Allogeneic Blood Stem Cell Transplantation after a Reduced-Intensity, Preparative Regimen

A Pilot Study in Patients with Refractory Malignancies

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BACKGROUND. The immune-mediated graft-versus-tumor (GVT) effect plays a therapeutic role in the treatment of patients with hematologic malignancies who undergo allogeneic hematopoietic stem cell transplantation (HSCT). More recently, it was reported that a GVT effect also occurred in patients who underwent transplantation for metastatic renal carcinoma. The authors carried out a pilot trial of allogeneic transplantation after a reduced-intensity, preparative regimen in patients with refractory malignancies, including solid tumors. The objectives of the current study were to evaluate the feasibility of this approach in terms of toxicity and engraftment and to document evidence of GVT effects.

METHODS. Seventeen patients with Stage IV malignancies (7 patients with renal cell carcinoma, 3 patients with sarcoma, 2 patients with breast carcinoma, 2 patients with Hodgkin disease, 1 patient with ovarian carcinoma, 1 patient with melanoma, and 1 patient with both melanoma and renal cell carcinoma) that were not amenable to further conventional treatment were enrolled. The median patient age was 43 years (range, 10–60 years). The Eastern Cooperative Oncology Group performance status (PS) was 0–1 in 11 patients and 2–3 in 6 patients. Preparative treatment consisted of reduced-intensity chemotherapy with fludarabine (30 mg/m^2 per day for 4 consecutive days) and cyclophosphamide (30 mg/Kg per day for 2 consecutive days) prior to allogeneic HSCT from a human leukocyte antigen-identical sibling. The median number of CD34+ cells infused was 6.06×10^6/kg (range, 1.5–14.0 $\times$ 10^6/kg). Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporin-A and short-term methotrexate.

RESULTS. Patients who had a PS of 2–3 prior to undergoing HSCT experienced Grade 4 hematologic toxicities and Grade $\geq$ 3 organ toxicities and died of either treatment-related complications or disease progression within 100 days from transplantation. By contrast, 10 of 11 patients who had a PS of 0–1 prior to undergoing HSCT experienced only short-lasting, Grade $\leq$ 3 neutropenia and thrombocytopenia and no organ toxicity; 1 of 10 patients died of graft failure on Day +29 after undergoing HSCT. By Day +90, 100% donor chimerism was documented in all patients with a past history of heavy chemotherapy, whereas mixed donor chimerism was observed in the 4 patients with a past history of only 1 line of chemotherapy and/or immunotherapy prior to entering the HSCT program. Grade 2–3 acute GVHD occurred in 5 patients. Among patients with a follow-up $>$ 100 days, 2 complete responses and 3 transitory partial responses were recorded.

CONCLUSIONS. With this conditioning regimen, full donor chimerism was achieved rapidly only in patients who had received previous intensive chemotherapy. In a proportion of patients with refractory malignancies, allogeneic transplantation resulted in tumor regression. This novel therapeutic strategy may provide little benefit in patients with poor PS and rapidly progressing disease. ***Cancer* 2002;94: 2409–15.** *© 2002 American Cancer Society.*

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Extensive clinical and experimental data support a favorable role for graft-versus-tumor (GVT) effects in promoting a cure for patients with hematologic malignancies who receive myeloablative therapy and undergo allogeneic hematopoietic stem cell transplantation (HSCT).[1–3] Recent reports suggest that donor lymphocytes transferred with the graft also may produce a clinically meaningful GVT effect in patients with refractory solid tumors.[4–6] However, because of the associated morbidity and mortality, myeloablative high-dose chemotherapy (HDC) and allogeneic transplantation are restricted to young, medically fit patients, thus excluding the vast majority of patients from this therapy. Studies in animal models and in humans have shown that sustained engraftment of donor hematopoietic stem cells can be accomplished with the use of preparative regimens that cause immunosuppression without ablating host hematopoiesis.[7–10] When this approach is used for patients with malignant disease, the clinical working hypothesis is to attack the tumor through the donor's immune system rather than trying to wipe out tumor cells through high-dose cytotoxic therapy.[11] Nonmyeloablative HSCT offers the advantage of low transplantation-related mortality and provides for the development of full donor lymphoid chimerism,[12,13] and it has been used successfully to treat patients with hematopoietic malignancies and patients with certain genetic diseases.[9,13] In view of experimental evidence demonstrating a GVT effect of allogeneic lymphocytes in animals who were pretreated with a nonmyeloablative, preparative regimen,[14,15] it is evident that this approach also may be considered for the immunotherapy of solid tumors. Childs et al. recently reported on the efficacy of a nonmyeloablative protocol followed by HSCT from human leukocyte antigen (HLA)-matched relatives in patients with metastatic renal carcinoma (RCC).[16] Ten of 19 patients who were enrolled in the latter study had measurable responses, and 3 patients enjoyed complete, long-lasting responses. Although those results were promising, the procedure was associated with severe graft-versus-host disease (GVHD) and rapidly progressive disease in a fraction of patients, and further refinement to minimize complications and improve efficacy are required. Furthermore, patients with other solid tumors that are resistant to conventional chemotherapy and radiotherapy also may be suitable for this approach.

We report the results of a pilot study carried out in 17 patients with advanced and refractory malignancies who were treated with a reduced-intensity, preparative regimen and underwent family donor allogeneic HSCT. The objective of this report was to evaluate the feasibility of this approach in terms of toxicity and engraftment and to assess the evidence of potential GVT effect.

MATERIALS AND METHODS

Patient Population

Eligible patients were age < 65 years and had histologically confirmed metastatic tumors that were documented radiographically as progressive despite prior therapy and that were not amenable to complete surgical resection or further systemic, conventional treatments. Patients were required to have an HLA-identical sibling and were excluded if they had bone metastases alone, if they had active brain metastases, or if they had received any treatment for their disease within 30 days before enrollment.

Seventeen consecutive patients underwent nonmyeloablative allogeneic HSCT for progressive disease. Seven patients had RCC, three patients had sarcoma (one patient had Ewing sarcoma, one patient had gastric sarcoma, and one patient had rhabdomyosarcoma), two patients had breast carcinoma (BC), two patients had Hodgkin disease (HD), and one patient each had ovarian carcinoma, melanoma, and both melanoma and RCC. The median age was 43 years (range, 10–64). Seven of 17 patients were female. All patients received transplantation from a molecularly typed, HLA-identical sibling. Seven patients had been treated previously with at least two lines of chemotherapy (CT), including HDC and autologous HSCT. Either immunotherapy alone or 1 line of CT with or without immunotherapy was recorded in the remaining 10 patients. All patients had multiple sites of metastatic tumor. The Eastern Cooperative Oncology Group performance status (PS) was 0–1 in 11 patients and 2–3 in 6 patients. Further details on patient characteristics are reported in Table 1.

HLA-identical sibling donors received 10 μg/kg of recombinant human granulocyte colony-stimulating factor (G-CSF; Filgrastim) subcutaneously daily for 4–6 days. Mobilized peripheral blood stem cells were collected by leukapheresis on Day 4 and on Days 5 and 6, if needed, to obtain a target dose of $> 4 \times 10^6$ CD34+ cells per kilogram of recipient body weight (Kg bw). In three patients, the target dose was not reached despite three consecutive leukaphereses. No positive selections or T-cell depletion procedures were performed. Patients or their legal guardians as well as donors gave written, informed consent to participate into this protocol, which was approved by the Internal Review Boards of the participating institutions.

TABLE 1
Characteristic of Patients and Outcome of Transplantation

Patient	Age (yrs)	Gender	Type of tumor	Metastatic sites	No of prior therapies	PS	Cells infused/kg ($CD34^+ \times 10^6$ $CD3^+ \times 10^8$)	Toxicity (hematologic Grade 4/ nonhematologic Grade ≥ 3)	aGVHD (Grade ≥ 2)	Outcome
1	17	M	Ewing sarcoma	Lung, bone	3 CT including HDC and auto-HSCT	1	6.5/2.4	No/no	skin, GI (3)	P, died in P on Day 390
2	29	F	Breast	Liver, lung, bone	3 CT including HDC and auto-HSCT	0	5.9/3.4	No/no	No	PR, died in P at Day 320
3	36	M	Renal	Lung, bone	2 (1 IT, 1 CT)	3	2.0/1,2	Yes/no	No	P, died on Day 80
4	44	M	Renal	Bone, soft tissue	1 IT	3	5.0/3.7	Yes/yes	No	P, died of MOF on Day 19
5	55	F	Melanoma	Bone marrow, LN, kidney	2 (1 IT, 1 CT)	2	6.6/1.9	Yes/no	Skin (2)	P, died on Day 97
6	58	M	Renal	Lung, bone	1 IT	3	6.1/2.8	Yes/yes	No	P, died on Day 47
7	45	M	Gastric sarcoma	Liver	1 CT	0	4.9/1.7	No/no	skin, GI (3)	P, alive on Day 240
8	10	M	Rhabdomyo sarcoma	LN, soft tissue	2 CT including HDC and auto-HSCT	0	2.4/0.5	No/no	No	CR, died of P on Day 231
9	54	F	Renal	Lung, LN, bone	2 (1 IT, I CT)	1	8.0/5.7	No/no	No	P, died on Day 47
10	36	F	Breast	Lung, brain	3 CT including HDC and auto-HSCT	1	3.8/3.8	No/no	Liver (2)	PR, died in P at Day 300
11	30	F	Hodgkin	Bone, soft tissue	RT, 4 CT including HDC and auto-HSCT	0	7.2/2.6	No/no	skin (2)	PR, alive at Day 240
12	16	M	Hodgkin	Bone, soft tissue	RT, 2 CT including HDC and auto-HSCT	0	6.8/5.3	No/no	No	CR, alive at Day 208
13	52	M	Melanoma and renal	Brain, LN, skin	1 IT	0	14.0/4.9	No/no	No	P, died on Day 110
14	60	M	Renal	Liver, bone, lung	1 IT	1	13.0/1.4	Yes/yes	NA	Died of graft failure on Day 29
15	35	M	Renal	Liver, bone, lung	1 IT	2	5.6/3.0	Yes/yes	No	P, died on Day 31
16	36	M	Renal	Liver	2 (1 IT, 1 CT)	1	3.8/1.4	No/no	No	P, died on Day 43
17	54	F	Ovarian	Bone marrow, peritoneal, LN	3 CT including HDC + auto-HSCT	2	1.5/4.6	Yes/yes	NA	Cardiac sudden death at Day 11

PS: performance status; aGVHD: acute graft-versus-host disease; M: male; F: female; CT: chemotherapy; HDC: high-dose CT: auto-HSCT: HDC with autologous hematopoietic stem cell transplantation; P: progression; PR: partial response; IT: immunotherapy; MOF: multiorgan failure; LN: lymph nodes; CR: complete response; RT: radiotherapy; GI: gastrointenstinal.

Preparative Regimen: HSCT and Post-Transplantation Immunosuppression

In selecting a low-intensity, preparative regimen, we chose two agents with proven immunosuppressive but nonmyeloablative effects.[10,12] The conditioning regimen consisted of intravenous cyclophosphamide (CY) 30 mg/Kg bw per day on Day −5 and Day −4 and fludarabine (FLU) 30 mg/m^2 per day from Day −5 to Day −2 before patients underwent HSCT. Cyclosporin-A (Cs-A), which was used to prevent both graft rejection and GVHD, was started 7 days before HSCT as an intravenous infusion at a dose of 3 mg/Kg per day. Subsequently, patients received oral Cs-A 6 mg/Kg per day in 2 divided doses. Methotrexate was administrated as part of GVHD prophylaxis at a dosage of 15 mg/m^2 intravenously on Day 1 and 10 mg/m^2 intravenously on Days 3, 6, and 11 after the allograft. Post-transplantation, Cs-A was decreased by 20% every week starting from Day +60 and was discontinued if severe GVHD had not developed.

Supportive Care

Antimicrobial therapy followed institutional protocols and consisted of itraconazole for antifungal prophylaxis, acyclovir for antiviral prophylaxis, and ciprofloxacin for antibacterial prophylaxis. Human cytomegalovirus (HCMV) serologic status was studied before transplantation in all patients and their donors. The expression of pp65 HCMV matrix protein was monitored to detect HCMV reactivation.[17] Patients who experienced reactivation of HCMV infection were treated with ganciclovir 10 mg/Kg per day until two negative controls were produced. For *Pneumocystis carinii* pneumonia prophylaxis, patients received oral cotrimoxazole starting from the day of engraftment. Empiric, broad-spectrum antibiotic therapy was started when patients became febrile, and antifungal therapy was employed in the presence of clinical evidence of fungal infection or fever persisting after 3 days of antibiotic therapy. G-CSF (Filgrastim; 5 μg/Kg per day subcutaneously) was administered if the white blood cell count fell to $< 1 \times 10^9$/L after HSCT. Platelet transfusions were given on a prophylactic basis when the platelet count was $< 10 \times 10^9$/L or in the presence of bleeding episodes, whereas red blood cell units were transfused in patients with hemoglobin levels $<$ 8 g/dL. All blood products were filtered and irradiated before transfusion.

Assessment of Chimerism, GVHD, and Tumor Response to Treatment

Myeloid and platelet engraftments were defined as the first of 3 consecutive days with an absolute neutrophil count (ANC) $> 0.5 \times 10^9$/L and unsupported platelets $> 50 \times 10^9$/L, respectively. Patients were considered assessable for engraftment if they survived for at least 14 days after undergoing transplantation. After transplantation, samples of blood were obtained monthly, and the degree of donor-recipient chimerism in both myeloid and T-cell lineages was assessed by polymerase chain reaction assay of minisatellite regions, as reported previously.[18]

Acute and chronic GVHD were classified according to previously described criteria.[19,20] Patients with sustained donor engraftment who survived for $>$ 14 days and $>$ 100 days after undergoing transplantation were evaluated for occurrence and severity of acute and chronic GVHD, respectively. Evaluation of treatment response was performed every month after transplantation according to the World Health Organization criteria.

RESULTS

HSCT and Engraftment

Patients received a median of 6.06×10^6 CD34+ hematopoietic cells/Kg (range, 1.5–14.0 $\times$ 10^6 CD34+ hematopoietic cells/Kg) and 2.96×10^8 CD3+ T cells/Kg (range, 0.5–5.7 $\times$ 10^8 CD3+ T cells/Kg). Complete neutrophil and platelet recovery was achieved at a median of 12 days (range, 5–19 days) and 15 days (range 11–30 days) after transplantation, respectively. In 4 patients, the ANC and platelet counts never fell below 1×10^9/L and 50×10^9/L, respectively.

At Day +90, full donor T-cell chimerism was documented in all patients who were treated previously with intensive chemotherapy. Stable mixed chimerism ($>$ 50% of donor cells) was observed in four patients who previously had received only one line of chemotherapy and/or cytokine-based therapy.

HSCT-Related Adverse Events

All patients with a PS of 2–3 experienced Grade 4 hematologic toxicity and Grade 3 and 4 organ toxicities in the post-transplantation phase. Among patients with a PS of 0–1, 10 patients presented with neutropenia and thrombocytopenia of short duration (never reaching Grade 4) and had no organ toxicity, and 1 patient died of graft failure on Day +29.

Grade 2–3, acute GVHD occurred in five patients after Cs-A withdrawal (see Table 1). In all patients, GVHD responded to steroids: Four patients experienced chronic GVHD involving the skin (four patients) and the gastrointestinal tract (two patients). HCMV reactivation occurred only in four patients, all of whom responded promptly to ganciclovir therapy.

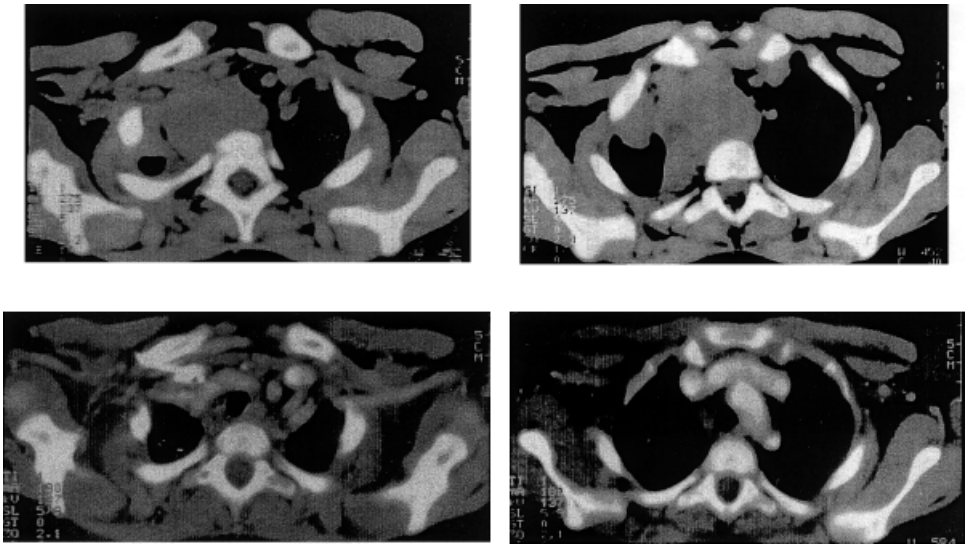

FIGURE 1. Computed tomography (CT) scan from a patient with rhabdomyosarcoma before (A,C) and 3 months after (B,D) the patient underwent allogeneic hematopoietic stem cell transplantation. CT scan sections are not fully superimposable; however, the overall interpretation of the images shows the regression of mediastinal tumor masses.

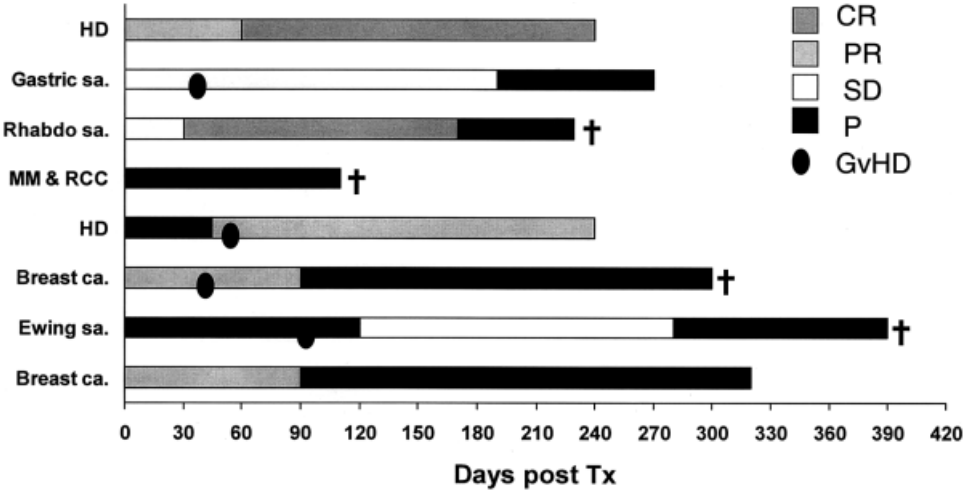

FIGURE 2. Tumor course and outcome of patients who had a follow-up $>$ 100 days. Patients are listed according to their underlying disease (for details, see text and Table 1). Crosses indicate patient deaths. HD: Hodgkin disease; sa.: sarcoma; MM & RCC: malignant melanoma and renal cell carcinoma; ca.: carcinoma; CR: complete response: PR: partial response: SD: stable disease: P: progression; GvHD: graft-versus-host disease $\geq$ Grade 2.

Patient Outcome

Table 1 shows that there were 13 patient deaths. Nine patients died in the early post-transplantation phase (i.e., within 100 days from transplantation) due to transplantation-related complications ($n = 3$ patients; 17%) or progressive disease ($n = 6$ patients; 35%), and four additional patients died of tumor progression a median of 254 days post-transplantation (range, 110–390 days). Overall, seven patients had evidence of rapidly progressing tumor after undergoing HSCT, all of whom had large tumor loads at baseline and died without signs of tumor regression. Two patients with BC had early regression of metastases, possibly due to response to the conditioning regimen, but progressed thereafter. One patient with Ewing sarcoma had initial radiographic evidence of tumor growth and subsequently had 5 months of stabilization after Cs-A withdrawal and the development of GVHD. Eventually, he died of progressive disease on Day +390. One patient with gastric sarcoma in progression at the time of HSCT had stable disease for 6 months post-transplantation and subsequently had low progressing disease; he was alive at Day +280. One patient with rhabdomyosarcoma achieved complete remission (Fig. 1) that lasted for 5 months, but he subsequently experienced disease recurrence and died on Day 231. The two patients with HD were in complete response and partial response at Day +208 and Day +240 after HSCT, respectively. The outcome of patients who had a follow-up $>$ 100 day is reported in detail in Figure 2.

DISCUSSION

Because allogeneic donor lymphocytes have been reported induce a GVT reaction in patients with leukemia and selected solid tumors, mostly metastatic RCC,[1–6,16,13,21] we evaluated the feasibility of alloge-

neic HSCT after a reduced-intensity, preparative therapy regimen in patients with Stage IV malignancies that were refractory to conventional management. Seventeen patients received a reduced-intensity, highly immunosuppressive, preparative regimen consisting of CY and FLU, followed by the transplantation of Filgrastin-mobilized blood hematopoietic stem cells from an HLA-identical sibling. Cs-A and short-term methotrexate were used to favor engraftment and to prevent GVHD. The objective of our experimental protocol was to determine whether this regimen had acceptable toxicity while establishing a competent donor immune system capable of exerting a GVT effect.

The data presented in this article indicate that allogeneic HSCT after a reduced-intensity, CY-FLU preparative regimen is a feasible procedure for the treatment of patients with advanced-stage malignancies. The incidence and severity of acute GVHD was limited, probably due to the effective prophylaxis that was used. HCMV reactivation, which responded promptly to antiviral treatment, occurred in only four patients (24% of the overall population), compared with a significantly greater proportion of patients who were given conventional myeloablative therapy in another study.[17] With only one exception, none of the medically fit patients experienced relevant extrahematologic toxicity or life-threatening neutropenia/thrombocytopenia. However, major transplantation-related toxicity and rapidly progressive disease were recorded in patients with a poor initial presentation (PS, 2–3), often related to the presence of large tumor masses. In such patients, severe post-transplantation immunodeficiency has the potential to inhibit any immune antitumor mechanisms, thus promoting disease progression. This may explain the extremely poor outcome of our patients with RCC and indicates that patients with a poor PS and/or rapidly progressing, metastatic disease are not likely to live long enough for the generation of a GVT effect and should not be treated with allogeneic HSCT.

To succeed, immunotherapy with allogeneic lymphocytes seems to require the full engraftment of the donor lymphocytes.[12] In the current series, this goal was obtained only in heavily pretreated patients, whereas patients who previously had received mild myelotoxic therapy and/or cytokine-based therapy (typically patients with melanoma and RCC) achieved mixed T-cell chimerism. Higher doses of CY or, alternatively, post-transplantation donor lymphocyte infusions may aid in achieving full chimerism in a larger proportion of patients.[16] The optimal nonmyeloablative preparative regimen for patients with solid tumors, as well as for patients with hematologic malignancies, undergoing allograft depends on several factors, including the aggressiveness of the patient's malignancy and the immunocompetence of the recipient. We combined CY with the potent immunosuppressive purine analogue FLU, which has been a component of most other reported reduced-intensity, preparative, allografting regimens. Previous studies have shown that FLU-based preparative regimens are well tolerated and are used currently in patients who are not eligible for conventional myeloablative conditioning because of advanced age and/or poor PS.[8,9,13,16,21] Immune-compromised patients, such patients who were treated previously with intensified chemoradiotherapy, may require less intensive immunosuppression to achieve engraftment than a fully immunocompetent recipient.[10,13,16] By contrast, in patients with highly proliferative malignancies, a more aggressive conditioning regimen, along with a rapid tapering of post-transplantation immunosuppression therapy, may favor the generation of a GVT effect more rapidly. In our experience, some patients have shown a response or disease stabilization after allografting, confirming that a GVT effect can be generated in solid tumors other than RCC. In particular, patients with sarcoma have shown some promising results. However, due to the limited number of patients enrolled in our study, data on the clinical outcome should be considered with great caution.

The results published to date in animal models[14,15] and, more recently, in human patients,[4–6,16] including our own experience, provide evidence of the therapeutic usefulness of the allogeneic GVT effect also in patients with nonhematologic malignancies. Therefore, it is likely that increasing numbers of investigators will perform allogeneic HSCT in patients with a widening variety of solid tumors. Because solid tumors show a wide diversity of etiology and antigen characteristics, it will be necessary to evaluate this approach in patients with each disease individually. In this regard, it is mandatory to treat patients in institutions that have proven experience in this setting and in the context of specific clinical trials that are designed to address major clinical and biologic issues in this field. In particular, great attention should be paid to developing a better understanding of the mechanisms of immune escape used by tumor cells and how to restore immune competence against malignant disease.[22]

Clinical studies reported to date, along with the current data, have recognized that proof of the two principles already is at hand: allogeneic T cells can induce clinically relevant responses in patients with RCC and other solid tumors, and donor lymphocytes can survive in the host after nonmyeloablative condi-

tioning. In the near future, a clinical improvement in this setting is likely to depend on the possibility of driving the donor immune system in a specific fashion against antigens exclusively or preferentially presented by tumor cells without damaging normal somatic host cells.

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