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Review Article

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 allo-immunity 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%

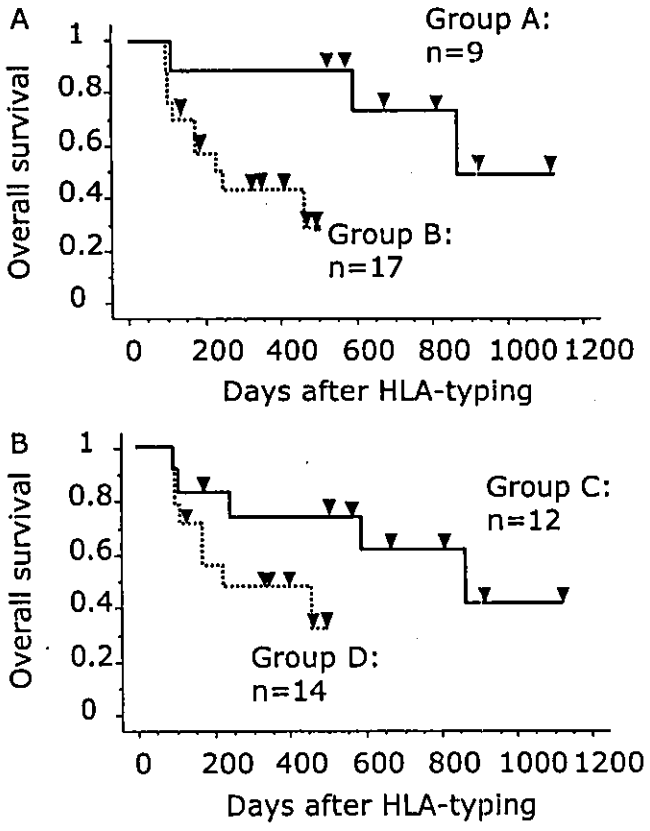

Figure 1. Kaplan–Meier estimates of the overall survival rates following HLA typing. (A) A comparison of overall survival rates between transplanted and non-transplanted patients. The overall survival rate was significantly higher in transplanted patients than in non-transplanted patients (P = 0.016). (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.

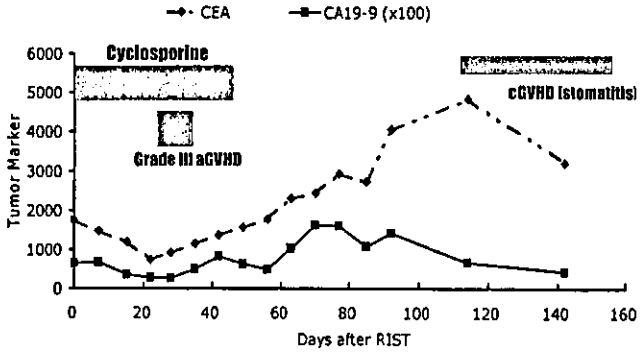

Figure 2. Clinical course of a patient with metastatic cholangiocellular carcinoma. A 56-year-old male with cholangiocarcinoma showed objective tumor shrinkage but not sufficiently satisfactory to regard it as partial remission.

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

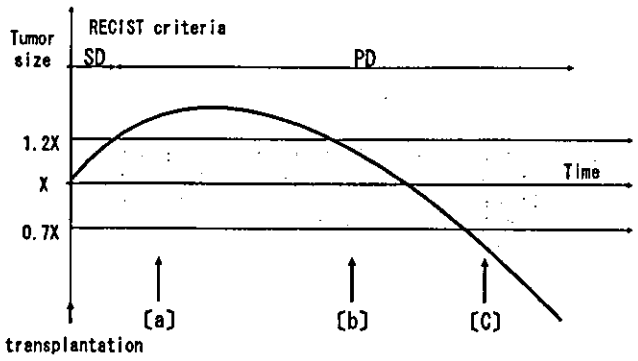

Figure 3. Course of tumor size after transplantation. Primary solid tumors are progressive despite chemoradiotherapy prior to transplantation. (a) Most tumors continue their natural growth until the development of a GVT effect, which usually occurs several months after transplantation. (b) If the tumor 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.

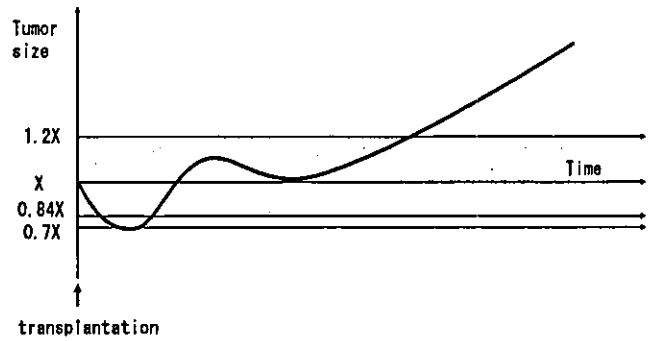

Figure 4. Fluctuation of tumor size after donor lymphocyte infusion or adjustment of immunosuppressive agents. It is difficult to handle patients in whom the tumor size fluctuates in response to post-transplant immunotherapy such as donor lymphocyte infusion and adjustment of immunosuppressive agents. 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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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 $> 1.0 \times 10^9$/liter or absolute neutrophil counts $> 0.5 \times 10^9$/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

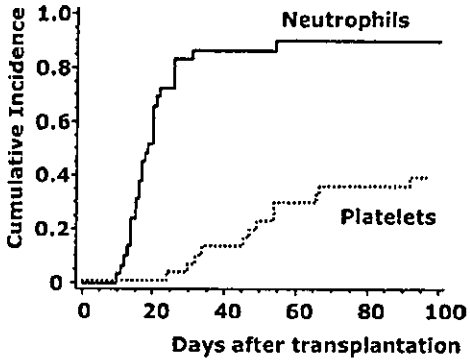

Fig. 1 Engraftment of neutrophils and platelets. Twenty-six (87%; 95% confidence interval, 75–99%) and 16 patients (40%; 95% confidence interval, 25–57%) achieved primary neutrophil and platelet engraftment, respectively.

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

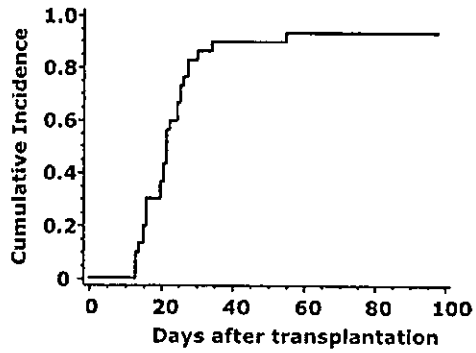

Fig. 2 Achievement of complete donor chimerism. Cumulative incidence of complete donor chimerism at day 60 after reduced-intensity unrelated cord-blood transplantation (*RI-UCBT*) was 93% (95% confidence interval, 84–100%), and median time to complete donor chimerism was day 22 (range, day 13–56).

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.

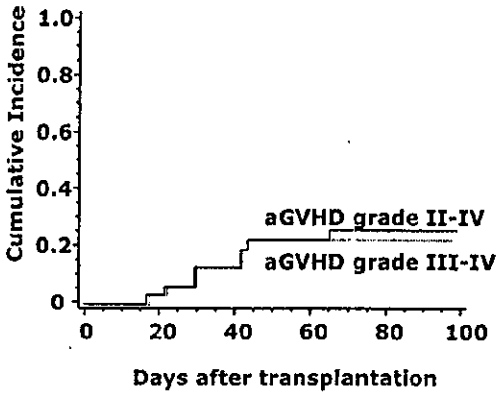

Fig. 3 Development of acute graft-*versus*-host disease (GVHD). Grade II-IV and III-IV acute GVHD developed in 27% (95% confidence interval, 11–43%) and 23% (95% confidence interval, 7.4–39%) of the patients, respectively. Median onsets of grade II-IV and III-IV acute GVHD were day 36 (range, day 17–66) and day 30 (range, day17–44), respectively.

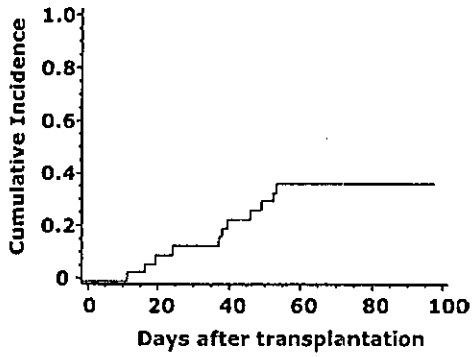

Fig. 4 Development of cytomegalovirus reactivation. Reactivation of cytomegalovirus was documented in 11 patients (37%) on a median of day 40 (range, day 13–55).

corticosteroids at the onset of infections. Reactivation of CMV was documented in 11 patients (37%) on a median of day 40 (range, day 13–55; Fig. 4). Eight of them had been treated with corticosteroids at the onset of CMV antigenemia. None of them developed CMV-related diseases. One patient developed hemorrhagic cystitis with adenovirus and BK virus infection.

Pre-Engraftment Noninfectious Fever. Seven patients with documented infection before engraftment were excluded from the analysis of pre-engraftment reaction (Table 4). Eighteen patients developed noninfectious fever before neutrophil engraftment (Fig. 5). Noninfectious high-grade fever often coexisted with eruption, diarrhea, and weight gain, starting on a median of day 9. Pathological examination of eruption from 8 patients revealed nonspecific inflammatory reactions and was not compatible with GVHD.

Survival. As of January 2004, a total of 11 patients remained alive. Median follow-up of the survivors and all of the enrolled patients were 238 days (range, 169–485) and 125 days (range, 26–485), respectively. Primary diseases recurred in 3 patients. Estimated 1-year OS and EFS were 32.7% (95% CI, 14.3–51.1%; Fig. 6) and 22.2% (95% CI, 5.9–38.5%; Fig. 7), respectively. Neither cell dose nor HLA disparity was associated with OS (Table 2).

DISCUSSION

Because CB contains a small amount of hematopoietic stem cells and stem cell boost or donor lymphocyte infusion is not available after UCBT, graft failure has been a major concern in adult UCBT. The present study demonstrated the feasibility of RI-UCBT for adult patients, in addition to pediatric patients (21). In this study, 26 of the 30 patients (87%) achieved durable engraftment, and 28 patients achieved complete donor chimerism by day 60, including 2 patients who died before engraftment. Interestingly, 4 patients with severe aplastic anemia, which has been associated with a high incidence of graft rejection (22), achieved complete chimerism after our reduced-intensity regimen. These findings suggest that the combination of fludarabine, melphalan, and low-dose TBI might be more immunosuppressive than conventional myeloablative regimens, creating niche for CB to engraft. Alternatively, CB may exert a strong graft-*versus*-host effect, making room for stable engraftment of stem cells.

Delayed hematopoietic recovery and infection during neutropenia are the significant concerns in adult UCBT. Laughlin *et*

Table 4 Characteristics of pre-engraftment reaction (n = 23)

Temperature	
38.0–38.9°C	2
39.0–39.9°C	10
≥40.0°C	7
Day of peak body temperature	9 (5–12)
Serum levels of CRP[a] (mg/dl)	13.8 (0.5–18.9)
Day of peak serum levels of CRP	10 (8–16)
Diarrhea	11
Eruption	10
Jaundice	5
Use of corticosteroid	13
Good response to corticosteroid	7

[a] CRP, C-reactive protein.

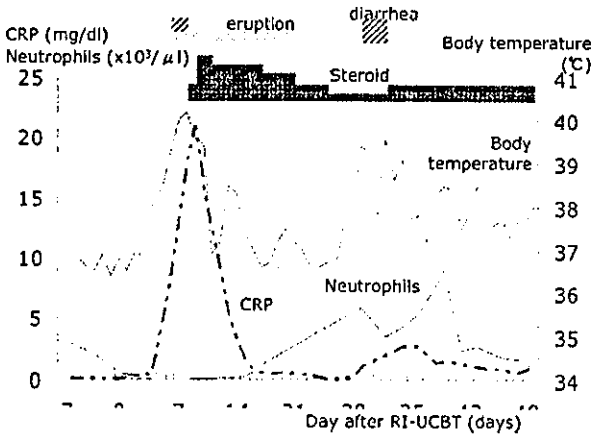

Fig. 5 Clinical course of a patient who developed pre-engraftment fever. Immune-reactions display two peaks, at around day 9 and day 18.

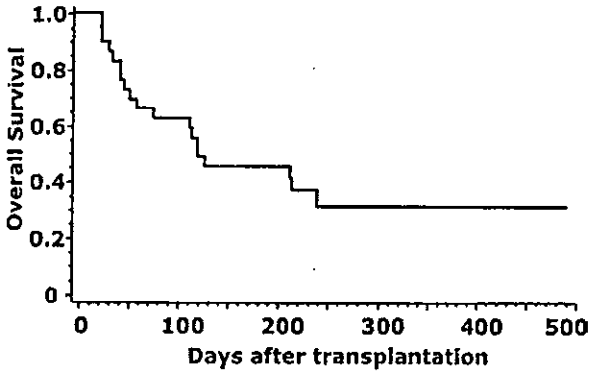

Fig. 6 Probability of overall survival after reduced-intensity unrelated cord-blood transplantation. Estimated 1-year overall survival was 32.7% (95% confidence interval, 14.3–51.1%).

al. (23) reported neutrophil recovery in 90% of patients by a median of 27 days after UCBT, which was significantly delayed compared with allo-HSCT. The delay has been attributed to the limited cell dose in the reports on myeloablative UCBT. The median nucleated cell dose in our study (3.1×10^7/kg) was greater than those in some reports from Western countries (2.1×10^7/kg; Ref. 9). The low median body weight (52 kg) in the Japanese population may favor neutrophil engraftment, whereas our results showed no association between the cell dose and engraftment in the small sample size. In the present study, median time to engraftment was 17.5 days (range, 10–54 days), which was much faster than that reported in previous studies on myeloablative UCBT (7–9). Our results were comparable with the report on adult RI-UCBT by Barker *et al.* (21). Their results showed neutrophil engraftment on a median of 26 days after busulfan/fludarabine/TBI 2 Gy and 9.5 days after cyclophosphamide/fludarabine/TBI 2 Gy. Whereas the reason for the difference remains unclear, these findings suggest that fludarabine-based reduced-intensity regimens enable rapid and stable engraftment.

TRM within 100 days was 27% in this study, which is lower than those reported on myeloablative UCBT (Refs. 7, 9, 24; 32–51% in pediatric patients and 56–63% in adults). Given the relatively old age (median, 58.5 years) and advanced stages of the primary diseases, our reduced-intensity preparative regimen probably decreased TRM. Our TRM within 100 days is comparable with that of 28% in adult RI-UCBT by Barker *et al* (21).

All of the patients tolerated our preparative regimen without grade IV RRT (Bearman's criteria; Ref. 2). Four patients developed grade III RRT with common involvements of the gut, kidney, and liver (Table 3). We used melphalan, which has dose-limiting toxicities of the gut and liver (25). These remained mild without hepatic veno-occlusive disease. Because renal toxicities of fludarabine, busulfan, and TBI 4 Gy are reportedly minimal, the high incidence of renal toxicity might be attributable to concomitant administration of nephrotoxic agents such as cyclosporin and antibiotics. Elderly patients might be susceptible to RRT. We plan to investigate optimal dosages of cyclosporin in RIST for elderly patients. Because TBI, even at a low dose, sometimes causes significant late toxicities in the lung (22), long-term follow-up is required.

Little information on GVHD after RI-UCBT is available. In the present study, the incidences of grade II-IV and III-IV acute GVHD and chronic GVHD were 27%, 23%, and 23%, respectively, whereas some reported those to be 33–44%, 11–22%, and 0–25%, respectively, in myeloablative UCBT (7, 8, 26). There are no significant differences in the incidences of GVHD between myeloablative UCBT and RI-UCBT. This is similar to the GVHD incidences in myeloablative allo-HSCT and RIST (27). Median onset of acute GVHD was 36 days (range, 17–66 days) in the present study, which was comparable with that of myeloablative UCBT (7, 8, 26). In contrast, the achievement of complete donor chimerism and the onset of acute GVHD are delayed in RIST compared with myeloablative allo-HSCT (27, 28). CB might have a potential of intense graft-*versus*-host effect, allowing niche for early engraftment. The characteristics of GVHD after RI-UCBT remain to be investigated, including different organ involvements and response to immunosuppressive treatment.

Interestingly, 20 patients developed inflammatory reactions before engraftment (Table 4). These reactions included noninfectious high-grade fever, eruption, diarrhea, and jaundice, starting on a median of day 9. Because the reactions preceded engraftment (median, day 17.5), we speculated that some form of immune reaction that is not categorized as acute GVHD occurs after RI-UCBT without achieving engraftment. The pre-engraftment fever has been reported on rare occasions in previous reports of UCBT and might be similar to those observed after haploidentical transplantations. Antithymocyte globulin and corticosteroids, which have strong immunosuppressive properties, were commonly used in previous studies on UCBT (9), whereas neither was used in the present study. Immunosuppressive treatment with corticosteroids was effective for the pre-engraftment fever. These findings support that immune-mediated reactions after UCBT might manifest easily with the present regimen. The doubling time of cultured CB $CD34^+$ cells is 7–10 days, which is several hundred-fold faster than that of cultured adult marrow cells (29). Mononuclear cells from CB display a unique cytokine profile such as comparable levels of

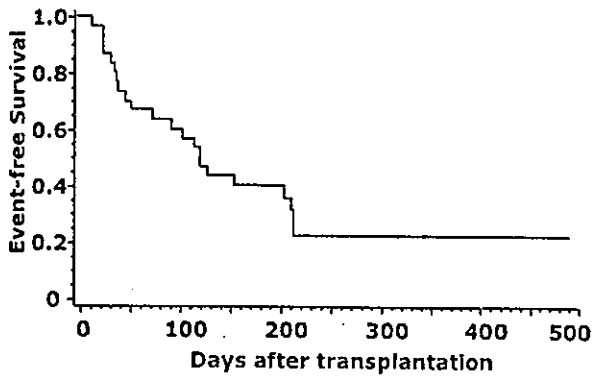

Fig. 7 Probability of event-free survival after reduced-intensity unrelated cord-blood transplantation. Estimated 1-year event-free survival was 22.2% (95% confidence interval, 5.9–38.5%).

interleukin (IL) 2, IL-6, and tumor necrosis factor α, reduced levels of IFN-γ and IL-10, and complete absence of IL-4 and IL-5 (30, 31). Pre-engraftment fever is possibly attributable to a cytokine storm induced by massive proliferation of cells with a unique cytokine profile. Another possibility is homeostasis-driven proliferation of naive T cells in highly immunosuppressed individuals, as demonstrated in murine models (32, 33). This reaction is reportedly associated with cytotoxic cytokines (32, 33). Fever as a transient response to contamination with maternal blood or cells during CB collection cannot be excluded (34). Reactivation of human herpesvirus 6 might be associated with this complication (35). If pre-engraftment fever exerts some antitumor effects, it is reasonable that patients with advanced and chemorefractory hematological diseases achieved long-term remission after RI-UCBT in the present study.

Infection is a common and significant problem in myeloablative UCBT (8, 9, 24), but little is known in RI-UCBT. The present study demonstrated that infection is also problematic in RI-UCBT. Twelve patients developed infection in this study, 9 of whom had been on corticosteroid therapy. Eight of 11 patients with CMV antigenemia had received corticosteroids. Delayed immunological reconstitution with or without GVHD, pre-engraftment fever, and corticosteroids may be risk factors for infection. Appropriate management of GVHD and pre-engraftment fever warrants additional investigation.

One-year OS was 35% in the present study, showing that some patients with advanced hematological malignancies can achieve durable remission after RI-UCBT. Contrary to our prediction, primary diseases recurred only in 3 patients. The candidates for RI-UCBT have extremely poor prognosis with conventional salvage chemotherapy. These findings suggest that RI-UCBT exerts strong antitumor activity and is promising for patients with refractory hematological malignancies without an HLA-identical sibling or an unrelated donor. In contrast, it is premature to apply RI-UCBT to low-risk diseases.

In conclusion, our study demonstrated the feasibility of RI-UCBT for adult patients with advanced hematological diseases, although the limitations included the small sample size and short follow-up. If CB is feasible for adults as an alternative stem cell source, RI-UCBT may become the choice of treatment for patients with advanced hematological diseases that are incurable with conventional treatments. RI-UCBT is particularly appealing for patients who require urgent treatments. Although RI-UCBT is currently associated with a high TRM, this study provided a rationale for continuing our clinical trials. Additional investigations need to focus on minimizing adverse effects including RRT, GVHD, and pre-engraftment immune reactions, whereas preserving graft-*versus*-leukemia effects.

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Reduced-Intensity Allogeneic Hematopoietic Stem-Cell Transplantation as an Immunotherapy for Metastatic Colorectal Cancer

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Background. Allogeneic stem-cell transplantation (allo-SCT) can induce curative graft-versus-leukemia reactions for hematologic malignancies through allogeneic immunity. Because the gastrointestinal tract is a target of graft-versus-host disease (GvHD), colorectal cancer might be a candidate for allo-SCT.

Methods. Four patients with metastatic colorectal cancer underwent reduced-intensity stem-cell transplantation (RIST) in the National Cancer Center Hospital between July 2002 and February 2003. Three patients received transplants from an human leukocyte antigen (HLA)-identical related donor, and the remaining patient received selected CD34-positive cells from a two-loci HLA-mismatched donor. The basis of preparative regimen was busulfan 4 mg/kg for 2 days and fludarabine 25 mg/kg for 6 days.

Results. All the patients tolerated the preparative regimen and achieved engraftment without significant toxicities. All developed acute or chronic GvHD. Although serum levels of CA19–9 and carcinoembryonic antigen were transiently elevated after RIST in all the patients, the levels subsequently decreased below the levels from before RIST in all but one patient. Three had measurable lesions before RIST, one achieved partial response, and the others stable disease, which was durable for 120 and 60 days. Three patients died; the causes of death were progressive disease, GvHD, and accident. Postmortem examination was obtained for two patients; in one patient, the peritoneal metastatic lesions macroscopically disappeared, and in the other patient, the supraclavicular lymph node disappeared while the other measurable lesions remained stable.

Conclusions. All the patients showed some evidence suggesting the presence of a graft-versus-tumor effect for colorectal cancer, which should be confirmed in a future prospective trial.

Keywords: Graft-versus-tumor effect, Graft-versus-host disease, Allogeneic immunity, Fludarabine, Carcinoembryonic antigen.

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A new strategy of allogeneic hematopoietic stem-cell transplantation (allo-SCT) using a reduced-intensity preparative regimen (reduced-intensity stem-cell transplantation [RIST]) was developed to decrease regimen-related toxicity (RRT) while preserving an adequate antitumor effect (*1, 2*). Different pioneering conditioning regimens for RIST have been investigated: those including purine analogs (*1–3*) and total body irradiation (TBI) combined with potent immunosuppressants (*4*). Because clinical studies on RIST have focused in hematologic malignancies, limited information is available on solid tumors (*5*), including renal cell carcinoma (RCC) (*6*), breast (*7, 8*), lung (*9*), ovarian (*10*), and colon cancer (*11*).

Because the epithelium is the target of graft-versus-host disease (GvHD), any types of carcinoma arising from the epithelial tissues such as keratinocytes, fibroblasts, exocrine glands, hepatobiliary trees, and gastrointestinal tract are theoretically susceptible to a graft-versus-tumor (GvT) effect. Murine models have provided some evidence for an allogeneic immune-mediated antisolid-tumor effect (*12*). Porter et al. (*13*) conducted a phase I clinical trial to determine whether a GvT effect could be observed after primary donor lymphocyte infusion (DLI) without stem-cell support in patients with primary cancers. Three of the four patients with acute GvHD and late chimerism responded to DLI. Eibl et al. (*14*) demonstrated that allogeneic T cells collected during GvHD could mediate a cytotoxic effect against breast cancer cell lines. Childs et al. (*15*) reported the results of 19 patients who underwent RIST for metastatic RCC. Seven patients achieved complete response and seven partial response (PR). The tumor response was associated with the development of GvHD. These results suggest that a GvT effect does exist in RIST for a variety of solid tumors, although long-term prognosis remains unknown.

Colorectal cancer is the second cause of cancer death (*16*), and prognosis for patients with unresectable and refractory to chemotherapy metastasis is poor. Development of novel therapeutic strategy is required. Because the gastrointestinal tract is the common target of GvHD, colorectal cancer might be sensitive to allogeneic immunity. We report four

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patients who underwent RIST for metastatic colorectal cancer.

PATIENTS AND METHODS

Patients

Between July 2002 and February 2003, four patients with colorectal cancer underwent RIST. All the patients had progressive metastatic disease, which was refractory to any conventional anticancer therapies including fluorouracil and irinotecan. Peripheral blood stem-cells were mobilized by subcutaneous injection of granulocyte colony-stimulating factor, 10 μg/kg, and harvested with the target cell dose of greater than 2.0×10^6/kg CD34+ cells.

The RIST program was approved by the Institutional Review Board of the National Cancer Center Hospital. A written informed consent was obtained from all the patients and donors.

Conditioning and Donors

The basis of preparative regimen was busulfan 4 mg/kg for 2 days and fludarabine 30 mg/kg for 6 days. Patient 1 received additional TBI 4.0 Gy in two fractions. Because a human leukocyte antigen (HLA)-identical donor was not available for patient 1, he underwent RIST from his two-loci mismatched brother-in-law with CD34+ cell selection. The number of simultaneously infused CD3+ cells was 2.5×10^4/kg. The remaining three patients had HLA-identical related donors: a sibling (patient 2 and 4) and an offspring (patient 3). Patient 2 received additional rabbit antithymocyte globulin (ATG) 2.5 mg/kg for 2 days to ensure durable engraftment. Since October 2002, ATG was omitted from the preparative regimen in the protocol of RIST for solid tumors, and ATG was not given to patient 3 and 4.

Engraftment and Management of GvHD

Recipient-donor chimerism in peripheral blood mononuclear cells (PBMC), T cells, and granulocytes were analyzed monthly after transplantation using polymerase chain reaction of informative short tandem repeat (*17*). GvHD prophylaxis was intravenous cyclosporine 3 mg/kg or oral cyclosporine 6 mg/kg from day −1. Because the incidence of acute GvHD is approximately 10% in RIST from an HLA-matched sibling after an ATG-containing regimen in our institution (*18*), we tapered cyclosporine early and rapidly over a 2-week period to enhance a GvT effect. The diagnosis of GvHD was made in concert with biopsy of the skin or the gastrointestinal tract. Acute and chronic GvHD were graded according to the consensus criteria (*19, 20*). Grade II to IV acute GvHD was treated with 2 mg/kg per day of methylprednisolone in addition to cyclosporine.

Supportive Measures

All the patients stayed in reverse isolation in a laminar airflow-equipped room and received prophylaxis with trimethoprim/sulfamethoxazole or pentamidine inhaler and ciprofloxacin against *Pneumocystis carinii* and bacterial infection, respectively. Fluconazole was administered for antifungal prophylaxis with a dose ranging from 200 to 400 mg/day. Herpes virus prophylaxis with acyclovir was also given as previously described (*21*). Cytomegalovirus pp65 antigenemia was routinely monitored once a week. When antigenemia was detected, preemptive therapy with ganciclovir was initiated as previously reported (*22*).

Evaluation of Tumor Response

All patients underwent computed tomography (CT) scanning before RIST and monthly after RIST to evaluate the tumor response. Serum levels of CA19–9 and carcinoembryonic antigen (CEA) were monitored weekly using chemiluminescent enzyme immunoassays (Lumipulse CA19–9-N, and Lumipulse CEA-N, Fujirevio, Tokyo, Japan, respectively).

Three patients (patient 2, 3, and 4) had measurable lesions before RIST, and tumor response was defined according to the response evaluation criteria in solid tumor (RECIST) (*23*) in these patients. Postmortem examination was available in the other two patients (patient 1 and 4).

RESULTS

Engraftment, Regimen-Related Toxicities, and GvHD

All the patients tolerated the preparative regimens with minimum RRTs. The neutrophil count reached 0.5×10^9/L on day 10 in patients 1, 3, and 4 and on day 12 in PATIENT 2. On day 30, chimerism analysis in PBMC showed full donor-type chimerism in all the patients. Grade II to IV acute GvHD developed in 3 patients, on median of 23 (20–47) days after RIST. Patient 1 developed grade IV acute GvHD after DLI to induce a GvT effect. The patient died of multiorgan failure caused by acute GvHD on day 62. The signs of GvHD in the remaining two patients spontaneously disappeared without additional immunosuppressant. Among two patients who survived over 100 days, both had chronic extensive GvHD.

Tumor Responses and Outcomes

Tumor responses were shown in Table 1. Although serum levels of CA19–9 and CEA transiently elevated after RIST in all the patients, the levels subsequently decreased below the levels before RIST in all but one patient (patient 4). Three patients had measurable lesions before RIST; one (patient 3) achieved PR, one (patient 2) stable disease (SD), which was durable for 120 days, and one (patient 4) progressive disease (PD).

Three patients died; the causes of death were GvHD (patient 1), PD (patient 2), and accident (patient 4). Postmortem examination was obtained for two patients; in patient 1, the peritoneal metastatic lesions macroscopically disappeared while microscopically detectable lesions remained. In patient 4, the supraclavicular lymph node metastasis disappeared while the other measurable lesions remained stable.

CASE PRESENTATION

Patient 1

A 44-year-old man underwent RIST from his two-loci mismatched brother-in-law in July 2002 for the treatment of metastatic rectal cancer. He had peritoneal metastasis, and no measurable lesions were documented before RIST. After preparative regimen consisting of fludarabine, busulfan, and 4 Gy TBI, he received CD34-positive stem cells.

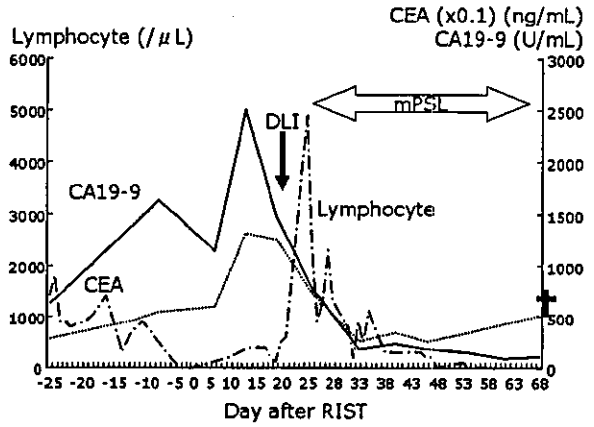

FIGURE 1. Clinical courses of patient 1. After engraftment and donor lymphocyte infusion (DLI), serum levels of tumor markers declined, and necropsy revealed that residual tumors in the peritonea shrunk. CEA, carcinoembryonic antigen; methylprednisolone, mPSL; RIST, reduced-intensity stem-cell transplantation.

His clinical courses was uneventful until day 21, when we added 4.0×10^6/kg CD3+ cells to improve immune recovery. The patient developed maculopapular rash, watery diarrhea, and jaundice on day 23. Based on the histopathologic examination of the skin, a diagnosis of grade IV acute GvHD was made. We initiated steroid-pulse therapy, but his condition deteriorated rapidly. He finally died of multiorgan failure caused by acute GvHD on day 62. Postmortem examination showed that the metastatic lesions in the peritoneum disappeared, whereas residual adenocarcinoma cells were observed by histopathologic examination.

Serum levels of CEA and CA19–9 increased from 45.7 ng/mL and 1388 U/mL before transplant to 131.0 ng/mL and 2507 U/mL on day 15, respectively. After DLI, serum levels of both values decreased rapidly (Fig. 1).

Patient 2

A 52-year-old woman underwent RIST from an HLA-identical sibling in August 2002 for metastatic rectal cancer. The metastatic lesions involved the liver and the both lungs. The liver lesions were measurable targets (Fig. 2). Because she had allergic reaction to the contrast agents on day 30, she received CT after day 60 without contrast agents. The patient had not developed acute GvHD, and we tapered off cyclosporine from day 35 until day 49 to enhance a GvT effect. Her clinical courses were uneventful until day 90, when she developed mucositis caused by chronic GvHD. To further augment the GvT effect, we withheld any immunosuppressive agents. The oral lesions had subsided spontaneously until day 120. Sequential abdominal CT scans failed to show progression until day 120 (Fig. 2). However, repeated CT scan on day 150 revealed an extensive progression of the metastatic lesions in the liver and the pleura (Fig. 2). She died of disease progression on day 172. An autopsy was denied by her family. The best and final responses were SD and PD, respectively. The duration of SD was 120 days.

Serum levels of CEA and CA19–9 were increased with transient decrease after engraftment until chronic GvHD developed and continued to decrease while the oral lesions persisted. Their serum levels were inversely associated with the severity of chronic GvHD (Fig. 3).

Patient 3

A 59-year-old woman underwent RIST from her HLA-identical offspring in October 2002. She presented metastatic

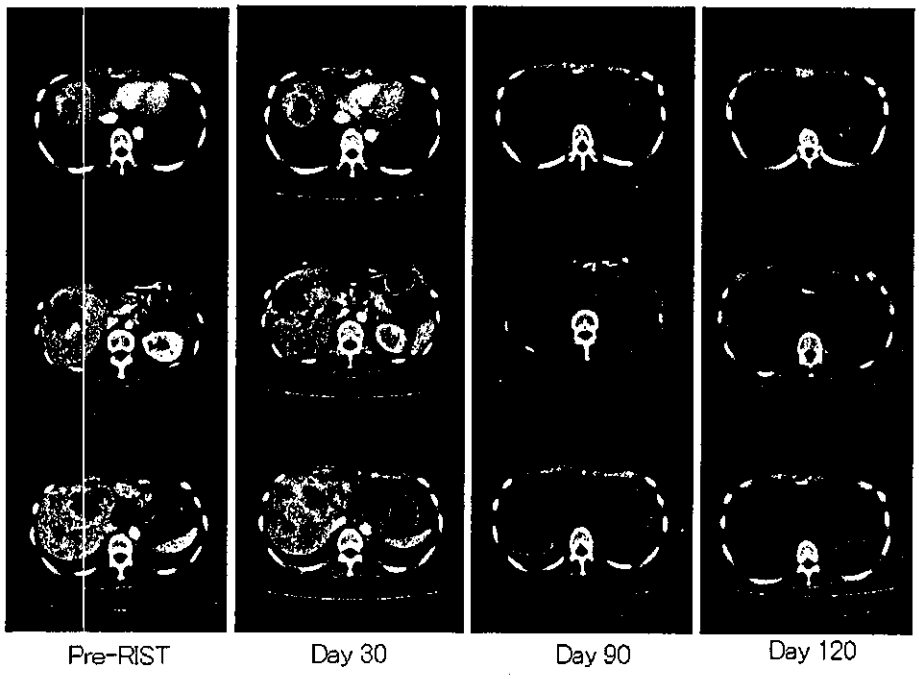

FIGURE 2. Changes of the metastatic lesions of the liver of patient 2. The metastatic lesions in the lung remained stable till day 120. However, metastatic lesions expanded with carcinogenic pleuritis.