Table 2. Transplantation outcomes

Patient no.	Status at study entry	$CD34^+$ cells infused ($\times 10^6$/kg)	Engraftment (days)	Accomplishment of complete donor chimerism (days)	Acute GVHD [grade (sites)]	Chronic GVHD [grade (sites)]	Interventions: IFN-α (date of beginning)	Interventions: DLI (date, cells*)	Best responses [duration (days)]	Outcomes
1	PD	4.4	11	30	None	None	Day 281-	Day 390, 1×10^7 day 420, 3×10^7 day 447, 3×10^7	PD	PD, died on day 733
2	PD	3.0	10	30	I (skin)	Extensive (mouth, eye)	None	None	SD (336)	PD, died on day 413
3	PD	5.5	NE†	30	None	None	Day 159-	Day 138, 1×10^7	SD (160)	PD, alive on day 1,040
4	PD	3.1	11	30	None	None	Day 143-	Day 255, 1×10^7	SD (760+)	SD, alive on day 760
5	PD	5.0	10	30	II (skin)	Extensive (skin, mouth)	None	None	PR (791+)	PR, alive on day 791
6	PD	3.2	11	30	II (skin, gut)	NE	None	None	PD	PD, died on day 74
7	PD	4.1	10	60	III (skin, liver, gut)	Limited (mouth)	Day 281-	None	PD	PD, alive on day 602
8	PD	2.8	11	60	None	Extensive (liver, mouth)	Day 71-	None	SD (477+)	SD, alive on day 477
9	PD	3.8	10	60	None	None	Day 62-	None	SD (108)	PD, alive on day 460

DLI = donor lymphocyte infusion; GVHD = graft-vs-host disease; IFN-α = interferon-α; NE = not evaluable; PD = progressive disease; PR = partial remission; SD = stable disease.

*Now of infused $CD34^+$ cells (/kg).

†Patient 3 did not develop neutropenia 0.5×10^9/L.

Table 3. Adverse events

Adverse events	No. of patients (%)
Acute GVHD	4 (44)
Grade I	1 (11)
Grade II	2 (22)
Grade III	1 (11)
Chronic GVHD	4 (50)
Limited	1 (13)
Extensive	3 (38)
Febrile neutropenia*	7 (78)
Bacterial infection†	3 (33)
Perianal abscess	1 (11)
Venous catheter infection	2 (22)
CMV antigenemia	7 (78)
Other‡	2 (22)

CMV = cytomegalovirus; GVHD = graft-vs-host disease.

*Febrile neutropenia was defined as >38.0°C with absolute neutrophil count 1×10^9/L.

†Bacterial infection was defined as >38.0°C with positive culture of bacteria in blood or discharged pus.

‡Other includes immune-mediated thrombocytopenia and hypothalamic hypothyroidism.

who developed grade II emesis. There was no regimen-related mortality within the first 100 days of transplantation, but patient 6 died of rapidly progressive disease on day 74. Seven patients developed CMV antigenemia at a median onset of day 32 (range 17–45), and four patients received preemptive therapy with ganciclovir. No patients developed CMV disease.

Graft-vs-host disease

The incidences of acute and chronic GVHD are given in Tables 2 and 3. Four patients developed acute GVHD; grade I in 1, grade II in 2, and grade III in 1. Three of these 4 patients subsequently developed chronic GVHD (1 limited and 2 extensive), and the remaining patient died of rapidly progressive liver metastases on day 74. Among the 5 patients without acute GVHD, one developed chronic extensive GVHD following the administration of low-dose IFN-α after transplantation. The remaining 4 patients did not develop chronic GVHD throughout their clinical courses despite treatment with IFN-α and/or DLI.

Administration of IFN-α and DLI following RIST

To augment the GVT effect, 6 patients received IFN-α, and 3 received additional DLI. Patient 8 developed hepatocellular and cholangiocellular liver injury, which was pathologically diagnosed as autoimmune hepatitis and hepatic involvement of chronic GVHD. The remaining 5 patients did not develop chronic GVHD while receiving IFN-α therapy and after DLI, and none showed obvious tumor regression.

Clinical responses

The best response was evaluated. PR, SD, and PD were observed in 1, 5, and 3 patients, respectively, with an overall response rate of 11%. In those whose best response was SD, the median duration of SD was 336 days (range 108–760).

As of July 2003, 6 patients are alive with a median follow-up of 681 days (range 460–1040 days). Consequently, the final outcomes were PR in 1, SD in 2, and PD in 6, and 3 patients died of disease progression. The actuarial 1-year and 2-year overall survival rates were 89% and 74%, respectively.

The correlation between GVHD and overall survival was not significant ($p = 0.40$), although patient 5 attained PR (Fig. 1) following both acute and chronic GVHD. His tumor began to regress 5 months after RIST despite immunosuppressive therapy for GVHD with cyclosporine and low-dose corticosteroid. He remained in PR on day 791. Among the remaining 4 patients with acute or chronic GVHD, best response was SD in 2 and PD in 2.

The pulmonary lesions of patient 2 rapidly progressed during corticosteroid therapy for acute GVHD and immune-mediated thrombocytopenia. After discontinuation of corticosteroid on day 132, his metastatic lesions started to regress without flaring of GVHD. He developed chronic GVHD on day 266, for which prednisolone 30 mg was resumed. Although chronic GVHD remained despite low-dose prednisolone, he maintained SD until day 336. He died of progression of lung metastases on day 413. Patient 6 received corticosteroid therapy for acute skin GVHD from day 45. His small metastatic lesions in the liver showed slow progression on computed tomographic (CT) scan on day 52. Tapering of steroid was followed by a marked elevation of serum transaminases and bilirubin, and corticosteroid was resumed on day 63 for probable acute liver GVHD. However, CT scan on day 66 showed a marked progression of the metastatic lesions in the liver, and he died of liver failure on day 74. The progression of liver metastases was confirmed at pathologic examinations of autopsy specimens without evidence of acute liver GVHD. In patients 7 and 8, no tumor reduction was observed despite the presence acute and/or chronic GVHD.

Although those patients without GVHD (no. 1, 3, 4, and 9) were given IFN-alpha and/or DLI, neither GVHD nor tumor regression was observed. Their best responses were SD in 3 and PD in 1.

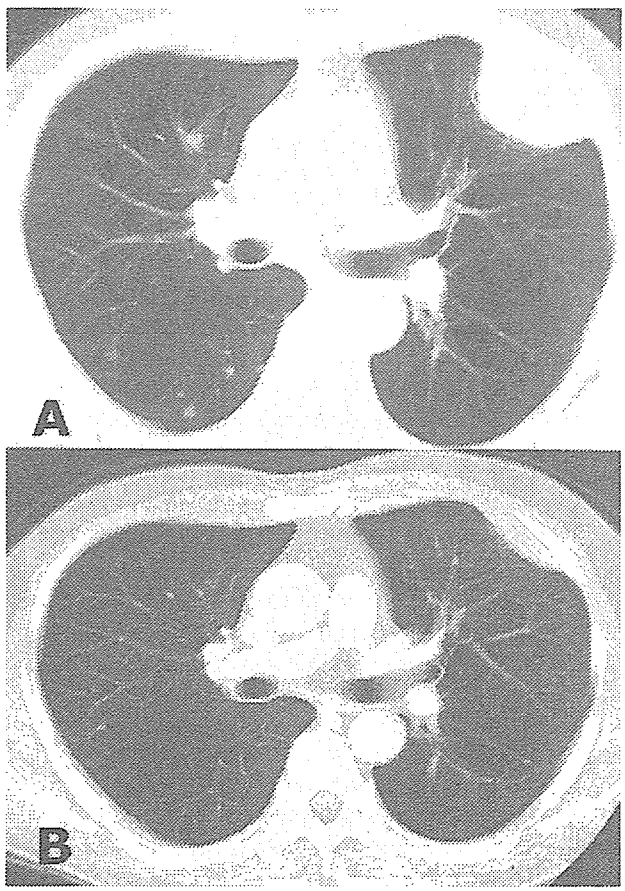

Figure 1. Computed tomography of the chest before (**A**) and 722 days after (**B**) transplantation in patient 6. Pleural and pulmonary metastases were reduced after transplant. The patient achieved partial remission.

Retrospective analysis of all patients undergoing HLA typing

Characteristics of transplanted or nontransplanted patients are summarized in Table 4. All patients were in stage IV with metastatic lesions. There were no significant differences

Table 4. Characteristics of transplanted and nontransplanted patients

	Transplanted patients	Nontransplanted patients	*p* Value
No. of patients	9	17	
Sex (male/female)	5/4	15/2	0.16*
Age at HLA typing [years, median (range)]	38 (25–61)	50 (36–63)	0.075†
Predominant histology (no. of patients)			0.41*
Clear cell carcinoma	6	6	
Non–clear cell carcinoma‡	3	7	
Unknown	0	4§	
Previous therapy (no. of patients)			
Nephrectomy	9	15	0.53*
Metastatectomy	5	7	0.68*
Radiation therapy	3	3	0.63*
IFN-α	9	17	0.99*
IL-2	3	4	0.66*
Chemotherapy (tegafur)	4	2	0.14*
No of metastatic organs [median (range)]	3 (1–5)	1 (1–3)	0.034†
Days from nephrectomy to HLA-typing [median (range)]	957 (0–3689)	658 (128–1382)	0.32†

IFN-α = interferon-α; IL-2 = interleukin-2.

*Fisher's exact test.

†Mann-Whitney U-test.

‡Includes granular cell carcinoma, papillary carcinoma, and spindle cell carcinoma.

§Includes two patients who did not undergo nephrectomy.

in characteristics between them, except for the number of metastatic sites. Histologic nuclear grade of RCC was not compared because most of the cases exhibited a mixture of histologic grades. Transplanted patients (n = 9) showed a **significantly higher overall survival rate than those who had** not received RIST (n = 17) (Fig. 2A, $p = 0.016$).

We compared the overall survival rates among 12 patients with matched donors and the other 14 patients without matched donors (Fig. 2). Characteristics of each group were summarized in Table 5. The 1-year actuarial survival rates were 74% and 48% in patients with and those without donors, respectively (Fig. 2B, $p = 0.088$).

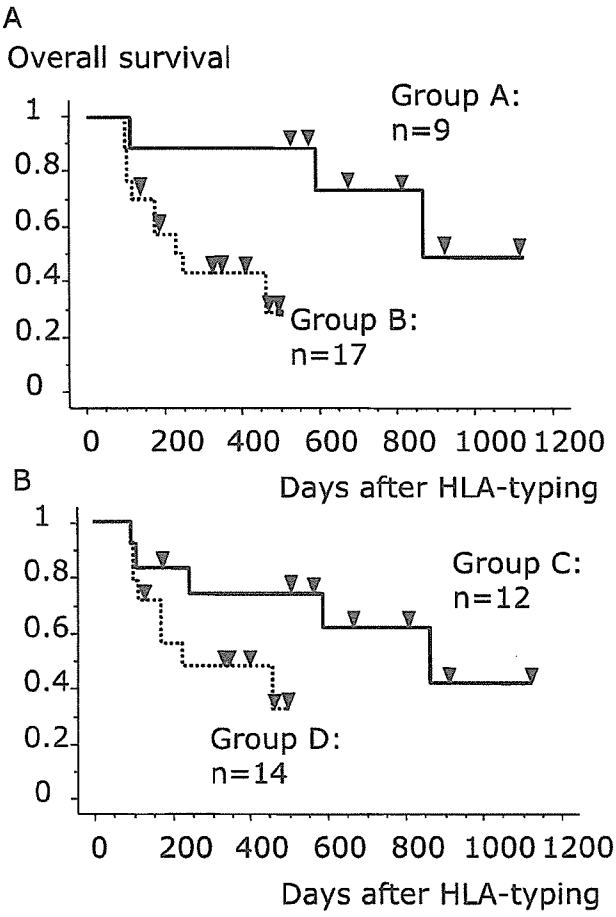

Figure 2. Kaplan-Meier estimates of the overall survival rates following HLA-typing. **(A)** Comparison of overall survival rates between transplanted **and nontransplanted patients. The overall survival rate was significantly** higher in transplanted patients than in nontransplanted patients ($p = 0.016$). **(B)** Comparison between patients with and patients without an HLA-matched donor. A trend toward a better survival was observed in patients with an HLA-matched donor ($p = 0.088$). Group A = transplanted patients (n = 9); group B = patients who had not received transplantation (n = 17); group C = patients with an HLA-matched donor (n = 12), including 9 transplanted patients; group D = patients without an HLA-matched donor (n = 14).

Table 5. Characteristics of patients with or without an HLA-matched donor

	Patients with an HLA-matched donor	Patients without an HLA-matched donor	*p* Value
No. of patients	12	14	
Sex (male/female)	8/4	12/2	0.36*
Age at HLA typing [years, median (range)]	47 (25–62)	51 (36–63)	0.16†
Predominant histology (no. of patients)			0.99*
Clear cell carcinoma	7	5	
Non-clear cell carcinoma‡	5	5	
Unknown	0	4§	
Previous therapy (no. of patients)			
Nephrectomy	12	12	0.48*
Metastatectomy	6	6	0.99*
Radiation therapy	3	3	0.99*
IFN-α	12	14	0.99*
IL-2	4	3	0.67*
Chemotherapy (tegafur)	5	1	0.065*
No. of metastatic organs [median (range)]	2 (1–5)	1.5 (1–3)	0.20†
Days from nephrectomy to HLA-typing [median (range)]	829 (0–3689)	561 (128–1382)	0.38†

IFN-α = interferon-α; IL-2 interleukin-2.
*Fisher's exact test.
†Mann-Whitney U-test.
‡Includes granular cell carcinoma, papillary carcinoma, and spindle cell carcinoma.
§Includes two patients who did not undergo nephrectomy.

Discussion

Our reduced-intensity regimen was well tolerated with minimal RRT. All of the patients achieved stable engraftment without DLI. Although previous pilot studies suggested that the risk of graft rejection decreases in heavily pretreated patients because of the carryover of immunosuppression and myelosuppression [16,29], stable engraftment could be established with our regimen in patients who had not received intensive chemotherapy prior to transplantation.

GVHD is the most significant complication in allogeneic HSCT. Our regimen and prophylactic procedure seem to be effective in preventing GVHD: grade II to IV acute GVHD developed in only three patients, and none developed fatal GVHD. Our regimen was different from others regarding the routine use of ATG. In RIST against hematologic malignancies, use of ATG promotes achievement of complete donor-type chimerism without increasing the risk of GVHD [30]. ATG may offer an advantage in RIST against RCC in terms of achieving stable engraftment and complete donor chimerism, as well as preventing GVHD.

The response rate was low in this study compared with other reports on RIST for RCC [14–19]. There are three possible explanations for these results. First, all of the patients had advanced metastatic RCC. The 1-year overall

survival rate of such patients was reported to be approximately 20% [8]. The poor patient backgrounds might have influenced the outcome of this study. Second, the extent of GVHD and the GVT effect may differ among different ethnic backgrounds, with possibly lower GVHD and associated GVT effects in Japanese populations [20,31]. Third, the low rate of acute GVHD in this study might have interfered with the curative potential of alloimmunity. The only patient who achieved PR had developed acute and chronic GVHD, whereas those without GVHD had no obvious tumor regression. It is likely that GVHD is closely associated with the clinical response, as previously suggested [14]. Although use of an ATG-containing preparative regimen does not increase relapses in RIST for low-risk myeloid leukemia [32], the role of ATG should be critically investigated in RIST for RCC.

The precise mechanism of the GVT effect remains unknown. Disease regression associated with cyclosporine withdrawal, complete donor chimerism, and GVHD provides evidence that cytotoxic donor T cells play an important role in this response. Recent studies have suggested that distinct T-cell populations recognizing tumor-specific antigens and/or minor histocompatibility antigens are involved in the GVT effect [33,34]. T-cell clones attacking both recipient's RCC cells and hematopoietic cells were isolated from responding patients [35]. On the other hand, some investigators suggested that the local cytokine storm associated with the early phase of allogeneic transplantation plays an important role in GVHD [36]. Tumor progression and regression in concordance with corticosteroid use observed in this study are compatible with this suggestion, since the cytokine production is readily suppressed by corticosteroid. It should be stressed that acute GVHD is the leading cause of death in allogeneic HSCT against RCC [14,17,18], and that prevention of GVHD is an extremely important consideration. It frequently is difficult to strike a balance between GVHD and GVT effect, as shown in patients 2 and 6. The close correlation between tumor progression and the time course of corticosteroid therapy suggests that it is difficult to modulate immune reactions following allogeneic HSCT using currently available maneuvers. A better understanding of the exact mechanism of the GVT effect should develop the formula for GVHD prophylaxis and improve the clinical efficacy and safety of this procedure.

It is difficult to evaluate clinical responses against solid tumors in allogeneic HSCT [37]. Previous reports noted that tumor regression often occurs several months after transplant [14,15,17,19]. In our study, some metastatic lesions progressed during the maximum immunosuppressive period with cyclosporine and subsequently showed stable disease or even regression after its discontinuation. However, these patients were evaluated as PD under the current RECIST criteria. A more accurate evaluation of treatment efficacy would be based on improvement of overall survival. The actuarial overall survival of the 12 patients with a matched donor was better than that of the other 14 patients without a donor (Fig. 2B). These findings suggest the efficacy of allogeneic HSCT with our regimen despite its low "response rate." However, these analyses are retrospective, and even the genetic allocation analysis might have contained unrecognized bias, leading to overestimation of the results. Phase II and III study is warranted to clarify the efficacy of RIST for RCC.

Acknowledgments

This study was supported by a Grant-in-Aid for the Second-Term Comprehensive 10-Year Strategy for Cancer Control and a Grant-in-Aid for Cancer Research from the Ministry of Health, Labor and Welfare of Japan.

References

1. Storb R. Allogeneic hematopoietic stem cell transplantation—yesterday, today, and tomorrow. Exp Hematol. 2003;31:1–10.
2. Slavin S, Nagler A, Naparstek E, et al. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. Blood. 1998;91: 756–763.
3. Giralt S, Estey E, Albitar M, et al. Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus-leukemia without myeloablative therapy. Blood. 1997;89:4531–4536.
4. Saito T, Kanda Y, Kami M, et al. Therapeutic potential of a reduced-intensity preparative regimen for allogeneic transplantation with cladribine, busulfan, and antithymocyte globulin against advanced/refractory acute leukemia/lymphoma. Clin Cancer Res.. 2002;8:1014–1020.
5. Giralt S, Thall PF, Khouri I, et al. Melphalan and purine analog-containing preparative regimens: reduced-intensity conditioning for patients with hematologic malignancies undergoing allogeneic progenitor cell transplantation. Blood. 2001;97:631–637.
6. Motzer RJ, Bander NH, Nanus DM. Renal-cell carcinoma. N Engl J Med. 1996;335:865–875.
7. Motzer RJ, Russo P. Systemic therapy for renal cell carcinoma. J Urol. 2000;163:408–417.
8. Kankuri M, Pelliniemi TT, Pyrhonen S, Nikkanen V, Helenius H, Salminen E. Feasibility of prolonged use of interferon-alpha in metastatic kidney carcinoma: a phase II study. Cancer. 2001;92:761–767.
9. Bay JO, Fleury J, Choufi B, et al. Allogeneic hematopoietic stem cell transplantation in ovarian carcinoma: results of five patients. Bone Marrow Transplant. 2002;30:95–102.
10. Eibl B, Schwaighofer H, Nachbaur D, et al. Evidence for a graft-versus-tumor effect in a patient treated with marrow ablative chemotherapy and allogeneic bone marrow transplantation for breast cancer. Blood. 1996;88:1501–1508.
11. Moscardo F, Martinez JA, Sanz GF, et al. Graft-versus-tumour effect in non-small-cell lung cancer after allogeneic peripheral blood stem cell transplantation. Br J Haematol. 2000;111:708–710.
12. Ueno NT, Rondon G, Mirza NQ, et al. Allogeneic peripheral-blood progenitor-cell transplantation for poor-risk patients with metastatic breast cancer. J Clin Oncol. 1998;16:986–993.
13. Zetterquist H, Hentschke P, Thorne A, et al. A graft-versus-colonic cancer effect of allogeneic stem cell transplantation. Bone Marrow Transplant. 2001;28:1161–1166.

14. Childs R, Chernoff A, Contentin N, et al. Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. N Engl J Med. 2000;343:750–758.
15. Bregni M, Dodero A, Peccatori J, et al. Nonmyeloablative conditioning followed by hematopoietic cell allografting and donor lymphocyte infusions for patients with metastatic renal and breast cancer. Blood. 2002;99:4234–4236.
16. Pedrazzoli P, Da Prada GA, Giorgiani G, et al. Allogeneic blood stem cell transplantation after a reduced-intensity, preparative regimen: a pilot study in patients with refractory malignancies. Cancer. 2002; 94:2409–2415.
17. Rini BI, Zimmerman T, Stadler WM, Gajewski TF, Vogelzang NJ. Allogeneic stem-cell transplantation of renal cell cancer after nonmyeloablative chemotherapy: feasibility, engraftment, and clinical results. J Clin Oncol. 2002;20:2017–2024.
18. Hentschke P, Barkholt L, Uzunel M, et al. Low-intensity conditioning and hematopoietic stem cell transplantation in patients with renal and colon carcinoma. Bone Marrow Transplant. 2003;31:253–261.
19. Ueno NT, Cheng YC, Rondon G, et al. Rapid induction of complete donor chimerism by the use of a reduced-intensity conditioning regimen composed of fludarabine and melphalan in allogeneic stem cell transplantation for metastatic solid tumors. Blood. 2003;102:3829–3836.
20. Morishima Y, Morishita Y, Tanimoto M, et al. Low incidence of acute graft-versus-host disease by the administration of methotrexate and cyclosporine in Japanese leukemia patients after bone marrow transplantation from human leukocyte antigen compatible siblings: possible role of genetic homogeneity. The Nagoya Bone Marrow Transplantation Group. Blood. 1989;74:2252–2256.
21. Sasazuki T, Juji T, Morishima Y, et al. Effect of matching of class I HLA alleles on clinical outcome after transplantation of hematopoietic stem cells from an unrelated donor. Japan Marrow Donor Program. N Engl J Med. 1998;339:1177–1185.
22. Van Lint MT, Uderzo C, Locasciulli A, et al. Early treatment of acute graft-versus-host disease with high- or low-dose 6–methylprednisolone: a multicenter randomized trial from the Italian Group for Bone Marrow Transplantation. Blood. 1998;92:2288–2293.
23. Kanda Y, Mineishi S, Saito T, et al. Long-term low-dose acyclovir against varicella-zoster virus reactivation after allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant. 2001;28:689–692.
24. Kanda Y, Mineishi S, Saito T, et al. Response-oriented preemptive therapy against cytomegalovirus disease with low-dose ganciclovir: a prospective evaluation. Transplantation. 2002;73:568–572.
25. Shulman HM, Sullivan KM, Weiden PL, et al. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. Am J Med. 1980;69:204–217.
26. Bearman SI, Appelbaum FR, Buckner CD, et al. Regimen-related toxicity in patients undergoing bone marrow transplantation. J Clin Oncol. 1988;6:1562–1568.
27. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus conference on acute GVHD grading. Bone Marrow Transplant. 1995;15:825–828.
28. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst. 2000;92:205–216.
29. Porter DL, Connors JM, Van Deerlin VM, et al. Graft-versus-tumor induction with donor leukocyte infusions as primary therapy for patients with malignancies. J Clin Oncol. 1999;17:1234–1243.
30. Nakai K, Mineishi S, Kami M, et al. Antithymocyte globulin affects the occurrence of acute and chronic graft-versus-host disease after a reduced-intensity conditioning regimen by modulating mixed chimerism induction and immune reconstitution. Transplantation. 2003;75: 2135–2143.
31. Morishima Y, Kodera Y, Hirabayashi N, et al. Low incidence of acute GVHD in patients transplanted with marrow from HLA-A, B, DR-compatible unrelated donors among Japanese. Bone Marrow Transplant. 1995;15:235–239.
32. Kroger N, Zabelina T, Kruger W, et al. In vivo T cell depletion with pretransplant anti-thymocyte globulin reduces graft-versus-host disease without increasing relapse in good risk myeloid leukemia patients after stem cell transplantation from matched related donors. Bone Marrow Transplant. 2002;29:683–689.
33. Storb RF, Lucarelli G, McSweeney PA, Childs RW. Hematopoietic cell transplantation for benign hematological disorders and solid tumors. Hematology (Am Soc Hematol Educ Program). 2003;372–397.
34. Drachenberg D, Childs RW. Allogeneic stem cell transplantation as immunotherapy for renal cell carcinoma: from immune enhancement to immune replacement. Urol Clin North Am. 2003;30:611–622.
35. Mena O, Igarashi T, Srinivasan R, et al. Immunologic mechanisms involved in the graft-vs-tumor (GVT) effect in renal cell carcinoma (RCC) following nonmyeloablative allogeneic peripheral blood stem cell transplantation (NST). Blood. 2001;98:356. Abstract 3555.
36. Teshima T, Ordemann R, Reddy P, et al. Acute graft-versus-host disease does not require alloantigen expression on host epithelium. Nat Med. 2002;8:575–581.
37. Hori A, Kami M, Kim SW, et al. Urgent need for a validated tumor response evaluation system for use in immunotherapy. Bone Marrow Transplant. 2004;33:255–256.