Nonmyeloablative Transplantation: An Allogeneic-Based Immunotherapy for Renal Cell Carcinoma

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ABSTRACT

High-dose chemotherapy followed by autologous hematopoietic stem cell transplantation has been explored as a method to enhance the efficacy of chemotherapy for advanced solid tumors. The failure of autologous hematopoietic stem cell transplantation to prolong survival in patients with metastatic solid tumors has sparked interest recently in studies exploring the potential of allogeneic hematopoietic stem cell transplantation for such patients. Allogeneic hematopoietic stem cell transplantation is widely accepted as a potent form of immunotherapy capable of curing patients with chemotherapy-refractory hematologic malignancies. However, it was not until the end of the 20th century that investigators initiated trials to test the potential of allogeneic hematopoietic stem cell transplantation as immunotherapy in malignancies of epithelial origin. Early pilot trials have established proof-of-principle that graft-versus-tumor effects can induce complete or partial remission in some treatment-refractory metastatic solid tumors. In this review, we discuss the rationale for pilot trials investigating the potential of nonmyeloablative allogeneic hematopoietic stem cell transplantation in advanced cytokine-refractory renal cancer, highlighting the preliminary success, limitations, and future clinical directions of this approach.

RENAL CANCER: A TARGET FOR THE IMMUNE SYSTEM

Metastatic renal cell carcinoma is associated with an extremely poor prognosis, with median survivals in the range of 12 to 15 months and 5-year survival of <5% (1). Conventional cytotoxic chemotherapy-based treatment of metastatic disease results in response rates of <15% and fails to prolong survival (2). However, renal cell carcinoma is unusual among solid tumors in that it appears susceptible to killing by immune cells. Early pilot trials of immunotherapy in renal cell carcinoma patients attempted to activate nonspecific immunity to illicit an antitumor response (3, 4). A number of investigators in the 1980s demonstrated that metastatic renal cell carcinoma would regress in 15% to 20% of patients treated with immunomodulators such as interleukin 2 (IL-2) and/or interferon α. Most impressive were responses reported after treatment with high-dose IL-2, where complete regression lasting >10 years was observed in a subset of patients with extensive metastatic disease. Unfortunately, high-dose IL-2 treatment proved to have considerable toxicity, with antitumor effects limited to a relatively small subset of patients.

In the past decade, investigators have begun to focus on methods to target immune cells specifically against cancer cells. One of the goals of conventional cancer immunotherapy is to identify tumor antigens that can induce T-cell-mediated cancer rejection (5). Such antigens could be used to magnify the immune response specifically against the tumor through cancer vaccine treatment. Most cancer antigens that have been identified to date are self-antigens expressed not only on tumor cells but also on normal cells found in the skin or testes (6). The goal of cancer vaccine strategies targeting these antigens is to break tolerance to these antigens, resulting in the induction of an effective antitumor immune response. Although these vaccine strategies may ultimately prove to have therapeutic value, at present only a small number of patients have benefited from this type of therapy. Most vaccine regimens have used a MHC class I restricted peptide-based approach. As a consequence, immune responses are CD8 restricted and limited to a single antigenic epitope without an important CD4+ helper T-cell component. Perhaps the greatest limitation of conventional peptide-based immunotherapy regimens is that they attempt to enhance an immune system rendered dysfunctional by prior chemotherapy treatment or the long-standing immunosuppressive effects of metastatic tumors (7). Furthermore, only a handful of antigens overexpressed or restricted to renal cell carcinoma have thus far been identified (8), additionally limiting vaccine-based strategies designed to enhance an innate immune response in this malignancy.

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR HEMATOLOGIC MALIGNANCIES: A POTENT AND CURATIVE FORM OF IMMUNOTHERAPY

Allogeneic hematopoietic stem cell transplantation has been used successfully for decades to treat patients with lethal hematologic malignancies. Two components contribute to the curative capacity of the procedure. First, dose-intensive chemotherapy or radiotherapy mediates a direct cytotoxic effect on malignant cells while suppressing the immune system of the recipient to allow for the engraftment of donor stem cells required to restore hematopoietic function. Second, engrafting donor immune cells [i.e., T cells, natural killer (NK) cells, and so forth] mediate potent antitumor effects on malignant cells that survive the effects of the conditioning regimen (9, 10). This donor immune-mediated antitumor process, called graft-versus-


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leukemia or graft-versus-tumor, is now recognized to have a curative capacity that is independent from the cytoreductive effects of the conditioning regimen. Definitive evidence supporting the existence of the graft-versus-leukemia/graft-versus-tumor effect was first demonstrated in the late 1980s in patients with relapsed chronic myeloid leukemia after allogeneic transplantation who were successfully induced back into a complete and durable remission after the infusion of donor lymphocytes (10). Similar curative graft-versus-tumor effects have now been demonstrated in a growing number of hematologic cancers, including acute leukemias, post-transplantation Epstein-Barr virus–associated lymphoproliferative disorder, chronic lymphocytic leukemia, Hodgkin’s and non-Hodgkin’s lymphoma, and multiple myeloma (11–20). A heightened appreciation for the curative capacity of the graft-versus-tumor effect has recently shifted the focus of allogeneic hematopoietic stem cell transplantation regimens away from studies exploring “high-dose” conditioning to transplantation approaches that capitalize on donor immune-mediated graft-versus-tumor effects.

In vitro and in vivo evidence suggests that minor histocompatibility antigens that are polymorphic between the patient and donor expressed on malignant cells are the dominant antigens that are targeted during a graft-versus-tumor effect. Recent studies (21–24) have discovered a number of minor histocompatibility antigens that are restricted to hematopoietic lineages or are broadly expressed on a variety of different normal tissues that can be recognized by donor-derived cytotoxic T lymphocytes.

Inspired by the power of the allogeneic antimalignancy effect, investigators were motivated recently to explore whether graft-versus-tumor alone without “mega-dose” chemoradiotherapy might be sufficient to cure some hematologic malignancies. To test this hypothesis, reduced-intensity, nonmyeloablative conditioning regimens were initiated in the mid-1990s as a method to avoid the substantial and frequently fatal toxic effects associated with conventional myeloablative allogeneic hematopoietic stem cell transplantation (25–28). Clinical results from a variety of different transplant centers have been encouraging, showing that nonmyeloablative conditioning is well tolerated, associated with a high likelihood of engraftment, and carries a reduced risk of morbidity and mortality compared with a historical cohort receiving a myeloablative transplant. Several studies have reported durable responses lasting >5 years in a variety of hematologic malignancies. Importantly, the reduced risk of toxicity with nonmyeloablative transplantation has finally provided a safe platform to test the potential of allogeneic immunotherapy in treatment-refractory solid tumors.

**GRAFT-VERSUS-SOLID TUMOR: NO LONGER JUST A HYPOTHESIS**

In hematologic malignancies, transplanted donor immune cells (particularly T cells) target minor histocompatibility antigens present in the patient but absent in the donor, resulting in graft-versus-tumor effects with or without concomitant graft-versus-host disease (GVHD). Because many solid tumors arise from epithelial tissues that are target tissue of GVHD (e.g., gastrointestinal mucosal cells, hepatobiliary tissues, keratinocytes, fibroblasts, and exocrine glands), investigators hypothesized that minor histocompatibility antigens expressed on malignantly transformed epithelial cells could serve as a target for a graft-versus-tumor effect.

The first evidence supporting the existence of a graft-versus-solid tumor effect came from animal studies demonstrating that allogeneic T cells could induce graft-versus-tumor effects in allogeneic murine mammary adenocarcinoma (29, 30). These experiments provided preliminary evidence that minor histocompatibility antigens were expressed on tumor cells, allowing them to serve as targets for a graft-versus-tumor effect in the allogeneic transplantation setting.

Early evidence of graft-versus-tumor effects in solid tumors in humans came from pilot trials of allogeneic transplantation being conducted in women who were not eligible for an autologous transplantation due to the inability to collect an autograft. Partial regression of metastatic disease was observed by a number of investigators during periods of acute GVHD. Eibl et al. (31) reported a case of a woman with metastatic breast cancer who underwent a myeloablative allogeneic bone marrow transplantation and had regression of liver metastasis during acute GVHD. Donor cytotoxic T lymphocytes specific for patient hematopoietic minor histocompatibility antigens were subsequently expanded from the blood of the patient that killed partially HLA-matched breast carcinoma cell lines. However, failure of patients to achieve complete responses and the considerable risks, including mortality, associated with myeloablative conditioning hindered enthusiasm for allogeneic transplantation in this setting.

**NONMYELOABLATIVE HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR METASTATIC RENAL CELL CARCINOMA**

**Trial Design.** Because myeloablative conditioning is associated with considerable risk of morbidity and mortality because renal cell carcinoma is typically refractory to chemotherapy, a nonmyeloablative transplantation strategy seemed optimal to test the potential of allogeneic immunotherapy against this tumor. The goals of pilot transplantation trials included the following: (1) to minimize toxicity of allogeneic hematopoietic stem cell transplantation by using a reduced intensity conditioning regimen, and (2) to optimize the induction of a graft-versus-tumor effect by the early withdrawal of GVHD prophylaxis and administration of post-transplantation donor lymphocyte infusions and/or immunomodulatory cytokines. Initial transplantation trials were restricted to patients with cytoreductive refractory metastatic disease who had an HLA-identical sibling to serve as donor. The conditioning regimen and transplantation approach first used at the National Heart, Lung, and Blood Institute is shown in Fig. 1. Fludarabine and cyclophosphamide were selected as conditioning agents because of their low toxicity profile and profound immunosuppressive effects that would allow the engraftment of the donor immune system (32). Because of concerns that immunosuppression in the early transplantation phase might accelerate disease progression, a number of strategies to rapidly enhance donor immune effects against the tumor were incorporated into the transplantation protocol: (1) a granulocyte colony-stimulating factor mobilized allograft rich in donor lymphocytes was used, (2) post-transplantation immunosup-
pression (e.g., cyclosporine) was tapered rapidly in patients with mixed T-cell chimerism or disease progression, (3) one or more donor lymphocyte infusions were given to patients with persistent mixed T-cell chimerism or disease progression, and (4) patients failing to respond to cyclosporine withdrawal with or without donor lymphocyte infusions were treated with s.c. interferon to up-regulate MHC class I or tumor antigen expression to enhance a graft-versus-tumor effect.

Preliminary Transplant Results for Renal Cell Carcinoma. Evidence supporting the existence of graft-versus-tumor effects against metastatic renal cell carcinoma has been reported recently by a number of transplant centers using a variety of different nonmyeloablative transplantation approaches (33–38). At the National Heart, Lung, and Blood Institute, 10 of the first 19 patients (54%) treated had tumor regression, including 7 partial responses and 3 complete responses (33). The first patient treated remains without evidence of disease 6 years after transplantation. Responses occurred most commonly in patients with a clear cell histologic subtype that was limited to the lungs, although regression of tumor lesions in multiple sites, including the bones, liver, and lymph nodes, was occasionally observed. The induction of a graft-versus-tumor effect was usually delayed in onset, occurring a median of 4 months after the transplantation, usually after the withdrawal of cyclosporine and after T-cell chimerism had converted from mixed to full donor in origin. Delayed regression of renal cell carcinoma is typically shown in Fig. 2. Therefore, high degrees of donor lymphocyte engraftment appear to be a prerequisite for a successful transplantation outcome, with transition from mixed toward full donor T-cell chimerism appearing to optimize the generation of a graft-versus-tumor effect (39). In some patients, delayed tumor regression ultimately followed early periods of disease progression. Graft-versus-tumor effects occurred most commonly in patients who had a history of acute GVHD, an observation reported previously in patients with hematologic malignancies (Fig. 3). However, we occasionally observed delayed disease regression in patients who never developed clinically overt acute or chronic GVHD. These observations suggested that donor T cells were likely the effector population that mediated disease regression, with broadly expressed minor histocompatibility antigens (i.e., disease regression with GVHD) or antigens restricted to the tumor (i.e., disease regression without GVHD) serving as targets for allogeneic T cells. A summary of trials reporting the results of nonmyeloablative allogeneic hematopoietic stem cell transplantation for metastatic renal cell carcinoma is shown in Table 1. Although published data are currently limited, regimens associated with a higher incidence of GVHD also appear to be associated with higher response rates. Furthermore, because most patients with metastatic renal cell carcinoma have not received prior treatment with chemotherapy (known to facilitate the engraftment of donor immune cells), reduction in the intensity
of transplantation conditioning appears to significantly increase the risk of graft rejection (34). Furthermore, these preliminary studies have shown that careful patient selection is critical for a successful outcome. Because graft-versus-tumor effects are delayed by months after transplantation, it is not surprising that patients with rapidly progressive metastatic disease typically fail to benefit from this approach (i.e., survival is shorter than the time required for a delayed graft-versus-tumor effect to occur).

Fig. 2 Delayed and sustained regression of metastatic renal cell carcinoma following a nonmyeloablative hematopoietic stem cell transplantation. A, 4 months after transplantation; B, 2.5 years after transplantation.
Mechanisms of Graft-versus-Tumor in Renal Cell Carcinoma. As discussed previously, the observation that graft-versus-tumor effects were more likely to occur in patients with a history of acute GVHD suggests that minor histocompatibility antigens broadly expressed on normal tissues and renal cell carcinoma cells might serve as a target for graft-versus-tumor effects. Indeed, minor histocompatibility antigen-specific T-cell clones that lyse renal cell carcinoma cells in vitro have been isolated from patients with metastatic renal cell carcinoma undergoing nonmyeloablative transplantation (40). However, disease regression observed in some patients who never developed GVHD also supports the hypothesis that antigens restricted to the tumor might also serve as targets for a graft-versus-tumor effect. Although this remains an active area of research, in vitro data supporting the existence of renal cell carcinoma-specific CD8+ T-cell clones that have already been observed in one responding patient. Although donor-derived CD8+ T cells are the most likely candidates mediating graft-versus-tumor effects against renal cell carcinoma, these data do not exclude a potential contribution from other donor cells such as CD4+ T cells, B cells, and NK cells. Indeed, some allogeneic NK cell subsets have been shown recently to have enhanced cytotoxicity against renal cell carcinoma tumor cells in vitro compared with autologous NK cell populations (41, 42).

Toxicity and Limitation of Nonmyeloablative Hematopoietic Stem Cell Transplantation. Although significant and occasionally complete regression of metastatic disease has been observed, a number of factors currently limit the application of allogeneic immunotherapy for renal cell carcinoma. Unfortunately, most responses have been partial, with complete responses occurring in a small subset of patients. Although toxicity appears less compared with conventional myeloablative approaches, regimen-related mortality rates of 10% to 15% persist, largely as a consequence of severe grade III to IV acute GVHD. Older patients (i.e., >55 years) appear particularly susceptible to morbidity and mortality associated with acute GVHD (43). The use of new and potent immunosuppressive agents that target pathways critical to GVHD holds the promise to decrease this risk (44), although possibly at the expense of inhibiting graft-versus-tumor effects. Another major limitation of this approach is its requirement for an HLA-identical sibling to serve as a stem cell donor. Only 25% of patients will have an HLA-matched sibling, limiting the procedure to a relatively small percentage of patients. However, ~60% of Caucasians in North America would be expected to have an HLA-matched unrelated donor in The National Marrow Donor Program. Therefore, trials investigating the feasibility of using unrelated HLA-matched donors are currently being pursued and, if successful, could expand the application of allogeneic transplantation for this tumor.

FUTURE DIRECTION
Nonmyeloablative allogeneic hematopoietic stem cell transplantation trials in renal cell carcinoma have provided additional proof of the strength of allogeneic immunotherapy. Furthermore, they have provided the first convincing evidence that the graft-versus-tumor effect can extend beyond hematologic malignancies to neoplasms of epithelial origin. Animal studies have demonstrated recently that graft-versus-tumor effects against solid tumors after allogeneic hematopoietic stem cell transplantation can be enhanced by post-transplantation tumor vaccination. Furthermore, some allogeneic NK cell subsets have been shown to have enhanced cytotoxicity against tumor cells compared with autologous NK cell populations (45). On the basis of these data, it is reasonable to speculate that

<table>
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<th>Investigator</th>
<th>Patient number</th>
<th>Conditioning regimen</th>
<th>GVT effect/%</th>
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<tr>
<td>Childs</td>
<td>19</td>
<td>Flu + Cy</td>
<td>Yes—53%</td>
</tr>
<tr>
<td>Rini</td>
<td>15</td>
<td>Flu + Cy</td>
<td>Yes—33%</td>
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<tr>
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<td>7</td>
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<td>Yes—56%</td>
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<td>10</td>
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</tr>
<tr>
<td>Ueno</td>
<td>15</td>
<td>Flu + Mel</td>
<td>Yes—53%</td>
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NOTE. Summarized from refs. 32–37.
Abbreviations: RCC, renal cell carcinoma; GVT, graft-versus-tumor; Flu, fludarabine; Cy, cyclophosphamide; Thio, thiotepa; TBI, total body irradiation; Mel, melphalan.
second-generation allogeneic hematopoietic stem cell transplantation trials that incorporate methods to enhance the donor immune system against the tumor through the use of adoptively infused tumor-reactive donor T cells and NK cells, as well as post-transplantation tumor vaccination strategies will likely be investigated in the near future.

OPEN DISCUSSION

Dr. Michael Atkins: There seems to be a large diversity in response rates between different centers that have done this procedure. What are your thoughts about this? Also, in hematologic malignancies there is a diverse response to this strategy based on the disease type. How do you see renal cell cancer in that setting? Do you think debulking or trying to start at a minimal disease state will make a difference?

Dr. Richard W. Childs: That is an important consideration, since whatever happens with hematologic malignancies we think could potentially apply to solid tumors. ALL, for instance, is thought of as being less immunoresponsive than CML. However, if you have ALL patients who have chemotherapy-refractory disease that is Philadelphia chromosome positive and you give them imatinib mesylate, you can get them into a transient remission and cure them with a nonmyeloablative transplant. I think the presence of active tumor, especially tumor that is growing quickly, is a major limitation with this approach. In the future, we need to incorporate strategies that bring people into transplant with minimal disease.

Dr. Robert Figlin: It would seem prudent to see what you can identify about the biology of the responders that could predict for successful transplantation. One might hypothesize that there is a group of immunologically responsive patients out there whose characteristics can be defined. Have you looked at CA-IX in your 25 responders to try and see whether there is something about the biology?

Dr. Childs: We have started sequencing VHL in responders versus nonresponders, which is an ongoing project.

Dr. Figlin: Have you stopped transplanting everything other than clear cell patients?

Dr. Childs: No, but I haven’t had a referral in the last 2 years for a patient with anything other than clear cell. We looked at survival in the papillaries, but they all had died before 4 months, before responses usually occur.

D. James Yang: Have you ever seen a complete remission in somebody who has failed high-dose IL-2?

Dr. Childs: Yes, two.

Dr. Yang: Have you ever measured serum cytokines sequentially to see whether or not there are distinct patterns in the serum?

Dr. Childs: Yes, we are doing that.

Dr. David Avigan: What would complicate any relationship between GVHD, cytokine release, and clinical benefit is that patients with GVHD frequently receive immunosuppressive therapy. Furthermore, the amount of therapy depends on the extent of GVHD, so you have a dampening effect on cytokine production that is variable between different patients.

Dr. Yang: Not everyone even will respond to a cytokine environment.

Dr. Childs: We see a lot of patients with bad GVHD who initially don’t respond. Then we get it under control, and 3 to 4 months later they respond in the absence of GVHD. So, clinically, it does not go along with a cytokine response.

Dr. Yang: Have you seen NK activity in circulating cells or eosinophilia?

Dr. Childs: We have seen eosinophilia. We saw a complete response in a patient who ended up having almost 50% eosinophilia at the time of response. NK cell populations are currently being evaluated. We see mostly activated T cells, CD8
t, DR positive, and CD57 positive at response; the problem is that is the exact same profile you see with GVHD. So we can’t use this as evidence that an antigen-specific response is occurring.

Dr. Janice Dutcher: When you say that you had 2 people who failed IL-2 who responded to transplant, was there a period of stable disease?

Dr. Childs: Everybody who was enrolled onto the trial was required to have progressive disease. They had received cytokines. I don’t know if any would have met criteria for stable disease, but when they came to us they did not have stable disease, and it was usually a relatively short window from IL-2 to us treating them.

Dr. Atkins: You had a low response rate in liver metastases. How much of that was from patients dying before they got off of immunosuppression versus just not being able to respond once they were off immunosuppression?

Dr. Childs: Death before coming off immunosuppression was a substantial component, but I don’t know if it reflects the general kidney cancer population. Most of our patients who had liver involvement got into trouble quicker than folks who did not have it.

Dr. Avigan: Was there a different experience with patients who were debulked versus not?

Dr. Childs: We only debulked a few patients, but we had a good experience with some of them.

REFERENCES

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Clin Cancer Res 2004;10:6353S-6359S.