 0.05 level. No interim analyses of efficacy were performed prior to termination of the study.

Results

Patient characteristics

Fifty-nine patients with a median age of 49 years (range 32–66 years) were treated. Thirty patients were randomized to receive immunotherapy and the remaining 29 patients to receive standard aPBSC transplant therapy. Table 1 presents patient demographics and the comparability of the two treatment groups. In addition to stage which was a stratification factor, patients had comparable distributions of age group, ER and PR status, and race.

Engraftment

All patients engrafted, so backup stem cell infusion was not needed. The median number of days required for the absolute neutrophil count (ANC) to maintain $500/mm^3$ for 32 days was 10 days in both treatment groups (range 8–12 days). Platelet engraftment occurred at median of 9 days post-transplant (range 6–22 days) for the no-immunotherapy arm and day 10 (range 6–25 days) in the immunotherapy arm ($p = 0.03$).

Withdrawal from immune therapy

Six of the 30 patients randomized to receive immunotherapy were removed from IL-2 due either to infection ($n = 5$) or patient's desire ($n = 1$). The median duration of IL-2 therapy for these six patients was 14 days (range: 4–21 days).

Progression-free Survival, overall survival, and post-progression survival

The median follow-up time for surviving patients was 56 months. Figure 2a gives the PFS curves by

Table 1. Comparability of patients by treatment Arm

Patient Characteristics	Treatment			
	All N (%)	Immunotherapy N (%)	No Immunotherapy N (%)	*p*-value*
All Patients	59 (100)	30 (51)	29 (49)	
Stage				
II–IIIA	35 (59)	18 (60)	17(59)	
IIIB	13 (22)	6 (20)	7 (24)	
IV	11 (19)	6 (20)	5 (17)	
Age (Yrs.)				>0.99
30–39	10 (17)	5 (17)	5 (17)	
40–49	24 (41)	12 (40)	12 (41)	
50–59	20 (34)	10 (33)	10 (34)	
60–69	5 (8)	3 (10)	2 (7)	
Treatment Site				0.18
LCC	45 (76)	24 (80)	21 (72)	
HCH	6 (10)	1 (3)	5 (17)	
UMA	8 (14)	5 (17)	3 (10)	
ER Status				0.20
+	29 (49)	15 (50)	14 (48)	
–	24 (41)	14 (47)	10 (34)	
UNK	6 (10)	1 (3)	5 (17)	
PR Status				0.21
+	27 (46)	14 (47)	13 (45)	
-	26 (44)	15 (50)	11 (38)	
UNK	6 (10)	1 (3)	5 (17)	
Race				0.17
African-American	7 (12)	2 (7)	5 (17)	
Asian	2 (4)	0 (0)	2 (7)	
Caucasian	48 (81)	26 (87)	22 (76)	
Hispanic	1 (2)	1 (3)	0 (0)	
Other	1 (2)	1 (3)	0 (0)	

LCCC = Lombardi Comprehensive Cancer Center, Georgetown University Medical Center; HCH = Holy Cross Memorial Hospital; UMA = University of Massachusetts Memorial Medical Center; ER = Estrogen Receptor; PR = Progesterone Receptor; UNK = Unknown or Missing.

*The *p*-value is provided to indicate a level of difference between the two groups. However, the interpretation is not based on determining if these differences occurred at random since the study was randomized, so all differences occurred at random.

treatment. Although the PFS curves appeared to separate during the first 18 months, they converged by 24 months and there was no statistical evidence of differing PFS times between groups ($p = 0.61$). The 3-year PFS was 53% (SE = 9%) in the immunotherapy group and 48% (SE = 9%) in the no-immunotherapy group.

Figure 2b gives the OS curves by treatment group and, subsequent to 1 year, the immunotherapy group had superior survival. At 3 years, the OS was 83% (SE = 7%), in the immunotherapy group and 69% (SE = 9%) in no-immunotherapy group. The apparent visual advantage in OS for the immunotherapy group did not achieve statistical significance at the 5% level ($p = 0.08$). Of the 23 deaths on the study, 14 were in the no-immunotherapy group.

Figure 2c gives the PPS curves for progressed patients. The median post-progression follow-up time among surviving patients was 18 months. The 18-month survival post-progression was 62% (SE = 12%) in the immunotherapy group and 44% (SE = 13%) in the no-immunotherapy group. The difference between curves was not significant ($p = 0.13$), and the *p*-value was identical to that in Figure 2b for the OS curves. The apparent difference in OS experience from the time of randomization between the immunotherapy and no immunotherapy arms was due primarily to longer survival post-progression in the immunotherapy group.

Progression-free survival and overall survival based on disease stages

Figure 3a and 3b provide the PFS and OS experience by treatment for the 35 Stage II–IIIA patients, the largest subgroup. While the initial trial design included a stratification by disease stage, the actual number of patients in each disease stage was quite small given the early termination of the study secondary to poor accrual to transplant trials. In Figure 3a, the estimated PFS at

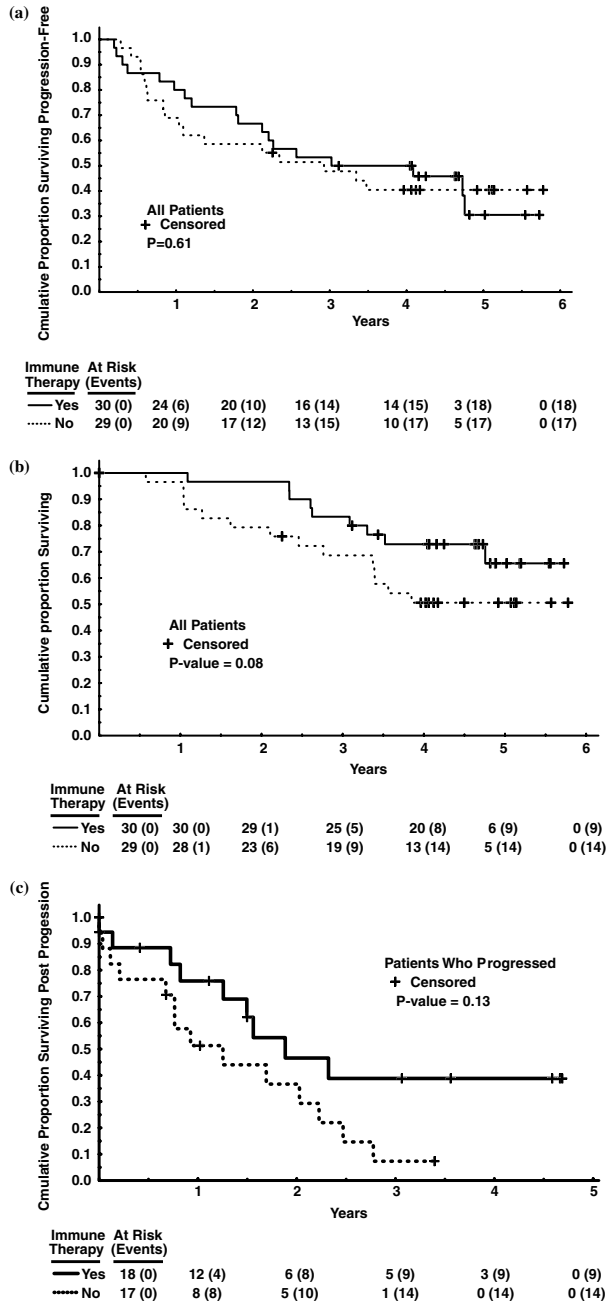

Figure 2. All patients. Progression-free (a), overall (b), and post progression (c) survival for the immunotherapy (solid), and no-immunotherapy (dashed) groups.

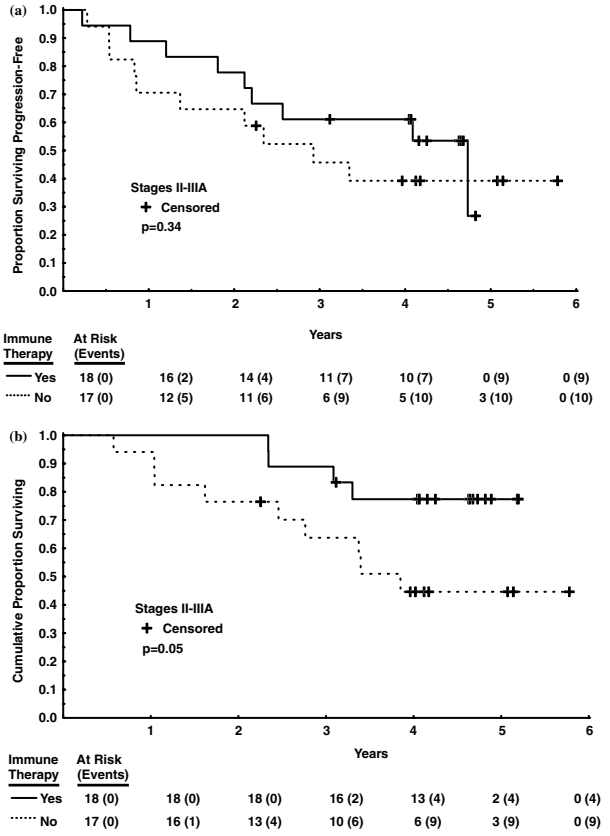

Figure 3. Stages II–IIIA. Progression-free (a) and overall (b) survival for patients with stages II–IIIA in the immunotherapy (solid), and no-immunotherapy (dashed) groups.

3 years in the immunotherapy group was 61% (SE = 11%) compared with 46% (SE = 12%) in the no-immunotherapy group, but the difference between curves was not statistically significant ($p = 0.34$). Figure 3b gives the survival curves by treatment in Stage II–IIIA patients, showing some advantage in survival for patients receiving immunotherapy ($p = 0.05$). At 3 years, 83% (SE = 9%) of patients were surviving in the immunotherapy group compared with 64% (SE = 12%) in the no-immunotherapy group.

The PFS at 3 years for the 13 stage IIIB patients was 50% (SE = 20%) in the immunotherapy group and 43% (SE = 19%) in the no-immunotherapy group ($p = 0.84$). The percent of stage IIIB patients surviving at 3 years was 83% (SE = 15%) in the immunotherapy group and 86% (SE = 13%) in the no-immunotherapy group ($p = 0.91$; curves not shown).

There were only 11 patients with Stage IV disease in the study. Patients with Stage IV disease receiving immunotherapy had 33% PFS at 2 years (33%; SE = 19%) compared to 60% (SE = 22%) in the no-immunotherapy arm, but the difference between curves was not significant ($p = 0.38$). The OS curves were similar for the immunotherapy and no-immunotherapy groups, with 3-year estimates of the percent surviving of 63% (SE = 21%) and 60% (SE = 22%), respectively ($p = 0.87$; curves not shown).

Evaluation of cutaneous aGVHD

Of the 45 patients treated at GUMC, 33 received 4 mm punch skin biopsies upon engraftment, 16 (48%) who were classified as having aGVHD and 17 patients without. There was a higher percent of patients with aGVHD in the no-immunotherapy arm (67%; 8/12) compared to the immunotherapy arm (38%; 8/21), but this difference was not significant ($p = 0.16$). Figure 4a shows a statistically significant advantage in PFS for patients with aGVHD ($p = 0.02$). The 3-year PFS was 75% (SE = 11%) for the aGVHD patients compared with 29% (SE = 11%) for the patients without

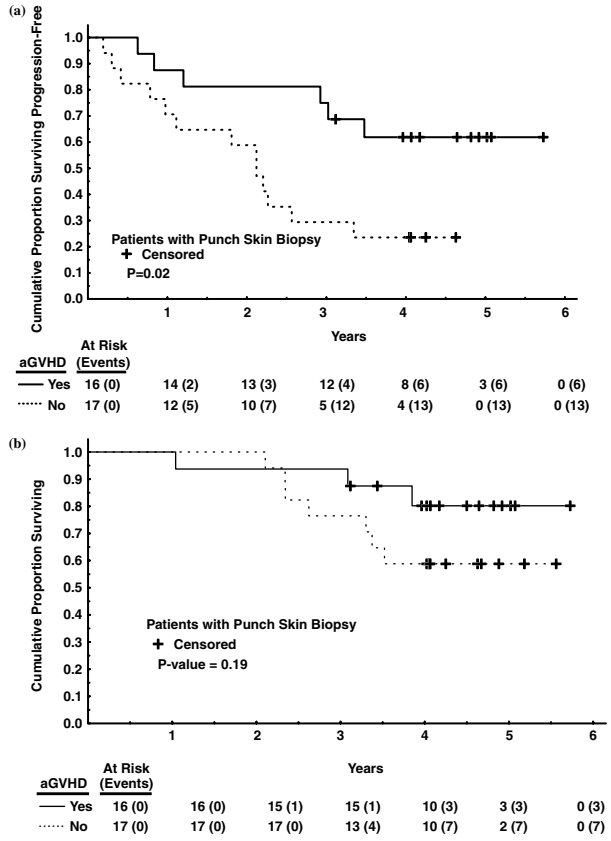

Figure 4. aGVHD. Progression-free (a) and overall (b) survival for patients with punch biopsies with (solid), and without (dashed) aGVHD.

aGVHD ($p = 0.02$). Survival curves for patients who had or did not have aGVHD are shown in Figure 4b. There was no statistically significant difference between the curves ($p = 0.19$), the percent of patients surviving 3 years being 94% (SE = 6%) for the aGVHD patients and 76% (SE = 10%) for patients not having aGVHD.

Cox regression models were fit to both the PFS and OS data including factors for treatment and occurrence of aGVHD (no or yes). In the model for PFS, the type of treatment was not statistically significant ($p = 0.67$), however occurrence of aGVHD was statistically significant ($p = 0.02$). Neither treatment nor aGVHD status was significantly associated with OS in the Cox model. With only 33 patients that were classified for aGVHD, additional studies are needed to confirm the prognostic value of aGVHD.

The incidence of in-hospital moderate (grade 3) or severe toxicities (grade 4)

There were no toxic deaths and all side effects were self-limiting. Toxicities are presented by treatment arm with severity in Table 2. There was no statistical difference in incidence of grades 3 and 4 toxicities between the two groups. No grade 4 non-hematologic toxicities occurred in the immunotherapy arm. Three patients experienced Grade 4 toxicities in the no-immunotherapy arm, consisting of diarrhea, dysphagia, stomatitis, or skin rash.

Cause of death

With the median follow-up time among surviving patients of 56 months, a total of 23 patients died, 9

Table 2. Numbers (and percentages) of patients experiencing moderate (grade 3) or severe (grade 4) toxicities

Toxicity	Immunotherapy N = 30		No Immunotherapy N = 29	
	Grade		Grade	
	3 N (%)	4 N (%)	3 N (%)	4 N (%)
Cardiac	1 (3)	0 (0)	1 (3)	0 (0)
Esophagitis/stomatitis	2 (7)	0 (0)	2 (7)	2* (7)
Flu-like symptoms	4 (14)	0 (0)	2 (7)	0 (0)
GI symptoms	2 (7)	0 (0)	3** (10)	1 (3)
Infection	3 (10)	0 (0)	4 (14)	0 (0)
Liver transaminase	1 (3)	0 (0)	0 (0)	0 (0)
Metabolic abnormalities	4 (14)	0 (0)	3 (10)	0 (0)
Coagulation	0 (0)	0 (0)	0 (0)	1 (3)
Phlebitis/ thrombosis Embolism	0 (0)	0 (0)	1 (3)	0 (0)
Pulmonary	1 (3)	0 (0)	1 (3)	0 (0)
Skin	1 (3)	0 (0)	1 (3)	1 (3)

Metabolic abnormalities = Hyperglycemia, hypocalcemia, hypomagnasemia, or hyponatremia. Flu-like symptoms = Fever without infection, Malaise/fatigue, or pain. GI symptoms = Anorexia, diarrhea, or nausea.

*One patient had grade 4 toxicities for both esophagitis and stomatitis; she is counted only once here, but there are a total of 3 grade four toxicities in this category.

**Two patients each had grade 3 toxicities for both anorexia and nausea. Each patient is only counted once here, but there are a total of 5 grade three toxicities in this category. Grade 4 = severe/life-threatening toxicity.

patients randomized to immunotherapy and 14 patients who were not. Of the patients expiring on immunotherapy, 7 patients died of tumor and 2 patients had unknown cause of death. Of the 14 patients not receiving immunotherapy, 13 died a known tumor-related death.

Discussion

The regimen using IL-2 activation of PBSCs and IL-2 following transplantation demonstrated some improvement in OS compared to standard aPBSC therapy in these high risk breast cancer patients that was not statistically significant ($p = 0.08$). This trial was planned to accrue 195 patients in order to detect a significant difference between the treatment arms (odds ratio for PFS of 2.0 or greater with statistical power of 80% at a 5% significance level). Due to the marked drop in enthusiasm for high dose chemotherapy and stem cell transplant in patients with breast cancer, only 59 patients were accrued before the study termination was recommended by the independent data safety monitoring board. Thus, any conclusions reached from this trial are underpowered, and must be viewed as tentative. There was no difference in PFS between the treatment groups so the difference in OS was due primarily to patients who received IL-2 living longer post-progression than patients without IL-2. For skin biopsy patients, aGVHD was associated with longer PFS ($p = 0.02$), but not OS ($p = 0.19$). Toxicities, aGVHD, and neutrophil engraftment were similar between arms. Platelet engraftment was delayed one day in the immunotherapy arm.

Immunotherapy following aPBSC transplantation has been shown to generate aGVHD in patients with breast cancer, multiple myeloma, lymphoma and acute myelogenous leukemia [11,13,21–28]. In the allogeneic bone marrow transplant setting, the presence of GVHD is associated with a GVT effect, possibly contributing to lower relapse rates [29,30]. The co-existence of an autologous GVT effect among patients experiencing aGVHD remains unknown.

The mechanism of action for the immunotherapy in this protocol differs from other regimens. Researchers at Johns Hopkins have used cyclosporin and γ-IFN post-transplant. It is postulated that the γ-IFN upregulates MHC Class II expression, while the cyclosporin prevents the deletion of auto-reactive lymphocytes. As a result, the auto-reactive lymphocytes recognize and attack the patient's cells with the enhanced MHC Class II expression.

Incubation of hematopoietic cells with IL-2 *in vitro* for 24 h generates cytotoxic effector cells [21,22,26,31]. These activated cells lyse NK-sensitive and NK-resistant tumor cells and result in *in vitro* purging [32,33]. Additionally, infusion of IL-2 activated aPBSC followed by low doses of parenteral IL-2 reduces tumor cell contamination within the graft and generates cytotoxic effector cells that may mediate a GVT effect *in vivo* [34–36].

Prior to implementing IL-2 activation of stem cells in clinical trials, growth and differentiation of hematopoietic progenitor cells had to remain unaffected. When bone marrow or peripheral blood stem cells are activated in IL-2 in long-term (7 days) and short-term (1 days) culture, there is no decrease in colony-forming cells, when analyzed by clonogenic assays [15,36]. A previous Phase I–II Clinical Trial with 61 patients demonstrated that the *in vitro* activation of aPBSC with IL-2 for transplantation followed by the same day parenteral administration of IL-2 did not delay engraftment and was associated with mild to moderate toxicities in Stage II–IV breast cancer patients [13]. In addition, some of these patients demonstrated signs and symptoms of cutaneous aGVHD [12]. The IL-2 activated bone marrow has significant anti-tumor activity *in vitro* and *in vivo* with no detrimental effect on hematopoiesis [35]. Previous breast cancer clinical trials using IL-2 activation of PBSC combined with parenteral IL-2 or the combination of IL-2 with α-IFN post-transplant, revealed no detrimental effect on hematopoiesis or engraftment [12,37].

Forty-eight hours or more of IL-2 incubation of peripheral blood mononuclear cells is required to generate lymphokine activated killer (LAK) cells [15,26,38]. This clinical trial utilized 24 h of IL-2 activation of PBSC to induce cytotoxic effector cells, suggesting that different cells may be involved. Although others have demonstrated the importance of T cell subsets in short-term IL-2 activation, especially $CD4^+$ and $CD8^+$ T cells [38], our results demonstrate that the majority of effector cells generated with 24-h IL-2 incubation are $CD8^+$ T cells, $CD56^+$ NK cells, and possibly, $CD8^+CD56^+$ cells [39]. Recent evidence shows that 5–30% of $CD8^+$ T cells co-express CD56 NK marker [40]. The cytokine-induced killer (CIK) cell, a cell possessing the combination of T cell markers ($CD3^+$) and NK cell marker ($CD56^+$) may also play a role, since the $CD3^+CD56^+$ cells also contributed to cytotoxicity [41,42]

The role of aPBSC transplantation for high-risk breast cancer patients has not yet been clearly defined [43]. Because of publications questioning the role of transplantation for breast cancer, accrual to this trial dropped markedly, causing the investigators to terminate patient accrual early, as recommended by the DMC. Although patients receiving immunotherapy experienced improved OS with median follow-up of 56 months in surviving patients, especially patients with Stage II–IIIA disease, the number of patients is small and there was no evidence of an improvement in PFS. These intriguing results from combining immunotherapy with myelosuppressive chemotherapy should be considered in the design of future clinical trials for high-risk breast cancer patients. Additionally, the relationship of outcome with aGVHD deserves further exploration.

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