Nonmyeloablative stem cell transplantation as immunotherapy for kidney cancer and other metastatic solid tumors

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Abstract

Over the past few decades, great strides have been made to advance the field of allogeneic hematopoietic stem cell transplantation. The donor immune mediated graft-vs-tumor effect that follows the procedure is now widely accepted as the most effective form cancer immunotherapy available for patients with a variety of advanced hematological malignancies. Recognition that a transplanted immune system could cure patients with treatment refractory leukemia led to the development of 'low-intensity' conditioning regimens, which have improved the safety of the procedure and broadened the application of allogeneic immunotherapy to a growing list of neoplastic diseases. Here we discuss the investigational use of allogeneic transplantation as immunotherapy for patients with metastatic, treatment-refractory solid tumors.

Introduction

For decades now, steady albeit slow progress in the treatment of patients with metastatic genitourinary malignancies has been made. During this time, researchers have sought to investigate whether immunotherapy might play a complementary role to surgery, radiotherapy and chemotherapy in the treatment of patients with advanced metastatic kidney cancer. Although only a minority of patients have responded to immunotherapy based approaches using cytokines (i.e. interleukin-2, interferon-alpha) or the adoptive infusion of tumor-infiltrating lymphocytes (TIL), proof of concept of the unusual vulnerability of kidney cancer to immune attack has been established (Rosenberg et al., 1994; Yannelli et al., 1996).

For about 2 decades investigators have appreciated that anti-leukemia effects are generated by donor immune cells transplanted following allogeneic stem cell transplantation for hematological malignancies (Weiden et al., 1979, 1981; Fefer et al., 1987; Goldman et al., 1988; Sullivan et al., 1989a). An increased

appreciation of the power of this immune effect has recently led investigators to initiate pilot trials of allogeneic transplantation in patients with advanced renal cell carcinoma and other metastatic solid tumors, predominantly based on the hypothesis that a graft versus tumor (GVT) effect, analogous to the graft-vs-leukemia (GVL) effect seen in hematological malignancies, might be generated against metastatic cancer following the transplantation of an allogeneic immune system. The relatively recent observation of regression of metastatic RCC following allogeneic blood cell transplantation has established that RCC, besides being susceptible to treatments that boost innate immunity, may also be a target for attack by allogeneic immune cells (Childs et al., 2000a). Here we discuss the rationale, design and results of pilot trials applying allogeneic immunotherapy to patients with treatment refractory metastatic kidney cancer and other treatment refractory solid tumors.

Allogeneic bone marrow/blood cell transplantation

Allogeneic bone marrow/hematopoietic blood cell transplantation (HCT) has been utilized as a clinical modality to treat patients with hematological malignancies for more than 30 years. Allogeneic HCT was first introduced as a treatment that allowed the use of 'super' high-dose chemo-radiotherapy in an attempt to eradicate all of the underlying leukemia. The function of the transplanted bone marrow or peripheral blood stem cells was to restore hematopoietic function which had been ablated following dose intensive conditioning (Thomas et al., 1975). In 1956, Barnes and colleagues first suggested the existence of donor immune mediated 'graft-versus-tumor' (GVT) effect from a murine model in which mice were cured after receiving allogeneic rather than syngeneic marrow (Barnes et al., 1956). However, it was much more difficult to conclusively demonstrate that GVT effects occurred in humans after this procedure. In the late 1970s and 1980s, a number of observations began to provide evidence that clinically meaningful and sometimes curative immune effects could be mediated through the transplanted donor's immune system against cancer (Weiden et al., 1979, 1981; Fefer et al., 1987; Goldman et al., 1988; Sullivan et al., 1989a, b; Horowitz et al., 1990; Gale & Horwitz, 1990). Weiden and colleagues observed that in patients with leukemia the relapse rates after HCT were significantly lower in patients who had a history of graft-versus-host disease (GVHD), the clinical sequelae of donor immune cells attacking normal host tissues (Weiden et al., 1979). Subsequently, other studies found that more than 60% of chronic phase CML patients and patients with AML in first remission relapsed after a syngeneic (monozygous twin donor) HCT compared with only a 10–20% relapse rate for HCT recipients of allogeneic (HLA-identical sibling) marrow (Fefer et al., 1987). It was inferred that GVT effects were being generated against minor histocompatibility antigens (disparate between patient and donor) and/or tumor-associated antigens expressed on the leukemia.

Evidence for GVT was also inferred by the slow disappearance of minimal residual CML post-HCT, often requiring 6–12 months to occur in the setting of tapering GVHD prophylaxis (Lee et al., 1992; Beguin et al., 1996; Suzuki et al., 1997; Mehta et al., 1997). These findings culminated in investigators treating patients with relapsed leukemia after allogeneic transplant with infusions of donor lymphocytes. In 1990, Kolb and colleagues treated 3 patients with relapsed

CML with donor lymphocyte infusions (DLI), all of whom subsequently achieved a complete cytogenetic remission. Follow-up data has shown that 60–70% of such patients can be anticipated to achieve a durable molecular remission following DLI therapy. Data obtained from patients cured following treatment with DLI has established allogeneic HCT to be the most potent form of immunotherapy available for patients with hematological malignancies (Kolb et al., 1995; Mattei et al., 2001; Baron & Beguin, 2000).

Mechanism of graft versus tumor (GVT) effect

The mechanism of GVT, although not entirely understood, is mediated through donor T-cells and NK cells (Barrett, 1997). Animal and clinical studies have demonstrated that donor CD8+ cytotoxic T cells and CD4+ T-helper cells are most likely the primary mediators of this effect (Truitt & Atasoylu, 1991; Appelbaum, 2001). CD8+ and CD4+ cells recognize antigenic peptides expressed on the surface of target cells bound to class I or class II MHC molecules respectively. These peptides may be of several varieties: (a) minor histocompatibility antigens (MHA) derived from proteins that differ between patient and donor due to polymorphisms in their genome (allo-antigens) (Goulmy, 1997; Warren et al., 1998a, b); (b) tumor peptides derived from mutated proteins resulting from mutations or translocations in genes specific for the malignancy (Gambacorti-Passerini et al., 1997); (c) normal proteins that are over-expressed on tumor cells (Ohminami et al., 2000; Oka et al., 2000). Although the latter two categories are not allo-antigens, it is possible that immune cells from the donor may illicit an immune response to these antigens based on a higher level of expression on target cells (Gambacorti-Passerini et al., 1997; Ohminami et al., 2000; Oka et al., 2000). In addition to T-cells, cytokines, antibodies and other cellular immune populations may play a role in mediating GVT effects. NK cells are particularly effective against AML cells following T-cell depleted haplo-identical transplantation in patients receiving allografts that have disparity between immunoglobulin like receptors (KIR) on donor NK cells with their HLA class I ligands (KIR ligands) expressed on leukemic cells (Ruggeri et al., 2002). Gamma-delta T-cells and cytokine-activated killer lymphocytes may also mediate anti-tumor effects (Lamb Jr et al., 1996; Morecki et al., 1997). Finally T-cells may also recruit non-specific effector cells like macrophages, NK and neutrophils to

kill tumor cells (Jiang et al., 1993; Asai et al., 1998; Ruggeri et al., 1999).

Myeloablative versus non-myeloablative regimens

Myeloablative allogeneic bone marrow transplantation has traditionally used high doses of chemotherapy with or without total body irradiation (TBI) to condition patients for transplantation (Thomas et al., 1975). Unfortunately, the high toxicity profile and 25–35% risk of mortality associated with the procedure has limited HCT to patients younger than 55 years and to those in good medical condition. To minimize regimen related toxicity while conserving GVT effects, reduced intensity ‘nonmyeloablative’ conditioning regimens have recently been developed (Table 1). By definition, a nonmyeloablative regimen does not eradicate host hematopoiesis, consequently allowing autologous bone marrow recovery to occur (Champlin et al., 2000). Furthermore, after engraftment, mixed chimerism (both patient and donor cells detectable) is typically present for a variable period of time (Childs et al., 1999) (Figure 1). Nonmyeloablative transplants (NST) appear to be associated with reduced infectious complications as a consequence of the neutropenic period being shortened because of autologous bone marrow recovery (Junghanss et al., 2002).

Nonmyeloablative preparative regimens are designed to provide enough immunosuppression to achieve donor engraftment and prevent rejection while minimizing toxicity. Unlike a myeloablative transplant, NST approaches use conditioning regimens that are not primarily designed to cyto-reduce malignant cells. Rather, the GVT effect which follows the approach is intended to eradicate the underlying malignancy. A variety of innovative NST approaches are currently being investigated in clinical trials in humans (Table 1) (Childs et al., 1999; McSweeney et al., 2001; Slavin et al., 1998; Giralt et al., 2001; Pedrazzoli et al., 2002a; Kottaridis et al., 2000). Nonmyeloablative regimens are generally well tolerated, even in older patients (McSweeney et al., 2001). The engraftment capacity of such regimens are directly impacted by the intensity of immunosuppression achieved by each individual conditioning regimen. In general, most regimens are associated with a less than 10% risk of graft rejection (McSweeney et al., 2001; Slavin et al., 1998).

Transplant related mortality following this approach is reported to range between 7.5–20% and is

impacted by variables such as the patient’s age, performance status, underlying malignancy, and type of donor used i.e., HLA-identical versus mismatch versus unrelated donor (McSweeney et al., 2001; Giralt et al., 2001). The incidence and severity of acute GVHD varies among regimens and remains the most important cause of non-relapse mortality following this approach (Table 2). Importantly however, curative GVT effects have been demonstrated against a variety of chemotherapy refractory hematological malignancies following NST.

NST for solid tumors

GVT effects were initially investigated and described in hematologic malignancies. In 1984, Moscovitch and colleagues published one of the first studies demonstrating the existence of graft versus tumor effect against a solid tumor in a murine model (Moscovitch & Slavin, 1984; Slavin et al., 2000). One of the first reports in a human of a GVT effect against a tumor of epithelial origin noted the incidental regression of a metastatic breast adenocarcinoma lesion following allogeneic HCT for relapsed acute myelogenous leukemia (Ben-Yosef et al., 1996).

Subsequently, reports of patients having regression of metastatic breast carcinoma which occurred concomitant with the onset of acute GVHD following a conventional myeloablative transplant were described by others (Eibl et al., 1996; Ueno et al., 1998). Unfortunately, the high risk of transplant related mortality associated with myeloablative allogeneic HCT largely precluded investigators from proceeding with systematic studies for GVT effects in other solid tumors. NST, a procedure associated with a reduced risk of regimen related morbidity and mortality, provided transplantors with the vehicle required to more safely conduct such trials.

Results by tumor

Renal cell carcinoma (RCC)

Metastatic RCC seemed an attractive initial target to investigate for GVT effects following NST given its refractoriness to chemotherapy (Motzer & Russo, 2000) and its proclivity for being susceptible to immune based therapies such as IL-2 or interferon-alpha (Rosenberg et al., 1994).

Table 1. Nonmyeloablative regimens under investigation

Reference	Conditioning regimen	Number of patients	Median age (yr)	Disease	TRM (%)
Giralt et al., 2001	FLU + MEL	86	52	CML, AML, NHL	37
McSweeney et al., 2001	TBI + FLU	45	56	CML, AML, CLL, ALL, NHL, HD, MM	4
Kottaridis et al., 2000	FLU + MEL + C1H	44	41	CML, AML, CLL, ALL, NHL, HD, MM	9
Childs et al., 2000a	FLU + CY	19	48	Solid tumors, and hematological diseases	11
Slavin et al., 1998	FLU + BU + ATG	26	31	CML, AML, NHL	15
Pedrazzoli et al., 2002a	FLU + CY	17	43	Solid tumors and hematological malignancies	N.R.

FLU: Fludarabine; MEL: Melphalan; TBI: total body irradiation; C1H: campath; BU: Busulfan; CY: cyclophosphamide; CML: chronic myeloid leukemia; ALL: acute lymphocytic leukemia; AML: acute myeloid leukemia; NHL: non-hodgkin's lymphoma; MM: multiple myeloma; HD: Hodgkin disease; CLL: chronic lymphocyte leukemia; N.R.: not reported.

Table 2. Acute and chronic GVHD

Reference	Acute GVHD Grade 2–4(%)	Acute GVHD Grade 3–4(%)	Chronic GVHD (%)
Giralt et al., 2001	49	29	68
McSweeney et al., 2001	47	11	74
Kottaridis et al., 2000	4	0	2
Childs et al., 2000a	53	16	21
Slavin et al., 1998	46	23	42
Pedrazzoli et al., 2002a	29	12	NR ^a

^a NR: Not reported.

In 1997, the National Institutes of Health (NIH) initiated a clinical protocol investigating NST in patients with advanced, cytokine refractory RCC (Childs et al., 2000a, b). Eligible patients were 18 to 75 years old with radiographically evaluable and progressive metastatic RCC who failed prior immunomodulatory therapy. Patients were required to have an HLA-identical sibling to serve as a blood stem cell donor. The preparative regimen consisted of IV Cyclophosphamide given as 60 mg/kg on days –7 and –6, Fludarabine 25 mg/m given on days –5, –4, –3, –2, –1, followed by infusion of a T-cell replete G-CSF mobilized PBSC allograft from the HLA matched donor. Cyclosporine alone or in combination with mycophenolic acid or with low-dose methotrexate was used as GVHD prophylaxis. To enhance the chances of achieving a GVT effect, we sought to achieve rapid and complete donor T-cell chimerism by early withdrawal of post-transplant immunosuppression followed by DLI infusion if necessary (Figure 2).

While NST remains an investigational approach for the treatment of advanced RCC, several groups including our own have observed convincing evidence demonstrating the susceptibility of this tumor to a GVT effect. Ten of the first 19 patients and subsequently 23 of the first 58 patients (40%) treated had radiographic evidence of disease regression consistent with a GVT effect. A minority of responders achieved a complete response including the first treated patient who remains in remission 5 years after transplantation. Although regression of metastasis has been observed in multiple metastatic locations, pulmonary responses have occurred most frequently (Figure 3). At present, responses have only been observed in patients with the clear cell histologic subtype of RCC. The median time to a disease response has been 5 months (range 1 to 15 months) with disease regression typically not occurring until the withdrawal or tapering of CSA in the setting of full donor T-cell chimerism. Patients with a prior history of acute GVHD were four times more likely to achieve a disease response than those who

never had GVHD. Approximately 59% of patients ultimately developed grade II-IV acute GVHD including 4 patients who died from GVHD related complications. The probability of transplant related mortality following this approach is age related and ranges from 0% in patients less than 40 years of age to 7 and 20% in patients between the ages of 40–49 and 50–59 years, respectively (unpublished data).

Several other groups have reported responses in patients with metastatic RCC undergoing NST using a variety of conditioning regimens (Table 3) (Rini et al., 2002; Pedrazzoli et al., 2002a; Bregni et al., 2002; Sandmaier et al., 2002; Blaise et al., 2002). Acute GVHD remains the major toxicity associated with this approach across all regimens. Proof of concept of the susceptibility of RCC to a GVT effect, has clearly been established and will likely lead to the development of second generation transplant trials. These forthcoming trials will focus on methods to decrease the incidence of acute GVHD while enhancing the efficacy GVT through post-transplant vaccination strategies or *in vitro* tumor 'primed' DLI that target the donor immune system specifically to the tumor.

Melanoma

A significant amount of *in vitro* and *in vivo* data support the susceptibility of melanoma to immune attack (Rosenberg et al., 1994; Melioli et al., 1994). As a consequence, we and others initiated similar trials of NST for patients with metastatic melanoma (Childs et al., 2000b). From November 1997 to June 2001, 12 patients were treated using the same approach employed for RCC. Although several responses were observed, they occurred in proximity to the conditioning regimen, were non-durable, and were likely the result of a chemotherapeutic effect of the conditioning regimen. Unfortunately, we observed long-term survivors with the vast majority of patients succumbing to rapid disease progression within 4 months of the procedure. These disappointing results may be attributed at least in part to advanced patient disease and rapid tumor kinetics in which death occurs before a GVT effect could be induced. The high risk of death from rapid disease progression discourages this approach and highlights the need for alternative investigational immune based strategies for treatment-refractory melanoma.

Breast

In 1996, Eibl et al published a case report of a 32 year old woman with metastatic inflammatory breast cancer who received an allogeneic BMT (Eibl et al., 1996). Resolution of metastatic lesions occurred simultaneously with acute GVHD. Cytotoxic minor antigen reactive T-cells, expanded from blood collected at the time of GVHD, were shown to kill partially HLA matched breast cancer cell lines *in vitro* consistent with the patient having had a GVT effect.

Subsequently, Ueno and colleagues reported their results of 10 patients with metastatic breast cancer that were treated with high dose chemotherapy followed by allogeneic BMT (Ueno et al., 1998). Remarkably, after progressing in the first months after the transplant, two patients had a late partial response associated with discontinuation of immunosuppression consistent with a GVT effect. Recently, Bregni and colleagues published their experience with 6 patients with advanced breast cancer treated with a Thiotepa/Fludarabine based NST. Two patients had a delayed partial response that occurred following treatment with a DLI consistent with a GVT effect (Bregni et al., 2002). Despite these encouraging results, complete responses as a consequence of a GVT effect have not yet been described in breast carcinoma (Ben-Yosef et al., 1996; Pedrazzoli et al., 2002a; Blaise et al., 2002).

Ovarian cancer and other tumors

Experience with HCT for ovarian cancer also has been restricted to a handful of cases (Bay et al., 2000). The first reported case of a GVT effect in this tumor was a woman with chemo-resistant ovarian cancer who underwent a myeloablative allogeneic HCT. At day 28, GVHD was noted followed by normalization of CA-125 on day 60 and remission of radiologically evaluable disease by day 90. Unfortunately, disease relapse occurred approximately 2 years later. Subsequently, the same group reported responses in 3 out of 4 patients with metastatic ovarian cancer treated with reduced intensity allogeneic transplant. Although these responses were reported to occur in association with acute GVHD, regression was noted in proximity to transplant conditioning raising the possibility that some of these anti-tumor effects were related to a chemotherapy effect. Longer follow-up will likely clarify the etiology of these responses as chemotherapeutic effects in such heavily treated patients are rarely

Figure 1. Mixed chimerism occurring after nonmyeloablative transplantation. A PCR based chimerism analysis using fluorescent labeled primers to a single short tandem repeat (STR) that is polymorphic between the patient and donor. The patient (blue band) and donor (red band) each have one specific informative STR and share one non-informative (white band) STR. Blood samples obtained at multiple time points post-transplant were sorted into myeloid and T-cells lineages using magnetic beads. As shown here, mixed chimerism was observed in both lineages although donor T-cell engraftment occurred more rapidly than donor myeloid engraftment.

Table 3. Nonmyeloablative stem cell transplantation for RCC

Investigator	Number of patients	Conditioning regimen	Response rate (%)
Childs (2000a)	19	FLU + CY	53
Rini (2002)	15	FLU + CY	33
Pedrazzoli (2002a)	7	FLU + CY	0
Bregni (2002)	7	THIO + FLU + CY	71
Sandmaier (2002)	5	FLU + TBI	20
Blaise (2002)	13	FLU + BU + ATG	NR

FLU: Fludarabine; CY: cyclophosphamide; TBI: total body irradiation; BU: Busulfan. N.R. not reported.

COMPONENTS TO NON-MYELOABLATIVE ALLO-TRANSPLANTATION

Figure 2. Components of NST required to needed the induction of a graft-vs-tumor effect.

Figure 3. Graft-vs-RCC effect following nonmyeloablative stem cell transplantation. Delayed regression of multiple RCC pulmonary metastasis following NST in a 46 year old woman who had previously failed IL-2 based immunotherapy.

durable (Bay et al., 2002). Two other cases of ‘chemo-resistant’ ovarian cancer regressing after NST have been presented in abstract form (Bornhauser et al., 2002), including a complete response in one patient. Despite these promising results, further investigation with longer patient follow-up will be required before definitive conclusions regarding the usefulness of allogeneic HCT for metastatic ovarian carcinoma can be discerned.

Anecdotal reports exist of several other tumors regressing after NST including soft-tissue and bony sarcomas, colon cancer, prostate carcinoma and lung carcinoma (Pedrazzoli et al., 2002a,b; Blaise et al., 2002; Moscardo et al., 2000). However until a sufficient number of transplants have been performed

generalizable statements regarding the susceptibility of individual tumors to an allogeneic GVT effect can not be made.

Future directions

Considerable research effort is currently in progress to the donor immune system specifically at the tumor (Morecki & Slavin, 2000). Murine experiments have shown that vaccines to recipient minor antigens, when administered to mice after the transplant, enhance GVT effects without increasing the risk of GVHD (Anderson Jr et al., 2000).

Teshima et al. have recently developed a novel approach to stimulate specific anti-tumor activity while

preserving tolerance to host antigens after T-cell depleted allogeneic HCT by vaccinating of recipients with irradiated melanoma cells engineered to secrete granulocyte-macrophage colony stimulating factor. In this murine model, DLI from a donor immunized with the recipient-derived melanoma vaccines enhanced the clinical activity of the tumor vaccine without increasing GVHD (Teshima et al., 2002).

The observation that cytokine refractory metastatic RCC may sometimes regress following allogeneic transplantation has provided proof of concept attesting to the powerful nature of the graft vs. tumor effect in this tumor. Although toxicity related to the sequelae of GVHD remains a major limiting factor, early pilot trials have provided data that clinically meaningful regression of a metastatic disease may occur in some RCC patients following this approach. The development of methods to prevent acute GVHD while targeting the allogeneic immune system to the tumor will hopefully improve the safety and efficacy of this promising investigational therapy.

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