Phase I Trial of Adoptive Cell Transfer with Mixed-Profile Type-I/Type-II Allogeneic T Cells for Metastatic Breast Cancer

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Abstract

Purpose: Metastatic breast cancer (MBC) response to allogeneic lymphocytes requires donor T-cell engraftment and is limited by graft-versus-host disease (GVHD). In mice, type-II–polarized T cells promote engraftment and modulate GVHD, whereas type-I–polarized T cells mediate more potent graft-versus-tumor (GVT) effects. This phase I translational study evaluated adoptive transfer of ex vivo costimulated type-I/type-II (T1/T2) donor T cells with T-cell–depleted (TCD) allogeneic stem cell transplantation (AlloSCT) for MBC.

Experimental Design: Patients had received anthracycline, taxane, and antibody therapies, and been treated for metastatic disease and a human leukocyte antigen (HLA)-identical–sibling donor. Donor lymphocytes were costimulated ex vivo with anti-CD3/anti-CD28 antibody–coated magnetic beads in interleukin (IL)-2/IL-4–supplemented media. Patients received reduced intensity conditioning, donor stem cells and T1/T2 cells, and monitoring for toxicity, engraftment, GVHD, and tumor response; results were compared with historical controls, identically treated except for T1/T2 product infusions.

Results: Mixed type-I/type-II CD4+ T cells predominated in T1/T2 products. Nine patients received T1/T2 cells at dose level 1 (5 × 10^6 cells/kg). T-cell donor chimerism reached 100% by a median of 28 days. Seven (78%) developed acute GVHD. At day +28, five patients had partial responses (56%) and none had MBC progression; thereafter, two patients had continued responses. Donor T-cell engraftment and tumor responses appeared faster than in historical controls, but GVHD rates were similar and responders progressed early, often following treatment of acute GVHD.

Conclusion: Allogeneic T1/T2 cells were safely infused with TCD-AlloSCT, appeared to promote donor engraftment, and may have contributed to transient early tumor responses. Clin Cancer Res; 17(21); 6878–87. ©2011 AACR.

Introduction

Metastatic breast cancer (MBC) remains incurable while novel therapeutic targets and investigational agents are identified at an unprecedented pace, with little progress on survival after first-line treatment failure (1). Several immunotherapeutic approaches have activity against breast cancer, including cellular therapies (2); synergy with cytotoxic agents might improve treatment outcomes (3). We have previously reported a clinically relevant graft-versus-tumor (GVT) effect after allogeneic stem cell transplantation (AlloSCT) in MBC, attributable to allogeneic lymphocytes (4). While prior reports suggested possible graft-versus-MBC (5–7), our study design distinguished immune-mediated responses from chemotherapy effects with reduced intensity conditioning (RIC) and T-cell–depleted (TCD) stem cell allografts with delayed donor lymphocyte engraftment.
The clinical benefit of allogeneic lymphocyte therapy for MBC. Large numbers of donor T cells costimulated/expanded ex vivo with anti-CD3/anti-CD28 antibody–coated magnetic beads have been safely administered to patients, including type-II–activated CD4+ T cells (24, 25) and activated DLI (23).

We initiated a phase I clinical study to evaluate donor T1/T2-cell therapy (BB-IND 11720; NCT00082953). Primary aims were to determine the feasibility and safety of administering ex vivo generated donor T1/T2 cells. Secondary aims were to assess the effects of T1/T2 cells on donor T-cell engraftment and tumor response through comparisons with historical control patients treated on a previous trial (NCT00020176). Both trials used the identical AlloSCT regimen except donor T1/T2-cell infusion (4).

Materials and Methods

Patients

Subjects had an eligible, human leukocyte antigen (HLA)-identical (6/6 antigen) sibling donor and MBC with measurable disease for which they had received a taxane, an anthracycline, appropriate targeted therapy (e.g., hormonal agents and/or trastuzumab for receptor-positive and/or HER2-overexpressing tumor) and at least 1 cytotoxic drug for metastatic disease. Eligibility allowed treated central nervous system (CNS) metastases that had not progressed for at least 4 weeks. The National Cancer Institute Institutional Review Board approved this study in 2004; all patients and donors provided informed, written consent. The trial permitted accrual of up to 36 recipient–donor pairs and treatment of 3 patient cohorts with escalating T1/T2-cell doses.

Cell processing

**Donor T1/T2 cells.** Donor peripheral blood mononuclear cells (PBMC) and plasma were collected from 5 to 15 L of whole blood processed (Fenwal CS 3000 Plus blood cell separator, Baxter Healthcare Corporation or COBE Spectra, Gambro BCT, Inc.). A portion of the fresh collection was ACK lysed, incubated with anti-CD3/anti-CD28 antibody–coated magnetic beads at a 3:1 bead/cell ratio (courtesy of C. H. June, University of Pennsylvania, BB-IND 6675), and T-cell enriched (MPC-1, Dynal, Invitrogen Corporation).

Bead-bound cells were seeded in gas-permeable cell culture bags (Lifecell, Baxter) in type-II culture media [X-VIVO 20, Cambrex, with 5% heat-inactivated, filtered autologous (donor) plasma, recombinant human IL-4 (rhIL-4), 1,000 IU/mL, BB-IND 4348, and rhIL-2, 20 IU/mL, Chiron Therapeutics]. Cells were cultured at 37°C in 5% to 7% CO2, humidified incubators for 12 days. Type-II media were added on days 2 and 4 (10×) and as needed (1×) to maintain a concentration of 0.5 × 10^6 cells/mL through at least day 8 of culture. Release criteria included attainment of 5 × 10^6 CD3+ cells/kg recipient weight and standard sterility assays. T1/T2-cell products were cryopreserved in 5 × 10^6 CD3+ cells/kg aliquots in Plasmalyte A (Baxter) with 4% human serum albumin, 5% dimethyl sulfoxide.
Peripheral blood stem cells. Following PBMC collection, donors underwent peripheral blood stem cell (PBSC) mobilization with filgrastim (8 mg/kg, subcutaneous injection, twice a day) and collection with daily apheresis starting on day 5 (Fenwal CS 3000 Plus) until at least 10^6 CD34^+ cells/kg were collected. Donor cells were subjected to a 4 to 5 log T-cell depletion ex vivo, using 1 of 2 methods: a combined positive selection of CD34^+ cells and negative selection of T cells, using CD2-, CD6-, and CD7-targeted antibodies (8), or following modification of institutional cell-processing procedures, a positive selection of CD34^+ cells (CliniMACS CD34 Reagent and Immunomagnetic Selection System, Miltenyi Biotec). A lymphocyte add-back to 1 x 10^5 CD3^+ cells/kg was performed to minimize T-cell dose variability, and allografts were cryopreserved as described.

Study design

Recipients underwent AlloSCT as previously described (Fig. 1; ref. 26). Briefly, patients received one or two 21-day cycles of TLD followed by restaging and a peripheral blood CD4^+ T-cell count. Absent disease progression, patients with circulating CD4^+ count greater than 50 cells/µL received a second cycle of TLD. After the second cycle, or after cycle one, if restaging showed disease progression, patients proceeded to RIC and donor cell infusions, irrespective of the CD4^+ T-cell count. Cyclosporine prophylaxis was begun on day −1. On day 0, donor PBSCs were infused, followed 1 hour later by T1/T2 cells. Filgrastim (10 µg/kg/d) was given from day 0 through neutrophil recovery and cyclosporine was given from day −1 through day +28 (200–300 µg/L), and then, in the absence of acute GVHD, rapidly tapered off over 2 weeks. Patients were monitored for toxicity in-hospital through neutrophil recovery then as outpatients biweekly through day +100 and monthly through day +180.

Donor chimerism was assessed with PCR-based analysis of short tandem repeats (STR) using commercially available probes (Perkin–Elmer Cetus). Bone marrow chimerism was assessed at transplant days +28 and +98, and PBMC chimerism, including CD3^+ and CD14^+ CD15^+ mononuclear cell subsets, was assessed every 2 weeks through day +98.
and at 4, 5, and 6 months posttransplant. The severity of acute and chronic GVHD was graded according to Keystone Consensus Criteria (27) and the International Bone Marrow Transplant Registry Consensus Criteria (28), respectively. During the course of the trial, NIH Consensus Criteria for scoring chronic GVHD (29) were adopted in our institution and are reported when available. Tumor response was assessed with computed tomographic (CT) scanning after each cycle of TLD and at 4 and 6 weeks after day 0, then monthly through 6 months. Serial CT measurements were made by independent study radiologists, and responses were recorded according to Response Evaluation Criteria in Solid Tumors (RECIST; ref. 30). Responses of historical controls were retrospectively assigned according to RECIST to permit comparisons.

Characterization of donor T1/T2 products and recipient peripheral blood lymphocytes

Donor T1/T2 cells and recipient peripheral blood lymphocyte (PBL) populations were evaluated by flow cytometry to quantify CD3+ lymphocytes and CD4+ and CD8+ T-cell subsets. To assess type-I and type-II polarization, T1/T2 product CD4+ and CD8+ T-cell subsets were assessed with intracellular flow cytometry for nuclear expression of transcription factors T-bet and GATA-3 and for IFNγ, TNFα, FoxP3, IL-2, IL-4, IL-10, and IL-13. Cytolytic potential was assessed with intracellular flow cytometry of T-cell subsets for perforin and granzyme expression. Two weeks following donor PBSC and T1/T2 product infusions, recipient PBL was assessed for secretion of IFNγ, IL-2, IL-4, and IL-10 (MACS Cytokine Secretion Assay; Miltenyi Biotec).

Statistical methods

This phase I trial tested feasibility and safety of donor T1/T2 cells. The primary safety endpoint was development of grade II to IV acute GVHD through day +42, coinciding with planned discontinuation of cyclosporine. Secondary endpoints included time to full donor T-cell engraftment, tumor response, and progression-free and overall survival and included comparisons with historical controls who underwent AlloSCT for MBC without T1/T2 cells. Three dose levels were planned, treating up to 12 subjects per cohort; accrual to the subsequent cohort was permitted if no more than 6 patients in a given cohort developed grade II to IV acute GVHD by day +42. An early stopping rule for dose-limiting toxicity specified termination if greater than one third of patients in a cohort experienced unresponsive grade III acute GVHD or if there was any grade IV acute GVHD and/or treatment-related death.

Comparisons between T1/T2 recipients and historical controls included rates of grade II to IV acute GVHD using the Fisher exact test; time to full donor T-cell chimerism using an exact Wilcoxon rank-sum test; and ordered-response categories using the Cochran–Armitage test for trend.

Results

Patient characteristics

Eleven patients with MBC and sibling donors were enrolled on study (Table 1) at dose level 1 (5 × 10^6 T1/T2 cells/kg). Recipient median age was 45 years (range, 37–57) and median duration of MBC was 15 months (range, 5–58). All cancers showed one or more adverse
tern. One quarter of CD4 of polarizing transcription factors followed a similar pattern; T cells, less than 10% expressed T-bet and only 3. Patients had received a median of 4 chemotherapy regimens for metastatic disease (range, 2–7).

**Characterization of donor T1/T2 products**

Ten donors underwent steady-state lymphocyte collection; T1/T2 products were generated from each. T1/T2 products predominantly comprised CD4+ T cells (median, 97%; range, 77–100), with a median CD4/CD8 ratio of 3.41 (range, 1.3–22.7; Table 2). As shown in Fig. 2, approximately 40% of expanded CD4+ T cells expressed IL-2 and IL-13, whereas less than 10% expressed IFNγ and IL-10; none expressed TNFα. Less than 10% of CD8+ T cells expressed IL-2, IFNγ, and IL-13, rare cells expressed IL-4 or IL-10, and none expressed TNFα. Intracellular expression of polarizing transcription factors followed a similar pattern. One quarter of CD4+ T cells expressed T-bet and only rare GATA-3+ cells; FoxP3 expression was similar to T-bet. Within CD8+ T cells, less than 10% expressed T-bet and GATA-3 expression was essentially absent. Examination of intracellular cytolytic protein expression showed that 40% to 50% of CD4+ T cells expressed both perforin and granzyme, whereas only granzyme was expressed in significant numbers of CD8+ T cells.

**Treatment**

Patient number 6 (PN6) voluntarily came off study prior to evaluation of cycle 1 of TLD. In the remaining 10 patients, TLD achieved a median 95% reduction in circulating CD4+ T-cell counts (range, 26–98), from 491 CD4+ T cells/μL at baseline (range, 60–960) to 34 CD4+ T cells/μL after TLD (range, 9–80). PN4 was removed from study prior to RIC due to rapid disease progression resulting in hepatic failure. Nine patients completed RIC and received donor stem cells and T1/T2 cells. Five patients developed acute GVHD prior to completion of cyclosporine taper; 4 patients completed cyclosporine taper and received DLI for disease progression per protocol.

**Toxicity**

**GVHD.** Following administration of T1/T2 cells, no infusion reactions, organ-specific toxicities, or hyperacute GVHD were observed. At hematologic recovery from RIC, 3 patients developed engraftment syndrome requiring steroid treatment (Table 3). Grade II to IV acute GVHD developed in 7 patients (77.9%), 5 occurring on or before day +42; 6 patients required systemic steroid treatment and all responded to therapy. Chronic GVHD developed in 3 of 8 evaluable patients (37.5%). In historical controls, 9 of 18 evaluable patients (50%) developed grade II to IV acute GVHD (P = 0.23; excludes PN4, who died on day +2), of which 3 occurred on or before day +42. Chronic GVHD developed in 8 of 15 evaluable patients (53.3%), of which 4 were “late-acute” GVHD coincident with establishment of full donor T-cell chimerism after day +100 (Supplementary Data).

**Treatment-related mortality.** There was no treatment-related mortality attributable to the T1/T2 cells. A single treatment-related death (sepsis) occurred on day +152 (11.1%); there was 1 death due to disease progression prior to day +100 (Table 3). In historical controls, there were 4 treatment-related deaths (21.1%) and 1 death due to

### Table 2. Donor cell products

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Abbreviations: Allograft/CD34, donor stem cell dose per kilogram recipient weight; allograft/CD3, donor T-cell dose after lymphocyte add-back per kilogram recipient weight; DLI, cell dose as CD3+ cells per kilogram recipient weight and day of administration relative to day 0 (donor stem cell and T1/T2 infusions); T1/T2 cells/dose, donor T1/T2-cell dose per kilogram recipient weight; T1/T2 cells/4/8 ratio, ratio of CD4+ to CD8+ T cells in donor T1/T2-cell products.

aTaken off-study prior to T1/T2 product infusion.

bThe doses for patients 10 and 12 reflect errors in the lymphocyte add-back.
disease progression prior to day +100 (Supplementary Data; refs. 4, 31).

**Engraftment**

All patients engrafted, with rapid hematologic recovery and full donor chimerism (neutrophils > 5,000 cells/µL: median, 10 days; range, 9–11; platelets > 50,000/µL: median, 12 days; range, 8–48, 1 patient remained transfusion dependent). The median time to full donor engraftment was 28 days in circulating T cells (range, 14–84); in historical controls, the median time to full donor T-cell engraftment was 77 days (range, 14–365; P = 0.051, exact 2-tailed Wilcoxon rank-sum test). Donor chimerism in circulating myeloid cells reached 100% in a median of 14 days in T1/T2 recipients and in historical controls (ranges, 14–28 and 14–98, respectively; refs. 4, 8, 31).

**In vivo effects of donor T1/T2 cell infusion**

At day +14 after T1/T2 cell infusion, circulating CD4+ and CD8+ T cells expressed a mixed type-I/type-II cytokine profile, with relatively balanced frequencies of T cells secreting IFNγ, IL-2, IL-4, and IL-10 (Fig. 2). An increase in eosinophils, associated with type-II immune responses and antitumor activity (32–34), was observed in 7 of 9 patients (Fig. 3); absolute eosinophil counts exceeded the upper limit of normal (ULN) by day +42 in all patients who had not received systemic steroids. In some, the increase in eosinophils coincided with tumor response and acute GVHD, as illustrated by the clinical course of PN14 (Fig. 3). Clinical evaluation of a pleural effusion on day +42 (PN12, pleural tumor nodules) permitted analysis of the bloody exudate, in which, 44% of the cells were lymphocytes and 58% of those were CD3+ (75% CD4+ and 20% CD8+). By
Prior to pretransplant TLD, 1 patient had a partial response (PR) and the others had stable disease (SD). By day +28 postinfusion, 5 patients had achieved PR, 4 had SD, and none had progressive disease (PD). In 17 evaluable historical controls, the day +28 responses included 5 PR (4 in whom MBC had responded to pretransplant chemotherapy), 8 SD, and 4 PD (trend toward higher response rate in T1/T2-cell recipients: 89% in the peripheral blood).

In whom MBC had responded to pretransplant chemotherapy (TLD) and day +28 posttransplant; steroids/day and indication, initial systemic steroid administration.

**Discussion**

While allogeneic lymphocytes effectively mediate MBC responses, clinical benefit is limited by GVHD, which is closely associated with donor engraftment and GVT response (4, 8, 9, 11). This first-in-human phase I trial of cytokine-polarized allogeneic CD4+CD8- T-cell transfer established the feasibility of generating mixed-profile donor T cells, which were well tolerated with respect to infusion-related toxicity. Evaluation of this cell product on the same platform used in our earlier MBC trial (host immune depletion, RIC, and TCD allografts) allowed us to explore T1/T2 efficacy with respect to engraftment, GVHD prevention, and antitumor activity.

As expected, costimulated donor T1/T2 cells predominantly comprised activated CD4+ T cells (35); nonetheless, there was also significant expansion of CD8+ T cells. Both CD4+ and CD8+ subsets of the T1/T2 products expressed the type-I transcription factor T-bet, whereas very low frequencies of T cells expressed the type-II transcription factor GATA-3. Cytokine expression in CD4+ cells was more consistent with a mixed Th1/Th2 product, with both IL-2 and IL-13 production and minimal production of other type-I and type-II cytokines. A smaller proportion of activated CD8+ T cells produced cytokines with a similar mixed type-I/type-II pattern. We previously reported while this study was ongoing, that purified CD4+ T cells costimulated with type-II cytokines showed a similar mixed Th1/Th2 cytokine phenotype (24, 25). Recipients of T1/T2 cells also produced a mixed pattern of type-I and type-II cytokines at day +14, suggesting that the infused cell product may have influenced T-cell cytokine production in vivo. Frequent eosinophilia after T1/T2 cell infusion further suggested in vivo type-II effects.

Relative to historical controls (8), T1/T2-cell recipients appeared to have faster donor T-cell engraftment. T1/T2 recipients also appeared to have better disease control than historical controls by day +28. While it is not possible to precisely delineate immunologic versus chemotherapeutic contributions to earlier tumor responses observed in T1/T2 recipients, it is noteworthy that these patients had fewer tumor responses to induction chemotherapy (TLD) than historical controls. It seems plausible that the rapid engraftment observed in T1/T2-cell recipients expedited donor

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Abbreviations: 100% Donor, first day following donor stem cell and T1/T2-cell infusions that sustained full donor chimerism (greater than 90%) was observed in whole-blood, column-separated T-cell (CD3+ and myeloid (CD14+CD15+) cell fractions (MACS; Cell Separation Columns, Miltenyi); TTP, first progression from day 0; survival, from day 0; GI, gastrointestinal; GVHD/acute, day of onset, maximum organ-specific staging and grade of acute GVHD; GVHD/chronic/stage, both IBMTR/NCI Consensus Criteria provided; response, RECIST responses after induction chemotherapy (TLD) and day +28 posttransplant; steroids/day and indication, initial systemic steroid administration.
T-cell infiltration into tumor tissue and contributed to earlier antitumor effects. Our study design to evaluate T1/T2 cells provided a rigorous test for whether T1/T2 cells could separate therapeutic from toxic effects of allogreactivity. The combination of CD34 selection and cyclosporine is a standard regimen to prevent acute GVHD; early cyclosporine taper was used in an aggressive maneuver to elicit GVT responses, exploiting cyclosporine’s differential potency in suppression of regulatory T cells and activated effector T cells, with rapid tapering to unleash allogreactivity (36, 37).

At dose level 1, T1/T2 cells did not reduce the incidence of acute GVHD relative to historical controls, nor improve opportunities to use DLI for antitumor effect. Indeed, the onset of GVHD was prior to cyclosporine taper in one third of patients, with GVHD treatment rapidly followed by cancer progression (4). The timing of T1/T2 administration (day 0) may have contributed to the rate of acute GVHD (24), when lymphopenia from the preparative regimen may have its greatest effect on proliferation and rapid turnover of effector T-cell populations. As postulated in lymphopenia-induced autoimmunity (38), even polarized donor lymphocytes infused on day 0 may undergo rapid expansion and give rise to tissue–antigen-directed effector populations and promote GVHD.

Study enrollment was terminated when 7 patients treated at dose level 1 developed acute GVHD, thereby precluding our ability to evaluate whether higher doses of T1/T2 cells might result in enhanced GVT effects. The patients on this study had very advanced breast cancer with metastatic disease progression on a median of 4 prior chemotherapy regimens; in addition, most patients carried multiple adverse prognostic factors. Furthermore, the current patient

Figure 3. In vivo effects of donor T1/T2-cell infusion. A, percentage change in sum-of-product tumor measurements from baseline (per RECIST) for T1/T2-cell recipients (top) and historical controls (bottom). Black bars denote patients with a tumor response at day ±28; gray bars denote patients with stable or progressive disease at day ±28. The day relative to donor T1/T2-cell infusion is shown on the horizontal axis for both graphs (middle). B, eosinophilia, a type-II inflammatory response, was observed in most patients. The relative percentage change in absolute eosinophil counts (AEC) from baseline at peak are shown for each patient using the left vertical axis; black columns identify patients who received steroids on or before day ±42, gray columns represent patients who did not. Using the right vertical axis, each patient’s peak AEC is shown (‡); the horizontal axis identifies the patient number and day of peak relative to donor T1/T2-cell infusion. The horizontal dotted line indicates the ULN for the AECs. C, in PN14, the rise in eosinophils coincided with early tumor response and the peak with onset of acute GVHD (day ±42). The line graph depicts AECs; the bar graph shows percentage change in sum-of-product tumor measurements from baseline at each time point; the ULN is shown by the horizontal dotted line; the vertical dashed line indicates when systemic steroids were initiated (day ±48).
cohort appeared to be more chemotherapy refractory relative to our previous trial; that is, we observed a lower rate of response to pretransplant induction chemotherapy. It is important to note that this combination of adverse disease characteristics is infrequently represented in CIBMTR-registered trials of AlloSCT for MBC (10). Therefore, it is possible that donor T1/T2 cells might mediate improved tumor control in patients with disease characteristics previously associated with GVT responses, including chemotherapy-sensitive disease, lower tumor burden, and/or bone-only metastases (9, 11). It is also possible that the current approach, whereby T-cell therapy is delayed relative to conventional transplant approaches that do not involve a prolonged course of induction chemotherapy, may have proven disadvantageous in the current patient population. Nonetheless, it seems unlikely that the T1/T2 cells we evaluated would sufficiently address the significant limitations of allogeneic immunotherapy of MBC.

Successful allogeneic immunotherapy for MBC will require a robust immune response and precise discrimination between the therapeutic GVT effect and GVHD toxicity. For allogeneic approaches to improve treatment outcomes in MBC, novel strategies to modulate alloreactivity are essential. The counter-regulatory potential of the type-II immune response appears promising in preclinical murine models. In an ongoing trial in patients with high-risk hematologic malignancies, we are evaluating the clinical potential of using rapamycin to stabilize type-II function in ex vivo polarized T cells (39–41). If early promise is sustained in promoting engraftment and early tumor response in high-risk hematologic malignancies, it would be reasonable to test rapamycin-enhanced type-II donor T cells in MBC.

Harnessing intrinsic regulatory mechanisms of T-cell immune responses may provide sufficient separation of therapeutic (GVT) from pathologic (GVHD) effects to extend the curative potential of allogeneic cell therapy in hematologic malignancies to individuals with MBC. Ultimately useful approaches must be applicable in a broad range of clinical venues; whereas complex study designs may be useful in early-phase trials such as this, the translation of effective cell therapies will require studied modification to increase clinical utility.

Disclosure of Potential Conflicts of Interest

C.H. June has royalties from U.S. Government-owned patents, and patent applications in the field of adoptive immunotherapy. This arrangement is under compliance with the policies of the University of Pennsylvania. No potential conflicts of interest were disclosed by other authors.

Acknowledgments

The authors thank Catherine Chow for her contribution to radiologic response evaluation, our clinical and research staff for their essential contributions and, especially, the patients and their donors and families for their participation and commitment to breast cancer research.

Grant Support

This research was supported by the Intramural Research Program of the Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD.

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Received June 26, 2011; revised September 1, 2011; accepted September 9, 2011; published OnlineFirst September 26, 2011.

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Phase I Trial of Adoptive Cell Transfer with Mixed-Profile Type-I/Type-II Allogeneic T Cells for Metastatic Breast Cancer

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Clin Cancer Res 2011;17:6878-6887. Published OnlineFirst September 26, 2011.

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