Allogeneic hematopoietic stem cell transplantation for metastatic breast cancer

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MICHAEL R. BISHOP

A B S T R A C T

The prognosis is poor and the options are limited for patients with metastatic breast cancer (MBC), especially for those patients who have previously received taxanes and anthracyclines; treatment strategies are primarily palliative. Murine models have demonstrated that allogeneic T cells are capable of eliciting graft-versus-tumor (GVT) effects against breast cancer, inhibiting growth of breast cancer cell lines *in vivo*, providing the rationale to pursue allogeneic adoptive cellular therapy as a strategy to treat MBC. However, the clinical application of allogeneic hematopoietic stem cell transplantation (alloHSCT) was limited by concerns over toxicity and unproven efficacy. The development of non-myeloablative (a.k.a. reduced-intensity) conditioning regimens, which have less treatment-related mortality but preserve the T-cell mediated GVT effects, led to increased investigation of alloHSCT in MBC. Early reports of non-myeloablative alloHSCT indicate that a clinical GVT effect against breast cancer does exist. The responses, observed in 20-40% of patients, appear to be associated with the development of complete donor lymphoid chimerism and may be delayed. In its current form, alloHSCT by itself is unlikely to result in complete eradication of MBC; however, it may serve as a therapeutic platform to complement and enhance the effects of existing cytotoxic therapies and immunotherapies (e.g. trastuzumab), as well as therapies under development (e.g. vaccines). Current data on alloHSCT for MBC should be interpreted cautiously and carefully used for the design of future studies to fully determine the clinical efficacy of this form of adoptive cellular therapy in MBC.

Key words: graft-versus-tumor, reduced intensity, graft-versus-host disease, chimerism, T cells, adoptive cellular therapy

From the Experimental Transplantation and Immunology Branch National Cancer Institute USA.

Correspondence: Michael R. Bishop, MD, Experimental Transplantation and Immunology Branch, National Cancer Institute Building 10, Room 12N226 Bethesda, MD 20892, USA E-mail: mbishop@mail.nih.gov

Metastatic breast cancer (MBC) is a relatively common and clinically significant worldwide health problem; approximately 41,000 women die each year from MBC in the United States alone.[1] The biological characteristics of MBC are extremely heterogeneous and affect the clinical course of the disease.[2,3] Treatment of MBC is based on age, disease-free interval from initial diagnosis, hormone-receptor status, and extent of disease.[4] The goals of treatment are maintenance of quality of life and prolongation of survival, as MBC is almost invariably incurable.[5] Chemotherapy, hormonal therapy, radiotherapy, and limited surgery are all used in the treatment of women with MBC. Almost all women will eventually become refractory to hormonal therapy, necessitating the use of systemic chemotherapy. The two most active classes of chemotherapy against MBC are anthracyclines and taxanes; however, both of these are more commonly being used together as part of adjuvant therapy.[6] A variety of second and third-line chemotherapeutic agents are available, including vinorelbine, gemcitabine, and capecitabine either alone or in combination with biological agents such as trastuzumab. Responses to these therapies are relatively consistent (15-30%), but median survival after their administration is generally less than 24 months.[7]

Hematopoietic stem cell transplantation from both autologous and allogeneic stem cell sources has been employed in the treatment of MBC since the early 1980s.[8] The application of high-dose chemotherapy and autologous hematopoietic stem cell transplantation (autoHSCT) was based on the relationship of dose intensity and response in breast cancer.[9] Phase I and II trials of high-dose chemotherapy and

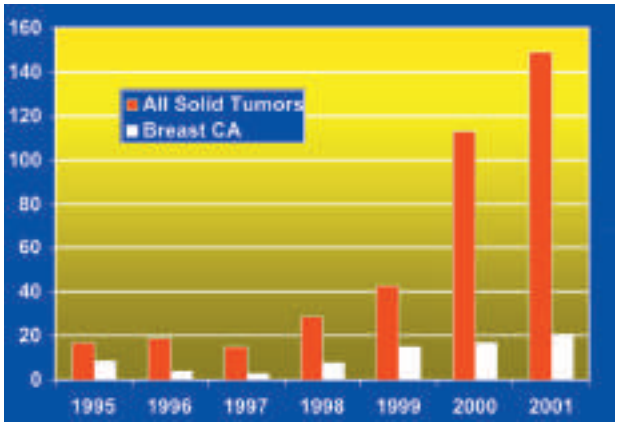

Figure 1. European Blood and Marrow Transplant Group: allogeneic hematopoietic stem cell transplantation for solid tumors activity (courtesy A. Gratwöhl, EBMT).

autoHSCT in breast cancer demonstrated a relatively high response rate for patients with measurable metastatic disease. This appeared to translate into an apparent improvement in survival when these patients were compared retrospectively with similar groups of patients treated with conventional therapy.[10] However, subsequent phase III trials that compared conventional chemotherapy to high-dose chemotherapy and autoHSCT failed to demonstrate a survival advantage for this modality.[11]

There had been limited enthusiasm to investigate allogeneic hematopoietic stem cell transplantation (alloHSCT) in MBC in light of the significant morbidity and mortality generally associated with this procedure. However, the introduction of less intense, non-myeloablative conditioning regimens, which were associated with a significant reduction in treatment-related mortality, led to the investigation of alloHSCT in solid tumors, which previously had been rarely considered for conventional alloHSCT.[12,13] The demonstration of a therapeutic effect of non-myeloablative alloHSCT in metastatic renal cell carcinoma has led to an increase in the investigation of alloHSCT in a variety of solid tumors including MBC (Figure 1).[14] This article reviews the rationale for and early clinical results of alloHSCT as adoptive cellular therapy in MBC.

Rationale for the use of alloHSCT as adoptive immuotherapy for MBC

The graft-versus-tumor effect. A significant part of the curative potential of alloHSCT in hematologic malignancies has been attributed to the reactivity of donor immune cells against host or tumor cell antigens, referred to as the *graft-versus-leukemia* or *graft-versus-tumor* (GVT) effect.[15,16]

The importance of these interactions between immunocompetent donor T lymphocytes and normal and tumor cells of host origin in mediating an anti-tumor response is supported by the clinical observations of an increased incidence of relapse in patients who receive allogeneic hematopoietic stem cell grafts from which T cells have been removed *ex vivo* (i.e. T-cell depletion), an inverse correlation between relapse and severity of clinical graft-versus-host disease (GVHD), and increased incidence of relapse after syngeneic (i.e. identical twin) or autologous hematopoietic stem cell transplants using the same conditioning regimen.[15-20] The most compelling evidence for a cell-mediated GVT effect comes from the observation that the infusion of donor lymphocytes (DLI), distant from the cytotoxic effects of the conditioning regimen, results in the remission of leukemic cells in patients who experienced relapse of disease after a myeloablative alloHSCT.[21-24]

T-cell reactivity against breast cancer

Breast cancer is potentially capable of eliciting tumor-specific responses by cytotoxic T lymphocytes (CTL).[25] However, there has been conflicting evidence regarding the clinical relevance of tumor-specific CTL populations in breast cancer patients. Lymphocytes that have been isolated from metastatic effusions of breast cancer patients are capable of recognizing and lysing autologous and allogeneic tumor cells in a tumor-specific, HLA-A2-restricted fashion.[26] Tumor-specific CTL have been generated from peripheral blood of breast cancer patients using HLA-class I-matched allogeneic breast cancer cells as stimulator cells.[25] Tumor-infiltrating lymphocytes (TIL) taken from breast cancer patients have been demonstrated to secrete cytokines upon interaction with autologous tumor cells, indicating that autologous tumor-reactive lymphocytes may also exist among TIL in breast cancer.[27] These data demonstrate the existence of a cell-mediated response to breast cancer and provide the rationale and impetus to pursue adoptive cellular therapy as a strategy to eliminate breast cancer.

Animal models of a graft-versus-tumor effect against breast cancer

Morecki *et al.* studied the effect of allogeneic adoptive cell therapy on tumor growth in a murine transplant model using the 4T1 mammary carcinoma cell line [H-2d].[28] Inoculation of 4T1 cells into syngeneic mice [BALB/c or (BALB/c X C57BL/6)F1] carrying H-2d histocompatible antigens resulted in lethal tumor colonies in the lungs. Sub-lethally irradiated F1 mice were inoculated with 4T1 cells to simulate minimal

residual disease. Mice then received immunocompetent splenocytes derived from naïve F1 donors, from BALB/c mice that were syngeneic to the tumor but semi-allogeneic to the host, or from C57BL/6 mice that were allogeneic to the tumor and semi-allogeneic to the host. The survival of F1 tumor-bearing mice that were treated with allogeneic C57BL/6 splenocytes was significantly longer than that of mice given F1 or BALB/c-derived splenocytes that were syngeneic to 4T1 tumor cells. These investigators further expanded on their results by testing whether GVT effects could be observed in secondary recipients of adoptively transferred lung cells derived from primary hosts that had previously been inoculated intravenously with 4T1 cells, and then injected with DBA/2 splenocytes immunized with host-derived BABL/c spleen cells.[29] An efficient GVT effect was demonstrated *in vitro* and *in vivo* with MiHC-mismatched DBA/2 splenocytes from mice pre-sensitized by multiple injections of irradiated tumor or BALB/c-derived spleen cells. All of the mice adoptively inoculated with lung cells from primary hosts that had previously been treated with these pre-sensitized effector cells were tumor free for more than 250 days. Secondary recipients inoculated with lung cells from mice given naive BALB/c or DBA/2 spleen cells died of metastatic tumors within 33 to 46 days. These results suggested to the authors that pre-immunized donor cells represent an effective tool against metastatic disease.

The Gress laboratory at the National Cancer Institute (NCI) of the United States investigated GVT effects against breast cancer in a murine model using TS/A, a transforming growth factor (TGF)-β1-secreting murine breast cancer cell line of BALB/c origin.[30] In the setting of disparate (parent into F1) allogeneic bone marrow transplantation (alloBMT), no appreciable GVT was identified. It was hypothesized that TGF-β1 secreted by the tumor might be inhibiting any antitumor response. To test this hypothesis, a TGF-β1 antisense vector was transfected into the TSA breast cancer cell line. Mice were then inoculated with either TGF-β1 antisense-transfected or a mock-transfected cell line and underwent syngeneic or alloBMT. No evidence of GVT was appreciated against the mock-transfected breast cancer cell line in syngeneic recipients; however, there was a statistically significant survival difference between allogeneic versus syngeneic bone marrow transplantation groups inoculated with the TGF-β1 antisense-transfected cell line (p = 0.00001). There was also a significant survival advantage for mice that received alloBMT and TGF-β1 antisense-transfected tumor versus mock-transfected tumor (p = 0.0008). These data suggested that the GVT effect does exist against antisense-transfected breast cancer cells and that TGF-β1 may be involved in suppressing antitumor responses in the setting of alloBMT for breast cancer.

Similary, the Fowler laboratory at the NCI used a murine transplant model to determine whether allo-specific donor T cells of type 2 cytokine phenotype (Tc2/Th2 cells) mediate a GVT effect with reduced GVHD, as compared to allo-specific donor $CD8^+$ cytotoxic T cells of type 1 cytokine phenotype (Tc1/Th1 cells) in the setting of metastatic breast cancer.[31] CB6F1 hosts [H-2b/d] were irradiated (1100 cGy) and then inoculated with the MMTV-breast cancer line, TSA [H-2d]. Mice then received bone marrow either depleted of T cells or with CD3, CD28-co-stimulated donor Tc1/Th1 or Tc2/Th2 cells. A GVT effect against breast cancer cells was observed with allogeneic, but not syngeneic, transplantation with Tc1/Th1 cells, as the median survival time increased from 25.6 to 69.2 days (p <0.0001). In contrast, allogeneic Tc2/Th2 cells mediated a modest, non-curative GVT effect. Tc1/Th1 allogeneic recipients had moderate GVHD that contributed to post-transplant deaths; in contrast, Tc2/Th2 recipients had minimal GVHD. These murine data indicate that immunocompetent cells allogeneic to mammary carcinoma cell lines are able to inhibit tumor development and in some cases eradicate tumor completely in the primary hosts and to prevent tumor growth in the adoptive recipients. Taken together they further suggest that adoptive cellular therapy with allogeneic T cells may provide a potentially clinically relevant GVT effect against human breast cancer.

Clinical data of alloHSCT in MBC

Myeloablative alloHSCT for MBC. There are anecdotal reports of myeloablative alloHSCT providing evidence that clinical GVT effects against MBC possibly do exist.[32-34] Eibl and colleagues reported on a 32-year old woman with metastatic inflammatory breast cancer who received an allogeneic bone marrow transplant from her HLA-identical sibling.[33] The patient received a myeloablative conditioning regimen consisting of cyclophosphamide, thiotepa, and carboplatin. Resolution of the patient's liver metastases was observed simultaneously with the development of clinical GVHD in the first weeks (day +27) after transplant. Cytotoxic T lymphocytes that were grown from the patient recognized host cells, but not HLA-identical donor cells. Recognition was restricted to major histocompatibility complex (MHC) class I antigens. In addition, minor histocompatibility antigen (MiHA)-specific and MHC class I antigen-restricted cytotoxic T cells recognizing breast carcinoma target cells were isolated from the blood of the patient. Similarly, Ueno and colleagues treated 10 MBC patients with high-dose chemotherapy (cyclophosphamide, carmustine,

and thiotepa) and allogeneic blood stem cell transplantation.[34] Shortly after transplantation, one patient achieved a complete remission, five achieved partial remissions, and four had stable disease. In two patients metastatic liver lesions regressed with the onset of acute GHVD, suggesting a GVT effect.

At the 2003 Annual Meeting of the American Society of Clinical Oncology a retrospective analysis of 39 patients with MBC who had received a myeloablative alloHSCT was presented by the International Bone Marrow Transplant Registry (IBMTR) and the European Group for Blood and Marrow Transplantation (EBMT).[35] The most commonly used myeloablative conditioning regimen consisted of cyclophosphamide, thiotepa, and BCNU. The complete and overall responses were 10% and 30%, respectively. Overall survival two years post-transplant was approximately 20%.

Or and colleagues investigated allogeneic adoptive cellular therapy in six patients with metastatic breast cancer.[36] The patients' disease was initially cyto-reduced with high-dose chemotherapy and autoHSCT. Patients were subsequently treated with HLA-matched DLI activated *in vivo* with human recombinant interleukin-2 at the time of disease progression or, if no evidence of disease, four months after autologous transplant. Following DLI, chimerism studies failed to demonstrate any evidence of donor cell engraftment. Two patients developed signs and symptoms compatible with GVHD. However, clear evidence of a GVT effect against breast cancer was not demonstrated. The authors concluded that techniques to boost chimerism without inducing GVHD were indicated.

Reduced-intensity and non-myeloablative alloHSCT for MBC

There have been approximately 60 cases reported in either manuscript or abstract form on the use of non-myeloablative alloHSCT.[37-45] Bregni and colleagues in Milan treated six patients with advanced metastatic breast cancer with non-myeloablative alloHSCT using a conditioning regimen consisting of thiotepa, cyclophosphamide and fludarabine.[38] All six patients experienced disease progression at a median of 69 days after transplantation. In order to induce a GVT effect, five patients received DLI after disease progression. Two partial responses occurred after the DLI; responses were not observed until four and two months after DLI, respectively. One patient died approximately 9 months after her response to DLI from progressive disease. The other patient was alive at the time of this report, and her disease has remained in remission for approximately six months since her response. The observation of responses to DLI following disease progression post-transplant provides further support that a clinically relevant GVT effect against breast cancer exists.

In June 2002 an international symposium on alloHSCT for solid tumors was held in Milan, Italy.[39-42] Of particular interest was a report on a novel approach to non-myeloablative alloHSCT taken by Carella and colleagues in San Giovanni Rotondo, Italy.[40] In this report 14 patients with heavily treated MBC first received high-dose therapy and autoHSCT to achieve maximal tumor reduction and provide significant immune suppression in the host (i.e. patient) before proceeding to a non-myeloablative alloHSCT. This tactic intended to provide the benefits of a conventional alloHSCT, but without the typical acute toxicities and associated mortality. There were no treatment-related deaths. Eight patients achieved either a clinical response (n = 5) or stabilization of disease. The median survival for all 14 patients was 539 days from the time of alloHSCT.

Based upon murine models, the NCI initiated a study investigating the use of allogeneic T cells as a form adoptive cellular therapy in patients with advanced breast cancer.[30,31,44] The specific aim of this trial was to determine whether a clinically relevant GVT effect can occur against MBC. In order to differentiate whether observed responses were due to the cytotoxic effects of chemotherapy in the conditioning regimen or to a GVT effect, the allografts were first T-cell depleted. Forty-two days after transplantation, patients were assessed for initial response; responses observed at this time were attributed to chemotherapy. Patients then received monthly, dose-escalated DLI starting on day +42 post-transplant. Subsequent responses were attributed to a true GVT effect. Twenty-four patients have been accrued to this ongoing trial, and fifteen patients are evaluable for response. There have been five partial responses (>50% reduction in overall tumor size) and three minor responses (>25-49% reduction). Among these eight patients, six responses were directly attributed to a GVT effect.

The IBMTR/EBMT report presented at the 2003 Annual Meeting of the American Society of Clinical Oncology also included 36 patients with MBC who received a non-myeloablative alloHSCT.[35] The non-myeloablative conditioning regimens most commonly used in this patient population were fludarabine-based in combination with an alkylating agent. The 100-day treatment-related mortality incidence was 6%. Complete and overall responses rates were 10% and 30%, respectively, identical to results with myeloablative conditioning. Overall survival two years post-transplant was approximately 25%.

Summary

Over the past five years alloHSCT has been increasingly investigated as a treatment for advanced MBC.

Table 1. Selected studies of allogeneic hematopoietic stem cell transplantation for metastatic breast cancer.

Investigator (Reference#)	*Patient Number*	*Conditioning Regimen*	*Tumor Response*	*Survival*
Bishop *et al*. National Cancer Institute, Bethesda, USA[44]	16	Fludarabine + Cyclophosphamide*	Partial responses = 5 Minor responses = 3	Median overall = 11 months Median progression-free = 3 months
Bregni *et al*. Istituto H San Raffaele, Milano, Italy[38]	6	Thiotepa + Fludarabine + Cyclophosphamide*	Partial responses = 2	3 alive (range: 417 - 1003 days)
Carella *et al*. San Giovanni Rotondo, Italy[40]	14	HDT/AutoHSCT♦ Fludarabine + Cyclophosphamide*	Clinical remissions = 4 Partial response = 1	Overall = 57%
Blaise *et al*. France, 8 institutions[41]	10	Fludarabine + Busulfan + ATG*	Partial response = 1	Not specified
Ueno *et al*. M.D. Anderson Cancer Center, Houston, USA[45]	8	Fludarabine and Melphalan*	Complete responses = 2 Minor response = 1	Overall = 75% at 1 year
IBMTR/EBMT[35]	75	Myloablative = 39 Non-myeloablative = 36	Complete responses = 6 Partial responses = 18	Not specified

**: non-myeloablative/reduced-intensity; HDT: high-dose therapy; AutoHSCT: autologous hematopoietic stem cell transplant; IBMTR: International Bone Marrow Transplant registry; EBMT: European Blood and Marrow Transplant Group.*

There is a growing amount of data that a true GVT effect exists against breast cancer after alloHSCT. Response rates vary (Table 1), but they are estimated to be approximately 25-40%, depending on the response criteria that are used. In the overwhelming majority of studies, responses were not observed until complete donor lymphoid chimerism had been established and responses were often delayed for periods up to several months.[38,44,45] At this time it is not known whether the lack of a response in certain patients reflects resistance of advanced breast cancer to cellular therapy or whether the bulk or proliferation of advanced breast cancer exceeds any potential benefit of an immunological effect. It is more than likely that it is a combination of the two, and it will be necessary to perform additional studies to determine whether the effects of alloHSCT could be more efficacious in a minimal residual disease state or earlier in the disease course before such resistance mechanisms develop.

It is unlikely that alloHSCT, in its present form, will be able to result in complete eradication of MBC by itself. It is more likely that this therapy will be used to enhance the effects of currently available treatments and immunotherapies such as trastuzumab, angiogenesis inhibitors, and vaccines.[46-51] There are murine data suggesting that conventional chemotherapeutic agents commonly used to treat MBC, such as paclitaxel, can actually enhance the effects of immunotherapy.[52] Similarly, it has been demonstrated that a cellular component can enhance the effects of monoclonal antibodies, such as trastuzumab, directed against HER2-neu.[46,47] Breast cancer expresses several unique antigens or over-expresses common antigens which are excellent vaccine targets. AlloHSCT provides a unique clinical research setting to enhance the effects of these vaccines in patients with MBC.[48,49] Recent murine data suggest that the administration of an oral DNA vaccine encoding murine vascular endothelial growth factor (VEGF) receptor 2 (a.k.a. FLK-1) resulted in T cells that specifically targeted endothelial cells in tumor vasculature that over-expressed VEGF receptors.[49] This vaccination strategy resulted in the regression of established solid tumors and was mediated by $CD8^+$ T cells. This effect could potentially be enhanced in the allogeneic setting.[51]

It remains unknown whether the responses observed after alloHSCT for MBC translate into improved survival. In the light of the extremely poor prognosis of MBC, the need for new therapies, and the suggestion of a GVT effect even in patients with chemo-refractory disease, it seems relatively reasonable to continue investigations on whether alloHSCT can make a significant clinical impact on this disease. However, such investigations need to be performed carefully in well

designed clinical trials. Appropriately, the results from the initial trials are being reported cautiously. The Accreditation Sub-Committee of the European Blood and Marrow Transplant Group has recommended that allogeneic hematopoietic stem cell transplantation for breast cancer only be performed using HLA-matched sibling donors and under the auspices of well designed developmental and pilot trials at specialized transplant centers that have a specific interest in this disease.[53] These recommendations seem very reasonable, as we hope that ongoing and future studies will provide relevant data on the specific role that alloHSCT could play in the treatment of metastatic breast cancer.

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