Table V. Multivariate Cox regression analysis and inverse probability of treatment weighted (IPTW) method analysis comparing transplant-related mortality (TRM), progression-free survival (PFS) and overall survival (OS) after peripheral blood stem cell transplantation (PBSCT) and bone marrow transplantation (BMT).

Outcomes	Analysis	Variables	HR (95% CI)	*P*-value
TRM at 100 d	Cox	Stem cell source: PBSCT	1·18 (0·66–2·12)	0·584
		Acute GvHD: grades II–IV	4·92 (2·57–9·42)	<0·001
	IPTW	Stem cell source: PBSCT	1·33 (0·84–2·10)	0·230
TRM at 1 year	Cox	Stem cell source: PBSCT	1·07 (0·69–1·66)	0·773
		Donor age: 40 years or older	1·98 (1·03–3·80)	0·040
		Acute GvHD: grades II–IV	2·58 (1·65–4·05)	<0·001
	IPTW	Stem cell source: PBSCT	1·17 (0·82–1·66)	0·381
Relapse	Cox	Stem cell source: PBSCT	0·95 (0·64–1·41)	0·806
		Disease risk: high	3·97 (2·66–5·94)	<0·001
		ECOG PS: 2–4	3·42 (1·73–6·77)	0·004
	IPTW	Stem cell source: PBSCT	0·95 (0·73–1·23)	0·676
PFS	Cox	Stem cell source: PBSCT	1·03 (0·77–1·37)	0·868
		Disease risk: high	2·41 (1·82–3·21)	<0·001
		ECOG PS: 2–4	2·83 (1·63–4·92)	<0·001
		Acute GvHD: grades II–IV	1·33 (1·00–1·78)	0·05
	IPTW	Stem cell source: PBSCT	1·05 (0·87–1·27)	0·589
OS	Cox	Stem cell source: PBSCT	0·99 (0·73–1·36)	0·972
		Disease risk: high	2·45 (1·79–3·34)	<0·001
		ECOG PS: 2–4	3·31 (1·88–5·84)	<0·001
		Acute GvHD: grades II–IV	1·57 (1·15–2·13)	0·004
	IPTW	Stem cell source: PBSCT	1·05 (0·85–1·29)	0·659

The following covariates were included in the Cox models as explanatory variables; patient and donor age (less than or more than 40 years), sex, sex matching, Eastern Cooperative Oncology Group performance status (ECOG PS), disease risk, cytomegalovirus (CMV) serology, stem cell source, conditioning regimen, doses of methotrexate (MTX), grades II–IV acute graft *versus* host disease (GvHD) and chronic GvHD. The values of stem cell source and significant covariates are shown in this table. Grades II–IV GvHD and chronic GvHD were included as time-dependent covariate (HR, hazard ratio).

Table VI. Causes of mortality and time of death.

	BMT (n = 104)	PBSCT (n = 75)
Number of TRM	51 (49·0)	44 (58·7)
Causes of TRM		
GvHD	4 (3·8)	13 (17·3)
Non-infectious pneumonia	6 (5·8)	6 (8·0)
Veno-occlusive disease of the liver	5 (4·8)	1 (1·3)
Infection	25 (24·0)	14 (18·7)
Haemorrhage	1 (1·0)	3 (4·0)
Others	10 (9·6)	7 (9·3)
Time of TRM		
Days 0–30	7 (6·7)	4 (5·3)
Days 31–100	14 (13·5)	20 (26·7)
After day 100	30 (28·8)	20 (26·7)
Number of deaths in relapse	53 (51·0)	31 (41·3)

TRM, transplant-related mortality; BMT, bone marrow transplantation; PBSCT, peripheral blood stem cell transplantation; GvHD, graft *versus* host disease.
Values are given as n (%).

three doses of MTX (day +1: 10 mg/m^2; day +3 and day +6: 7 mg/m^2) rather than four doses of MTX routinely used in other countries, because of the lower frequency of GvHD in Japan (Morishima *et al*, 1989). An RCT from the European Group for Blood and Marrow Transplantation (EBMT) study, in which increased incidence of acute and chronic GvHD was shown, also gave three doses of MTX (Schmitz *et al*, 2002). Omission of day +11, MTX may influence the incidence of acute and chronic GvHD (Nash *et al*, 1992; Cutler *et al*, 2001; Mehta & Singhal, 2002), although we did not find any difference among the different MTX dose groups. A recent report from the EBMT suggested that post-transplant G-CSF might increase the incidence of acute and chronic GvHD and TRM, resulting in lower leukaemia-free and OS rates after BMT (Ringden *et al*, 2004). Although the use of G-CSF postallografting is usually accepted as a standard care in Japan, we need to reconsider this indication, especially after BMT.

Notably, the observed cumulative incidence of grades II–IV acute GvHD in patients receiving HLA-identical transplants seemed lower in both groups (BMT 32·0%, PBSCT 37·4%) compared with rates reported from western countries (Powles *et al*, 2000; Bensinger *et al*, 2001; Couban *et al*, 2002; Schmitz *et al*, 2002). These data are consistent with previous reports on Japanese BMT patients (Morishima *et al*, 1989; Oh *et al*, 2002). Oh *et al* (2002) reported a multivariate analysis for adult allogeneic BMT patients showing that a Japanese cohort had a significantly lower risk of acute GvHD than white American, black American and Irish cohorts [relative risk

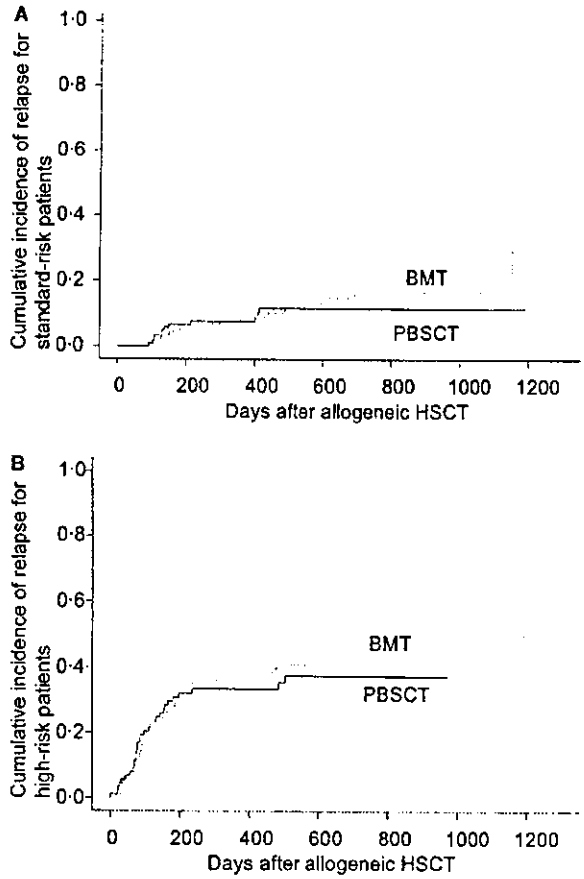

Fig 4. Cumulative incidences of relapse after allogeneic peripheral blood stem cell transplantation (PBSCT) compared with bone marrow transplantation (BMT). Cumulative incidence functions (A: standard-risk group; B: high-risk group) were predicted from the proportional subdistribution hazards model and adjusted for effects of significant covariates.

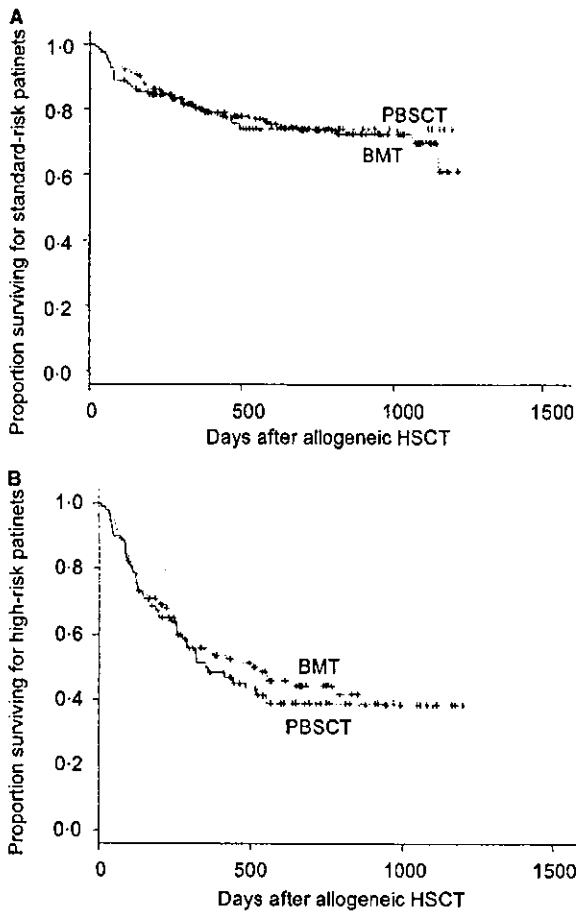

Fig 5. Probabilities of overall survival after allogeneic peripheral blood stem cell transplantation (PBSCT) compared with bone marrow transplantation (BMT). Probabilities were derived from Kaplan–Meier estimates [A: overall survival (OS) for standard risk group; B: OS for high-risk group].

(RR) = 1·77, $P < 0·01$; RR = 1·84, $P < 0·01$; RR = 2·22, $P < 0·01$ respectively]. Our data suggest that this trend might also apply to PBSCT. This difference has been speculated to reflect a lower degree of diversity for HLA and minor histocompatibility antigens among Japanese. However, a recent report revealed the influence of an interleukin-10 promotor polymorphism after allogeneic HSCT (Lin *et al*, 2003). The interleukin-10-592A/A genotype was associated with a decreased risk of grade III or IV acute GvHD. The frequency of this genotype is 67% in the Japanese population (Tegoshi *et al*, 2002), which is much higher than the frequency of 23% and 24% in two white populations (Lin *et al*, 2003). This finding may account for the decreased incidence and severity of acute GvHD in Japanese population than in white populations.

We found a significantly increased cumulative incidence of chronic GvHD among PBSCT patients in accord with several previous studies (Champlin *et al*, 2000; Bensinger *et al*, 2001; Cutler *et al*, 2001; Schmitz *et al*, 2002; Heldal *et al*, 2003). In particular, the extensive form of chronic GvHD was increased in the PBSCT cohort, whereas the incidence of the limited form was similar in the two cohorts. There is now considerable evidence that the preferential expansion of T-helper 2 (Th2) cells after allogeneic HSCT is associated with the development of chronic GvHD in both murine models and human beings (Doutrelepont *et al*, 1991; Umland *et al*, 1992; Allen *et al*, 1993; De Wit *et al*, 1993; Garlisi *et al*, 1993; Tanaka *et al*, 1997). A G-CSF-induced Th2 cytokine profile of donor T cells may be associated with increased incidence and severity of chronic GvHD (Pan *et al*, 1995). G-CSF also mobilized type 2 dendritic cells, which promote Th2 responses (Arpinati *et al*, 2000). Thus, G-CSF may have an important role in the development of chronic GHVD among PBSCT patients.

Another interesting point is the different distribution of organs affected by acute and chronic GvHD in BMT and PBSCT. Although previous reports demonstrated that skin and vaginal involvement (Bensinger *et al*, 2001; Flowers *et al*,

2002) or ocular involvement (Mohty *et al*, 2002) of chronic GvHD was more prevalent after PBSCT, the current study showed an increased incidence of skin, ocular sicca and oral mucositis, similar to Sjoegren syndrome. It is not well understood how selected organs become the targets of activated T cells. Inflammatory chemokines expressed in inflamed tissues upon stimulation by proinflammatory cytokines are specialized for the recruitment of effector cells (Moser & Loetscher, 2001). In mouse models, a comparative study of gene expression profiles of livers after experimental allogeneic and syngeneic BMT using oligonucleotide microarrays identified genes related to leucocyte trafficking that were upregulated at day 7 after allogeneic BMT when neither hepatic injury nor donor T-cell migration into the liver was evident (Ichiba *et al*, 2003). This study suggests that the interferon-γ produced by donor T cells in secondary lymphoid organs transactivates genes in target organs, stimulating the recruitment of effector cells to target organs and eventually rendering them vulnerable to effector cell attack. Thus, quantifiable and qualitative differences in immunological cells in PBSC grafts compared with bone marrow grafts may affect the chemokine environment, leading to the different distribution of affected organs. Alternately, increased numbers of affected organs in PBSC patients may simply reflect the increased severity of chronic GvHD.

Recent reports suggest that chronic GvHD with risk factors may negatively affect patients' survival (Akpek *et al*, 2001, 2003; Przepiorka *et al*, 2001). Long-term follow-up of an RCT showed that, although the cumulative incidence of chronic GvHD at 3 years was similar in BMT and PBSCT patients, chronic GvHD after PBSCT was more protracted and less responsive to treatment than after BMT (Bensinger *et al*, 2001; Flowers *et al*, 2002). With increasing numbers of long-term survivors, we need more information concerning the clinical characteristics of chronic GvHD after PBSCT (Przepiorka *et al*, 2001).

It has been postulated that a GVL effect may be observed, and the results of allogeneic HSCT may be improved in the presence of GvHD (Sullivan *et al*, 1989; Horowitz *et al*, 1990). However, the potential advantage of the GVL effect of allogeneic HSCT is often reduced by the GvHD-related morbidity and mortality (Weiden *et al*, 1981; Sullivan *et al*, 1989; Horowitz *et al*, 1990; Przepiorka *et al*, 2001; Lee *et al*, 2002). In most of the previous RCTs comparing BMT and PBSCT, the sample sizes were too small to detect meaningful survival increases (Schmitz *et al*, 1998; Blaise *et al*, 2000; Heldal *et al*, 2000; Powles *et al*, 2000). Even in the larger RCTs, survival was evaluated as a secondary end point (Bensinger *et al*, 2001; Couban *et al*, 2002; Schmitz *et al*, 2002). Bensinger *et al* (2001) and Couban *et al* (2002) have reported an OS benefit of PBSCT in patients with advanced disease. The former study included miscellaneous diseases and the observed advantage was derived from subgroup analysis, in which we were unable to draw reliable conclusions. The latter study, which involved 228 patients, included only myeloid malignancy but the improved survival was due to lower TRM with similar relapse rates, suggesting that faster haematological recovery accounts for this benefit. A meta-analysis reported by Cutler *et al* (2001), which involved 16 studies, and a large RCT from the EBMT (Schmitz *et al*, 2002) included 350 patients, and showed an increased incidence of acute and chronic GvHD, with no significant difference in relapse (Cutler *et al*, 2001; Schmitz *et al*, 2002) and survival rate (Schmitz *et al*, 2002). A recent meta-analysis suggested that any survival advantage of PBSCT is limited to patients with advanced disease (Horan *et al*, 2003). Thus, allogeneic PBSCT offered the prospect of a better outcome, but evidence for a survival benefit has been inconclusive. We must explicitly state that caution is highly advisable when interpreting *post hoc* subgroup analyses. These cannot be used for recommendations on treatment selection for individual patients, although they can be used in the development of new, empirically based research hypotheses. In addition, there might be a different impact on patient outcome after allogeneic HSCT according to stem cell source in this particular ethnic group, if the incidence of acute GvHD is lower than western countries. In the present study, multivariate analyses revealed that differences in stem cell source was not a significant factor for acute GvHD, relapse, TRM, PFS and OS despite the increased incidence of chronic GvHD after PBSCT. Early mortality within day 100 of PBSCT could be reduced because of faster engraftment (Champlin *et al*, 2000; Couban *et al*, 2002) but we did not observe this advantage. Our data showed that grades II–IV acute GvHD were significant adverse prognostic factors for TRM. The advantages of PBSCT may thus be counterbalanced by the increased incidence of GvHD. Treatment of acute and chronic GvHD was performed at the physician's discretion and immunosuppressive treatment may hamper the GVL effect in some cases. This may indicate the difficulty of separating GVL effects from GvHD clinically. We analysed the data according to each disease category and risk status, although there were no apparent differences between the two groups (data not shown). Therefore, in contrast to general belief, whether the GVL effect will improve survival after PBSCT remains unknown. Assessment of the overall benefits of PBSCT compared with BMT will require long-term follow-up of the morbidity of patients associated with chronic GvHD.

The retrospective nature, the heterogeneity of the diagnoses and the relatively short follow-up limit the power of this analysis. We cannot exclude the possibility that there are unmeasured confounders that could cause a bias between two groups. Analysis of the $CD34^+$ and $CD3^+$ cell dose was not performed because these are generally dependent on the source of stem cells, and in addition, we could not obtain enough data, especially in the BMT group. In multicentre studies, there is likely to be a variation among centres in both baseline risks and treatment effects that cannot be explained by the known prognostic factors (Frassoni *et al*, 2000; Matsuo *et al*, 2000; Loberiza *et al*, 2003). To resolve the limitations described

above, we needed an RCT in Japan. We have therefore launched a prospective, open-label RCT comparing allogeneic BMT *versus* PBSCT for adult patients with leukaemia. The primary end point of this trial is leukaemia-free survival based on time-to-event analysis. We plan the sample size per one arm to be 160, in order to detect the difference of 1·6 to 1·7 in HR for leukaemia-free survival. If this study can be completed, the impact of stem cell source on survival will be defined more accurately than the previous studies.

In summary, we observed faster engraftment and increased incidence of chronic GvHD in PBSCT compared with BMT for Japanese patients. The incidence of GvHD was lower than the western populations, but there were no differences in relapse, TRM, PFS and OS between PBSCT and BMT. These results suggest that the choice of haematopoietic stem cell source should be considered based on the data for individual ethnic populations. More detailed analysis and future trials may reveal the differential applicability of stem cells from these different sources in each disease category and hence enable us to choose appropriately between BMT and PBSCT based on reliable evidence.

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Appendix A

This study was conducted at the following institutions under the auspices of the following investigators in Japan: M. Sakai (Tokyo Metropolitan Hospital, Tokyo), T. Hamaki (National Cancer Centre, Tokyo), T. Karasuno (Osaka Medical Centre for Cancer and Cardiovascular diseases, Osaka), M. Kasai (Japanese Red Cross Nagoya first Hospital, Aichi), K. Kishi (Tokai University School of Medicine, Kanagawa), S. Okamoto (Keio University School of Medicine, Tokyo), N. Maseki (Saitama Cancer Centre Hospital, Saitama), S. Morishima (Meitetsu Hospital, Aichi), S. Yamasaki (Municipal Kitakyushu Medical Centre, Fukuoka), M. Kasai (Sapporo Hokuyu Hospital, Hokkaido), T. Kamimura (Harasanshin Hospital, Fukuoka), K. Shinagawa (Okayama University Medical School, Okayama), T. Yamane (Osaka City University, Osaka), S. Miyawaki (Saiseikai Maebashi Hospital, Gunma), Y. Miyazaki (Kansai Medical University, Osaka), T. Yamashita (National Medical Defence College, Saitama), N. Uike (National Kyushu Cancer Centre, Fukuoka), A. Maruta (Kanagawa Cancer Centre, Kanagawa), M. Misawa (Hyogo College of Medicine, Hyogo), K. Mitani (Dokkyo University School of Medicine, Tochigi), K. Kamezaki (Kyushu University Graduate School of Medical Sciences, Fukuoka), M. Masuda (Ryukyu University, Okinawa), J. Ishikawa (Osaka University, Osaka), A. Wake (Kokura Memorial Hospital, Fukuoka), A. Kohno (JA Aichi Showa Hospital, Aichi), M. Hara (Ehime Prefectural Central Hospital, Ehime), M. Kuroiwa (Hamanomachi Hospital, Fukuoka), E. Kusumi (Toranomon Hospital, Tokyo), K. Nishiwaki (Jikei University School of Medicine, Tokyo), M. Imamura (Hokkaido University Graduate School of Medicine, Hokkaido), Y. Takemoto (Jiaikai Imamura Hospital, Kagoshima), K. Fujimaki (Yokohama City University School of Medicine, Kanagawa), T. Tamaki (Rinku General Medical Centre, Osaka), Y. Takamatsu (Fukuoka University School of Medicine, Fukuoka), T. Murayama (Hyogo Medical Centre for Adults, Hyogo), M. Hirokawa (Akita University School of Medicine, Akita), T. Kobayashi (Tsuchiura Kyodo General Hospital, Ibaraki), K. Ozawa (Jichi Medical School, Tochigi), T. Ashida (Kinki University School of Medicine, Osaka), S. Imamura (Fukui Medical University, Fukui), Y. Kimura (Tokyo Medical University, Tokyo), K. Hodohara (Shiga Medical University, Shiga), H. Ago (Shimane Prefectural Central Hospital, Shimane), C. Shimazaki (Kyoto Prefectural University of Medicine, Kyoto), H. Teshima (Osaka City General Hospital, Osaka), A. Kubota (National Kyushu Medical Centre, Fukuoka), J. Tsukada (University of Occupational and Enviromental Health, School of Medicine, Fukuoka), C. Hashimoto (Yokohama City University Medical Centre), A. Yokota (Chiba Municipal Hospital, Chiba), H. Tsurumi (Gifu University, Gifu), M. Yamaguchi (Ishikawa Prefectural Central Hospital, Ishikawa), T. Endo (Hokkaido University Graduate School of Medical Sciences, Hokkaido), T. Chujo (Kanazawa University Graduate School of Medical Sciences, Ishikawa), M. Masuda (Tokyo Women's Medical College, Tokyo), S. Murakami (Social Insurance Kyoto Hospital, Kyoto), N. Emi (Nagoya University School of Medicine, Aichi), T. Fujisaki (Matsuyama Red Cross Hospital, Ehime), E. Matsuishi (Saga Prefectural Hospital Koseikan, Saga), F. Sano (St Mariannna University School of Medicine, Yokohama City Seibu Hospital, Kanagawa), Y. Torimoto (Asahikawa Medical College, Hokkaido), K. Yakushiji (Kurume University School of Medicine, Fukuoka), N. Uoshima (Matsushita Memorial Hospital, Osaka), H. Takamatsu (Kurobe City Hospital, Toyama), Y. Kobayashi (Kyoto Prefectural University of Medicine, Kyoto), K. Sunami (National Okayama Medical Centre, Okayama), K. Naito (Hamamatsu University School of Medicine, Shizuoka), H. Taguchi (Kochi Medical School, Kochi), S. Tsuchiya (Institute of Development, Aging and Cancer, Tohoku University, Miyagi), Y. Itoh (National Beppu Hospital, Oita), S. Doi (Kyoto Katsura Hospital, Kyoto), H. Kobayashi (Kyoto Prefectural Hospital, Kyoto), K. Tanimoto (Shin-koga Hospital, Fukuoka), K. Hayashi (Hoshigaoka Koseinenkin Hospital, Osaka), K. Kawachi (Takamatsu Red Cross Hospital, Kagawa), A. Urabe (NTT Kanto Medical Centre, Tokyo), R. Okamoto (Tokyo Metropolitan Komagome Hospital, Tokyo), T. Nishiura (National Kure Medical Centre, Hiroshima), H. Kimura (Kita-Fukushima Medical Centre,

Fukushima), T. Matsunaga (Sapporo Medical University School of Medicine, Hokkaido), N. Masauzi (Hakodate Municipal Hospital, Hokkaido), and T. Ishida (Sapporo Medical School, Hokkaido).

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Allogeneic hematopoietic stem cell transplantation with a reduced-intensity conditioning regimen for treatment of metastatic renal cell carcinoma: single institution experience with a minimum 1-year follow-up

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Objective. **The aim of this study was to evaluate the safety and efficacy of allogeneic hematopoietic stem cell transplantation with a reduced-intensity conditioning regimen (RIST) for interferon-α–refractory metastatic renal cell carcinoma (RCC).**

Patients and Methods. **Of 26 patients referred to the National Cancer Center Hospital for possible RIST between June 2000 and April 2002, an HLA-identical relative was identified for 12 patients. Nine patients underwent RIST. The conditioning regimen consisted of fludarabine 180 mg/m^2 or cladribine 0.66 mg/kg, plus busulfan 8 mg/kg and rabbit antithymocyte globulin 5 mg/kg. Graft-vs-host disease (GVHD) prophylaxis was cyclosporine alone.**

Results. **All patients achieved engraftment without grade III to IV nonhematologic regimen-related toxicity. All patients achieved complete donor-type chimerism without donor lymphocyte infusion by day 60. Four patients developed acute GVHD, and four developed chronic GVHD. One patient (11%) achieved partial response. As of July 2003, six patients were alive at median follow-up of 681 days. The actuarial overall survival rate was 89% at 1 year and 74% at 2 years. The overall survival rate tended to be higher in the 12 patients with a matched donor than in the other 14 patients without a matched donor ($p = 0.088$).**

Conclusion. **Our RIST procedure is feasible without severe toxicity. The efficacy of RIST for RCC should be confirmed in phase II/III clinical trials.** © **2004 International Society for Experimental Hematology. Published by Elsevier Inc.**

Allogeneic hematopoietic stem cell transplantation (HSCT) has been established as a standard therapy for various hematologic malignancies [1]. In allogeneic HSCT, malignant cells are eradicated through 1) myeloablation by irradiation or cytotoxic agents in the pretransplant conditioning regimen, and 2) an immunologic antitumor effect mediated by donor-derived immune competent cells [graft-vs-leukemia (GVL) effect] [1]. Although complete myeloablation once was considered essential for the engraftment of infused donor cells, recent investigations have proven that intense immunosuppression is sufficient for durable engraftment [2,3].

Reduced-intensity hematopoietic stem cell transplantation (RIST), which is expected to work mainly through a GVL effect rather than myeloablation, is associated with less regimen-related toxicity (RRT) compared to conventional HSCT [2,4]. Fludarabine and cladribine (2-chlorodeoxyadenosine), purine analogues with intense immunosuppressive

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but moderate myelosuppressive activities, have been commonly used as backbone agents in many current RIST regimens [2,4,5].

Renal cell carcinoma (RCC) is highly resistant to systemic therapy with hormonal and chemotherapeutic agents [6,7]. Although some patients show a durable response to immunotherapy with interferon-α (IFN-α) or interleukin-2 (IL-2), its response rate usually remains around 10–20% [6,7]. When they become refractory to IFN-α, the prognosis is uniformly grim, and no effective salvage therapies have been established [8]. Recently, the allogeneic immune-mediated antitumor effect has been proven to work against some solid tumors [graft-vs-tumor (GVT) effect] [9–13], including RCC [14–19]. Childs et al. [14] reported their treatment results in 19 patients with metastatic RCC using allogeneic HSCT; the response rate was 53%. However, there have been large differences in the conditioning regimen, immune regulatory maneuver after transplantation, and patients' backgrounds among studies on allogeneic HSCT against RCC [14–19]. In particular, differences in patient selection criteria and ethnic considerations [20,21] make it difficult to compare these reports. A suitable regimen and procedure for allogeneic RIST against metastatic RCC remain to be established.

To obtain additional information on the feasibility and efficacy of RIST against metastatic RCC, we report the results of our Japanese phase I study on RIST against metastatic RCC. The patients were followed for a minimum of 1 year.

Patients and methods

Patients

Patients with measurable metastatic RCC that was refractory to treatment with IFN-α and who had an HLA-identical or one antigen-mismatched healthy related donor were eligible to participate in the phase I protocol. Between September 1999 and October 2002, 26 patients with metastatic RCC who were referred to our hospital underwent HLA typing for donor screening. The median number of relatives examined per patient was 2 (range 1–14). HLA-matched donors were available in 12 patients, but 3 donor/recipient pairs were found to be ineligible for transplantation during further examinations. The remaining 9 patients were enrolled in this study, which was approved by the Institutional Review Board of National Cancer Center Hospital in Tokyo, Japan. Written informed consent was obtained from all patients/donors.

Eligibility criteria were as follows: 1) younger than 70 years; 2) life expectancy of at least 6 weeks; 3) Karnofsky performance score ≥70% or Eastern Cooperative Oncology Group score ≤2; 4) satisfactory cardiac function as evidenced by ejection fraction ≥45% by echocardiogram, diffusion capacity ≥40%, forced expiratory volume ≥50%, and PaO_2 in room air ≥60 mmHg; 5) serum bilirubin ≤2.5 mg/dL and serum aspartate aminotransferase <3 times upper reference limit; and 6) serum creatinine ≤2.0 mg/dL or creatinine clearance ≥50 mL/min. Patients were excluded if they had active infection, cardiac insanity including unstable angina pectoris and heart failure, uncontrolled diabetes mellitus, or a mental disorder that required treatment. Although nephrectomy was not a condition of enrollment, all of the patients had, in fact, previously undergone radical nephrectomy.

Stem cell collection

All patients received granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood stem cell (PBSC). Donors were injected with G-CSF at 5 μg/kg subcutaneously twice daily starting 3 days before the first collection of PBSC until the end of collection. Leukapheresis was performed daily until $>3.0 \times 10^6$ $CD34^+$ cells/kg of recipient body weight were collected. Collected cells were then cryopreserved using standard techniques. Cryopreserved PBSC were thawed and infused after the conditioning regimen was completed on day 0.

Conditioning regimen and transplantation procedures

The conditioning regimen in the first three patients consisted of cladribine 0.11 mg/kg/day by 2-hour intravenous infusion for 6 days (days −8 to −3), oral busulfan 4 mg/kg for 2 days (days −4 and −3), and rabbit antithymocyte globulin (ATG, Thymoglobulin; IMTIX-SANGSTAT, Lyon, France) 2.5 mg/kg by 12-hour intravenous infusion for 2 days (days −2 and −1). In the remaining six patients (no. 4–9), cladribine was replaced by fludarabine at 30 mg/m^2/day because cladribine was no longer available. Graft-vs-host disease (GVHD) prophylaxis consisted of cyclosporine alone, initiated on day −1 at a dose of 3 mg/kg/day by continuous intravenous infusion to maintain serum levels of 250 ng/mL. This was changed to an oral form when it could be tolerated. Withdrawal of cyclosporine was started on day 30 and completed by day 100 in the absence of acute GVHD. However, in the three most recently treated patients, cyclosporine was discontinued on day 45 in an attempt to induce a GVT effect earlier. Methylprednisolone (1–2 mg/kg) was added for patients who developed grade II to IV acute GVHD [22].

Engraftment and supportive care

Engraftment was defined as the first of 2 consecutive days with an absolute neutrophil count (ANC) of 0.5×10^9/L or more. Patients received G-CSF at a dose of 5 μg/kg/day by intravenous injection from day 6 after transplant until engraftment. Packed platelets and red blood cells were transfused to maintain the platelet level above 20×10^9/L and the hemoglobin level above 8 g/dL. All blood products were irradiated and filtered before transfusion.

Patients received antibacterial and antifungal prophylaxis consisting of oral ciprofloxacin 600 mg/day and fluconazole 200 mg/day, beginning 3 days before the start of the conditioning regimen. As prophylaxis against *Pneumocystis carinii* pneumonia, sulfamethoxazole/trimethoprim was given for at least 14 consecutive days (1600 mg of sulfamethoxazole and 320 mg of trimethoprim daily) before transplantation and was resumed after engraftment on a 2-day per week schedule. As prophylaxis against herpes simplex virus infection and varicella zoster virus reactivation, acyclovir was given 1000 mg/day orally or 750 mg/day intravenously from days −7 to 35, followed by long-term low-dose (400 mg/day) oral administration until the end of immunosuppressive therapy [23]. All patients received cytomegalovirus (CMV) high-titer intravenous immunoglobulin 5 g weekly for the first 3 months after transplantation. A CMV antigenemia assay with C7-HRP monoclonal antibody (Teijin, Tokyo, Japan) was performed at least once per week, and antigenemia-guided preemptive therapy with ganciclovir was performed as previously described [24].

Assessment of chimerism
Chimerism assay was performed with peripheral blood $CD3^+$ cells or mononuclear cells by the short tandem repeat method on days 30, 60, 90, and 120 after transplantation, and every 60 days thereafter, as described previously [4]. Complete donor-cell type chimerism was defined as 90% or more donor-type DNA. Donor lymphocyte infusion (DLI) was planned when patients failed to achieve complete donor chimerism after discontinuation of cyclosporine.

Treatment for progressive disease following RIST
DLI or low-dose subcutaneous IFN-α therapy was planned for patients with persistent or progressive disease in the absence of GVHD after discontinuation of cyclosporine.

Outcome measures
The primary endpoint was achievement of sustained engraftment with the induction of complete donor-type chimerism, without death during the first 100 days. All deaths within the first 100 days of transplant were defined as failure regardless of the cause of death, because it often is difficult to distinguish between transplantation-related mortality and death due to progressive disease during the early posttransplant period. RRT and acute and chronic GVHD were evaluated according to standard criteria [25–27]. Treatment response was evaluated monthly after transplantation according to the Response Evaluation Criteria in Solid Tumors (RECIST) [28]. Briefly, if the longest diameters of measurable lesions were reduced by 30% or more for at least 4 weeks compared with those before RIST, the patients were determined as partial remission (PR). If the diameters showed 20% or greater increase compared with the smallest diameters or if new lesions appeared, the patients were determined as progressive disease (PD). Patients were determined as stable disease (SD) when they did not met either PR or PD criteria. We also evaluated the duration of SD.

Statistical analysis
The characteristics of the patient groups were compared using Fisher's exact test or Mann-Whitney U-test. The actuarial survival rate was calculated by the Kaplan-Meier method. To calculate the survival of transplanted cases, the date of transplantation was defined as day 0 of the survival period. To compare the survival of transplanted and nontransplanted patients or patients with and without an HLA-matched donor, the date of HLA typing was defined as day 0 of the survival period. Differences between survival rates were calculated using Wilcoxon's log rank analysis. $p < 0.05$ were considered significant.

Results

Transplantation, engraftment, and chimerism analysis
Characteristics of the enrolled patients and transplantation outcomes are given in Tables 1 and 2, respectively. All nine patients received a stem cell graft from an HLA-matched sibling donor. Engraftment was achieved a median of 10.5 days after transplantation (range 10–11). Six patients did not develop a platelet count $<20 \times 10^9$/L, and the other three achieved an unsupported platelet count $>20 \times 10^9$/L on a median of 11 days (range 9–11). Three patients received transfusion of packed red blood cells with a median of 12 units during the first month (range 10–18), and seven patients received packed platelets with a median of 24 units during the first month (range 10–100). Complete donor chimerism was achieved without additional DLI by day 30 (n = 6) or day 60 (n = 3).

Toxicities
Transplantation-related adverse events are listed in Table 3. No acute phase nonhematologic RRT of grade III/IV was observed. Most patients could maintain oral intake throughout the transplantation course, except for patient 8

Table 1. Patient characteristics

Patient no.	Age/sex	Histology of primary tumors	Prior surgeries	Prior treatments	Days from nephrectomy to transplantation	Metastatic organs	
						No.	Sites
1	29/M	Granular + spindle	Nephrectomy	IFN-α	734	4	Liver, lung, skin, renal fossa
2	38/M	Clear + granular	Nephrectomy, lung resection	IFN-α, IL-2	1282	2	Lung, LN
3	32/F	Papillary	Nephrectomy, LN dissection	IFN-α	1045	1	Lung
4	48/F	Clear	Nephrectomy, parotidectomy	IFN-α, IL-2, tegafur	3746	5	Lung, bone, salivary gland, LN, renal fossa
5	59/M	Clear + granular + spindle	Nephrectomy	IFN-α, tegafur	1083	2	Lung, pleura
6	35/M	Clear + granular	Nephrectomy, liver resection	IFN-α, IL-2, tegafur, XRT (bone)	322	4	Lung, liver, bone, LN
7	25/F	Papillar	Nephrectomy	IFN-α, tegafur, XRT (bone)	62	3	Liver, bone, LN
8	47/F	Clear	Nephrectomy	IFN-α, TAE (kidney)	3722	5	Lung, pancreas, kidney, adrenal gland, LN
9	61/M	Clear + granular + spindle	Nephrectomy, lung resection	IFN-α, XRT (bone)	427	1	Bone

clear = clear cell carcinoma; granular = granular cell carcinoma; IFN-α = interferon-α; IL-2 = interleukin-2; LN = lymph node; papillary = papillary carcinoma; spindle = spindle cell carcinoma; TAE = transarterial embolization; XRT = radiation therapy.

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.

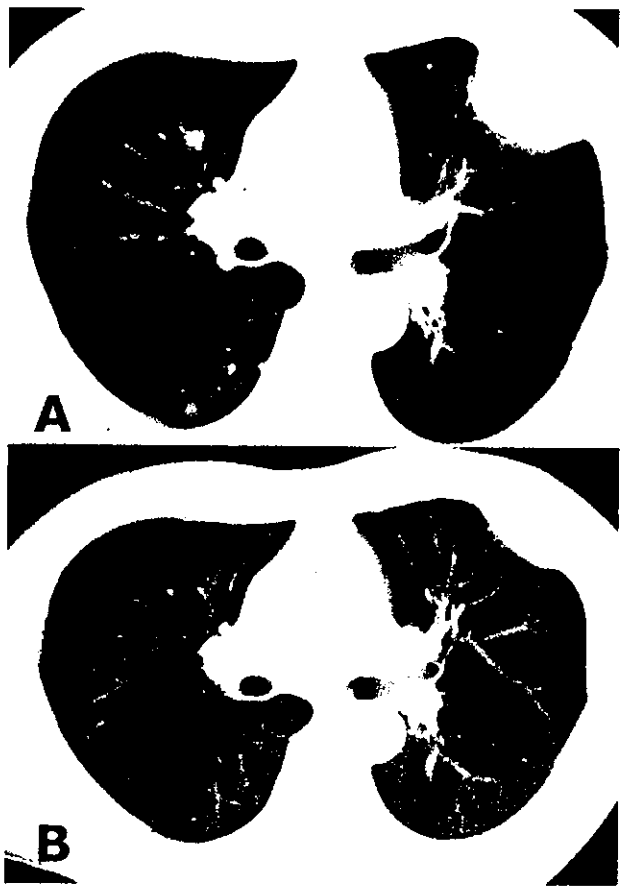

Figure 1. Computed tomography of the chest before (A) and 722 days after (B) transplantation in patient 6. Pleural and pulmonary metastases were reduced after transplant. The patient achieved partial remission.

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, $p = 0.088$).

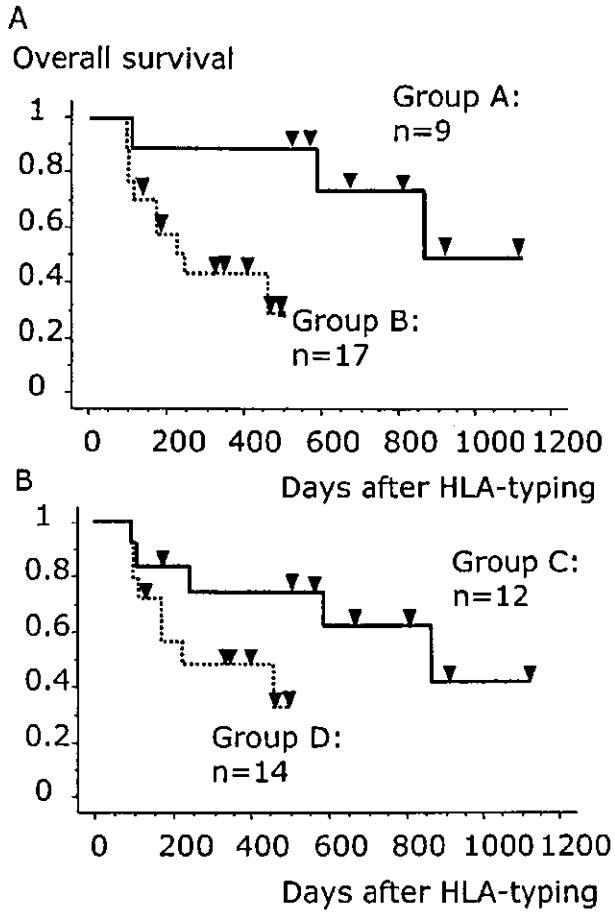

Figure 2. Kaplan-Meier estimates of the overall survival rates following HLA-typing. (A) Comparison of overall survival rates between transplanted and nontransplanted patients. The overall survival rate was significantly higher in transplanted patients than in nontransplanted patients ($p = 0.016$). (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.

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