Table 1 Regimen-related toxicity according to the Seattle criteria

Toxicity	*Grade 1*	*Grade 2*	*Grade 3*
Heart	Mild electrocardiogram abnormality, not requiring medical intervention; or noted heart enlargement on CXR with no clinical symptoms	Moderate electrocardiogram abnormalities requiring and responding to medical intervention; or requiring continuous monitoring without treatment; or congestive heart failure responsive to digitalis or diuretics	Severe electrocardiogram abnormalities with no or only partial response to medical intervention; or heart failure with no or only minor response to medical intervention: or decrease in voltage by more than 50%
Bladder	Macroscopic hematuria after 2 days from last chemotherapy dose with no subjective symptoms of cystitis and not caused by infection	Macroscopic hematuria after 7 days from last chemotherapy dose not caused by infection; or hematuria after 2 days with subjective symptoms of cystitis not caused by infection	Hemorrhagic cystitis with frank blood, necessitating invasive local intervention with installation of sclerosing agents, nephrostomy or other surgical procedures
Kidney	Increase in creatinine up to twice the baseline value	Increase in creatinine above twice baseline but not requiring dialysis	Requirement of dialysis
Lung	Dyspnea without CXR changes not caused by infection or congestive heart failure; or CXR showing isolated infiltrate or mild interstitial changes without symptoms not caused by infection or congestive heart failure	CXR with extensive localized infiltrate or moderate interstitial changes combined with dyspnea and not caused by infection or CHF; or decrease of PO_2 (>10% from baseline) but not requiring mechanical ventilation or >50% O_2 on mask and not caused by infection	Interstitial changes requiring mechanical ventilatory support or >50% oxygen on mask and not caused by infection or CHF
Liver	Mild hepatic dysfunction with 2.0 mg/dl <bilirubin <6.0 mg/dl or weight gain >2.5% and <5% from baseline, of noncardiac origin; or serum AST increase more than two-fold but less than five-fold from lowest preconditioning	Moderate hepatic dysfunction with bilirubin >6 mg/dl <20 mg/dl; or serum AST increase >five-fold from preconditioning; or clinical ascites or image-documented ascites >100 ml; or weight gain >5% from baseline of noncardiac origin	Severe hepatic dysfunction with bilirubin >20 mg/dl; or hepatic encephalopathy; or ascites compromising respiratory function
CNS	Somnolence but the patient is easily arousable and oriented after arousal	Somnolence with confusion after arousal; or other new objective CNS symptoms with no loss of consciousness not more easily explained by other medication, bleeding, or CNS infection	Seizures or coma not explained (documented) by other medication, CNS infection, or bleeding
Stomatitis	Pain and/or ulceration not requiring a continuous i.v. narcotic drug	Pain and/or ulceration requiring a continuous i.v. narcotic drug (morphine drip)	Severe ulceration and/or mucositis requiring preventive intubation; or resulting in documented aspiration pneumonia with or without intubation
GI toxicity	Watery stools >500 ml but <2000 ml every day not related to infection	Watery stools >2000 ml every day not related to infection; or macroscopic hemorrhagic stools with no effect on cardiovascular status not caused by infection; or subileus not related to infection	Ileus requiring nasogastric suction and/or surgery and not related to infection; or hemorrhagic enterocolitis affecting cardiovascular status and requiring transfusion

Grade IV regimen-related toxicity is defined as fatal toxicity.
CXR = chest X ray; i.v. = intravenous; CNS = central nervous system; GI = gastrointestinal; CHF = congestive heart failure.

Table 2 Regimen-related toxicity according to NCI-CTC version 2.0

Toxicity		*Grade 1*	*Grade 2*	*Grade 3*	*Grade 4*
Heart	Arrhythmia	Asymptomatic, not requiring treatment	Symptomatic, but not requiring treatment	Symptomatic and requiring treatment	Life-threatening (eg, arrhythmia associated with CHF, hypotension, syncope, shock)
	Ischemia/infarction	Nonspecific T-wave flattening or changes	Asymptomatic, ST- and T-wave changes suggesting ischemia	Angina without evidence of infarction	Acute myocardial infarction
	Left ventricular function	Asymptomatic decline of resting ejection fraction of >10% but <20% of baseline value; shortening fraction >24% but <30%	Asymptomatic but resting ejection fraction below LLN for laboratory or decline of resting ejection fraction >20% of baseline value; <24% shortening fraction	CHF responsive to treatment	Severe or refractory CHF or requiring intubation
Bladder	Hematuria	Microscopic only	Intermittent gross bleeding, no clots	Persistent gross bleeding or clots; may require catheterization or instrumentation, or transfusion	Open surgery or necrosis or deep bladder ulceration
Kidney	Renal dysfunction	Not defined	Not defined	Requiring dialysis, but reversible	Requiring dialysis and irreversible
	Creatinine levels	>ULN–1.5 × ULN	>1.5–3.0 × ULN	>3.0–6.0 × ULN	>6.0 × ULN
Lung	Hypoxia	Not defined	Decreased O_2 saturation with exercise	Decreased O_2 saturation at rest, requiring supplemental oxygen	Decreased O_2 saturation, requiring pressure support (CPAP) or assisted ventilation
	Pneumonitis/pulmonary infiltrates	Radiographic changes but asymptomatic or symptoms not requiring steroids	Radiographic changes and requiring steroids or diuretics	Radiographic changes and requiring oxygen	Radiographic changes and requiring assisted ventilation
Liver	Bilirubin	>ULN–1.5 × ULN	>1.5–3.0 × ULN	>3.0–10.0 × ULN	>10.0 × ULN
	Aspartate aminotransferase (AST)	>ULN–2.5 × ULN	>2.5–5.0 × ULN	>5.0–20.0 × ULN	>20.0 × ULN
	Alanine aminotransferase (ALT)	>ULN–2.5 × ULN	>2.5–5.0 × ULN	>5.0–20.0 × ULN	>20.0 × ULN
	Alkaline phosphatase (ALP)	>ULN–2.5 × ULN	>2.5–5.0 × ULN	>5.0–20.0 × ULN	>20.0 × ULN
	Weight gain/asites	2–5%	5–10%	⩾10% or as ascites	⩾10% or fluid retention resulting in pulmonary failure
CNS	Depressed level of consciousness	Somnolence or sedation not interfering with function	Somnolence or sedation interfering with function, but not interfering with activities of daily living	Obtundation or stupor; difficult; interfering with activities of daily living	Coma
Stomatitis	Stomatitis/pharyngitis	Painless ulcers, erythema, or mild soreness in the absence of lesions	Painful erythema, edema or ulcers but can swallow	Painful erythema, edema, or ulcers preventing swallowing or requiring hydration or parenteral (or enteral) nutritional support	Severe ulceration requiring prophylactic intubation or resulting in documented aspiration pneumonia
GI toxicity	Diarrhea	Increase of <4 stools/day over pretreatment	Increase of 4–6 stools/day, or nocturnal stools	Increase of ⩾7 stools/day or incontinence; or need for parenteral support for dehydration	Physiologic consequences requiring intensive care; or hemodynamic collapse

ULN = upper limit of normal values; LLN = lower limit of normal value; WNL = within normal limits; CHF = congestive heart failure; GI = gastrointestinal.

Table 3 Toxicity grading using the Seattle criteria in 86 patients undergoing RIST

Grade	*0*	*1*	*2*	*3*	*4*
Gut	69	17	0	0	0
Stomatitis	47	29	10	0	0
Central nervous system	78	4	2	2	0
Liver	23	31	31	1	0
Lung	75	4	3	3	1
Kidney	51	31	3	1	0
Bladder	84	2	0	0	0
Heart	70	8	7	1	0
Maximal grades	8	38	35	4	1

Table 4 Toxicity grading using NCI-CTC ver. 2.0 in 86 patients undergoing RIST

Grades	*0*	*1*	*2*	*3*	*4*
Gut	50	18	10	7	1
Stomatitis	35	8	32	1	0
Central nervous system	74	5	2	2	3
Liver	5	27	27	22	5
Lung	56	12	0	17	1
Kidney	47	26	11	2	0
Bladder	61	23	1	1	0
Heart	72	6	3	3	2
Maximal grades	2	16	25	35	8

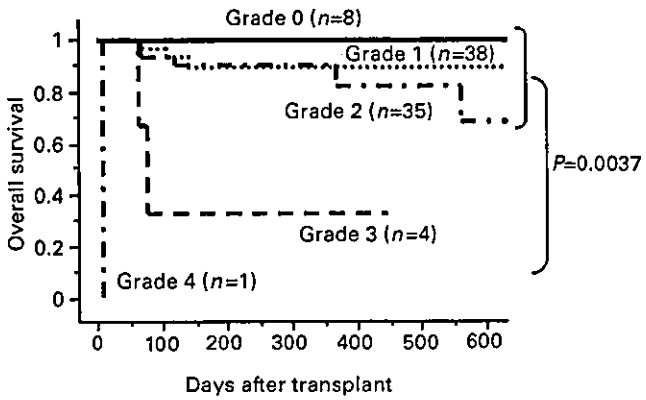

Figure 1 Overall survival evaluated by the Seattle criteria. Five patients had grade 3–4 toxicity in at least one organ, of whom three died (60%). The estimated 1-year OS was 25.0% (95% confidence interval (CI) 0.0–61.7%). In contrast, of the 81 patients with grade 0–2 toxicity in all the organ systems, eight died (9.9%). The estimated 1-year OS was 74.2% (95% CI, 64.2–84.2%). The one-year OS was significantly lower in the patients with grade 3–4 toxicity ($P=0.0037$).

(2%), 16 (19%), 25 (29%), 35 (41%), and eight patients (9%), respectively.

The lung toxicity on day 100 using either the Seattle criteria or NCI-CTC ver. 2.0 was not maximal in any of the 86 patients.

There were 10 cases of the 'up-staging' of the toxicity from the Seattle criteria to NCI-CTC version 2.0: liver ($n=6$), and kidney ($n=4$).

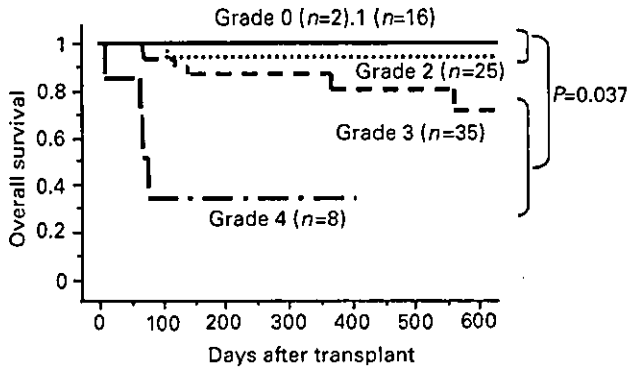

Figure 2 Overall survival evaluated by NCI-CTC version 2.0. In total, 43 patients had grade 3–4 toxicity in at least one organ, of whom 10 patients (23.2%) died of TRM. The estimated 1-year OS was 64.7% (95% CI, 50.2–79.2%). In contrast, of the remaining 43 patients with grade 2 toxicity in all the organ systems, one died of GVHD, resulting in 78.5% (95% CI, 64.8–92.2%) of estimated 1-year OS and 8.8% of TRM. The 1-year OS was significantly lower in the patients with grade 3–4 toxicity ($P=0.037$).

Table 5 Variables influencing the grades of regimen-related toxicity according to NCI-CTC ver. 2.0

Variables	*Grade 0–2 (n=43)*	*Grade 3–4 (n=43)*	*P-value*
Age			
Median (range)	53 (4–65)	50 (19–67)	0.459
Sex			
Male/female	26/17	31/12	0.362
Risk of primary diseases			
High/low	13/30	20/23	0.183
Preparative regimens			
Fludarabine-based/cladribine-based	34/9	30/13	0.459
ATG-containing yes/no	22/21	27/16	0.384
TBI-containing yes/no	1/42	2/41	0.999
GVHD prophylaxis			
Cyclosporine alone/ cyclosporine and methotrexate	38/5	32/11	0.165
Donors			
Related/unrelated	41/2	40/3	0.999
Matched/mismatched	37/6	32/11	0.278

ATG = anti-thymocyte globulin, TBI = total body irradiation, GVHD = graft-versus-host disease.
Any variables were significant on multivariate analysis.

Association between toxicity grading and survival following RIST

A total of 27 patients died: 16 of disease progression (19%) and 11 of TRM (13%). The 11 patients who died of TRM had the maximal toxicity of grade 2 ($n=1$), grade 3 ($n=6$), and grade 4 ($n=4$) by NCI-CTC ver. 2.0, which was also graded with the Seattle criteria to be grade 1 ($n=3$), grade 2 ($n=5$), grade 3 ($n=2$), and grade 4 ($n=1$). The causes of TRM were GVHD/steroid-related infection ($n=6$), GVHD (bronchiolitis obliterans) ($n=1$), infection ($n=2$), and

Table 6 Regimen-related toxicities (RRT) in studies reported previously

Authors/Reference	*n*	*Preparative regimens*	*Age years*	*Primary diseases*	*GVHD prophylaxis*	*RRT*				*Transplant-related mortality (TRM)*
						Criteria	*III–IV*	*III*	*IV*	
Carella *et al*[15]	15	Flu/CY[a]	34 (19–60)	HD, NHL	CSP/MTX	Details not described				Not described
Childs *et al*[3]	19	Flu/CY[b]	48 (37–65)	RCC	CSP	Details not described				2/19 (11%)
Giralt *et al*[16]	86	Flu/Mel[c] ($n=78$)	52 (22–70)	AML/MDS, CML, ALL/ lymphoma	FK506/MTX	Bearman's criteria	19	13	6	38.0%
		Clad/Mel[d] ($=8$)					12	5	7	88.0%
Khouri *et al*[4]	6	Flu/CY[a]	62 (51–71)	CLL	FK506/MTX	Details not described	0%	0%	0%	
	4	PFA[e]	55 (47–61)	Intermediate-grade lymphoma or in Richter's transformation						
	5	Flu/CY[f]	50 (47–57)	Low-grade lymphoma						
Khouri *et al*[17]	20	Flu/CY[a] or Flu/CY[f]	51 (31–68)	Follicular lymphoma	FK506/MTX	Bearman's criteria	0%[g]	0%	0%	3/20 (15%)
Slavin *et al*[2]	26	Flu/BU/ATG	33.5 (1–61)	Acute leukemia, chronic leukemia, NHL, MDS, MM, and genetic diseases	CSP	WHO criteria				4/26 (15%)
Nagler *et al*[18]	23	Flu/BU/ATG	41 (13–63)	HD, NHL	CSP	Bearman's criteria	17%	13%	4%	7/23 (30%)
McSweeney *et al*[6]	45	TBI 200 cGy	56 (31–71)	ALL, AML, CLL, CML, HD, MM, NHL, WM, CLL, MDS	CSP/MMF	Details not described[i]	0%	0%	0%	3/56 (5%)[h]
This study	86	Clad or Flu/ BU ± ATG[j]	51 (4–67)	AML, MDS, CML, ALL, lymphoma, TLBL, ATL, solid tumor	CSP or CSP/ MTX	Bearman's criteria	6%	5%	1%	11/86 (13%)
						NCI-CTC ver. 2.0	50%	41%	9%	

HD = Hodgkin's lymphoma; NHL = non-Hodgkin's lymphoma; RCC = renal cell carcinoma; AML = acute myeloid leukemia; MDS = myelodysplastic syndrome; CML = chronic myelogenous leukemia; ALL = acute lymphocytic leukemia; CLL = chronic lymphocytic leukemia, MM = multiple myeloma; WM = Waldenstrome's macroglobulinemia.

[a]Flu/CY: fludarabine 30 mg/m^2 with cyclophosphamide 300 mg/m^2 daily for 3 days.

[b]Flu/CY: fludarabine 25 mg/m^2 given daily for 5 days and cyclophosphamide 60 mg/kg for 2 days.

[c]Flu/Mel: fludarabine 25 mg/m^2 given daily for 5 days and melphalan 90 mg/m^2 or 70 mg/m^2 for 2 days.

[d]Clad/Mel: cladribine 12 mg/m^2 given daily for 5 days and melphalan 90 mg/m^2 or 70 mg/m^2 for 2 days.

[e]PFA: cisplatin 25 mg/m^2 daily for 4 days; fludarabine 30 mg/m^2; and cytarabine 500 mg/m^2 daily for 2 days.

[f]Flu/CY: fludarabine 30 mg/m^2 given daily for 5 days and cyclophosphamide 1000 mg/m^2 for 2 days.

[g]Moderate VOD was observed in two and was severe in two subjects.

[h]Four with disease progression died from DLI-induced GVHD and infections and three died of transplantation complications without disease progression.

[i]No patient experienced regimen-related painful mucositis, severe nausea and vomiting, pulmonary toxicity, cardiac toxicity, hemorrhagic cystitis, or new-onset alopecia.

[j]Flu/BU/ATG: fludarabine 30 mg/m^2 for 6 consecutive days, oral busulfan 4 mg/kg/day for 2 days, and anti-T-lymphocyte globulin 10 mg/kg/day for 4 days.

others ($n=2$). Maximal grades of GVHD in fatal cases were IV ($n=1$), III ($n=1$), II ($n=3$), and I ($n=2$).

Figure 1 demonstrates the association between the maximal toxicity of the Seattle criteria and OS. Five patients had grade 3–4 toxicity in at least one organ, of whom three died (60%). Estimated 1-year OS was 25.0% (95% confidence interval (CI) 0.0–61.7%). In contrast, of the 81 patients with grade 0–2 toxicity in all the organ systems, eight died (9.9%). The estimated 1-year OS was 74.2% (95% CI, 64.2–84.2%). The 1-year OS was significantly lower in the patients with grade 3–4 toxicity ($P=0.0037$).

Figure 2 demonstrates the association between the maximal toxicity of NCI-CTC ver. 2.0 and OS. In all, 43 patients had grade 3–4 toxicity in at least one organ, of whom 10 patients (23.2%) died of TRM. The estimated 1-year OS was 64.7% (95% CI, 50.2–79.2%). In contrast, of the remaining 43 patients with grade 2 toxicity in all the organ systems, one died of GVHD, resulting in 78.5% (95% CI, 64.8–92.2%) of estimated 1-year OS and 8.8% of TRM. The 1-year OS was significantly lower in the patients with grade 3–4 toxicity ($P=0.037$).

Variables influencing RRT

No variables were found to be associated with RRT of NCI-CTC ver. 2.0 by univariate (Table 5) or multivariate analysis.

Variables influencing overall survival

Patients who survived longer than 30 days were included in this analysis. Multivariate analysis showed that survival was significantly different between unrelated *vs* related donors (hazard ratio 7.5, 95% CI 1.7–32.8, $P=0.0074$), HLA-mismatched *vs* matched (hazard ratio 3.8, 95% CI 1.1–12.9, $P=0.0295$), and the maximal toxicity grade 3–4 *vs* grade 2–3 of NCI-CTC ver. 2.0 within day 30 post transplant (hazard ratio 3.0, 95% CI 1.2–7.3, $P=0.0177$).

Discussion

Evaluation of RRT after RIST is not uniform. As a result, toxicity grades vary among studies (Table 6) (2–4, 6, 15–18). Our study shows that both the Seattle criteria and NCI-CTC ver. 2.0 are significantly associated with outcome, and have predictive value.

The prognosis of grade 3 by the Seattle criteria is comparable to that of grade 4 by NCI-CTC ver. 2.0, and the prognosis of grade 2 by the Seattle criteria is comparable to that of grade 3 by NCI-CTC ver. 2.0 (Figures 1 and 2). However, neither criteria can offer a cutoff to predict death since the sensitivity and specificity are insufficient; the threshold of $\leqslant$grade 2 by Seattle criteria and $\leqslant$grade 3 by NCI-CTC ver. 2.0 would be sensitive but not specific to predict TRM, whereas the threshold of $\geqslant$grade 3 by the Seattle criteria and $\geqslant$grade 4 by NCI-CTC ver. 2.0 would be specific but not sensitive. These findings suggest that these criteria need to be modified for use in RIST.

There are two types of complications associated with allogeneic HSCT. One is the organ toxicity directly caused by preparative regimens. The other is immunological complications, represented by GVHD. When anti-T-cell antibodies are included in conditioning regimens, the frequency of GVHD is decreased[19,20] showing that GVHD is influenced by the types of preparative regimens. Given the fact that GVHD is the most common cause of nonrelapse death after RIST,[8] GVHD should be considered in the safety evaluation of conditioning regimens.

Another common complication after RIST is early progression of the underlying malignancy. This phenomenon could potentially be considered a consequence (and therefore toxicity) of the reduced intensity of the conditioning regimen.

Another consideration is the follow-up duration in evaluating immunological complications following RIST. The duration of observation after chemotherapy is usually 30 days. In contrast, the onset of GVHD can be delayed. The period of 30 days of observation is not long enough to evaluate the safety of RIST. Although the day 100 TRM has been used in RIST, it is not sufficient in evaluation of the immunological complications. We propose that TRM until day 200 should be used in the evaluation criteria for RIST-related toxicity.

Our study shows that both the Seattle criteria and NCI-CTC ver. 2.0 are useful in evaluating toxicity of RIST. Prospective studies are required to establish a proper toxicity grading system for RIST.

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Review Article

Reduced-intensity Hematopoietic Stem Cell Transplantation (RIST) for Solid Malignancies

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Reduced-intensity Hematopoietic Stem Cell Transplantation (RIST) for Solid Malignancies

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Reduced intensity stem cell transplantation (RIST) is a new approach of stem cell transplantation, which has shown promising features as reported in multiple phase I and II studies. Elderly patients, who are not eligible for conventional myeloablative hematopoietic stem cell transplantation (HSCT), are now treatable with RIST. It has also reduced regimen-related toxicity and provided better prognosis in short-term follow-up than conventional HSCT. Among solid tumors, metastatic renal cell carcinoma was found to respond well to RIST. Clinical studies are currently being conducted to evaluate the efficacy of RIST in other types of solid tumors. However, the mechanism of graft-versus-host disease (GVHD) and graft-versus-tumor (GVT) effects remains unclear. More knowledge on the mechanism is crucial to enhance the antitumor effect and to improve the prognosis further.

Key words: graft-versus-tumor effects – graft-versus-host disease – renal cell carcinoma – allogeneic hematopoietic stem cell transplantation – breast cancer

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION AS AN IMMUNE THERAPY

Allogeneic hematopoietic stem cell transplantation (allo-SCT) for the treatment of hematological malignancies was originally based upon the effect of a myeloablative preparative regimen. A preparative regimen using high-dose chemoradiotherapy would suppress the host's immune response and eradicate the residual tumor cells. Marrow was infused to restore hematopoiesis (1). In combination with preceding induction and consolidation cytotoxic chemotherapy, myeloablative preparative regimens followed by allo-SCT were supposed to eradicate the residual underlying diseases.

However, it was found that allogeneic cells were responsible for immunological responses against tumor cells. This is called a graft-versus-leukemia (GVL) or graft-versus-tumor (GVT) effect (2). Evidence supporting this hypothesis includes (i) lower incidences of relapse in patients receiving allo-SCT than in those receiving autologous SCT (3); (ii) higher risk of relapse in patients receiving syngeneic SCT (4); and (iii) lower risk of relapse in patients with acute and/or chronic graft-versus-host disease (GVHD) than those without these conditions (5). Furthermore, GVL or GVT effects were found to be mediated by lymphocytes, especially T cells, based on the clinical findings of (i) higher risk of relapse after T-cell depletion than non-depleted SCT (6); and (ii) therapeutic effects of donor lymphocyte infusion (DLI) (7). In particular, chronic myeloid leukemia (CML) responds well to DLI, and most patients with CML who relapse following allo-SCT can achieve remission with DLI (8). Based on these findings, allo-SCT is now regarded as one of the available immune therapies.

REDUCED-INTENSITY STEM CELL TRANSPLANTATION (RIST)

The high-dose chemotherapy and radiation used as preparative regimen for allo-SCT are associated with a considerable morbidity and mortality (9). This approach has therefore been restricted to young patients without co-morbidities. The majority of patients with hematological malignancies are ineligible for high-dose chemotherapy or radiotherapy because of their old age and co-morbidities. Although allo-SCT is the most powerful treatment for refractory hematological malignancies, only a small proportion of these patients have the opportunity to undergo this treatment.

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Recently, a new strategy for transplantation using a reduced-intensity or non-myeloablative preparative regimen has been developed to reduce regimen-related toxicity (RRT) while preserving adequate antitumor effects (10–14). Various regimens with different intensity can be categorized roughly into two intensity groups: (i) reduced-intensity regimens which retain a certain degree of RRT and require hospitalization; and (ii) minimally myelosuppressive regimens which rely on post-grafting immunosuppression to permit engraftment (15,16). The aim of post-grafting immunosuppression is to control GVHD and to suppress residual host-versus-graft (HVG) effects that would impede engraftment.

These reduced-toxicity regimens are frequently termed 'non-myeloablative' and 'reduced-intensity' regimens. At present, a variety of preparative regimens have been developed. Both myelosuppression and immunosuppression vary widely among them. According to a working definition, a truly non-myeloablative regimen should allow prompt hematopoietic recovery (within 28 days of transplantation) without stem cell rescue, and mixed chimerism usually occurs upon engraftment (15,16). These regimens do not ablate host immunity and depend on the activity of donor T cells to achieve engraftment. The regimen of 2 Gy total body irradiation (TBI) with or without fludarabine reported by the Seattle Transplantation Team (12) is classified as a truly non-myeloablative regimen. In contrast, autologous hematopoietic recovery does not occur without stem cell support after the other regimens such as fludarabine/busulfan and fludarabine/cyclophosphamide, and they are termed reduced-intensity preparative regimens.

PRECLINICAL MODEL OF NON-MYELOABLATIVE SCT

The Seattle Transplantation Team reported the results of preclinical canine studies on non-myeloablative SCT. The researchers considered that two immunological barriers must be overcome in the setting of allo-SCT (17). One is the GVHD, and the other is the rejection or HVG reaction. Both reactions are mediated by T lymphocytes, suggesting that immunosuppressive agents given after allo-SCT to control GVHD might modulate HVG reactions. The latter feature would allow minimization of the high-dose chemotherapy given before allo-SCT for host suppression.

Animal models demonstrated a dose–response relationship between TBI and engraftment (18). In random-bred dogs, a single fraction of 920 cGy TBI, corresponding to 1500 cGy fractionated TBI, resulted in engraftment of dog leukocyte antigen (DLA)-identical littermate marrow in all cases. When the dose was decreased by 50%, the majority of dogs rejected their grafts. At the reduced dose, the addition of post-grafting prednisone did not enhance engraftment, while cyclosporin given for 5 weeks led to engraftment in all of the animals. When the TBI dose was decreased further to 200 cGy, cyclosporin only allowed engraftment for 3–4 months, after which the grafts were rejected. The combination of methotrexate and cyclosporin resulted in engraftment in two out of five animals, but the rest rejected. A combination of mycophenolate mofetil (MMF) and cyclosporin given for 4 and 5 weeks after transplantation was evaluated for its effect on engraftment. The regimen was capable of both controlling GVHD and preventing graft rejection by suppressing a GVH reaction, with 11 of 12 dogs demonstrating stable engraftment of marrow from DLA-identical littermates (19).

They further investigated whether the major role of TBI is to create marrow space or to provide host immunosuppression (20). They irradiated the central lymph node chain from the neck to the upper abdomen with 450 cGy before allo-SCT, and administered MMF and cyclosporin after allo-SCT. At 6 weeks post-transplant, donor cells were present in non-irradiated marrow spaces, suggesting that radiation was not essential to create marrow space for engraftment. After 1 year, DLI was given to the animals and recipient cells disappeared within 9 weeks. These findings indicate that engraftment might be accomplished by blocking HVG reactions and inducing the GVH reaction, and that high-dose cytotoxic chemotherapy and radiotherapy could be eliminated from the preparative regimens.

RATIONALE OF ALLO-SCT FOR SOLID TUMORS

Several findings justify allo-SCT for solid tumors: (i) GVT effects can target tissue-specific polymorphic antigens which are not derived from hematopoietic lineages; (ii) some solid tumors are sensitive to immunotherapy, such as renal cell carcinoma (RCC), melanoma and ovarian cancer; (iii) antigens restricted to the tumor could stimulate tumor-specific alloimmunity in contrast to defective T cells in the tumor-bearing host; and (iv) in theory, all carcinomas arising from epithelial tissues such as keratinocytes, fibroblasts, exocrine glands, hepatobiliary trees and the gastrointestinal tract, which are targets of acute and chronic GVHD, should be susceptible to a GVT effect.

Before clinical trials were initiated, murine models have provided some evidence for a GVT effect (21,22). Among animals inoculated with mammary adenocarcinoma cells, the recipients of allo-SCT showed better survival than did those of syngeneic SCT (21). Further studies provided evidence that murine mammary adenocarcinoma cells expressed minor histocompatibility antigens (mHas) that could be targeted by alloreactive donor T cells in the setting of allogeneic but not autologous bone marrow transplantation (23). Prigozhina et al. demonstrated in animal models that effective eradication of tumor cells as well as leukemic cells can be achieved following allo-SCT using non-myeloablative preparative regimens (24).

The earliest clinical evidence supporting the existence of a GVT effect in a solid tumor was observed in a patient with metastatic breast carcinoma undergoing fully myeloablative SCT for relapsed acute myeloid leukemia. The incidental regression of a metastatic lesion of breast carcinoma raised the possibility of a responsible GVT effect (25). Regression of liver

metastasis in association with severe acute GVHD was reported in a woman transplanted for metastatic breast carcinoma. The researchers demonstrated that allogeneic T cells collected during GVHD and cultivated were able to mediate a cytotoxic effect against breast cancer cell lines (26), suggesting that disease regression resulted from donor T cells targeting broadly expressed mHas. Since then, similar anecdotal reports have been published concerning a possible GVT effect in lung cancer (27), ovarian cancer (28), colon cancer (29), neuroblastoma (30), pancreas cancer (31,32) and ependymoma (33). Porter et al. conducted a phase I clinical trial to determine whether a GVT effect could be observed after primary DLI without stem cell support in patients with primary cancers (34). Three of four patients with acute GVHD and late chimerism responded to primary DLI. These findings indicate that the GVT effect does occur in the setting of allo-SCT for solid tumors.

CLINICAL TRIALS FOR SOLID TUMORS

Metastatic Renal Cell Cancer (RCC)

In 1997, Childs et al. initiated a clinical trial to evaluate GVT effects in metastatic RCC (35). Chemotherapy is ineffective in the majority of cases and does not prolong survival. However, RCC has a distinct nature from that of other solid tumors. There is increasing evidence that they may be susceptible to T-cell immune responses. Biopsy of spontaneously regressing lesions has shown tumor-infiltrating lymphocytes with predominant $CD8^+$ T cells exhibiting major histocompatibilty complex (MHC) class I restricted cytotoxicity against autologous tumor targets (36). Furthermore, unlike most solid tumors, RCC is susceptible to cytokines such as interleukin-2 (IL-2) and interferon-α (37), suggesting that T cells represent the principle effector.

Childs' group treated 19 patients with metastatic RCC (35). The preparative regimen consisted of fludarabine 25 mg/m^2 for 5 days and cyclophosphamide 60 mg/kg for 2 days. Cyclosporin, used to prevent GVHD, was withdrawn early in patients with mixed T-cell chimerism and/or disease progression. Patients without response received up to three courses of DLI. At the time of the last follow-up, nine of the 19 patients were alive 287–831 days after transplantation (median follow-up, 402 days). Two died of transplantation-related causes, and eight from progressive disease. In 10 patients, metastatic disease regressed: three had a complete response, and seven had a partial response. The patients who had a complete response remained in remission 27, 25 and 16 months after transplantation. Results of this clinical trial were updated in 2002 (38). Clinical response is significantly associated with the development of GVHD. There is a 4–6 month interval between transplantation and development of a GVT effect, and patients with rapidly progressive diseases are unlikely to benefit from RIST. Disease response was observed most commonly in patients with pulmonary metastases of clear-cell histology without other organ involvement. Some patients who had failed to respond to interferon-α prior to transplantation achieved responses following administration of a low dose of this agent after transplantation.

After the first report on RIST for RCC, several phase I/II studies have been reported (Table 1) (39–44). Response rates varied widely from 0 to 57%, but it should be noted that some responses were reported in seven of the nine studies. While long-term prognosis remains unknown, response to allo-SCT has been confirmed in some independent studies. Rini et al. described regression of primary kidney tumors, a rare event among responders to cytokine-based therapy (39). According to a European retrospective survey (45), allo-SCT was used in <20 cases of solid tumors until 1997; since then it increased to 159 in 2002, mainly for RCC.

We also initiated a phase I clinical trial on RIST for metastatic RCC (46). From June 2000 to April 2002, nine patients received peripheral blood stem cell transplantation from a

Table 1. Clinical trials on RIST for metastatic renal cell carcinoma

Reference	Donor	No. of patients	Preparative regimen	GVHD prophylaxis	Response rates
Childs et al. (35)	An HLA-identical or one locus-mismatched related donor	19	CY/Flu	CSP	53%
Childs and Barrett (38)	HLA-identical and one locus-mismatched related	52	CY/Flu	CSP	48%
Rini et al. (39)	An HLA-identical sibling	12	CY/Flu	Tacrolimus and MMF	33%
Bregni et al. (40)	An HLA-identical sibling	7	CY/Flu	CSP and MTX	57%
Blaise et al. (42)	An HLA-identical sibling	25	ATG/BU/Flu	CSP	4%
Ueno et al. (43)	An HLA-identical related or matched unrelated donor	15	Melphalan/Flu	Tacrolimus and MTX	27%
Pedrazzoli et al. (41)	An HLA-identical sibling	7	CY/Flu	CSP and MTX	0%
Hentschke et al. (44)	An HLA-identical related or matched unrelated donor	10	2 Gy TBI/Flu*	CSP and MMF	0%
Nakagawa et al. (46)	An HLA-identical sibling	9	ATG/BU/Flu	CSP	11%

CY, cyclophosphamide; Flu, fludarabine; CSP, cyclosporin; MMF, mycophenolate mofetil; MTX, methotrexate; ATG, anti-thymocyte globulin; BU, busulfan; TBI, total body irradiation.
*Recipients receiving transplants from unrelated donors were given thymoglobulin.

human leukocyte antigen (HLA)-identical sibling donor. The conditioning regimen consisted of fludarabine 180 mg/m^2 or cladribine 0.66 mg/kg, plus busulfan 8 mg/kg and rabbit anti-thymocyte globulin (ATG). GVHD prophylaxis consisted of cyclosporin 3 mg/kg alone. All of the patients achieved engraftment, with no grade III–IV non-hematological RRT, and complete donor cell type chimerism was achieved without additional DLI by day 60. Acute and chronic GVHD was seen in four patients each. One patient achieved partial remission (response rate 11%) and, as of July 2003, six patients are alive with a median follow-up of 22.5 months. The actuarial overall survival rate was 74% at 2 years. We followed all the 26 patients who were referred to our institute for RIST and were subject to HLA typing. Transplanted patients (n = 9) showed significantly higher overall survival rate than those who had not received RIST (n = 17) (Fig. 1A, P = 0.016). We compared the overall survival rates between 12 patients with matched donors and the other 14 patients without them (Fig. 1B). The 1-year actuarial survival rates were 74 and 48%

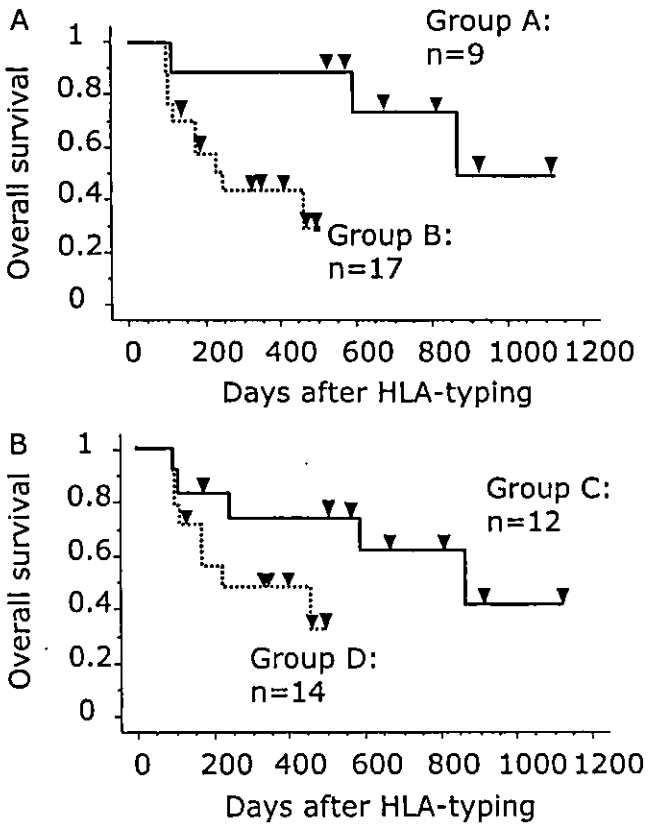

Figure 1. Kaplan–Meier estimates of the overall survival rates following HLA typing. (A) A comparison of overall survival rates between transplanted and non-transplanted patients. The overall survival rate was significantly higher in transplanted patients than in non-transplanted patients (P = 0.016). (B) A comparison between patients with an HLA-matched donor and those without. 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 nine transplanted patients; group D, patients without an HLA-matched donor (n = 14).

in patients with donors and those without them, respectively (P = 0.088). This study confirmed the feasibility of allo-SCT for metastatic RCC, and suggests that it might improve prognosis of patients with metastatic RCC. Further phase II or III studies are warranted.

Breast Cancer

After the first case report by Eibl et al. (26), Ueno et al. reported the results of a feasibility study on conventional myeloablative allo-SCT for metastatic breast cancer in 16 patients (47,48). This study included patients without progressive disease. The preparative regimen consisted of cyclophosphamide, carmustine and thiotepa. GVHD prophylaxis was mainly tacrolimus and methotrexate. The responses were complete response (n = 1), partial response (n = 5) and stable disease (n = 8) in the 15 evaluable patients. Two patients responded during acute GVHD following the withdrawal of immunosuppression.

Ueno et al. further investigated the feasibility of RIST for metastatic breast cancer (43). A total of eight patients received allo-SCT following fludarabine and melphalan. Three patients showed some clinical responses (complete response two, minor response one). Metastatic lesions resolved 3 months after development of chronic GVHD in one patient, and the other two patients demonstrated tumor response at 13 and 17 months after transplantation. The delayed response was comparable with that in RIST for RCC. Since fludarabine and melphalan produce little cytoreduction in metastatic breast cancer and the underlying disease progressed immediately after transplantation in more than half of the patients, it is reasonable to assume that the disease response was attributable to a GVT effect.

Since their reports, GVT effects against breast cancer have been confirmed by other researchers (40–42,49) (Table 2).

Melanoma

Childs and Srinivasan treated 11 patients with metastatic melanoma (50). This study highlights some of the potential problems in applying RIST for some solid tumors. Death from rapid disease progression occurred before day 100 in five patients. Although three patients achieved partial regression, their responses occurred early in the courses of RIST with a short duration, suggesting that these responses were attributable to chemotherapy effects related to preparative regimens rather than GVT effects. One patient had delayed regression of several subcutaneous metastatic nodules. The investigators speculated that RIST should be limited to a minority of melanoma patients who have slow-growing diseases.

Other Cancers

There is little information on the efficacy of allo-SCT for most solid tumors. Some anecdotal reports have been published on allo-SCT for a variety of cancers (28,31,44,51–54). A case report and a small case series of RIST for metastatic ovarian

Table 2. Experience on allo-SCT for metastatic breast cancer

Reference	Donor	No. of patients	Preparative regimen	GVHD prophylaxis	Response rates*
Ueno et al. (48)	An HLA-identical sibling	16	CBT	Tacrolimus and MTX†	40%
Ueno et al. (43)	An HLA-identical related or matched unrelated donor	8	Melphalan/Flu	Tacrolimus and MTX	25%
Bregni et al. (40)	An HLA-identical sibling	6	CY/Flu	CSP and MTX	33%
Blaise et al. (42)	An HLA-identical sibling	17	ATG/BU/Flu	CSP	12%
Pedrazzoli et al. (41)	An HLA-identical sibling	2	CY/Flu	CSP and MTX	100%
Hentschke et al. (44)	An HLA-identical related or matched unrelated donor	1	2 Gy TBI/Flu	CSP and MMF	0%

CY, cyclophosphamide; Flu, fludarabine; CSP, cyclosporin; MMF, mycophenolate mofetil; MTX, methotrexate; ATG, anti-thymocyte globulin; BU, busulfan; TBI, total body irradiation; CBT, cyclophosphamide, carmustine, thiotepa.
*Response includes complete and partial responses.
†Two patients received cyclosporin and methylprednisolone.

cancer and colorectal cancer have been published recently (28,42,44,53,54). These tumors may be promising candidates for allo-SCT; however, it should be noted that both ovarian and colorectal cancer are susceptible to chemotherapy, making it difficult to conclude that disease regression was attributable to a GVT effect.

We evaluated a total of 14 patients with refractory non-renal solid tumors (four rhabdomyosarcoma, two melanoma, two neuroblastoma, two cholangiocarcinoma, two other sarcomas and two other carcinomas) who underwent RIST according to our institutional phase I protocol (52,55). The conditioning regimen and GVHD prophylaxis were the same as those for metastatic RCC. All patients but one with melanoma achieved complete donor chimerism without DLI. Only three patients showed grade II–IV acute GVHD and two showed chronic GVHD. Four patients died before day 100 after RIST and another four after day 100. Seven out of the eight patients died of disease progression. Although comprehensive evaluation of the GVT effect is impossible due to the diversity of the diseases, it is remarkable that there are two patients with disease-free survival longer than 11 months after RIST. One is a 7-year-old female with metastatic neuroblastoma which recurred after autologous bone marrow transplantation. The other is a 16-year-old female with metastatic alveolar type rhabdomyosarcoma. Both were transplanted when they had a small volume of residual disease compared with other patients with sarcoma. Among patients with carcinomas, a 56-year-old male with cholangiocarcinoma showed objective tumor regression which did not satisfy the criteria for partial regression (Fig. 2). There was no apparent correlation between GVHD and a GVT effect.

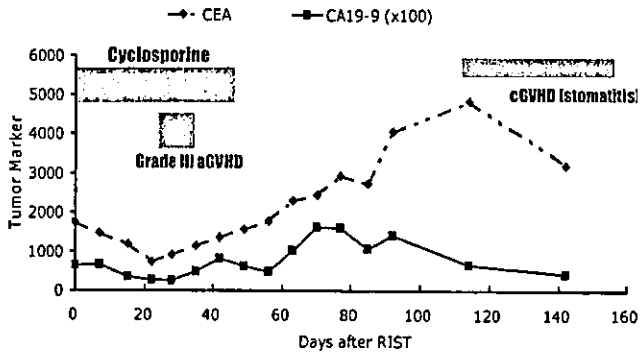

Figure 2. Clinical course of a patient with metastatic cholangiocellular carcinoma. A 56-year-old male with cholangiocarcinoma showed objective tumor shrinkage but not sufficiently satisfactory to regard it as partial remission.

MECHANISM

The precise mechanism of the GVT effect remains unknown. The lack of information on tumor target antigens and immune mediators for GVT effects does not allow us to predict which diseases will respond to RIST.

Disease regression associated with cyclosporin withdrawal, complete donor chimerism and GVHD provides evidence that cytotoxic donor T cells play an important role in this response. RCC cells express a broad range of mHas that could render them susceptible to a GVT effect (56). These findings suggest that both broadly expressed mHas and antigens restricted to RCC cells may be a target of a GVT effect. Recent studies have demonstrated that distinct T-cell populations recognizing tumor-specific antigens and/or mHas are involved in the GVT effect (57). T-cell clones attacking both RCC cells and hematopoietic cells of the recipients were isolated from responding patients (58). Retrospective clinical studies and *in vitro* studies using clinical samples demonstrated that cytotoxic T cells against leukemia-specific antigens or hematopoiesis-restricted mHas can induce remission in allo-SCT for acute leukemia (59–61). In animal models, adoptive transfer of HA-1- and HA-2-specific cytotoxic T lymphocytes generated *in vitro* can be used as immunotherapy to treat hematological malignancies relapsing after allo-SCT (62,63). Using these cytotoxic T cells, GVT effects can be separated from GVHD (64). In contrast to allo-SCT for hematological malignancies, little information is available concerning target

antigens and cytotoxic T cells in allo-SCT for solid malignancies, and further studies are warranted.

Some investigators suggested that innate immunity plays an important role in the development of a GVT effect. Natural killer (NK) cells have been studied intensively, since they are capable of mediating a GVL effect in acute myeloid leukemia without causing GVHD (65). Igarashi et al. reported that allogeneic NK cells with killer immunoglobulin-like receptor ligand incompatibility play an important role in cytotoxicity against melanoma and renal cell carcinoma cells (66). Furthermore, Teshima et al. reported that the local cytokine storm associated with the early phase of allogeneic transplantation plays an important role in GVHD (67). The tumor progression and regression in concordance with corticosteroid use and discontinuation observed in our study (46,68) are compatible with their suggestion, since the cytokine production is readily suppressed by corticosteroid.

Stelljes et al. recently reported an interesting animal study using allogeneic parent-into-F(1) murine transplantation models [BALB/c or C57BL/6→[C57BL/6 × BALB/c]F(1)] with different tumors derived from either parental strain (69). They provided experimental proof of a donor $CD8^+$ T cell-mediated tumor-associated antigen-specific anti-tumor response *in vivo* that is driven by GVHD. GVHD was identified as a driving force for GVT effects in RIST for solid tumors. It may represent one of the mechanisms contributing to GVT effects observed in allogeneic transplant recipients.

FUTURE DIRECTIONS

Control of Negative Aspects of RIST

Despite progressive improvement of transplant safety, the risk of significant transplant-related malignancy (TRM) limits the widespread application of allo-SCT for solid tumors. TRM remains 10–25% even in RIST. Without evidence of efficacy, most physicians considered this risk too high to justify studies of allo-SCT in patients with solid malignancies. The risk/benefit ratio is an important factor to decide the treatment plan in individual cases.

GVHD is the most significant concern in RIST as well as conventional allo-SCT (70). Approximately two-thirds of RIST recipients develop grade II–IV acute GVHD, and 10% of patients who receive RIST from an HLA-identical sibling died of GVHD in the National Cancer Center Hospital (70). Intensification of GVHD prophylaxis using potent immunosuppressive agents such as MMF, infliximab, ATG and CAMPATH-1H has contributed to improve GVHD-related outcomes (50,71,72); however, use of these agents might diminish GVT effects (50,68), and could increase the rate of serious infections (73). T-cell depletion can significantly reduce the risk of GVHD; however, it does not provide definite evidence of improving the outcomes of allo-SCT for solid or hematological malignancies. They might increase the risk of graft rejection and life-threatening infections (74). Several new strategies of T-cell depletion are currently under investigation, such as delayed T-cell add-back (75), the use of a suicide gene system (76), and selective $CD8^+$ depletion (77). Enhancement of the recovery of tissue damaged by GVHD is another promising approach. Some researchers showed that keratinocyte growth factor (KGF) administration is beneficial for the treatment and prevention of chemotherapy-induced gastrointestinal damage (78,79). It might ameliorate the organ damage caused by GVHD, leading to separation of GVHD from the beneficial GVL effects after allo-SCT (80). Since KGF has a possible risk of oncogenesis and cancer progression, further studies are required to investigate its safety in the setting of allo-SCT for solid tumors.

Another common immunological complication is the progression of the primary disease during immunosuppression. Preparative regimens of RIST have intense immunosuppressive effects to ensure the engraftment of donor cells. The half-life of antibodies such as ATG and CAMPATH-1H is so long as to maintain their immunosuppressive effects after RIST. Although these agents are effective in GVHD prophylaxis, they may deteriorate GVT effects and induce disease progression during immunosuppression (35). This phenomenon needs to be recognized as toxicity associated with conditioning regimens in RIST for solid tumors. However, when the primary disease is in progression at transplant, the possible association of conditioning regimens with early post-transplant progression cannot be distinguished from the natural course of the disease. This issue is troublesome in phase I or II clinical trials, particularly in solid tumors, as they are in progression at transplant. When the primary disease is in complete or partial remission, or stable disease at transplant, early post-transplant progression is more likely to be associated with conditioning regimens, requiring the clinician to be alert to this.

Enhancement of a GVT Reaction

Future studies should focus on directing the immune responses specifically to the tumors. In hematological malignancies, leukemia-specific cytotoxic T lymphocytes (CTLs) are frequently generated after allo-SCT, and are important in maintaining remission (81). Falkenburg et al. reported that treatment with *ex vivo*-generated leukemia-reactive T cells achieved remission in a patient with CML who relapsed after allo-SCT and was resistant to DLI (82). These results support the possibility of using DLI *ex vivo* primed against solid tumor cells. Several antigens targeted by alloreactive lymphocytes have been identified in allo-SCT for solid tumors. However, the expression of tumor-specific antigens varies considerably within the same tumor and at different stages of diseases. It is therefore difficult to produce antigen-specific CTLs in the treatment of solid tumors.

There are some possibilities to enhance tumor-specific allogeneic immunity prior to transplantation. One is to utilize donor cells activated against tumor alloantigens. While GVHD is a significant concern associated with pre-transplant immunization of allogeneic marrow donors with recipient-derived

tumor cells (83), some animal studies have shown that immunization of allo-SCT recipients with tumor cells can enhance GVT activities without exacerbating GVHD (84,85). It has been shown that CTLs can be generated using the whole tumor cells, which allows epitopes to be selected that are immunogeneic in the context of individual CTL repertoires (86). This approach can be applicable in allo-SCT for solid tumors with unknown target antigens. Morecki et al. reported that pre-immunization with mHa-mismatched tumor or spleen cells was capable of activating effector cells to induce GVT effects (87).

Post-transplant vaccination against tumor-specific or mHas or *ex vivo* generation of tumor-specific T cells followed by their adoptive transfer is another promising approach. Luznik et al. reported an animal model, showing a cooperation between host and donor T cells in the response to a tumor cell vaccine given after an RIST protocol that achieves stable mixed chimerism (88). GVT effects may be enhanced by the use of cytokines such as granulocyte–macrophage colony-stimulating factor (GM-CSF), which may improve antigen presentation, and interferons, which may increase tumor antigen presentation by upregulating MHC class I and class II HLA molecules. Animal studies demonstrate that other cytokines such as IL-1 (89), IL-11 (90), and procedures capable of interfering with immunoregulatory mechanisms (91,92) are effective for inhibiting GVHD while preserving GVT effects.

Besides immunological approaches, it is critical to clarify the best timing and patient conditions for allo-SCT against solid tumors. Disease progression kinetic and immune status of the hosts are major factors influencing the sensitivity to allogeneic immunity (42). The efficacy of tumor cell eradication by alloreactive lymphocytes depends on the initial ratio between the number of tumor-specific immunocompetent cells in the graft and tumor cell burden of the recipient. Tumor debulking by the preparative regimen or surgical procedures before transplant might be important to enhance GVT effects. Preclinical evidence suggests that a lymphopenic host may represent a favorable clinical setting for immunotherapy (93). Dudley et al. provided evidence of cancer regression by the adoptive transfer of autologous tumor-reactive T cells directed against melanoma antigens in patients receiving a non-myeloablative, highly immunosuppressive preparative regimen (94). This approach may be helpful in allo-SCT for solid tumors.

Evaluation of Tumor Responses

Evaluation methods of tumor response to RIST have not been established. Even in the article of RIST for RCC by Childs et al. (35), their method of tumor response evaluation was not clearly described. It is critical to develop a global method to evaluate tumor response to RIST to share RIST results worldwide (95). Although the RECIST (Response Evaluation Criteria in Solid Tumors) system has been used as a gold standard to evaluate the response of solid tumors to treatment mainly in the field of cancer chemotherapy (96), it has not been fully validated in the area of allo-SCT for solid tumors. Compared with hematological malignancies, solid tumors are generally more resistant to the cytotoxic agents used in conditioning regimens administered before transplantation. Consequently, there may be some important differences in evaluating the response of solid tumors between RIST and conventional chemotherapy.

First, the feasibility of applying RECIST should be critically validated before its extensive application in transplantation (97). Tumor regression occurs several months after transplantation, and most tumors continue their natural growth until the manifestation of effective alloimmunity to restrain tumor growth. If the original RECIST criteria (96) are applied to patients undergoing RIST for solid tumors, most of the GVT effect would be evaluated as progressive disease, which would preclude subsequent evaluation (Fig. 3). Therefore, RECIST may underestimate the efficacy of RIST. Secondly, the proper time to measure the tumor size as a baseline for evaluating a subsequent tumor response has not been defined. In contrast to the results with chemotherapy, the tumor often temporarily increases in size following RIST. Accordingly, when the size at transplantation is used as a baseline, as in chemotherapy, a therapeutic effect following the initial progression could be overlooked or underestimated (Fig. 3). On the other hand, evaluating regression from the largest size after transplant certainly overestimates the effect of treatment (Fig. 3), and gives an unacceptable bias. Thirdly, the tumor size after RIST often fluctuates in response to a *de novo* GVT effect, post-transplant immunotherapy including DLI, and adjustment of immunosuppressive agents (Fig. 4). In this situation, it is clear that any evaluation of the response duration, such as progression-free survival and the overall response duration, is essentially impossible using the current RECIST criteria. Improved overall survival will ultimately be evaluated in phase III trials. To reach this point, a global standard evaluation system, that enables the

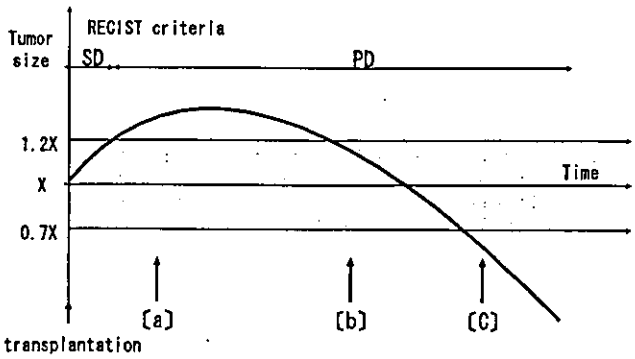

Figure 3. Course of tumor size after transplantation. Primary solid tumors are progressive despite chemoradiotherapy prior to transplantation. (a) Most tumors continue their natural growth until the development of a GVT effect, which usually occurs several months after transplantation. (b) If the tumor has increased in size compared with that at the time of transplant, regression from the largest size may overestimate the treatment effect. (c) If the tumor size at transplant is defined as a baseline, some treatment effects, observed in patients whose lesions show initial progression followed by regression with the development of GVHD, will be underestimated.

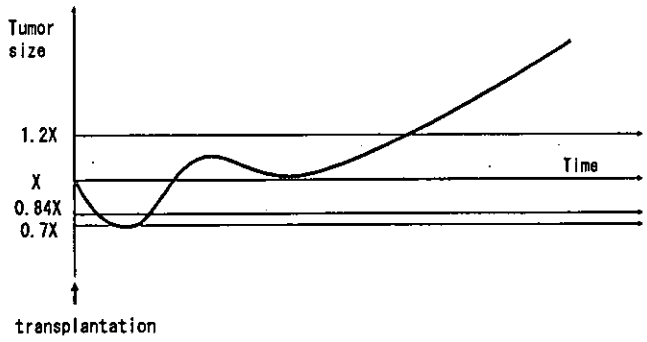

Figure 4. Fluctuation of tumor size after donor lymphocyte infusion or adjustment of immunosuppressive agents. It is difficult to handle patients in whom the tumor size fluctuates in response to post-transplant immunotherapy such as donor lymphocyte infusion and adjustment of immunosuppressive agents. Neither an appropriate timing of response evaluation nor an appropriate time to measure a baseline tumor size has been established in these cases.

effective screening of a therapeutic effect in an earlier phase II study, will need to be established. We hope that this review will inspire a productive discussion.

Use of Alternative Stem Cell Sources

Only 30–40% of patients in Japan have an HLA-identical sibling to serve as an allo-SCT donor. Unrelated bone marrow or umbilical cord blood may serve as an effective source of stem cells, thereby broadening the scope of patients who may benefit from allo-SCT. RIST using these stem cells is a promising alternative option. Some pilot studies have demonstrated the feasibility of allo-SCT from MUD (98,99) or using umbilical cord blood (100,101). Trials evaluating RIST using alternative stem cell sources have been started in many transplantation centers.

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Featured Article

Successful Engraftment After Reduced-Intensity Umbilical Cord Blood Transplantation for Adult Patients with Advanced Hematological Diseases

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ABSTRACT

Purpose: **The purpose of this research was to evaluate the feasibility of reduced-intensity unrelated cord-blood transplantation (RI-UCBT) in adult patients with advanced hematological diseases.**

Experimental Design: **Thirty patients (median age, 58.5 years; range, 20–70 years) with advanced hematological diseases underwent RI-UCBT at Toranomon Hospital between September 2002 and August 2003. Preparative regimen composed of fludarabine 25 mg/m^2 on days −7 to −3, melphalan 80 mg/m^2 on day −2, and 4 Gy total body irradiation on day −1. Graft-*versus*-host disease prophylaxis was composed of cyclosporin alone.**

Results: **Twenty-six patients achieved primary neutrophil engraftment after a median of 17.5 days. Median infused total cell dose was 3.1 × 10^7/kg (range, 2.0–4.3 × 10^7/kg). Two transplant-related mortalities occurred within 28 days of transplant, and another 2 patients displayed primary graft failure. Cumulative incidence of complete donor chimerism at day 60 was 93%. Grade II-IV acute graft-*versus*-host disease occurred in 27% of patients, with median onset 36 days. Primary disease recurred in 3 patients, and transplant-related mortality within 100 days was 27%. Estimated 1-year overall survival was 32.7%. Excluding 7 patients with documented infection, 19 patients displayed noninfectious fever before engraftment (median onset, day 9). Manifestations included high-grade fever, eruption, and diarrhea. The symptoms responded well to corticosteroid treatments in 7 of 13 treated patients.**

Conclusion: **This study demonstrated the feasibility of RI-UCBT in adults.**

INTRODUCTION

Allogeneic hematopoietic stem-cell transplantation (allo-HSCT) is a curative treatment for refractory hematological malignancies. The therapeutic benefits are attributable to myeloablative radiochemotherapy and graft-*versus*-leukemia effects (1), whereas the severe regimen-related toxicity (RRT; Ref. 2) limited allo-HSCT to young patients without comorbidities.

Reduced-intensity stem-cell transplantation (RIST) using a nonmyeloablative preparative regimen has been developed to decrease RRT, whereas preserving adequate antitumor effects (3–5). Different pioneering conditioning regimens for RIST have been investigated, such as those including purine analogs (3–6) and total body irradiation (TBI). Although RIST has been attempted in various diseases (5, 6), suitable preparative regimens with adequate immunosuppression have yet to be established.

Although allo-HSCT from an HLA-identical sibling is promising, only 30% of the patients have an HLA-identical sibling donor. The value of unrelated cord-blood transplantation (UCBT) was confirmed for pediatric patients (7, 8). It has seen recent application in adult patients (9). Whereas the potential graft-*versus*-leukemia effects by cord-blood (CB) without severe graft-*versus*-host disease (GVHD; Ref. 10) has been reported, current questions include whether CB provides a sufficient number of stem cells for adults and suitable graft-*versus*-leukemia effects.

Reduced-intensity (RI)-UCBT (11, 12) represents a promising treatment for advanced hematological malignancies. Wagner *et al.* (12) reported recently the feasibility of RI-UCBT for pediatric patients. However, the feasibility in adult patients remains unclear. We report 30 adult patients with advanced hematological diseases who underwent RI-UCBT after fludarabine, melphalan, and 4 Gy TBI since October 2003 at our institution.

PATIENTS AND METHODS

Study Patients and Donors. Thirty patients with hematological diseases underwent RI-UCBT at Toranomon Hospital between September 2002 and August 2003. All of the patients had hematological disorders that were incurable with conventional treatments and were considered inappropriate for conven-

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tional allo-HSCT due to the lack of an HLA-identical sibling or a suitable unrelated donor, age >50 years old and/or organ dysfunction (generally attributable to previous intense chemo- and/or radiotherapy).

All of the patients provided written informed consent in accordance with the requirements of the Institutional Review Board.

HLA Typing and Donor Matching. An unrelated donor was searched through the Japan Marrow Donation Program (13) for patients without an HLA-identical sibling donor. When no appropriate donor was identified, the Japan Cord Blood Bank Network (14) was searched. CB units, which were ≥4 of 6 HLA-antigen matched and contained at least 2×10^7 nucleated cells/kg of recipient body weight before freezing were used. CB units were not depleted of T lymphocytes.

Preparative Regimen. The preparative regimen was composed of fludarabine 25 mg/m^2 on days −7 to −3, melphalan 80 mg/m^2 on day −2, and 4 Gy TBI in 2 fractions on day −1.

Supportive Cares. All of the patients were managed in reverse isolation in laminar airflow-equipped rooms and received trimethoprim/sulfamethoxazole for *Pneumocystis carinii* prophylaxis. Fluoroquinolone and fluconazole were administered for prophylaxis of bacterial and fungal infections, respectively. Prophylaxis of herpes virus infection with acyclovir was also given (15). Neutropenic fever was managed according to the guidelines (16, 17). Cytomegalovirus (CMV) pp65 antigenemia was monitored once a week. If positive results were identified, preemptive therapy with foscarnet was initiated. Hemoglobin and platelet counts were maintained at >7 g/dl and $>10 \times 10^9$/liter, respectively, with in-line filtered and irradiated blood transfusions.

Management of GVHD. GVHD was clinically diagnosed in combination with skin or gut biopsies after engraftment or attainment of 100% donor chimerism. Acute and chronic GVHD were graded according to the established criteria (18, 19).

GVHD prophylaxis was a continuous infusion of cyclosporin 3 mg/kg from day −1 until the patients tolerated oral administration. It was tapered off from day 100 until day 150. If grade II-IV acute GVHD developed, 1 mg/kg/day of prednisolone was added to cyclosporin and tapered from the beginning of clinical response.

Chimerism Analysis. Chimerism was assessed using fluorescent *in situ* hybridization in sex-mismatched donor-recipient pairs. In sex-matched pairs, PCR for variable numbers of tandem repeats was used with donor cells detected at a sensitivity of 10% (20).

Whole blood and CD3-positive cell chimerism was assessed at the time of granulocyte engraftment. When engraftment was delayed, chimerism was assessed on day 30. For those who died before engraftment, chimerism was assessed at least once during life.

Engraftment. Engraftment was defined as WBC counts > 1.0×10^9/liter or absolute neutrophil counts > 0.5×10^9/liter for 2 consecutive days. Granulocyte colony stimulating factor (Filgrastim) 300 μg/m^2/day was administered i.v. from day 1 until neutrophil engraftment.

Graft failure was defined as peripheral cytopenia and marrow hypoplasia occurring later than day 60, without detection of donor markers by cytogenetic and/or molecular techniques.

Table 1 Patient characteristics (n = 30)

Age (y), median (range)	58.5 (20–70)
Weight (kg), median (range)	52 (38–75)
Male/female	16/14
Diagnosis	
Malignancy	
Acute myeloid leukemia	14
Myelodisplastic syndrome	1
Acute lymphoblastic leukemia	3
Adult T-cell leukemia	5
Plasma cell leukemia	1
Chronic myeloid leukemia	1
Malignant lymphoma	1
Benign	
Severe aplastic anemia	4
Disease status at transplantation (malignancy)	
Remission	1
Refractory to previous chemotherapy	25

RRT and Transplantation-Related Mortality (TRM). RRT was defined as any nonhematological organ dysfunction from day 0 to day 28 and was graded according to the Bearman's criteria (2). TRM was defined as death without the primary disease progression.

Endpoints and Statistical Analysis. Primary end points were composed of the rates of durable engraftment and TRM within day 100. Secondary end points were the rates of RRT, acute and chronic GVHD, infections, event-free survival (EFS), and overall survival (OS).

Acute GVHD was analyzed for engrafted patients. Chronic GVHD was analyzed for patients who survived ≥100 days.

EFS was defined as the duration of survival after transplantation without disease progression, relapse, graft failure, or death. The probabilities of OS and EFS were shown by the Kaplan-Meier method as of January 31, 2004. Surviving patients were censored on the last day of follow-up. Cox regression analysis was used to determine the effect of various variables on OS.

RESULTS

Patient Characteristics. Median age was 58.5 years (range, 20–70 years), and median weight was 52 kg (range, 38–75 kg; Table 1). All of the patients were CMV-seropositive.

The malignancies of 25 patients were refractory to cytotoxic chemotherapies except acute myeloblastic leukemia (n = 1) in first CR. The remaining 4 patients had transfusion-dependent severe aplastic anemia.

CB Characteristics. Twenty-four and 6 patients received 4 of 6 and 5 of 6 HLA-antigen-matched CB, respectively. Twenty-one patient CB pairs were sex-mismatched. Median infused total nucleated cell dose and CD34-positive cell dose before freezing were 3.1×10^7/kg (range, $2.0–4.3 \times 10^7$/kg) and 0.74×10^5/kg (range, $0.17–2.5 \times 10^5$/kg), respectively.

Engraftment. Twenty-six patients [87%; 95% confidence interval (95% CI), 75–99%] achieved primary neutrophil engraftment, among whom median day of engraftment was 17.5 days (range, 10–54 days; Fig. 1). Their engraftment was durable

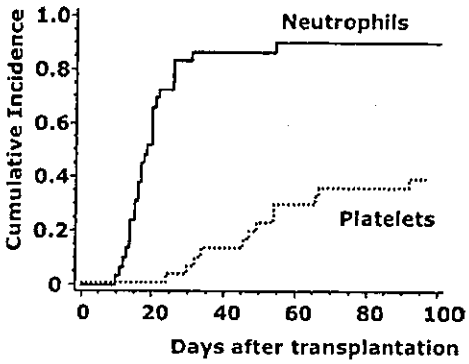

Fig. 1 Engraftment of neutrophils and platelets. Twenty-six (87%; 95% confidence interval, 75–99%) and 16 patients (40%; 95% confidence interval, 25–57%) achieved primary neutrophil and platelet engraftment, respectively.

Table 2 Neutrophil engraftment, chimerism, and overall survival

Neutrophil engraftment Variable	n	% (95% CI)[a]	*P*
Total cell dose			
≧3 × 10^7/kg	16	94% (82–100%)	
<3 × 10^7/kg	14	79% (57–100%)	0.25
HLA disparities			
HLA 5/6 match	6	67% (29–100%)	
HLA 4/6 match	24	92% (81–100%)	0.24
100% Donor chimerism			
Total cell dose			
≧3 × 10^7/kg	16	100%	
<3 × 10^7/kg	14	86% (67–100%)	0.63
HLA disparity			
HLA 5/6 match	6	83% (54–100%)	
HLA 4/6 match	24	96% (88–100%)	0.31
Overall survival			
Total cell dose			
≧3 × 10^7/kg	16	54% (24–83%)	
<3 × 10^7/kg	14	52% (6.6–87%)	0.70
HLA disparities			
HLA 5/6 match	6	63% (20–100%)	
HLA 4/6 match	24	51% (20–81%)	0.60

[a] CI, confidence interval.

without requiring readministration of Filgrastim. Two patients died of TRM within 28 days of transplant. Primary graft failure occurred in the remaining 2 patients, who underwent second RI-UCBT with the same preparative regimen and GVHD prophylaxis and achieved neutrophil engraftment and complete donor chimerism. No patients experienced a decrease in neutrophil <0.5 × 10^9/liter during the follow-up.

Platelet counts >20 × 10^9/liter were achieved by 16 patients (40%; 95% CI, 25–57%) on a median day of 39 days (range, 25–95 days). No other patient achieved platelet recovery until the last day of follow-up.

No significant association was found between neutrophil engraftment and either infused cell dose or HLA disparity (Table 2).

Chimerism Analysis. Chimerism data were obtained from all of the 30 patients. Cumulative incidence of complete donor chimerism at day 60 was 93% (95% CI, 84–100%), and median time to complete donor chimerism was 22 days (range, 13–56 days; Fig. 2). The 2 patients who died of TRM within 28 days had complete donor chimerism before neutrophil engraftment. All of the surviving patients were monitored for chimerism every 3 months, followed the cyclosporine tapering schedule from day 100 to day 150, and maintained complete donor chimerism during the follow-up even after the discontinuation of immunosuppressants.

No significant association was identified between complete donor chimerism and either infused cell dose or HLA disparity (Table 2).

RRT and TRM. Four patients (13%) developed grade III RRT. No patient had grade IV RRT. The most commonly involved organs were the gut and kidney (Table 3).

TRM within 100 days of RI-UCBT was 27%. Primary causes of death were interstitial pneumonitis (n = 2), acute GVHD (n = 2), gastrointestinal bleeding (n = 1), acute heart failure (n = 1), limbic encephalopathy (n = 1), and sepsis (n = 1).

GVHD. Grade II-IV and III-IV acute GVHD occurred in 27% (95% CI, 11–43%) and 23% (95% CI, 7.4–39%) of the patients, respectively. Median onset of grade II-IV acute GVHD was day 36 (range, day 17–66; Fig. 3).

Of the 13 patients who survived >100 days, 3 (23%) developed chronic GVHD.

Infection. Twelve patients developed infections: bacteremia (n = 8), invasive aspergillosis (n = 3), and pulmonary tuberculosis (n = 1). Nine of them had been treated with

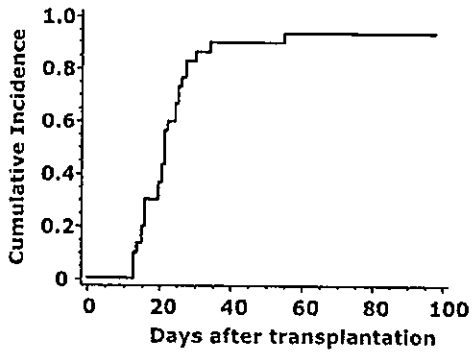

Fig. 2 Achievement of complete donor chimerism. Cumulative incidence of complete donor chimerism at day 60 after reduced-intensity unrelated cord-blood transplantation (*RI-UCBT*) was 93% (95% confidence interval, 84–100%), and median time to complete donor chimerism was day 22 (range, day 13–56).

Table 3 Regimen-related toxicity within 28 days (Bearman's score)

Score	Diarrhea	Kidney	CNS[a]	Liver	Lung
Grade 0	18	18	26	22	27
Grade 1	8	5	0	3	2
Grade 2	4	6	1	4	0
Grade 3	0	1	3	1	1
Grade 4	0	0	0	0	0

[a] CNS, central nervous system.