Successful Clinical Response to Irinotecan in Relapsed Neuroblastoma

Toshiji Shitara, MD,[1] Akira Shimada, MD,[1] Yoshiaki Tsuchida, MD,[2]* Norio Suzuki, MD,[2] Fumiaki Toki, MD,[2] and Minoru Kuroiwa, MD[2]

Key words: irinotecan; neuroblastoma; childhood cancer; phase II trials

Camptothecin (CPT), an alkaloid with a novel ring structure, was first isolated from the Chinese tree *Camptotheca acuminata*. A water-soluble derivative of CPT, 7-ethy-10-(4-[1- piperidino]-1-piperidino)-carbonyl-camptothecin hydrochloride trihydrate (irinotecan, CPT-11), was subsequently synthesized [1]. Irinotecan as well as topotecan [2] inhibits DNA topoisomerase I, which is an essential nuclear enzyme. It relaxes torsionally strained duplex DNA, enabling replication and transcription. This agent has been reported to be effective against various human malignancies including lymphoma, gastric cancer, small cell lung cancer, non-small cell lung cancer, cervical cancer, epithelial ovarian cancer, colorectal cancer, and desmoplastic round blue cell tumor [3–11]. Three Phase I trials of irinotecan were conducted in childhood tumors in the United States, France, and Japan [12–14]. Investigators in those three groups have proposed their respective appropriate drug doses and administration schedules for Phase II trials.

A variety of approaches to the treatment of advanced neuroblastoma have been employed, and the clinical results have improved in recent years [15,16]. Nevertheless, the prognosis is particularly dismal once patients with this tumor relapse after myeloablative therapy followed by stem cell transplantation. It therefore seems appropriate to investigate the activity of new agents, such as irinotecan in these patients. It was in this spirit that we administered irinotecan to a patient who had relapsed.

She was an 8-month-old Japanese girl who was referred to the Gunma Children's Medical Center for work-up because the mass-screening test for neuroblastoma performed at 7 months of age was positive. On admission, a hard irregular mass was palpable in the upper left abdomen. Urinary vanillylmandelic acid (VMA) was elevated to 93.3 μg/mgCr, urinary homovanillic acid (HVA) to 183.5 μg/mgCR, serum neuron-specific enolase (NSE) to 142 ng/ml, serum lactic dehydrogenase (LDH) to 1,611 IU/L, and serum ferritin to 72 ng/ml. Magnetic resonance imaging (MRI) revealed a tumor in the left abdomen, compressing the left kidney downward. MRI also disclosed tumor invasion the right mediastinum. A biopsy of the abdominal tumor revealed neuroblastoma; she was classified as INSS Stage 4 originating in the left adrenal gland. There was no infiltration to the bone marrow. Although the biopsy indicated a favorable histology according to the Shimada classification [17], the *MYCN* gene was amplified by as many as 120 copies.

Chemotherapy was started according to the protocol of the Study Group of Japan [16]. After five courses of chemotherapy, when MRI showed shrinkage of the tumor, radical excision of the abdominal tumor was carried out. At surgery viable tumor cells were detected in approximately 10% of the tumor tissue. Surgical removal of the mediastinal tumor extension was undertaken after another course of chemotherapy, but viable tumor cells were again detected in the tumor. The patient was considered to be in very good partial response, and detection of minimal residual disease using protein gene product 9.5 was negative. After 12 courses of chemotherapy, an autologous bone marrow transplantation was carried out. The conditioning regimen was that designated Hi-MEC [18], consisting of melphalan, etoposide, and carboplatin without total body irradiation, according to the standardized protocol of the Study Group of Japan. After recovery of the bone marrow, the patient was placed on a regimen of 13-*cis*-retinoic acid to minimize the risk of the possible regrowth of the tumor [15].

Two years after the bone marrow transplantation, her chest X-ray films and MRI revealed a mass lesion in the hilum of the right lung and the mediastinum (Fig. 1). Urinary VMA was negative although serum NSE was elevated to 45 ng/ml. A biopsy of the recurrent mass revealed neuroblastoma, which was histologically the same as the initial specimen. In addition, the *MYCN* gene was again amplified. Chemotherapy was started based on the schedule of irinotecan Phase II studies in Japan [14], consisting of irinotecan 180 mg/m^2/day for three consecutive days. This was repeated once after 25 days off. Bone marrow suppression and diarrhea as adverse effects developed after the first course. However, during the second course, these complications were minimized by supportive care. After two courses of irinotecan, a 52% reduction in tumor size was seen on chest radiographs (Fig. 2). The patient was then placed on alternative irinotecan regimen, with the same administration schedule for the first and second courses, followed by ifosfamide and carboplatin. After six courses of such chemotherapy, she received 30 Gy of radiotherapy to the hilum of the right lung and mediastinum. MRI showed a complete resolution of the mass. She is currently scheduled to undergo another myeloablative course of chemotherapy followed by stem cell transplantation with curative intent.

[1]Department of Hematology/Oncology, Gunma Children's Medical Center, Gunma, Japan

[2]Department of Surgery, Gunma Children's Medical Center, Gunma, Japan

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*Correspondence to: Yoshiaki Tsuchida, Department of Surgery, Gunma Children's Medical Center, 779 Shimohakota, Hokkitsu, Seta-gun, Gunma 377-8577, Japan. E-mail: tuchida@gcmc.pref.gunma.jp

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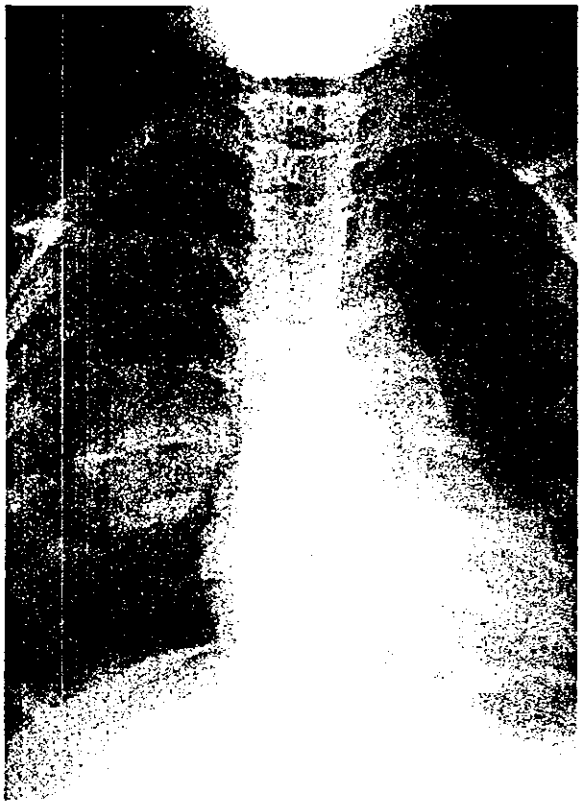

Fig. 1. Two years after bone marrow transplantation, a chest X-ray film showed a mass lesion in the hilum of the right lung and the mediastinum.

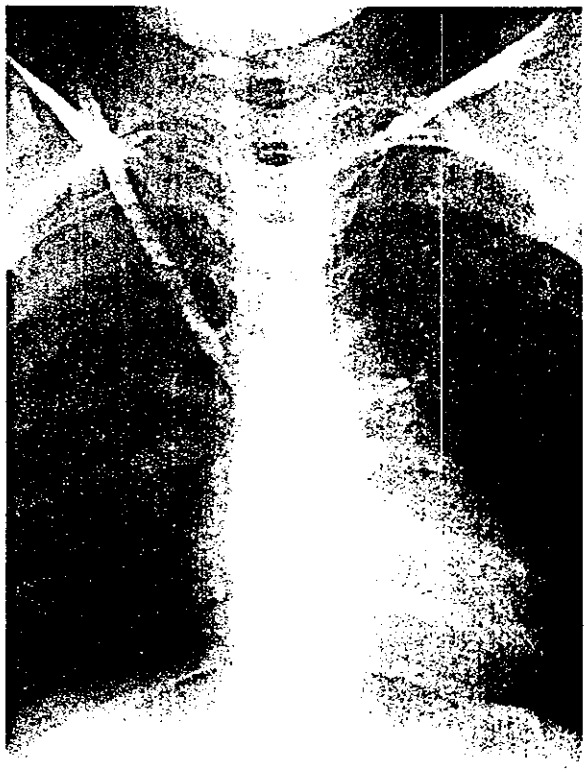

Fig. 2. Radiograph after two courses of irinotecan. Marked reduction in tumor size.

DISCUSSION

Among the camptotecin derivatives, topotecan and irinotecan are most widely used in Phase I and/or II trials in children. The recommended dose and administration schedule of irinotecan differ among researchers [12–14]. Furman et al. [12] recommend continuous administration of irinotecan 20 mg/m^2/day for five consecutive days, repeated once after 2 days off (10 days' administration in total), after determining the maximum tolerated dose (MTD) of irinotecan in an in vivo system. On the other hand, Vassal et al. [13] reported that the MTD of irinotecan for children was 600 mg/m^2 when given as a 120-min intravenous infusion every 21 days. Mugishima and others from Japan [14] determined that the MTD of irinotecan for children is between 160 and 180 mg/m^2/day administered over three consecutive days, repeated once after 25 days off. These MTDs are currently recommended for Phase II trials in the respective groups. These three regimens have merits and demerits. The Japanese group considers that the administration over three consecutive days may have an advantage over the others, and the irinotecan schedule in our patient is the one currently employed in the Phase II trials of the Japanese group. As a single, independent experience, Rosoff and Bayliff [11] administered irinotecan 50 mg/m^2/day for 5 days every 3–4 weeks in two patients with desmoplastic round blue cell tumors and saw significant responses.

There have been significance advances in the treatment of advanced neuroblastoma during recent years [15,16], but the clinical results are still poor compared with those in other childhood cancers, such as Wilms tumor, hepatoblastoma, etc. According to our recent analyses [17], the 5-year relapse-free survival rate was 36.0% for Stage 4 neuroblastoma patients with *MYCN* amplification, and 32.2% for those without *MYCN* amplification. Therefore, we currently lose more advanced neuroblastoma patients than we can save. The responses reported here in our patient suggest that irinotecan should be explored further in Phase II trials, and might be included as an active agent in the first-line treatment of advanced neuroblastoma.

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POSSIBLE BENEFITS OF HIGH-DOSE CHEMOTHERAPY AS INTENSIVE CONSOLIDATION IN PATIENTS WITH HIGH-RISK RHABDOMYOSARCOMA WHO ACHIEVE COMPLETE REMISSION WITH CONVENTIONAL CHEMOTHERAPY

Hiroshi Matsubara, MD, Atsushi Makimoto, MD, Takeshi Higa, MD, Hiroshi Kawamoto, MD, Jun Takayama, MD, and Mutsuro Ohira, MD □ Division of Pediatric Oncology, National Cancer Center Hospital, Tokyo, Japan

Ryohei Yokoyama, MD, and Yasuo Beppu, MD □ Division of Orthopedics, National Cancer Center Hospital, Tokyo, Japan

Yoichi Takaue, MD □ Division of Hematopoietic Stem Cell Transplantation, National Cancer Center Hospital, Tokyo, Japan

□ *The authors reviewed their single-center experience with autologous stem cell transplantation (SCT) in 22 patients with advanced rhabdomyosarcoma. Pathological subtypes included alveolar (n = 7) and embryonal types (n = 15). The conditioning regimen primarily consisted of etoposide, carboplatin, and melphalan. Fourteen, five, and three patients underwent SCT in CR, PR, and PD, respectively. Eight patients are currently alive without evidence of disease. The overall survival rate at 5 years was 70% for 14 patients who were in CR at the time of SCT. This limited experience warrants the examination of SCT in a prospective study.*

Keywords. high-dose chemotherapy, melphalan, rhabdomyosarcoma, stemcell transplantation

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children and arises in various sites. With the use of contemporary multidisciplinary approaches, including multiagent chemotherapy, radiotherapy, and surgery, more than 75% of children with local or regional involvement by RMS can be cured [1]. However, the prognosis of patients with unresectable or advanced metastatic tumors remains extremely poor [2, 3]. Since RMS is chemosensitive, increasing the dose of chemotherapy in the context of autologous hematopoietic stem cell transplantation (SCT) has been investigated over the past several years. The purpose of SCT is to eliminate micrometastatic residual disease in patients who have achieved a clinical complete remission (CR) after conventional chemotherapy [4]. Since previously published studies have included only small numbers of patients with widely varying eligibility

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Address correspondence to Hiroshi Matsubara, Department of Pediatrics, Kyoto University Hospital, 54 Kawahara-cho, Shogoin Sakyo-ku 606-8507, Kyoto, Japan. E-mail: hrmatsub@kuhp.kyoto-u.ac.jp

criteria and cytoreductive regimens, we believe that the accumulation of individual experience is still needed to evaluate the feasibility of this strategy [5–11]. This report presents a retrospective analysis of high-risk patients with RMS who were treated in a single institution with high-dose chemotherapy followed by autologous SCT.

PATIENTS AND METHODS

Twenty-two serially enrolled patients underwent autologous SCT between July 1990 and September 1999 at the National Cancer Center Hospital of Japan. The clinical characteristics of the patients are listed in Table 1. The median age of the patients at the time of SCT was 8.5 years (range, 2–22 years), and they consisted of 14 males and 8 females. The site of the primary tumor involvement was the limbs in 5 patients, orbit in 4, parameningeal in 7, head and neck in 4, pelvis in 1, and chest wall in 1. The histologic classification included alveolar type in 7 and embryonal type in 15 cases. The patient distribution at the primary diagnosis according to the group system used by the International Rhabdomyosarcoma Study (IRS) was clinical group I in 3 patients, II in 1, III in 16, and IV in 2. Using the Tumor-Node-Metastasis (TNM) staging system, there were 8 patients in stage 1, 4 in stage 2, 8 in stage 3, and 2 in stage 4.

Primary Treatment and Patient Status Before SCT

Eligibility criteria for the SCT included (1) clinical group III or IV disease at the primary diagnosis or (2) local relapse or distant metastasis in patient with clinical group I or II. All of the patients had received conventional chemotherapy as part of their primary therapy before SCT. Treatment regimens varied widely and included VAC (vincristine, dactinomycin, and cyclophosphamide), VAC-THP (pirarubicin in addition to VAC), VCA (vincristine, cyclophosphamide, and doxorubicin), and VAI (vincristine, dactinomycin, and ifosfamide), with or without cisplatin, etoposide, or methotrexate. Twelve patients had undergone surgery, including resection of the primary ($n = 11$) or both the primary and metastatic tumors ($n = 1$). Radiation therapy using 30–60 Gy was administered before SCT in 12 patients, after SCT in 2, and both before and after SCT in 1. Seven patients did not receive radiation therapy for the primary tumor.

Stem cells were obtained from bone marrow (2 were treated ex vivo with 4-hydroperoxycyclophosphamide and 2 were purified to $CD34^+$ cells) in 11 patients who were treated before 1995. Thereafter, peripheral blood stem cells (PBSC) were used as the source. Among 11 patients from whom we collected PBSC, 9 patients received PBSC transplantation (PBSCT) exclusively and 2 received both marrow and PBSC. CR and partial remission (PR) were defined, respectively, as a complete disappearance and at least a 50%

TABLE 1 Patient Characteristics

Patient no.	Age (year)	Sex	Primary site	Pathology	Surgery/LRx for primary site	Stage at diagnosis	Group at diagnosis	Group at SCT	Status at SCT	HDC regimens	Graft	Outcome	Site of relapse	EFS after SCT (month)	OS after SCT (month)
1	8	M	Extremity	Embryonal	+/−	3	III	III	CR	Hi-MEC	BM	REL	Nodes	5	137+
2	9	F	Orbit	Embryonal	+/+	1	III	III	CR	VP-16 + IFO + L-PAM	BM	NED	—	131+	131+
3	6	F	Orbit	Alveolar	+/+	1	I	III	PR	Hi-MEC	BM	REL	Primary	2	10
4	6	M	Head & neck	Embryonal	+/−	1	III	III	CR	Hi-MEC	BM	NED	—	127+	127+
5	15	F	Genitourinary	Embryonal	+/−	1	III	III	CR	Hi-MEC	BM	REL	Primary	17	124+
6	6	M	Parameningeal	Embryonal	+/+	2	III	III	CR	Hi-MEC	BM (4HC)	NED	—	110+	110+
7	2	M	Parameningeal	Embryonal	−/−	3	III	III	CR	Hi-MEC	BM	NED	—	115+	115+
8	14	M	Trunk	Embryonal	+/+	2	II	IV	PD	VP-16 + CDDP + L-PAM + EPI	BM	PD	Primary	—	9
9	8	M	Extremity	Alveolar	+/+	3	III	III	CR	Hi-MEC + THP	PBSC	REL	Primary	12	35
10	2	F	Parameningeal	Embryonal	−/+	2	III	III	CR	Hi-MEC + THP	BM	NED	—	83+	83+
11	6	F	Head & neck	Embryonal	−/−	1	III	III	CR	Hi-MEC + THP	PBSC	NED	—	78+	78+
12	11	F	Parameningeal	Embryonal	−/+	3	III	III	CR	Hi-MEC + THP	BM (CD34)	REL	Primary	8	32
13	8	M	Head & neck	Embryonal	−/+	4	IV	IV	PD	L-PAM	PBSC	PD	Primary	—	9
14	2	M	Head & neck	Alveolar	+/+	1	I	IV	CR	Hi-MEC + THP	BM (CD34)	REL	Nodes	13	31
15	17	F	Extremity	Alveolar	−/−	4	IV	IV	CR	Hi-MEC	PBSC	REL	Nodes	3	9
16	22	M	Extremity	Embryonal	−/+	3	III	IV	PR	Hi-MEC	PBSC	REL	Bone	7	9
17	14	M	Extremity	Embryonal	+/+	3	III	IV	PD	Hi-MEC + THP	PBSC	PD	Primary	—	2
18	4	M	Parameningeal	Embryonal	−/+	2	III	III	CR	Hi-MEC + THP	PBSC	NED	—	52+	52+
19	16	M	Parameningeal	Alveolar	+/+	3	III	IV	PR	Hi-MEC + THP	BM + PBSC	REL	Bone	2	7
20	20	F	Parameningeal	Alveolar	+/+	3	III	IV	PR	Hi-MEC	PBSC	REL	Bone	4	15
21	17	M	Orbit	Alveolar	−/+	1	I	III	CR	Hi-MEC	PBSC	NED	—	35+	35+
22	9	M	Orbit	Embryonal	−/+	1	III	III	PR	T T+ VP-16	BM + PBSC	REL	Primary	2	11

Note. HDC, high-dose chemotherapy; LRx, local radiotherapy; PFS, progression-free survival; OS, overall survival; CR, complete remission; PR, partial remission; PD, progression disease; Hi-MEC, melphalan + etoposide + carboplatin; VP-16, etoposide; IFO, ifosfamide; CDDP, cisplatin; EPI, epirubicin; L-PAM, melphalan; THP, pirarubicin; TT, thiotepa; BM, bone marrow; PBSC, peripheral blood stem cell; 4HC, 4-hydroperoxycyclophosphamide; REL, relapse; NED, no evidence of disease.

decrease of tumors. The disease status was evaluated by surgical procedures, pathological evaluation, and imaging studies, including CT scan, magnetic resonance imaging, and scintigraphy. Three patients in clinical group I, 1 in II, and 4 in III developed metastatic tumor and local recurrence during or after the initial therapy. As a result, there were 14 patients in group III and 8 in group IV at the time of SCT.

SCT/SCT Regimen

The primary regimen ($n = 10$) used for SCT was the Hi-MEC regimen, which consisted of etoposide 800 mg/m^2, carboplatin 1200 mg/m^2, and melphalan 180 mg/m^2 [12]. The regimens used in the remaining patients included (1) Hi-MEC + pirarubicin 80 mg/m^2 ($n = 8$), (2) etoposide 450 mg/m^2 + melphalan 180 mg/m^2 + ifosfamide 7.5 g/m^2 ($n = 1$), (3) etoposide 800 mg/m^2 + melphalan 140 mg/m^2 + cisplatin 100 mg/m^2 + epirubicin 45 mg/m^2 ($n = 1$), (4) etoposide 900 mg/m^2 + thiotepa 600 mg/m^2 ($n = 1$), and (5) melphalan 210 mg/m^2 alone ($n = 1$). No chemotherapy was given after SCT until the disease recurred. All patients were nursed in a room equipped with filtered air flow, and received intravenous hyperalimentation or blood products as needed. Granulocyte colony-stimulating factor (G-CSF) was used to assist granulocyte engraftment in 14 patients who were treated after 1993.

Engraftment

Granulocyte recovery was defined as the first of 2 consecutive days with an absolute granulocyte count of $>0.5 \times 10^9$/L. Platelet recovery was defined as the day when the platelet count rose to $>20 \times 10^9$/L without platelet transfusion support.

Statistical Analysis

No patients were lost to follow-up. Survival curves were generated according to the Kaplan–Meier method and the time was counted from the date of SCT [13].

RESULTS

The median duration of therapy from the onset of the primary or recurrent refractory disease to SCT was 9.5 months (2–20 months). Fourteen, five, and three patients underwent SCT in CR, PR and progressive disease (PD), respectively. No patients died of toxicity directly related to SCT. All of the patients experienced moderate nausea and vomiting during conditioning, but

this could be controlled with antiemetics. The major nonhematological toxicity was limited to the digestive tract, and included grade 2–3 mucositis and diarrhea. All of the patients experienced profound myelosuppression, and the median duration of neutropenia $<0.5 \times 10^9$/L and thrombocytopenia $<50 \times 10^9$/L was 13 (9–20) and 29 (13–71) days, respectively.

After SCT, 8 of the 14 patients who underwent SCT in CR remained in CR, with a median follow-up period of 99 months (35–131 months). Six patients developed recurrent disease: 3 patients locally and 3 at a regional site. Only 2 of the 6 patients are still disease-free at 85 and 79 months, respectively; they received salvage therapy, including surgical resection, chemotherapy, and radiotherapy. Only 2 of the 9 patients with no metastasis or recurrence before SCT and who underwent SCT in CR died of recurrence after SCT. All 3 patients with recurrent disease who underwent SCT in CR remained in CR. On the other hand, all of the 8 patients who underwent SCT in PR or PD died of progressive disease. Furthermore, all 8 patients in clinical group IV at the time of SCT died of recurrent or progressive disease after SCT. All recurrences after SCT were documented within 17 months after SCT.

The Kaplan–Meier estimates of the disease-free survival (DFS) and overall survival (OS) rates for all patients are shown in Figure 1. The 5-year DFS and OS rates were 36 and 45%, respectively. Figure 2 shows the OS rate of patients who had been in CR versus PR or PD at the time of SCT. The 5-year OS rate was 70% for 14 patients who received SCT in CR, and 0% for 8 patients in PR or PD. The 5-year OS rate according to tumor histology is shown in Figure 3. The difference between embryonal type (75%) and alveolar type (0%) was significant ($p = .015$). The 5-year OS rate was 90% for 10 patients with embryonal type tumor who underwent SCT in CR, and 82% for 12 patients

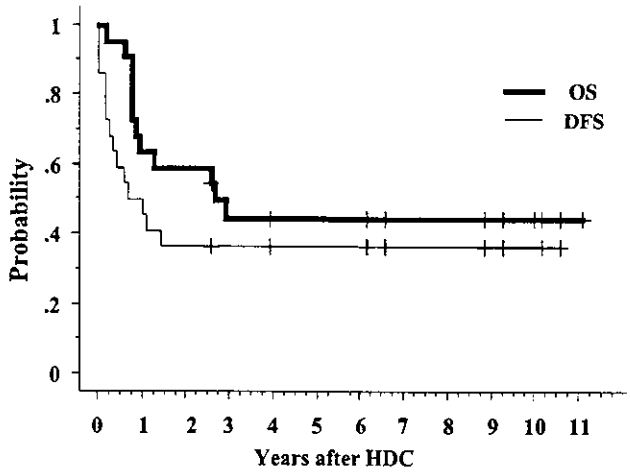

FIGURE 1 Overall survival and event-free survival in patients with advanced rhabdomyosarcoma.

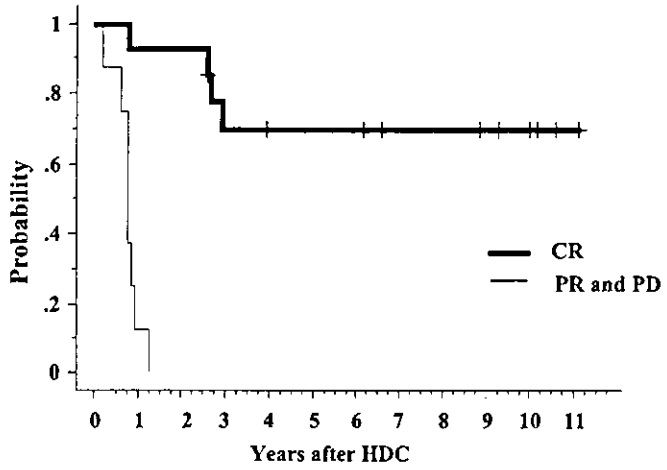

FIGURE 2 Overall survival in patients with advanced rhabdomyosarcoma according to status at the time of stem cell transplantation.

without distant metastasis who underwent SCT in CR. The 5-year OS rate for 11 patients more than 8 years old at the time of SCT was 18%, while this was 75% for 8 patients less than 8 years old. No patient developed secondary cancer during the observation period. However, 4 patients suffered from late effects related to therapy as follows: one patient has persistent edema in the right arm due to circulatory damage caused by the operation and radiation therapy, one has a secretion failure of the lacrimal gland secondary to radiation therapy, and two patients with a primary tumor on their ear have a hearing impairment on the same side.

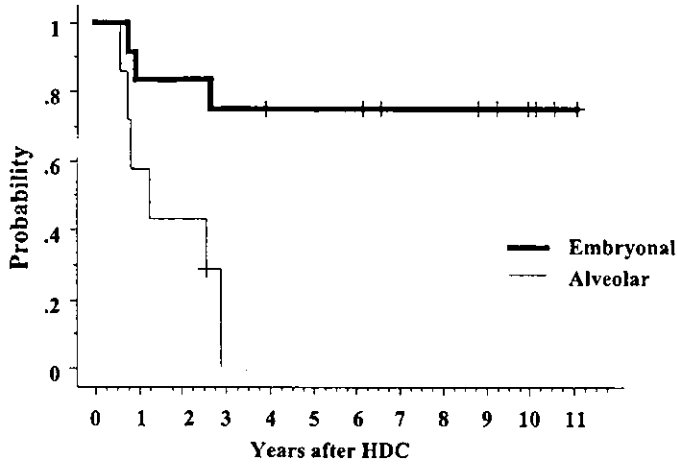

FIGURE 3 Overall survival in patients with advanced rhabdomyosarcoma according to pathological subtypes.

DISCUSSION

RMS is chemosensitive and the critical role of chemotherapy, mainly in an adjuvant setting, is well established. The intensification of conventional chemotherapy by escalating the dose and number of drugs has been related to improved therapeutic results [1, 14–16]. The overall survival rate is close to 75% even for locally advanced and primary nonresectable RMS in the IRS-IV group [1]. On the other hand, the prognosis of metastatic and/or recurrent RMS remains very poor, except for patients less than 10 years of age with stage IV embryonal RMS [2, 3, 11].

Although many clinical studies have been conducted to test the effectiveness of SCT followed by SCT in an attempt to overcome this problem, as summarized in Table 2, the results have been controversial [10, 11, 17–19]. Weigel et al. reviewed 64 published papers, including 389 patients with metastatic or recurrent rhabdomyosarcoma who received SCT. From this analysis, it does not appear to be a significant advantage to undergoing SCT for patients with relapsed or refractory high-risk RMS [20]. Carli et al. [11] reported that although the addition of SCT to consolidation therapy may prolong progression-free survival in high-risk patients, it did not significantly improve the ultimate outcome. However, their report emphasizes the importance of achieving CR before week 18, i.e., the third cycle of chemotherapy. They reported a statistically significant difference in both DFS and OS rates between patients who were in CR before the third cycle and those who were not.

Several other studies have shown a survival advantage in patients who received SCT in CR compared with those treated with conventional chemotherapy alone [8, 9, 17, 18]. However, a controversy still exists regarding the efficacy of SCT, probably because of the small numbers of patients, the heterogeneity of the patient's characteristics, and the widely varying cytoreductive regimens used in individual studies [10, 11]. SCT may offer several possible benefits: patients in CR pretransplant, which probably reflects the high chemosensitivity of the original disease, generally obtain more benefits from SCT than non-CR patients and the total duration of therapy is shorter with the SCT strategy.

Our analysis also suggests that SCT may be beneficial as an intensive consolidation therapy for select patients with high-risk RMS: unresectable or metastatic disease at diagnosis, refractory disease, or recurrent disease upon or after completion of the initial treatment. In our series, 4 of the 5 patients with local recurrent disease who received SCT in CR are still disease-free with a median follow-up of 119 months. Pappo et al. reported that the 5-year survival rate for 605 patients who experienced relapse after receiving treatment for IRS-III, IV pilot, or IV was no better than 20% [21]. Patients with an embryonal histology, local/regional residual disease, or local/regional recurrent disease may also benefit from SCT when they achieve CR before SCT. Hence,

TABLE 2 High-Dose Chemotherapy for Solid Tumors

First author	Year	No. of patients	Indication for HDCT	State in HDCT	Conditioning regimens	Result
This report	2003	22	Group III or IV RMS	CR: 14, PR: 5, PD: 3	Hi-MEC: 10, Hi-MEC + THP: 8, others: 4	5-year OS 44.6% 5-year OS 70.1% (HDCT in CR)
Boulad [10]	1998	26	Stage IV or stage II or III at unfavorable site (solid tumors)	CR: 13, PR: 6 NR: 3, PD: 4	L-PAM + VP-16: 26	2-year OS 56%
Carli [11]	1999	52	Metastatic RMS	CR[a]: 27, PR: 24, NR: 1	L-PAM: 42, Hi-MEC or L-PAM + BU + TEPA: 10	3-year OS 40% (standard chemo 27.7%)
Perentesis [17]	1999	24	Metastatic or relapsed or therapy-refractory disease (solid tumors)	CR: 9, PR: 15	VP-16 + TEPA + CY: 11 BU + L-PAM + TEPA: 13	4-year OS 78% (CR) 4-year OS 8% (PR)
Blay [18]	2000	30	Unresectable lung metastasis or two or more metastatic sites (solid tumors)	CR: 8, PR: 19, MR: 3	IFO + VP-16 + CDDP: 30	5-year OS 75% (CR) 5-year OS 5% (PR + MR)

Note. HDCT, high-dose chemotherapy; RMS, rhabdomyosarcoma; CR, complete remission; PR, partial remission; MR, minor response remission; NR, no response; PD, progression disease; OS, overall survival; Hi-MEC, melphalan + etoposide + carboplatin; THP, pirarubicin; L-PAM, melphalan; VP-16, etoposide; BU, busulfan; TEPA, thiotepa; CY, cyclophosphamide; IFO, ifosfamide; CDDP, cisplatin.

[a] Variable before third cycle (total third cycle before HDCT).

our observations suggest the need for a well-designed prospective clinical trial.

On the other hand, it appears to be very difficult to cure patients with a gross residual or refractory tumor, especially that with an alveolar histology. To improve the therapeutic outcome of such patients, strategies that include SCT must be more refined. Autologous SCT is the most promising consolidation chemotherapy for minimizing the volume of microscopic residual disease to below the level needed for a subsequent cure [9, 22]. In this regard, pretransplant treatments including conventional chemotherapy, radiation therapy, and surgery have to be well coordinated to achieve CR status prior to SCT. Regarding pretransplantation chemotherapy, possible strategies include (1) alternating multiagent combination chemotherapy, such as VAC and VIE (vincristine, ifosfamide and etoposide), and (2) aggressive incorporation of promising agents, such as camptothecins, gemcitabine, and possibly platinums. The application of tandem autologous SCT may also be useful for eradicating the residual tumor burden. To support these potential applications, therapy-related toxicities of SCT, including infections, organ toxicities, and increased risk of secondary cancer, should be reduced through the use of PBSC rather than bone marrow and sophisticated supportive therapies, including the use of G-CSF.

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ANTITHYMOCYTE GLOBULIN AFFECTS THE OCCURRENCE OF ACUTE AND CHRONIC GRAFT-VERSUS-HOST DISEASE AFTER A REDUCED-INTENSITY CONDITIONING REGIMEN BY MODULATING MIXED CHIMERISM INDUCTION AND IMMUNE RECONSTITUTION

KUNIHISA NAKAI,[1] SHIN MINEISHI,[1] MASAHIRO KAMI,[1] TAKESHI SAITO,[1,2] AKIKO HORI,[1] RIE KOJIMA,[1] OSAMU IMATAKI,[1] TAMAE HAMAKI,[1] SATOSHI YOSHIHARA,[1] MUTSUKO OHNISHI,[3] SUNG-WON KIM,[1] TOSHIHIKO ANDO,[1] ARIMA FUMITOH,[2] YOSHINOBU KANDA,[1] ATSUSHI MAKIMOTO,[1] RYUJI TANOSAKI,[1] SACHIYO KANAI,[3] YUJI HEIKE,[3] TOSHIHIRO OHNISHI,[4] YOSHIFUMI KAWANO,[5] HIRO WAKASUGI,[3] AND YOICHI TAKAUE[1,6]

Background. **There have been no detailed analyses of the induction of donor cell–type chimerism, the onset and incidence of acute and chronic graft-versus-host disease (GVHD), and the immune recovery kinetics after reduced-intensity stem cell transplantation (RIST).**

Methods. **To address these, with particular emphasis on the impact of the use of antithymocyte globulin (ATG) in RIST, we compared 39 consecutively registered patients who underwent RIST from an HLA-matched related donor and 33 patients who underwent conventional marrow-ablative transplantation.**

Results. **The incidences of grades II to IV acute and chronic GVHD tended to be less in RIST with ATG than in either RIST without ATG or conventional marrow-ablative transplantation. In a multivariate analysis, the predictive factors for acute and chronic GVHD included, respectively, ATG and grades II to IV acute GVHD. In a chimerism analysis, the achievement of complete donor chimera in T-cell lineage was delayed in RIST without ATG compared with RIST with ATG ($P=0.038$), which might explain the observed delayed onset of acute GVHD in RIST with ATG compared with the other two regimens. The ratio of type 1 and 2 dendritic cells did not affect the development of GVHD, whereas the number of naive $CD4^+$ T cells did. No difference was observed in the incidence of clinically definitive infection, including cytomegalovirus, among the three cohorts, regardless of the use of ATG.**

Conclusions. **We suggest that the conditioning regimen and immunosuppressive strategy after RIST should be carefully balanced against the risk of GVHD and of relapse of the basic disorder caused by the lack of a graft-versus-leukemia benefit.**

[1] Stem Cell Transplant Unit, National Cancer Center Hospital, Chuo-ku, Tokyo, Japan.

[2] Hematology Division, National Cancer Center Hospital, Chuo-ku, Tokyo, Japan.

[3] Pharmacology Division, National Cancer Center Research Institute, Chuo-ku, Tokyo, Japan.

[4] Department of Pediatrics, University of Tokushima, Tokushima City, Tokushima, Japan.

[5] Department of Pediatrics, University of Kagoshima, Kagoshima, Japan.

[6] Address correspondence to: Yoichi Takaue, M.D., Stem Cell Transplant Unit, National Cancer Center Hospital, 5-1-1, Tsukiji, Chuo-ku, Tokyo, 104-0045, Japan. E-mail: ytakaue@ncc.go.jp.

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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) has been established as a curative procedure for a variety of hematologic malignancies and other disorders (*1*). However, several complications after allo-HSCT remain lethal, such as regimen-related toxicities (RRT) caused by high-dose chemoradiotherapy, graft-versus-host disease (GVHD), and infections. To decrease their incidence, particularly RRT, reduced-intensity stem cell transplantation (RIST) has recently been developed for those who are not eligible for conventional stem cell transplantation because of old age or organ dysfunction (*2*). The primary concept of RIST is to enhance engraftment using intense immunosuppressive agents rather than myeloablative agents, with the expectation that donor-derived T cells will subsequently eradicate host-derived malignant cells, which is called a graft-versus-leukemia or graft-versus-tumor effect (*3*). Several immunosuppressive agents, such as purine analogs (cladribine, fludarabine) and antithymocyte globulin (ATG), have been investigated in the RIST procedure to facilitate engraftment (*2,4*). Both agents, especially ATG, have been shown to reduce the incidence of allograft rejection in allo-HSCT and solid organ transplantation by suppressing the regeneration of host T cells (*5*). Therefore, we conducted an initial phase I and II study of RIST against hematologic malignancies and found good results with a preimmunosuppressive regimen consisting of cladribine and ATG (*6*). All of the engrafted patients achieved the successful engraftment of complete donor chimerism, defined as more than 90% donor cells. After confirming the success of the initial trial, we performed RIST using a reduced immunosuppression regimen that did not include ATG, and this also led to successful engraftment (*7*). Although it is widely known that RRT is reduced by the application of RIST (*2,4,6*), the incidence and clinical impact of GVHD and infections after RIST have not been well characterized.

Initially, RIST is expected to contribute to reduced GVHD by decreasing the intensity of the "cytokine storm" that prepares the stage for the development of GVHD. However, there is a fine balance between the risk of GVHD or relapse and a graft-versus-leukemia or graft-versus-tumor effect. Hence, fine tuning of GVHD has become an important topic after a RIST procedure.

After nonspecific priming with a cytokine storm, the development of GVHD is initiated by donor T cells that recognize host peptides not present in the donor (*8*). In this immunologic setting, antigen-presenting cells could play a key role in the immune response after allogeneic trans-

plantation (*9*). Among antigen-presenting cells, dendritic cells (DC) are the most efficient stimulators of T cells (*10*). The peripheral blood contains two subsets of immature DC (i.e., DC1 and DC2) (*9,11*). Both DC1 and DC2 induce the proliferation of allogeneic naive $CD4^+$ T cells and lead to their differentiation into type 1 helper T cells (Th1) (DC1) or type 2 helper T cells (Th2) (DC2) (*12*). Polarization toward Th1 cells, which secrete interferon-α to promote the generation of cytotoxic T cells, could contribute to the development of GVHD, whereas polarization toward Th2 cells, which secrete interleukin (IL)-4 and IL-10, could result in the suppression of GVHD (*13*). Hence, it would be important to investigate the correlation between the kinetics of DC and T cells and the occurrence of GVHD after transplantation. In the setting of conventional marrow-ablative stem cell transplantation (CST), host DC and lymphocytes in most tissues are completely depleted by high-dose chemoradiotherapy (*14,15*); this is followed by the rapid establishment of donor DC chimerism (*16*). However, little is known about the kinetics of DC1 and DC2 after transplantation, particularly in the RIST setting.

Hence, we analyzed the onset and incidence of GVHD after RIST and compared the results with those observed after CST, particularly focusing on their correlation with the kinetics of the induction of mixed chimerism. Additionally, using flow cytometry analysis, we investigated whether the kinetics of DC and T cells was associated with GVHD. The fact that the background incidence and severity of GVHD are lower in Japan than in other countries (*17*) should enable a more precise analysis.

MATERIALS AND METHODS

Patients

This study was approved by the Institutional Review Board of the National Cancer Center Hospital. Patients with hematologic malignancies who were not eligible for CST because of their age or organ dysfunction and those with metastatic solid tumors were enrolled in the RIST protocol, while the other patients with hematologic malignancies underwent CST. A total of 72 patients underwent allogeneic peripheral blood stem cell (PBSC) transplantation from an HLA-matched related donor between June 1999 and September 2001. Overall, 39 patients underwent RIST and 33 underwent CST. The patient characteristics are shown in Table 1. We classified the patients into two populations based on preceding chemotherapy and the nature of the underlying disease: heavily pretreated (acute leukemias and non-Hodgkin's lymphoma) and less heavily pretreated (chronic leukemia, myelodysplastic syndrome, severe aplastic anemia, and paroxysmal nocturnal hemoglobinuria).

Blood Stem Cell Collection

Donors were injected with granulocyte colony-stimulating factor at 5 μg/kg subcutaneously twice daily starting 3 days before the first collection of PBSC until the end of collection. Leukapheresis was performed daily using conventional techniques, and the target value of $CD34^+$ cells was set at 3×10^6/kg of the recipient's body weight. Collected donor PBSC were then cryopreserved without T-cell depletion using standard methods for subsequent thawing and infusion.

Conditioning Regimens

In the RIST group (Fig. 1), 20 patients received the cladribine or Flu/Bu/ATG regimen ("RIST with ATG"), which consisted of cladrib-

TABLE 1. Patient characteristics

	RIST with ATG (n=20)	RIST without ATG (n=19)	CST (n=33)	*P* value
Median age (range)	47 (4–66)	55 (25–65)	41 (18–57)	0.0008
Disease				
AML	4	6	8	
MDS	3	4	3	
ALL	—	1	7	
CML	1	1	8	
NHL	1	7	7	
SAA	1	—	—	
PNH	1	—	—	
Solid tumor[a]	9	—	—	
Disease status				
CR	7	11	23	
NR	13	8	10	0.047
Pretreatment[b]				
Heavily	5	15	23	
Less heavily	15	4	10	0.0008
Infused cell dose ($\times10^6$/kg)				
$CD3^+$ cells median (range)	3.93 (2.02–7.05)	4.16 (1.24–5.59)	4.12 (1.92–6.37)	0.78
$CD4^+$ cells median (range)	2.16 (1.06–6.23)	2.33 (0.71–4.14)	2.14 (1.03–4.09)	0.89
$CD8^+$ cells median (range)	1.4 (0.08–3.14)	1.15 (0.46–2.06)	1.34 (0.57–3.52)	0.30
$CD34^+$ cells median (range)	4.21 (1.57–7.81)	3.35 (1.77–6.55)	4.41 (2.27–7.76)	0.0054
Duration of neutropenia median day (range)	11 (5–15)	11 (8–15)	14 (10–25)	<0.0001

AML, acute myeloblastic leukemia; MDS, myeodysplastic syndrome; ALL, acute lymphoblastic leukemia; CML, chronic myeloblastic leukemia; NHL, non-Hodgkin's lymphoma; SAA, severe aplastic anemia; PNH, paroxysmal nocturnal hemoglobinuria; CR, complete remission; NR, nonremission.

[a] Solid tumor category includes five renal cell carcinoma, two neuroblastoma, one rhabdomyosarcoma, and one melanoma.

[b] Heavily pretreated patients include acute leukemia and NHL, whereas less heavily include chronic leukemia, MDS, SAA, PNH, and solid tumor.

ine (Leustatin, Ortho Biotech, Raritan, NJ; 0.11 mg/kg for 6 days) or Flu (Fludara, Schering AG, Berlin, Germany; 30 mg/m^2 for 6 days), Bu (busulfan; 4 mg/kg for 2 days), and rabbit ATG (Thymoglobulin, IMTIX-SANGSTAT, Lyons, France; 2.5 mg/kg for 4 or 2 days), whereas 19 received the Flu/Bu regimen ("RIST without ATG"), which consisted of Flu (30 mg/m^2 for 6 days) and Bu (4 mg/kg for 2 days). In the RIST with ATG group, the dosage of ATG was decreased after confirming stable engraftment with a 4-day administration of ATG because of a previous observation of severely delayed recovery of CD4$^+$ T cells with the addition of ATG. In the CST group, 17 patients received the Bu/Cy regimen, which consisted of Bu (4 mg/kg for 4 days) and Cy (cyclophosphamide; 60 mg/kg for 2 days), whereas 16 received the Cy/total body irradiation regimen, which consisted of Cy (60 mg/kg for 2 days) and fractionated total body irradiation (2 Gy twice a day for 3 consecutive days).

Supportive Therapies

All patients received 300 μg/m^2 granulocyte colony-stimulating factor (filgrastim) from day 6 after transplantation until they achieved an absolute neutrophil count greater than 1.0×10^9/L. Hemoglobin was maintained at above 7.0 g/dL and the platelet count was maintained at above 20×10^9/L with filtered and irradiated blood products. Antibacterial and antifungal prophylaxis consisted of 600 mg per day ciprofloxacin and 200 mg per day fluconazole. Prophylaxis against herpes simplex virus was performed with acyclovir at a dose of 1,000 mg per day orally or 750 mg per day intravenously (IV) from day −7 to day 35, followed by low-dose (400 mg/day) oral administration until the end of immunosuppressive therapy. *Pneumocystis carinii* prophylaxis included trimethoprim/sulfamethoxazole for 14 days before transplantation and twice weekly after engraftment.

Chimerism Analysis

T-cell donor-host chimerism analysis was performed with CD3$^+$ cells by the polymerase chain reaction-based amplification of polymorphic short tandem repeat regions, as previously described (7). Peripheral blood mononuclear cells were separated by the Ficoll-Hypaque method and then by magnetic cell sorting, using anti-CD3 monoclonal antibody combined with immunomagnetic beads (Miltenyi Biotec, Germany), to give CD3-positive and -negative cells as final products.

GVHD Prophylaxis

Prophylaxis against acute GVHD was performed with cyclosporine (CsA) alone in RIST and with CsA and short-term methotrexate in CST. One patient who received RIST with ATG, four who received RIST without ATG, and three who received CST underwent rapid tapering of CsA in an attempt to induce a graft-versus-leukemia or graft-versus-tumor effect, because of chemotherapy-resistant acute leukemia, non-Hodgkin's lymphoma, or relapsing disease after their first allogeneic transplantation. Rapid tapering was defined as a tapering rate of greater than 25% per week after engraftment or cessation of CsA by day 40. In the rest of the patients, except for severe aplastic anemia and paroxysmal nocturnal hemoglobinuria, CsA was tapered during 5 to 7 weeks with discontinuation by 16 weeks if no GVHD developed. If the patient developed grades II to IV acute GVHD, CsA was resumed to achieve the therapeutic level and methylprednisolone therapy was added at a dose of 1 to 2 mg/kg per day IV.

Acute and Chronic GVHD

Acute GVHD was diagnosed both clinically and histologically in all patients and was classified as grade I to IV according to the criteria of the consensus conference on acute GVHD, as previously described (*18*). Liver involvement was diagnosed with a biopsy specimen whenever feasible. We inspected and evaluated all organs commonly involved in chronic GVHD, regardless of whether symptoms existed. Chronic GVHD was diagnosed clinically and was classified as either limited or extensive (*19*). A biopsy specimen of suspected organ was taken if possible.

Infectious Episodes and Cytomegalovirus (CMV) Antigenemia Assay

Clinically definitive infection was defined as an illness that was associated with symptoms and signs consistent with an infection and microbiologic documentation of a pathogen. Microbiologic documentation consisted of isolation of the pathogen by culture from sterile or from nonsterile sites or histologic or immunohistologic evidence.

An antigenemia assay was performed at least once a week after engraftment using the monoclonal antibody HRP-C7 (Teijin, Tokyo, Japan) raised against CMV immediate-early antigen, and preemptive therapy guided by antigenemia against CMV disease was conducted, as previously described (*20*). To evaluate the risk factors for CMV infection, we used the same definition as Nichols et al. (*21*) for rising antigenemia (i.e., an increase greater than or equal to twice the initial antigenemia).

Immune Phenotypic Assays

To investigate the recovery time course of DC and T cells after allo-HSCT, we monitored their surface markers as follows. Peripheral blood was collected in tubes containing sodium heparin at 30, 60, 90, 120, 180, 240, 300, and 360 days after transplantation. Three-color immunofluorescent staining was performed using the whole-blood lysis technique, and cells were analyzed on a fluorescence-activated cell sorter (FACSCalibur; Becton Dickinson Immunocytometry Systems [BDIS], San Jose, CA). Briefly, heparin-

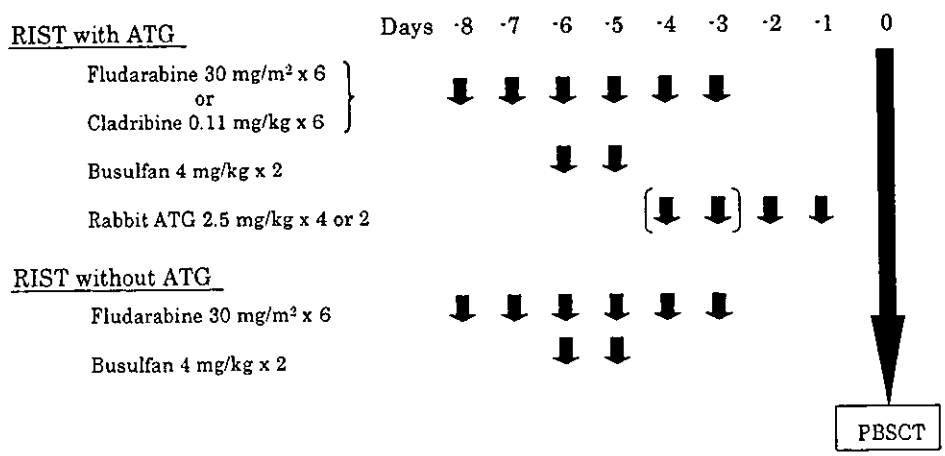

FIGURE 1. RIST protocol. In the RIST with ATG protocol, ATG was administered 2 days instead of 4 days (see text). PBSCT, peripheral blood stem cell transplantation.

ized peripheral blood was divided into 100-μl aliquots and stained with the three appropriate monoclonal antibodies for 30 min at 4°C in the dark. This was followed by the simultaneous lysis of erythrocytes and fixation of leukocytes using BD FACS Lysing Solution (BDIS) for 10 min at room temperature in the dark. Cells were then washed twice with CellWASH (BDIS). Finally, the cells were fixed with CellFIX (BDIS) for flow cytometry analysis. Data acquisition was performed with CellQuest software using a fluorescent or forward scatter threshold. Lymphocytes were identified by forward scatter and side scatter analysis based on 30,000 events, if possible. The following monoclonal antibodies were used in this study: mouse IgG1 (fluorescein isothiocyanate; FITC), mouse IgG1 (phycoerythrin; PE), IgG2b (PE), IgG1 (peridinin chlorophyll protein; PerCP), CD3 (PerCP), CD4 (PerCP), CD4/CD8 cocktail (FITC/PE), CD45 RA (FITC), CD45RO (PE), lineage cocktail 1 (FITC), HLA-DR (PerCP), CD11c (PE), and CD123 (PE). CD4/CD8 cocktail, CD45RA, and CD45RO were purchased from Immunotech (A Coulter Company, Marseilles, France), and the others were from BDIS. Lineage cocktail 1 consisted of CD3, CD14, CD16, CD19, CD20, and CD56 in one vial. CD4 cells were defined as $CD4^+CD3^+$, and CD8 cells were considered $CD8^+CD3^+$. Proportions of CD45RA and CD45RO were determined in $CD4^+$ cells only. DC were defined as $lineage^-$ and $HLA\text{-}DR^+$ lesions, and then separated into $CD11c^+$ (DC1) or $CD123^+$ (DC2) subsets.

DC Activation Assay

To investigate the immune function of DC, we analyzed the intracellular production of tumor necrosis factor (TNF)-α only for DC1, because we confirmed that few DC2 exist after allo-HSCT, as previously described (22). Briefly, heparinized whole blood was stimulated with 0.1 μg/mL lipopolysaccharide (Sigma Chemical Co, St. Louis, MO). Brefeldin (Sigma) was then added to inhibit cytokine secretion at a final concentration of 10 μg/mL, and the blood was incubated for 2 hours at 37° and 5% CO_2 in polypropylene tubes. Next, 2 mM EDTA was added to arrest activation and remove adherent cells, the blood was separated into FACS tubes (200 μl each), and appropriate anti-surface markers (lineage cocktail 1 [FITC], HLA-DR [PerCP], and CD11c allophycocyanin [BDIS]) were added. After incubation for 30 min at room temperature in the dark, the blood was lysed for 10 min by adding 2 mL of FACS lysing solution (BDIS). Cells were then washed and permeabilized using FACS permeabilizing solution 2 (BDIS) for 10 min. After an additional wash, anti-cytokine antibody (TNF-α; PE) was added and the cells were incubated for 30 min at room temperature in the dark. Finally, the cells were washed and fixed for flow cytometry analysis.

Clinical Endpoints

The primary endpoints of this study were (1) the onset and incidence of acute and chronic GVHD in RIST with or without ATG, in comparison with those in CST; (2) the correlation between the onset and incidence of acute GVHD and chimera status within RIST; and (3) the correlation between GVHD and the recovery kinetics of DC and T cells. The secondary endpoints were the incidence of infection in the three regimens and the correlation between infectious episodes and GVHD or GVHD-related steroid therapy.

Statistical Methods

Differences among the three regimens were evaluated using the chi-square test. Continuous data were compared using the Kruskal-Wallis test. The times to the onset of acute and chronic GVHD, clinically definitive infection, and positive CMV antigenemia were estimated by the Kaplan-Meier method and compared using the log-rank test. To evaluate predictive factors, we performed univariate and multivariate analyses using a Cox proportional hazard model to adjust the hazard ratio for patients who did not have covariates. To determine the risk factors related to rising antigenemia, we first analyzed the probability of event against variables in a univariate analysis and then in a backward stepwise logistic regression analysis. The time course of DC recovery and the intracellular cytokine of DC1 and T cells were evaluated using a two-way analysis of variance.

RESULTS

Patient Characteristics

Both of the conditioning regimens in RIST were tolerated by all of the respective patients. There were nine patients with solid tumors in the RIST with ATG group. Thus, more patients in this group were not in remission and they were less heavily pretreated, compared with those who were treated with RIST without ATG or CST ($P=0.047$, 0.0008, respectively; Table 1).

The median numbers of infused $CD3^+$, $CD4^+$, and $CD8^+$ cells were comparable among the three regimens (Table 1). Although the number of infused $CD34^+$ cells in RIST without ATG was less than in the other two regimens ($P=0.0054$), all of the former patients showed prompt hematopoietic recovery. Collectively, all 72 patients successfully achieved engraftment. The median number of days in the neutropenic period was 11 in the RIST regimens (either with or without ATG) and 14 in the CST regimen ($P<0.0001$).

Chimerism Analysis

Chimerism was assessed with regard to T-cell lineage in patients who received RIST with or without ATG (Fig. 2A). Complete donor-type chimera (>90%) was achieved within 30 days in all patients who received RIST with ATG, whereas this took much longer (up to 90 days) in RIST without ATG, particularly for the T-cell lineage ($P=0.038$). Complete donor-type chimera was seen in RIST without ATG after 90 days.

Acute GVHD

Grades II to IV acute GVHD were diagnosed in 10% (2/20) of RIST with ATG, 63% (12/19) of RIST without ATG, and 33% (11/33) of CST ($P=0.0068$, log-rank test; Table 2, Fig. 2A). Furthermore, a proportional hazard model showed that only ATG influenced the occurrence of grades II to IV acute GVHD (hazard ratio [HR]=0.16, $P=0.014$). These findings suggest that ATG is associated with a significantly lower incidence of acute GVHD. The numbers of patients who underwent rapid tapering of CsA were as follows: one in RIST with ATG, four in RIST without ATG, and three in CST. Among these, two in the RIST without ATG group developed grades II and IV acute GVHD, respectively, whereas one in the CST group developed grade II acute GVHD. The median number of days until the onset of acute GVHD in the three regimens was 53 in RIST with ATG, 74 in RIST without ATG, and 27 in CST ($P=0.064$; Table 2). Interestingly, the onset in RIST without ATG was delayed compared with that in CST. Moreover, there was a close correlation between the onset of acute GVHD and the timing of chimerism induction (Fig. 2A), suggesting that conversion to complete donor-type chimerism prepares the stage for the development of acute GVHD. The patients who received RIST without ATG also tended to be more likely to receive steroid therapy against GVHD compared with those with the other two regimens ($P=0.048$; Table 2). Overall, steroid therapy was effective and could be tapered immediately except in two patients in RIST with

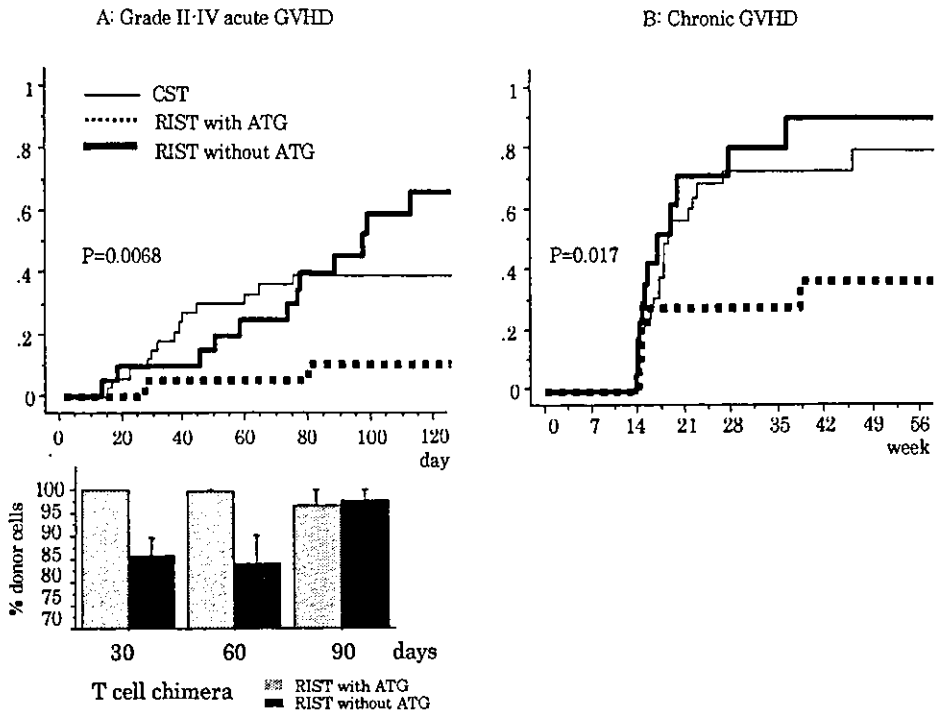

FIGURE 2. (A) Incidence of acute GVHD (grades II-IV) and chimerism analysis of T cells in RIST with ATG or without ATG. (B) Incidence of chronic GVHD.

TABLE 2. GVHD prophylaxis, incidence of GVHD, and steroid use

	RIST with ATG (n=20)	RIST without ATG (n=19)	CST (n=33)	*P* value
GVHD prophylaxis				
CsA	20	19	0	
CsA+MTX	0	0	33	
Rapid taper of CsA	1	4	3	
Acute GVHD	3	14	19	
I	1	2	6	
II–IV	2	12	13	0.0068
III–IV	2	4	5	
Median days to grade II–IV (range)	53 (26–79)	74 (12–111)	29 (14–74)	0.064
Chronic GVHD	6	12	21	0.017
Limited	4	2	8	
Extensive	2	10	13	
De novo	4	1	8	
Quiscent	0	3	3	
Progressive	2	8	8	
Steroid use ≥1 mg/kg	7	13	12	0.048

ATG, two in RIST without ATG, and two in CST. Among these six patients, five had received rapid tapering of CsA. Consequently, four patients died of GVHD or GVHD-related complications.

Chronic GVHD

Chronic GVHD was diagnosed in 30% (6/20) of RIST with ATG, 63% (12/19) of RIST without ATG, and 64% (21/33) of CST (P=0.017, log-rank test) (Table 2, Fig. 2B). Thus, the incidence of chronic GVHD was significantly lower in RIST with ATG than in the other two regimens. Progressive and extensive chronic GVHD was more frequent in RIST without ATG than in the other two regimens (Table 2), possibly because of the delayed onset of acute GVHD in RIST without ATG in a multivariate analysis (HR=2.63, P=0.0035). The therapeutic response against chronic GVHD with CsA, steroid, or both was satisfactory in all patients.

Immune Phenotype Analysis

In the analysis of T cells, there were fewer $CD3^+$ and $CD3^+/CD8^+$ cells in the RIST regimens than in the CST regimen at day 30, but these numbers quickly recovered to within the normal range after day 60 (Fig. 3A,B). The recovery kinetics of $CD3^+/CD4^+$ cells were in the following order: CST > RIST without ATG > RIST with ATG (P<0.0001; Fig. 3C). RIST without ATG and CST showed normal ranges within 360 days after transplantation, whereas RIST with ATG did not show normal ranges. Notably, there were significantly fewer $CD4^+/CD45RA^+$ cells, a marker of naive $CD4^+$ T cells, in RIST with ATG than in RIST without ATG

($P<0.0001$) or in CST ($P<0.0001$; Fig. 3D). Moreover, the recovery profiles in CST and RIST without ATG were similar throughout the initial year. On the other hand, $CD4^+$/$CD45RO^+$ cells, a marker of memory $CD4^+$ T cells, showed a profile similar to $CD3^+/CD4^+$ ($P<0.0001$; Fig. 3E), whereas all three regimens recovered to within the normal range by 240 days after transplantation. These findings suggested that ATG contributed to the delayed reconstitution of $CD4^+$ T cells after transplantation.

In infused grafts, there were significantly more DC2 than DC1 ($P=0.008$; Fig. 4A). After engraftment, the recovery kinetics of blood DC and the intracytoplasmic production of TNF-α in DC1 cells approached normal ranges, with no essential difference among the three regimens ($P=0.40$ and 0.61, respectively; Fig. 4B,C). Interestingly, the DC1/DC2 ratio was greater than 1.0 in all three regimens by day 30 after transplantation, despite the predominance of DC2 in infused cells (Fig. 4D).

Infections

The incidence of clinically definitive infection was not different among the three regimens (Table 3). Although the relative incidence of fungal and viral infection could not be meaningfully analyzed because of the small number of patients, the incidence of viral infection tended to be higher in CST than in the other two regimens. CMV diseases consisted

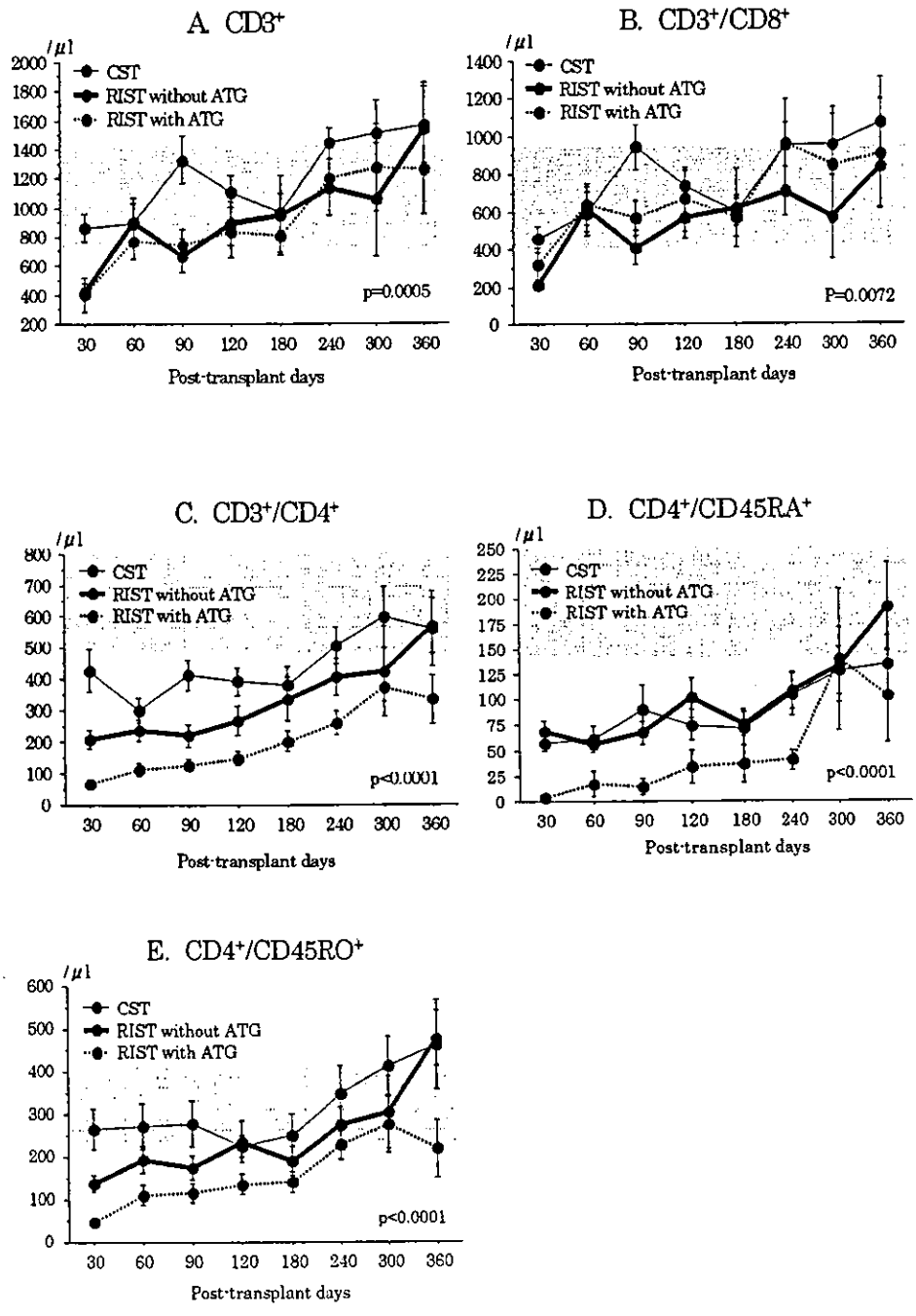

FIGURE 3. (A to E) Phenotypic recovery of T cells after RIST with ATG, RIST without ATG, and CST. $CD3^+$, $CD3^+/CD8^+$, $CD3^+/CD4^+$, $CD4^+/CD45RA^+$, and $CD4^+$/$CD45RO^+$, respectively. The mean value and standard error are shown by dot and whisker plots. The shaded area shows the reference range (25th to 75th percentile in 12 healthy adult donors).

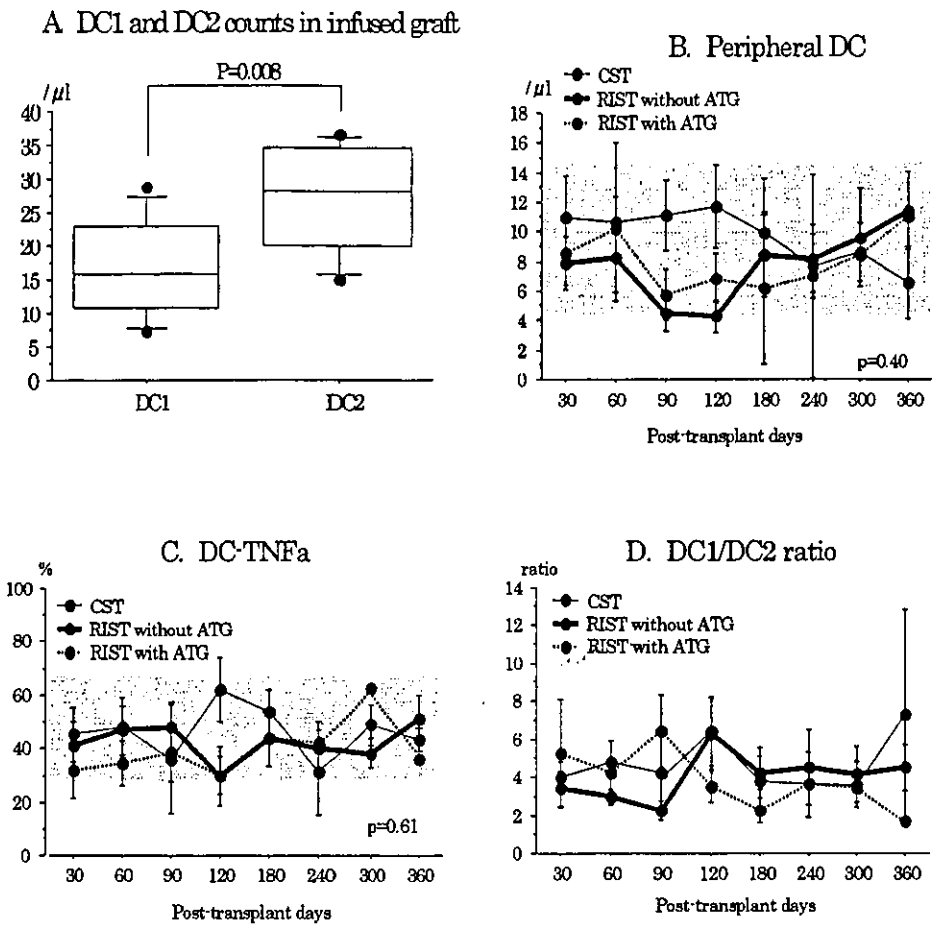

FIGURE 4. (A) DC1 and DC2 counts in infused graft. DC counts in peripheral blood (B), intracytoplasmic TNF-α of DC as a percentage (C), and the DC1/DC2 ratio (D) were measured after RIST with ATG, RIST without ATG, and CST. The mean value and standard error are shown by dot and whisker plots. The shaded area shows the reference range (25th to 75th percentile in 12 healthy adult donors).

of three cases of gastroenteritis and one case of cystitis, whereas adenovirus infection was manifested as hemorrhagic cystitis in all cases. Although there was no difference in the incidence of CMV antigenemia itself, the numbers of episodes of rising antigenemia were marginally different among the three regimens ($P=0.08$) (Table 3). In a univariate analysis using the Cox regression hazard model, factors identified as marginally significant were steroid therapy for clinically definitive infection [HR=1.8, 95% confidence interval (CI)=0.96–3.3, $P=0.066$] and grades II to IV acute GVHD and steroid therapy for CMV antigenemia (HR=1.8 and 1.8, 95% CI=0.9–3.3 and 0.95–3.3, $P=0.079$ and 0.073, respectively). Multivariate analysis did not show any independent significant factors. Grades II to IV acute GVHD and steroid therapy were identified as risk factors for rising antigenemia in a univariate analysis (odds ratio=17.2 and 26.7, 95%CI=3.4–86 and 3.2–219, $P=0.0005$ and 0.0022, respectively). A backward stepwise logistic regression analysis showed that only steroid therapy independently influenced the incidence of rising antigenemia.

DISCUSSION

Although RIST has been the subject of recent intense clinical research, the benefit of ATG, with regard to the occurrence of GVHD, immune kinetics, and infectious complications, has not been established. The effect of rabbit ATG in the prevention of GVHD was previously evaluated in two randomized studies on bone marrow transplantation from an HLA-matched unrelated donor after conditioning with a conventional Cy/total body irradiation regimen (*23*). As a result, the overall incidence of extensive chronic GVHD was lower in patients who received ATG than in those who did not (39% vs. 62%). In this study, we analyzed the contribution of ATG by focusing on the occurrence of acute and chronic GVHD and the recovery kinetics of T cells and DC. We also examined the incidence and clinical characteristics of infectious episodes after RIST with ATG. Consequently, we found that the incidence of acute and chronic GVHD in RIST was notably lower with ATG. Although it has been controversial whether the incidence of GVHD is lower after RIST or CST, it is noteworthy that in our study a lower dose of ATG in the RIST procedure suppressed the development of acute and chronic GVHD after allo-HSCT.

Using the same type of RIST regimen, Slavin et al. (*2*) reported that the incidences of grades I to IV and grades III and IV acute GVHD was 46% (12/26) and 25% (6/26), respectively, whereas the incidence of chronic GVHD was 35% (9/26); these are comparable to values previously published for the CST procedure (*24*). We think that the lower incidence of GVHD in our study reflects the more homogeneous distribution of HLA in Japan. Bornhauser et al. (*25*) used a Flu/Bu without ATG regimen, and the incidences of grades II to IV acute and chronic GVHD was 15% (3/20) and 25% (5/20), respectively, which were even lower than our findings. More-