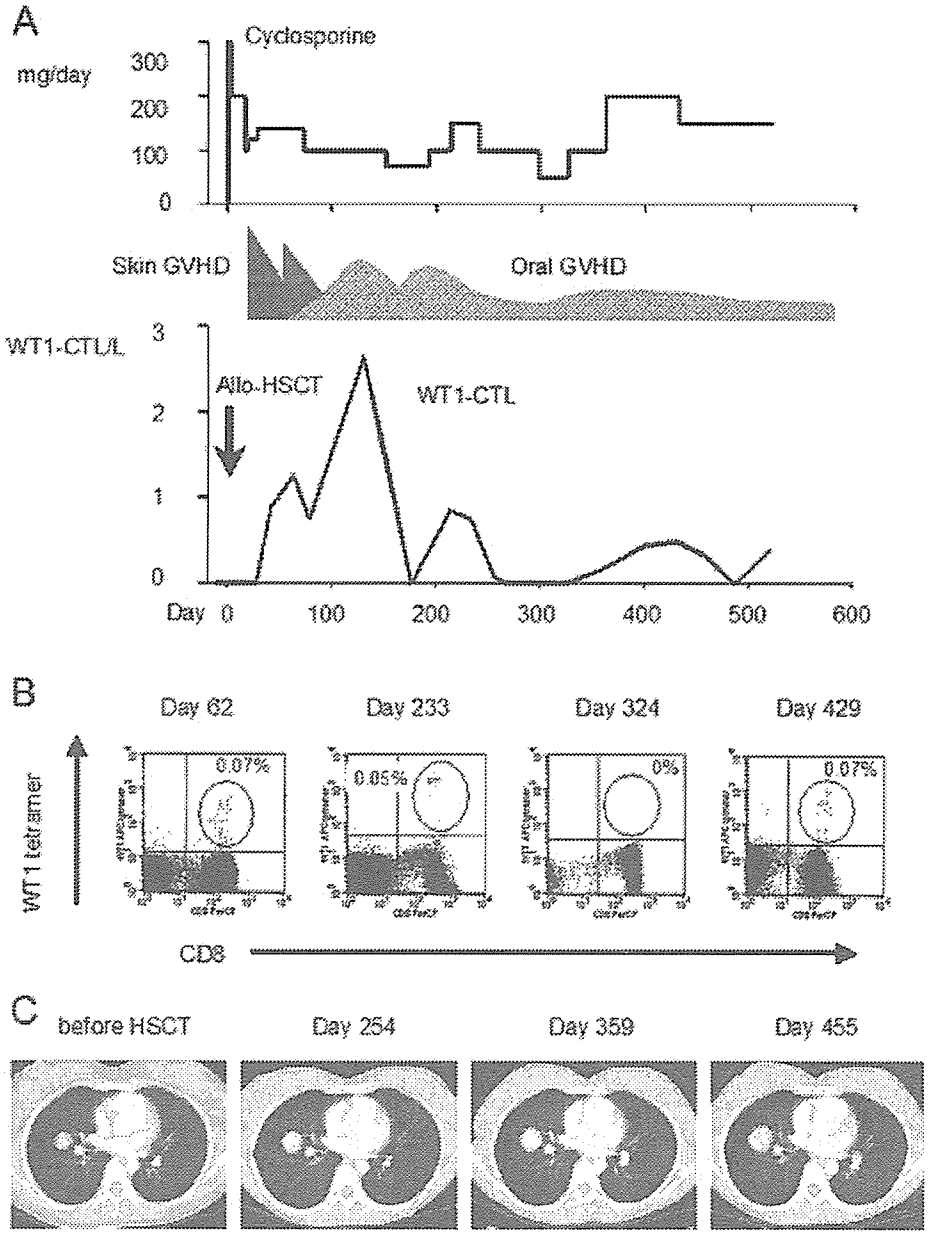

FIGURE 2 – Clinical course and tetramer analysis of Case 1 with RCC WT1-CTL was detected after the occurrence of skin GVHD, followed by a peak on day 128 when oral GVHD developed (*a*, *b*). Lung metastases slowly progressed while WT1-CTL disappeared (*c*).

peptide-containing cultures showed expansion of antigen-specific CTL, with all samples showing 0% for PR1-CTL and PRAME-CTL even after culture. The sample taken from a patient with RCC showed a meaningful expansion of WT1-CTL (Figs. 1*a*–1*h*).

Intracellular cytokine staining

The CMV-CTL and WT1-CTL, expanded by culture, were analyzed for intracellular IFN-γ. The cells were gated on tetramer-positive fraction of the lymphocyte gate, and the positive rate of CD8 and IFN-γ was analyzed. As for the CMV peptide cultured cells obtained from 7 patients, the mean rate of $CD8^+$/IFN-γ^+ in the CMV-$tetramer^+$ lymphocyte gate was 31.8% when stimulated with CMV peptide, whereas it was 1.72% when stimulated with PBS (negative control). A demonstrative result is shown in Figures 1*i* and 1*j*. For cells taken from a RCC patient and cultured with WT1 peptide, the rate of $CD8^+$/IFN-γ^+ in the WT1-$tetramer^+$ lymphocyte gate was 22.9% when stimulated with WT1 peptide, whereas it was 0.66% for negative control (Figs. 1*k* and 1*l*).

Serial analysis of WT1-CTL in patients with RCC

On the basis of Based on these results, we performed serial analyses of WT1-specific CTL during the clinical course of 2 patients with RCC who underwent RIST. The samples were obtained biweekly until day 200 and at longer intervals thereafter.

The first case is a 32-year-old female who had undergone resection of the primary disease, but had multiple lung metastases that were resistant to interferon therapy (Fig. 2). The histology of the primary disease was mixed RCC, which was positive for WT1 (Figs. 3*a* and 3*b*). She received RIST after conditioning with cladribine and busulfan, and cyclosporine (CSP) was administrated as GVHD prophylaxis. Engraftment was achieved on day 12, which was followed by skin GVHD that extended to the whole

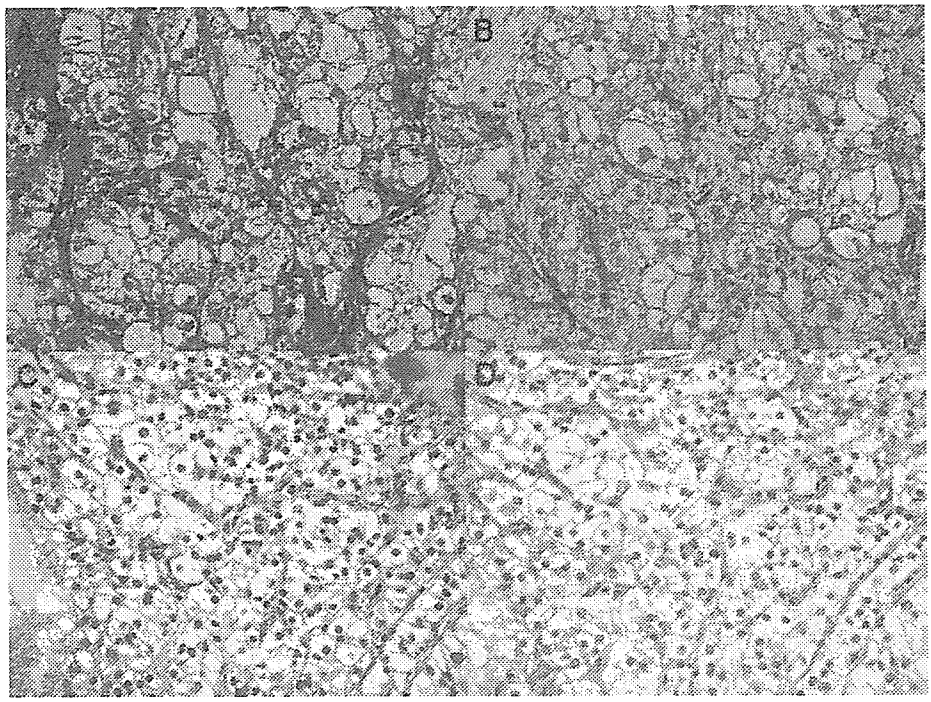

FIGURE 3 – Histology of resected RCC. The resected tumor sample in the first case with hematoxylin–eosin staining (*a*) confirmed a mixed cell carcinoma of the kidney. Immunostaining with WT1 (*b*) was positive for WT1. The second case with clear cell carcinoma (*c*) also showed positive staining for WT1 (*d*). Original magnification ×200 for (*a*, *b*) and ×100 for (*c*, *d*).

body. She was treated with topical corticosteroid after skin biopsy, which provided prompt resolution. WT1-CTL was detected at day 40 when the skin rash recurred, and the peak formation of WT1-CTL occurred on day 128 when oral chronic GVHD developed. The lung metastasis showed a stable disease until day 254, when the tumor started to grow with a slight improvement of oral GVHD, and WT1-CTL became undetectable on day 268. However, with a subsequent slight exaggeration of oral GVHD from day 359, a low titer of WT1-CTL once again became detectable from day 399. This patient is currently doing well at day 520 postHSCT, with a 24% increase in lung metastasis but with no new lesion.

The second case is a 43-year-old male patient who had the primary disease resected, but developed multiple lung metastases, which progressed despite interferon therapy. The histology of the primary disease was clear cell carcinoma that was positive for WT1 (Figs. 3*c* and 3*d*). The patient received RIST after conditioning with fludarabine busulfan, and anti-thymocyte globulin with CSP for GVHD prophylaxis (Fig. 4). He developed liver acute GVHD on day 83, after a rapid reduction in the dose of CSP. Liver GVHD was successfully treated by resuming CSP at a dose of 400 mg/body. He became positive for WT1-CTL on day 90; however, it disappeared along with the remission of liver GVHD. After CSP was tapered, skin GVHD occurred and WT1-CTL became detectable again. However, WT1-CTL disappeared from day 239 with the remission of skin GVHD, and the disease showed rapid progression. Donor lymphocyte infusion was performed on day 350 to induce a GVT effect, but WT1-CTL was not induced, and the patient died of respiratory failure because of disease progression on day 377 postHSCT.

Immunophenotype of WT1-CTL

The immunophenotype of the WT1-CTL in the RCC patients described earlier was analyzed (Fig. 5). The samples obtained at days 40, 77, 128, 149 and 233 posttransplantation from the first patient and at days 97, 146 and 196 in the second patient had adequate numbers of WT1-CTL for analysis. The phenotype did not differ significantly among samples taken from the same patient at different occasions. The WT1-CTL was effector phenotype in both patients, but different among the 2 patients as described later. In the first patient, WT1-CTL was mainly effector memory phenotype. Seventy percent of the WT1-CTL expressed $CD45RA^+/CD45R0^-$ phenotype, 53% were of $CD57^+/CD45R0^-$ phenotype and 22% were of $CD57^-/CD45R0^-$ phenotype. In the other classification, 38% were $CD27^-/CD45RA^+$ and 34% were $CD27^+/CD45RA^+$. In the second patient, 80% of the WT1-CTL had the $CD45RA^-/CD45R0^+$ phenotype and 57% expressed $CD57^+/CD45RO^+$. In the other classification, 66% were $CD45RA^-/CD27^-$ and 21% were $CD45RA^-/CD27^+$. In both patients, over 95% of the WT1-CTL were negative for CCR7.

Discussion

Our study showed that CTL with avidity for the WT1 antigen are present in the peripheral blood of patients who underwent allogeneic HSCT for malignant disease. A GVT effect is thought to be mediated by expanding donor T cells, and a relationship has been reported between GVHD and disease control.[24] However, an optimal immune-monitoring system for tumor antigen-specific CTL, which is thought to be the effector cell for the GVT effect, has not been well established. Few studies on WT1-CTL have been reported, and most have focused on patients with leukemia[25] or those who received peptide vaccination.[16,26] This is the first report of the kinetics of WT1-CTL in patients with RCC.

In the tetramer assay, we were able to reduce the background staining by sorting T cells with phenotypes, including CD4, CD13 and CD19, in addition to a lymphocyte gate using FSC and SSC. By this procedure, bright and distinct tetramer staining with fewer false-positive results was obtained, which made it possible to detect antigen-specific CTL present at very low levels. Tetramer assay using fresh peripheral blood is the best screening procedure, since it could be performed more easily and quickly than conventional procedures. Previous studies have used peptide stimulation and cytokine production analysis, such as ELISPOT assay or intracellular cytokine assay, to detect antigen-specific CTL.[25,27] However, they are only semiquantitative, as it is impossible to exclude nonspecific cytokine production. We have overcome these problems by simultaneously staining the cells with tetramer and intracellular cytokine, which visualized the IFN-γ production pattern of antigen-specific CTL.

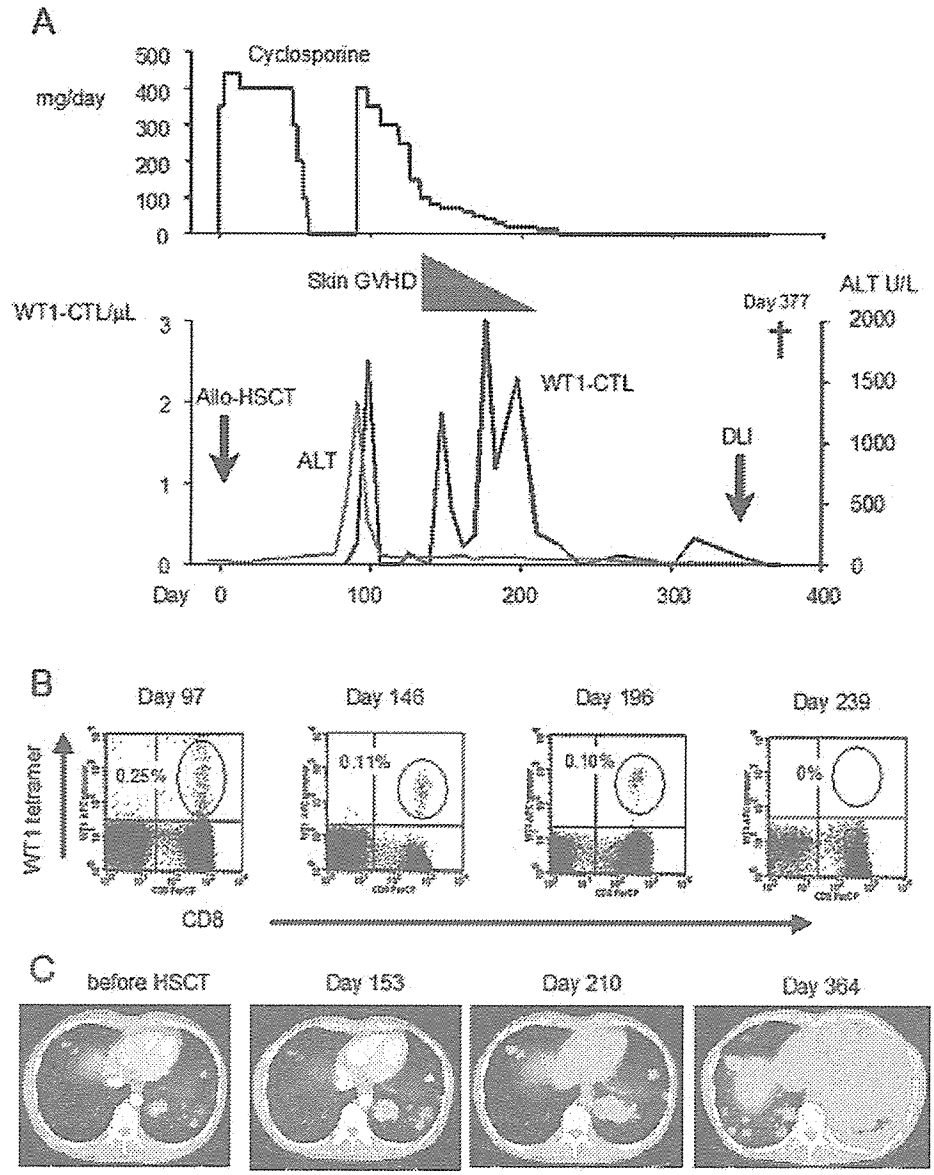

FIGURE 4 – Clinical course and tetramer kinetics in Case 2 with RCC. WT1-CTL emerged after the occurrence of liver and skin GVHD (*a*, *b*). The tumor rapidly grew after the disappearance of WT1-CTL (*c*).

The serial analysis of WT1 tetramer in 2 RCC patients clearly showed that WT1-CTL emerges after HSCT in relation with GVHD, and that they are associated with disease control/progression. It has been reported that the expansion of $CD8^+$ IFN-γ-producing T cells and the incidence of GVHD are associated with the clinical response to nonmyeloablative allogeneic HSCT for RCC.[2] Our results suggest that WT1-CTL can be included among these $CD8^+$ IFN-γ-producing T cells.

The immunostaining of WT1 showed a cytoplasmic pattern in both cases. Although WT1 is usually a nuclear protein, it is reported that some types of adenocarcinomas show cytoplasmic pattern.[4,28] Also, a recent study showed that WT1 shuttles between the nucleus and cytoplasm, and thereby 10–50% of total cellular WT1 can be detected in the cytoplasm.[29] From these evidences, we conclude that the RCC cells in the 2 patients expressed WT1, which was present as a tumor antigen.

The WT1-CTL was detected in a relatively short period after HSCT, when the patient obtained full donor chimerism, which may suggest that the precursor of WT1-CTL was already present in the donor graft. Since the WT1-CTL in the donor graft was under the level of detection of the tetramer assay and the WT1-CTL emerged soon after the occurrence of GVHD, it is quite likely that an immunological event associated with GVHD induced rapid expansion of the WT1-CTL. We can assume that GVHD drove the tumor-antigen to a peripheral circulation and stimulated WT1-CTL, together with a significant amount of cytokines, which were produced in the very early phase of HSCT and GVHD.

The immunophenotyping of antigen-specific CTL may be useful for predicting the function of CTL and disease prognosis.[30–34] In RCC patients, most of the WT1-CTL detected was $CCR7^-/CD57^+$, consistent with an effector memory phenotype. The first case showed a relatively high frequency of CD45RA+/$CD45RO^-$ phenotype with an equal ratio of $CD27^+$ and $CD27^-$, while most of the WT1-CTL in the second case had $CD45RA^-/CD45RO^+$ and $CD27^-$ phenotypes. From a previous report, a cytokine-producing memory T cell subset capable of rapidly inducing IFN-γ and TNF-α synthesis shows the $CD27^-$ phenotype, with varying degrees of CD45RA/CD45RO expression.[35] In another article, CTL with phe-

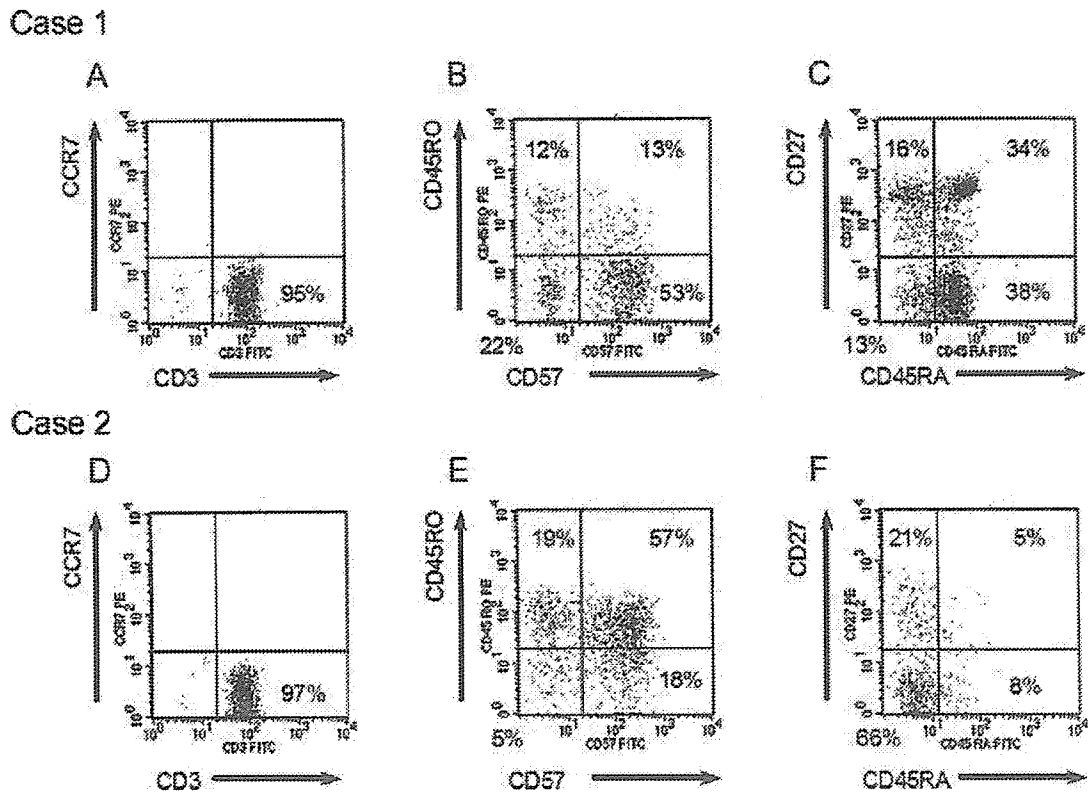

FIGURE 5 – Immunophenotype analysis of WT1-specific CTL. The immunophenotype of WT1-CTL in the first case of RCC was $CCR7^-$ and mainly $CD57^+/CD45RO^-/CDRA^+$ (*a–c*). The immunophenotype of WT1-CTL in the second case was $CCR7^-$ and $CD57^+/CD45RO^+$ and $CDRA^-/CD27^-$ (*d–f*).

notypes, including $CCR7^-$, $CD45RA^-$ and $CD45RO^+$, were shown to respond to antigen-specific peptide, while those with $CCR7^+$, $CD45RA^+$ and $CD45RO^-$ phenotypes were associated with a lack of response to antigen-specific peptide.[36] Hence, based on the phenotype analysis in correlation with the clinical course, WT1-CTL seemed to have played a major role in disease control in the second patient, while an antitumor cell other than WT1-CTL may have had an effect in the first patient, since the disease progression in this patient was slow even when the WT1-CTL disappeared. NK cells may be the predominant antitumor cells, since this patient had a high proportion of NK cells in the peripheral blood at day 296 (7.0%/lymphocyte), which had been only 0.72% on day 149. Further analysis of antigen-specific CTL is critically required to elucidate the precise relationship between the phenotype and cell function.

We have previously demonstrated that CMV epitope NLVPMVATV is presented in both HLA-A*0201 and HLA-A*0206.[37] It is quite likely that WT1 epitope RMFPNAPYL is also commonly presented in HLA-A02 phenotypes, since WT1-CTL was detected not only in HLA-A*0201 patients but also in those with HLA-A*0206. Monitoring of WT1-CTL by tetramer assay can be widely applied to the HLA-A02 phenotype, since over 95% of HLA-A02 are either A*0201 or A*0206.[38,39]

Although several studies on PR1-CTL detection in patients with leukemia have been reported,[19,25,27] PR1- and PRAME-specific CTL were not detected in our study. We considered that one time positivity of the tetramer assay is not sufficient, since there may be an interassay variability. Since neither PR1-CTL nor PRAME-CTL was detected even after cell culture, in which the expansion of WT1-CTL and CMV-CTL was successful, we speculate that PR1-CTL and PRAME-CTL were not induced in most of the patients after HSCT.

In conclusion, our results suggest that WT1-CTL is involved in a GVT effect and WT1 is currently the best antigen for immunomonitoring after HSCT, while PR1 and PRAME are less potent antigens to be used for wider application. Although WT1-CTL may occur after HSCT *per se* without additional immunotherapy, it would be ideal to induce GVT effect with minimal GVHD. Further development of a WT1-based immunotherapy is desired to induce optimal antitumor immune response.

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Myeloablative allogeneic hematopoietic stem cell transplantation for non-Hodgkin lymphoma: a nationwide survey in Japan

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We retrospectively surveyed the data of 233 patients who underwent myeloablative allogeneic hematopoietic stem cell transplantation (allo-HSCT) for non-Hodgkin lymphoma (NHL). Donors were HLA-matched relatives in 154 patients (66%) or unrelated volunteers in 60 (26%). Ninety patients (39%) were in complete remission. One hundred ninety-three (83%) received a total body irradiation (TBI)–based regimen, and 40 (17%) received a non–TBI-based regimen. Acute graft-versus-host disease (GVHD) occurred in 155 (67%) of the 233 evaluable patients; grade II to IV in 90 (39%), and grade III to IV in 37 (16%). Treatment-related mortality (TRM) was observed in 98 patients (42%), and 68% of them were related to GVHD. In a multivariate analysis, chemoresistance, prior autograft, and chronic GVHD were identified as adverse prognostic factors for TRM. Relapse or progression of lymphoma was observed in 21%. The 2-year overall survival rates of the patients with indolent (n = 38), aggressive (n = 111), and lymphoblastic lymphoma (n = 84) were 57%, 42%, and 41%, respectively. In a multivariate analysis, chemoresistance, prior autograft, and prior radiotherapy were identified as adverse prognostic factors for overall survival. Although myeloablative allo-HSCT represents an effective therapeutic option for patients with NHL, more work is still needed to decrease TRM and relapse. (Blood. 2006;108:382-389)

Introduction

Hematopoietic stem cell transplantation (HSCT) for patients with non-Hodgkin lymphoma (NHL) has been mainly focused on an autograft strategy. High-dose therapy with autologous HSCT (auto-HSCT) can increase remission rates and possibly prolong disease-free survival and overall survival (OS) in patients with chemotherapy-sensitive NHL at relapse.[1] This is also effective as first-line therapy for those with advanced aggressive lymphoma.[2] Nevertheless, relapse is a frequent cause of treatment failure after auto-HSCT.[1,3]

Allogeneic HSCT (allo-HSCT) has several advantages over auto-HSCT, because the former can avoid the reinfusion of malignant cells and can also be associated with a graft-versus-lymphoma (GVL) effect, which might reduce the risk of relapse. Most physicians believe that a small fraction of patients with end-stage aggressive lymphoma can still achieve prolonged lymphoma-free survival with the application of allo-HSCT. However, the high incidence of treatment-related mortality (TRM) (up to 55%) associated with allogeneic HSCT with a myeloablative regimen has prevented the wider application of this strategy.[4-8] Several reports on allo-HSCT for refractory or advanced lymphoma, as well as studies comparing auto- versus allo-HSCT for NHL, have been published over the past decade.[8-10] However, most of these studies were small and nonrandomized, and incorporated patients who had heterogeneous backgrounds. Thus, the role of allo-HSCT in the treatment of NHL remains controversial. Moreover, the outcome of allo-HSCT in each histologic subtype has not been fully determined. Previous studies have suggested that allo-HSCT improves the prognosis of patients with advanced follicular lymphoma (FL),[7,10,11] whereas few reports have been published on its benefit in aggressive lymphoma.[12,13] In particular, there has been very little information available on subtypes, including mantle-cell lymphoma[11,14]; peripheral T-cell lymphoma, unspecified (PTCL)[15]; natural killer (NK) cell lymphoma[16]; and anaplastic large cell lymphoma.

The application of reduced-intensity stem cell transplantation (RIST) or "nonmyeloablative" HSCT has been reported to decrease

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TRM.[17-19] Additionally, the recent development of supportive treatments may have decreased the risk of TRM and facilitated the application of allo-HSCT to NHL.[20] Therefore, we conducted a retrospective nationwide survey on Japanese patients with NHL who had undergone conventional allo-HSCT to establish a benchmark of myeloablative allo-HSCT in the treatment of NHL.

Patients, materials, and methods

Data sources

This survey collected the data of 233 consecutive patients who received myeloablative allo-HSCT for NHL between 1990 and 2001 in 56 participating hospitals. Data were derived from questionnaires distributed to each hospital. Additional questionnaires were sent to confirm the follow-up data, including the occurrence of graft-versus-host disease (GVHD). The indications for allo-HSCT were left to the discretion of each institution. The patients included in this study received a conditioning regimen with an intensity that was equivalent to that of total body irradiation (TBI) plus cyclophosphamide or busulfan plus cyclophosphamide. Patients who had previously received monoclonal antibody therapy or T-cell–depleted transplantation, those younger than 14 years, and those who received RIST were not included. Additionally, those with adult T-cell leukemia/lymphoma were excluded because their clinical course differed from that of other types of lymphoma. The minimum data required for the inclusion of a patient in this study were age, sex, histologic diagnosis, prior treatment details, status at transplantation, donor information, conditioning regimen, date of transplantation, therapy-related complications, date of last follow-up, disease status at follow-up, date of disease progression/death, and cause of death. Approval was obtained from the institutional review board. Informed consent was provided according to the Declaration of Helsinki.

Definitions

The initial institutional histologic diagnosis was further reviewed by a pathologist (K. Takeuchi) using the WHO classification.[21] Briefly, NHL was divided into 3 clinical subtypes: indolent, aggressive, and lymphoblastic lymphoma. Indolent lymphoma included all grades of FL and extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). Aggressive lymphoma included all lymphomas except for indolent and lymphoblastic lymphoma. Transformed indolent lymphoma and Burkitt lymphoma were classified as aggressive lymphoma. Furthermore, because most of the patients were evaluated before publication of the WHO classification, this analysis only included those who had tumors that formed lesions, such as T-cell lymphoblastic lymphoma (T-LBL), and all other patients who had features of leukemia were excluded. Those with chemosensitive disease included all patients who had shown a response to the last chemotherapy prior to transplantation (partial remission [PR], complete remission [CR] unconfirmed, and CR), whereas chemoresistant disease included those with primary refractory disease or refractory relapse prior to transplantation. Acute and chronic GVHD was graded according to the consensus criteria.[22,23] Patients who survived 100 days were evaluable for the assessment of chronic GVHD. OS was measured as the time from the day of transplantation until death from any cause, and progression-free survival (PFS) was the time from the day of transplantation until disease progression (PD)/relapse or death from any cause. Patients who died from transplantation-related causes were classified as TRM regardless of their disease status.

Statistical analysis

OS and PFS were calculated using the Kaplan-Meier method.[24] Surviving patients were censored on the last day of follow-up, in July 2002. The associations among patient-, disease-, and transplantation-related factors and OS were assessed by using univariate and multivariate Cox proportional hazards models. The associations between these factors and TRM were assessed by using univariate and multivariate logistic models. The variables analyzed included age, clinical subtype, histologic diagnosis, chemosensitivity, history of autograft or radiotherapy, years of transplantation, donor, source of stem cells, TBI-containing regimen, GVHD prophylaxis, and acute and chronic GVHD. Acute GVHD was treated as a time-dependent covariate in the Cox model. Stepwise variable selection at a significance level of .05 was used to identify covariates associated with outcomes. TRM and disease progression/relapse were calculated by using cumulative incidence. The statistical analysis was performed with the SAS 8.2 program package (SAS Institute, Cary, NC).

Results

Patients' characteristics

The patients' characteristics are listed in Table 1. All patients were younger than 60 years at the time of transplantation, with a median age of 31 years. Thirty-eight patients (16%) had indolent lymphoma, 111 (48%) had aggressive lymphoma (diffuse large B-cell, n = 44; PTCL, n = 22; extranodal NK/T-cell, n = 19; anaplastic large cell, n = 7; mantle cell, n = 5; Burkitt, n = 4; angioimmunoblastic T cell, n = 2; blastic NK cell, n = 2; hepatosplenic T-cell, n = 2; subcutaneous panniculitis like T cell, n = 2; mycosis fungoides with visceral dissemination, n = 2), and 84 (36%) had lymphoblastic lymphoma. Ninety patients (39%) were in CR, 38 (16%) were in PR, 42 (18%) were in primary refractory, and 63 (27%) had refractory relapse at the time of allo-HSCT. Ninety patients (39%) had received 4 or more chemotherapy regimens before allo-HSCT. Forty patients (17%) had received prior autograft, and 81 (35%) had received prior radiotherapy. One hundred fifty-four patients (66%) received a transplant from a human leukocyte antigen (HLA)–matched related donor, 19 (8%) from a 1-antigen–mismatched related donor, 43 (19%) from a matched unrelated donor, and 17 (7%) from a 1-antigen–mismatched unrelated donor. One hundred fifty-nine (68%) patients received bone marrow (60 from an unrelated donor) and 70 (30%) received granulocyte colony-stimulating factor (G-CSF)–mobilized peripheral blood. One hundred ninety-three patients (83%) received TBI-based myeloablative regimens, including TBI 12 Gy plus cyclophosphamide (n = 60); a combination of TBI, cyclophosphamide, and etoposide (n = 47); or TBI, cyclophosphamide, and cytarabine (n = 40). Forty patients (17%) received a non–TBI-based myeloablative regimen, including a combination of busulfan and cyclophosphamide with or without other agents (n = 27); melphalan, thiotepa, and busulfan (n = 3); cytarabine, ranimustine, carboplatin, cyclophosphamide, and total lymphoid irradiation (n = 2); or cytarabine, etoposide, and busulfan (n = 2). The remaining 6 patients received individualized regimens. GVHD prophylaxis included a combination of cyclosporin and methotrexate in 204 (88%) or tacrolimus and methotrexate in 22 (9%). Two hundred twenty-six patients (97%) were treated with G-CSF, starting at days +1 to +6 after graft infusion until engraftment.

GVHD

Acute GVHD occurred in 155 (67%) of the 233 patients: grade I in 65 (28%), grade II to IV in 90 (39%), and grade III to IV in 37 (16%) patients. Of the 165 patients who survived the initial 100 days after allo-HSCT, chronic GVHD occurred in 79 (48%), with extensive type in 48 (29%). In allo-HSCT from related (n = 173) and unrelated (n = 60) donors, grade II to IV acute GVHD occurred, respectively, in 61 (35%) and 30 (50%), grade III to acute GVHD occurred in 25 (15%) and 12 (20%), chronic GVHD occurred in 54 (31%) and 25 (42%) patients, and chronic extensive

Table 1. Patient-, disease-, and transplantation-related characteristics

Variable	No. (%)*
Patient characteristics	
Younger than 40 y	158 (68)
40 y or older	75 (32)
Male sex	150 (64)
Disease characteristics at diagnosis	
Histology	
Indolent	38 (16)
Follicular	37 (16)
MALT	1 (0)
Aggressive	111 (48)
Diffuse large B cell	44 (19)
Peripheral T cell, unspecified	22 (9)
Extranodal NK/T cell, nasal type	19 (8)
Anaplastic large cell	7 (3)
Mantle cell	5 (2)
Others	14 (6)
Lymphoblastic	84 (36)
Precursor B cell	7 (3)
Precursor T cell	77 (33)
Stage I	9 (4)
Stage II	25 (11)
Stage III	30 (13)
Stage IV	150 (64)
No data	19 (8)
Disease characteristics at transplantation	
Response to chemotherapy†	
Sensitive	128 (55)
Complete remission‡	90 (39)
Partial remission	38 (16)
Resistant	104 (45)
Primary refractory disease	41 (18)
Refractory relapse	63 (27)
No. of prior chemotherapy regimens†	3 (0-11)
Fewer than 4 regimens	143 (61)
At least 4 regimens	90 (39)
Prior autograft	40 (17)
Prior radiotherapy	81 (35)
Transplantation characteristics	
Year of transplantation	
1990-1995	46 (20)
1996-2001	187 (80)
No. of patients receiving a transplant per hospital	
Fewer than 9 patients	146 (63)
At least 9 patients	87 (37)
Donor	
HLA-matched related	154 (66)
HLA-1 antigen-mismatched related	19 (8)
HLA-matched unrelated	43 (19)
HLA-1 antigen-mismatched unrelated	17 (7)
Donor-recipient sex match	
Male-male	80 (34)
Male-female	66 (28)
Female-male	33 (14)
Female-female	46 (20)
Donor-recipient CMV status§	
+/+	131 (57)
−/+	14 (6)
+/−	14 (6)
−/−	11 (5)
Source of stem cells	
Bone marrow	159 (68)
Peripheral blood cells	70 (30)
Bone marrow + peripheral blood cells	2 (1)
Cord blood	2 (1)
Conditioning regimen	
TBI-containing	193 (83)
Non-TBI	40 (17)
GVHD prophylaxis	
Cyclosporin + methotrexate	204 (88)
Tacrolimus + methotrexate	22 (9)
Others	7 (3)

The study included 233 patients. The median age was 31 years (range, 15-59 years). Age was a continuous variable.

MALT indicates extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue; NK, natural killer; HLA, human leukocyte antigen; CMV, cytomegalovirus; TBI, total body irradiation; GVHD, graft-versus-host disease.

*Categoric variable.

†One patient with mediastinal B-LBL did not receive prior chemotherapy for an unknown reason but did receive prior radiotherapy.

‡Includes 2 patients in complete remission, unconfirmed.

§Sixty-three pairs were not evaluated for CMV status.

GVHD occurred in 33 (19%) and 16 (27%). In allo-HSCT from HLA-matched (n = 197) and mismatched (n = 36) donors, grade II to IV acute GVHD occurred, respectively, in 76 (39%) and 15 (42%), grade III to IV acute GVHD occurred in 30 (15%) and 7 (19%), chronic GVHD occurred in 65 (33%) and 14 (39%), and chronic extensive GVHD occurred in 41 (21%) and 7 (19%). The distribution pattern of the incidences of acute and chronic GVHD by background factors was analyzed by using a chi-square test. Although none of the factors correlated with acute GVHD, the incidence of chronic GVHD was higher in patients who had GVHD prophylaxis with tacrolimus plus methotrexate than in those with cyclosporin plus methotrexate ($P = .015$, chi-square test; $P = 0.023$, Fisher exact test).

Disease response

Of the 143 patients who had measurable disease at allo-HSCT, 89 (62%) achieved CR, 7 (5%) PR, 6 (4%) stable disease (SD), and 12 (8%) PD, whereas 29 (20%) were not evaluable because of early death. Of the 90 patients who were in CR at transplantation, 80 (89%) maintained CR, 4 (4%) showed PD, and 6 (7%) were not evaluable because of early death. Thirty-five patients died before the first response evaluation, with a median survival of 29 days (range, 0-72 days) after allo-HSCT. In the 27 patients with indolent lymphoma who had measurable disease at allo-HSCT, 22 (81%) achieved CR or PR. In the 72 patients with aggressive lymphoma who had measurable disease at allo-HSCT, 49 (68%) achieved CR or PR. In the 41 patients with lymphoblastic lymphoma who had measurable disease at allo-HSCT, 26 (63%) achieved CR.

TRM, disease relapse, and progression

Ninety-eight patients (42%) died of TRM, and its cumulative incidence is shown in Figure 1. Of the 98 patients who died of therapy-related complications, 60 (61%) died within day 100 of transplantation and 38 (39%) died thereafter. The major causes of TRM included GVHD (n = 11), infection (n = 29), interstitial pneumonitis (n = 16), venoocclusive disease of the liver (n = 11), thrombotic microangiopathy (n = 8), heart failure (n = 7), hemorrhage (n = 4), renal failure (n = 3), and others (n = 9), as shown in Table 2. The causes of infection-related mortality (n = 29) were bacterial (n = 13), fungal (n = 11), or viral (n = 5). Seventeen (59%) of 29 patients died of infections within 100 days of allo-HSCT, 7 (24%) from 101 days to 1 year and 5 (17%) thereafter. Fourteen patients died of TRM before engraftment. Of the 98 patients who died of TRM, 67 (68%) had GVHD, and 11 of

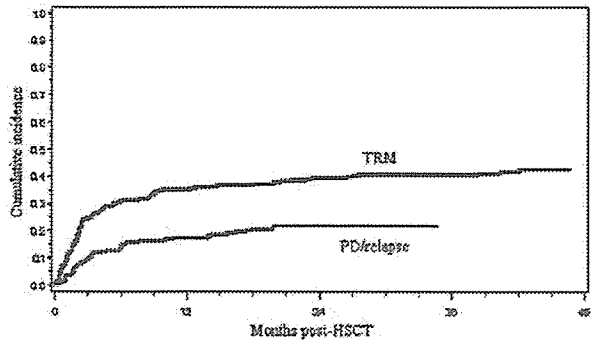

Figure 1. Cumulative incidences of treatment-related mortality (TRM) and disease relapse/progression (PD/relapse).

these died of GVHD (6 acute, 5 chronic) itself. The 14 factors shown in Table 3 were assessed with regard to their relation to TRM. A univariate analysis revealed that 6 factors, including older patient age, chemoresistant disease, prior autograft, prior radiotherapy, aggressive lymphoma other than PTCL, and chronic GVHD, were associated with a significantly increased risk of TRM. In a multivariate analysis using a logistic model, chemoresistant disease, prior autograft, and chronic GVHD remained significant.

The cumulative incidence of relapse and PD is shown in Figure 1. Relapse or progression of lymphoma after allo-HSCT was observed in 49 patients (21%; 5 indolent, 19 aggressive, 25 LBL), and 32 (14%; 3 indolent, 13 aggressive, and 16 LBL) died of PD. Of the 105 patients with chemoresistant disease before allo-HSCT, 61 (58%) died of treatment-related complications, 19 (18%) died of PD, and 25 (24%) are alive with a median follow-up of 20.9 months (range, 1.8-136.0 months). Of the 128 patients with chemosensitive disease before allo-HSCT, 37 (29%) died of treatment-related complications, 12 (9%) died of PD, and 79 (62%) are alive with a median follow-up of 35.2 months (range, 4.4-140.2 months). Eight (16%) of the 49 patients who showed PD died of treatment-related complications such as infection (n = 4), interstitial pneumonitis (n = 3), and GVHD (n = 1). Only 6 of the 70 patients who had passed 2 years after transplantation developed relapse thereafter.

Donor lymphocyte infusion

Donor lymphocyte infusions (DLIs) were given after the withdrawal of immunosuppressive therapy to those who relapsed or showed evidence of disease progression or persistent disease without any sign of GVHD. A total of 7 patients, including 5 with T-LBL, received DLI after allo-HSCT from an HLA-matched related donor (n = 6) or a -matched unrelated donor (n = 1). Two patients who received DLI from an HLA-matched related donor developed grade II acute GVHD, which subsequently extended to extensive chronic GVHD; one of them with T-LBL died without a response, whereas the other with T-cell lymphoma is still alive without disease progression 3.8 years after allo-HSCT. Five patients did not develop GVHD following DLI; 3 patients subsequently died of disease progression, but 2 patients with T-LBL are still alive without disease progression at 361 and 783 days after allo-HSCT.

OS and PFS

One hundred four (45%) of the 233 patients are currently alive with a median follow-up of 31 months (range, 1.8-138 months). The OS and PFS are, respectively, 45% and 40% at 2 years, and 39% and 36% at 5 years after allo-HSCT (Figure 2). Median OS and PFS are, respectively, 15.6 months (95% confidence interval, 9.6-27.6 months) and 9.6 months (6-18 months). The 2-year OS of those with indolent, aggressive, and lymphoblastic lymphoma was, respectively, 57%, 42%, and 41%. Patients with indolent lymphoma tended to have a better survival ($P = .131$, log rank test; $P = .064$, G. Wilcoxon test) (Figure 3). Kaplan-Meier estimates of OS of patients with 4 histologic subtypes of aggressive lymphoma, including diffuse large B-cell lymphoma (n = 44), PTCL (n = 22), extranodal NK/T-cell lymphoma, nasal type (n = 19), and others (n = 26), are shown in Figure 4.

The 14 clinical factors shown in Table 4 were assessed with regard to their relation to OS. A univariate analysis revealed that 5 factors, including chemoresistant disease, prior autograft, prior radiotherapy, aggressive lymphoma other than PTCL, and clinical subtype (aggressive versus indolent), were associated with a significantly worse OS. In a multivariate analysis using Cox proportional hazard models, chemoresistant disease, prior autograft, and prior radiotherapy were associated with a worse OS (Table 4). Acute GVHD, which was treated as a time-dependent variable, was not a significant factor for OS in both univariate and multivariate models. The relation between OS and response to chemotherapy is shown in Figure 5.

Discussion

This report describes the general outcome of patients with NHL who underwent modern allo-HSCT with a myeloablative regimen

Table 2. Causes of treatment-related mortality

Causes of TRM	Patients, no. (%)	No. of patients with GVHD	No. of patients without GVHD	Early death, no.*
GVHD	11 (11)			
Infection	29 (30)	15	8	6
Interstitial pneumonitis	16 (17)	15	0	1
Venoocclusive disease	11 (11)	5	4	2
Thrombotic microangiopathy	8 (8)	7	1	0
Heart failure	7 (7)	3	1	3
Hemorrhage	4 (4)	3	1	0
Renal failure	3 (3)	2	1	0
Others†	9 (9)	6	1	2
Total	98 (100)	56	17	14

GVHD indicates graft-versus-host disease.

*Early death was defined as treatment-related death before engraftment.

†Others (n = 9) were acute respiratory distress syndrome (n = 2), hepatic failure (n = 2), leukoencephalopathy (n = 1), secondary solid cancer (n = 1), suicide (n = 1), and unknown cause (n = 2).

Table 3. Univariate and multivariate analyses of treatment-related mortality

Variable	No.	Univariate analysis Hazard ratio (95% CI)	Univariate analysis P	Multivariate analysis Hazard ratio (95% CI)	Multivariate analysis P
Age at transplantation			.035		—
Younger than 40 y	158	1.00		—	
40 y or older	75	1.82 (1.04-3.17)		—	
Clinical subtype			.349		—
Indolent	38	1.00		—	
Lymphoblastic	84	1.47 (0.66-3.32)		—	
Clinical subtype			.103		—
Indolent	38	1.00		—	
Aggressive	111	1.91 (0.88-4.16)		—	
Aggressive lymphoma			.045		—
PTCL	22	1.00		—	
Non-PTCL	89	2.85 (1.02-7.94)		—	
Response to chemotherapy			< .001		< .001
Sensitive	128	1.00		1.00	
Resistant	105	3.41 (1.97-5.88)		2.95 (1.66-5.25)	
Prior autograft			< .001		< .001
No	193	1.00		1.00	
Yes	40	4.74 (2.23-10.07)		4.09 (1.85-9.04)	
Prior radiotherapy			.010		—
No	152	1.00		—	
Yes	81	2.05 (1.18-3.55)		—	
Years of transplantation			.225		—
1996-2001	187	1.00		—	
1990-1995	46	1.49 (0.78-2.86)		—	
Donor			.295		—
HLA-matched	197	1.00		—	
HLA-mismatched	36	1.46 (0.72-2.98)		—	
HLA-matched donor			.437		—
Related	154	1.00		—	
Unrelated	43	1.24 (0.72-2.15)		—	
Source of stem cells*			.544		—
BM	159	1.00		—	
PBSCs	70	1.09 (0.82-1.46)		—	
Conditioning regimen			.144		—
TBI-containing	193	1.00		—	
Others	40	1.67 (0.84-3.30)		—	
GVHD prophylaxis†			.169		—
Cyclosporin + methotrexate	204	1.00		—	
Tacrolimus + methotrexate	22	1.86 (0.77-4.51)		—	
Acute GVHD			.537		—
No	78	1.00		—	
Yes	155	1.19 (0.69-2.06)		—	
Chronic GVHD			< .001		.029
No	79	1.00		1.00	
Yes	154	2.76 (1.53-4.98)		2.02 (1.07-3.77)	

CI indicates confidence interval; PTCL, peripheral T-cell lymphoma; HLA, human leukocyte antigen; BM, bone marrow; GVHD, graft-versus-host disease; and —, not applicable.

*Those who received cord blood (n = 2) or BM + PBSC (n = 2) were excluded because of the small number of patients.

†Seven patients using other GVHD prophylaxis were excluded.

in Japan, focusing on the background problems of myeloablative therapy and the identification of risk factors for TRM and OS. We showed that long-term, lymphoma-free survival could be achieved in approximately 40% of patients. Patients with FL had a better prognosis, consistent with previous reports.[8,10] Even in patients with aggressive lymphoma or LBL, long-term survival of 35% was identified, consistent with previous reports.[8,9] However, there were no significant differences between clinical subtypes (eg, aggressive versus indolent or PTCL versus non-PTCL) in a multivariate analysis. Because rituximab became commercially available after 2001 in Japan, patients with B-cell NHL who received anti-CD20 antibody therapy were not included in this study. The clinical effect of the introduction of rituximab on outcome after allogeneic transplantation should be carefully evaluated in a future study.

Our study confirmed a high TRM rate (42%) after conventional allo-HSCT with a myeloablative regimen, consistent with previous reports.[4-8,25] One of the major causes of death was severe regimen-related toxicities, which included interstitial pneumonitis, venoocclusive disease, cardiac and renal toxicity, and organ hemorrhage. Although TBI-based regimens are frequently chosen because lymphoma cells are considered to be sensitive to irradiation, they have also been associated with long-term complications, including

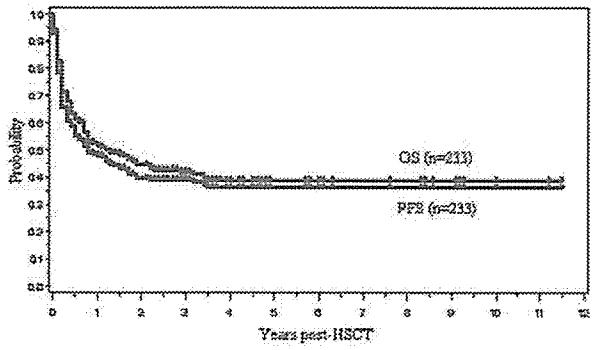

Figure 2. Overall survival (OS) and progression-free survival (PFS) for all 233 patients.

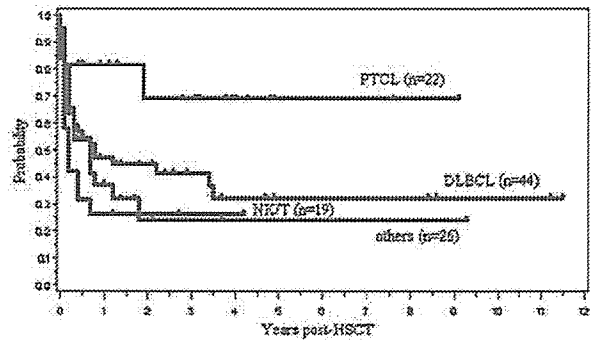

Figure 4. Overall survival for patients with 4 histologic subtypes of aggressive lymphoma. PTCL indicates peripheral T-cell lymphoma, unspecified; DLBCL, diffuse large B-cell lymphoma; NK/T, extranodal NK/T-cell lymphoma, nasal type.

interstitial pneumonitis.[26,27] Because most patients received TBI-based regimens as reported,[4,5,7] we failed to detect any significant differences in TRM between those who received or did not receive TBI.

Another major cause of death in our study was GVHD and/or infection. Of the 98 patients who died of treatment-related complications in our study, 29 (30%) died of infection. At least half of the patients (15 of 29) who died of infectious complications also had GVHD. In a prospective trial of allo-HSCT for patients with NHL, infection accounted for 63% of all TRM,[28] whereas other studies, including ours, have reported an incidence of 25% to 30%.[4,6] In practical transplantation procedures, complications are usually multifactorial, and it is always very difficult to define the exact cause of death, which may account for the wide variations in the incidence of infections among those who died of TRM (18%-63%) in previous reports.[4,5,28,29]

In this study, the incidence of chronic GVHD was high (48%), and chronic GVHD was a risk factor for TRM. The reason for the higher incidence of chronic GVHD in our study compared with the IBMTR report[9,30] was that the IBMTR study included data of patients who died within 100 days after allo-HSCT, whereas we excluded these patients. Unexpectedly, the incidence of chronic GVHD was higher in patients who had GVHD prophylaxis with tacrolimus plus methotrexate than in those with cyclosporin plus methotrexate. In Japan, there is a clear tendency to select tacrolimus rather than cyclosporine for GVHD prophylaxis in unrelated or HLA-mismatched transplantation.[31,32] In addition, PBSCT is not yet permitted for unrelated transplantation. Altogether, the higher incidence of GVHD observed in the tacrolimus group may simply reflect that patients with a higher risk of GVHD were selected to receive tacrolimus.

We found that the incidence of disease relapse/progression of NHL was low (21%). High TRM in the early phase of the transplantation course may mask later disease relapse/progression, and this made it difficult to estimate the relapse rate in this study. OS and PFS were not affected by the severity of acute GVHD. Our limited analysis failed to confirm a GVL effect after myeloablative allo-HSCT. Although the risk of relapse for patients with acute or chronic GVHD was not significantly different from that of patients without acute or chronic GVHD in previous studies with malignant lymphoma,[8,10,30] a study from the Japan Marrow Donor Program showed that the development of grade II to IV acute GVHD was associated with a lower incidence of disease progression after unrelated HSCT.[31] It has been reported that a low level of acute GVHD was associated with improved OS, and all levels of acute GVHD were associated with a decrease in the relapse rate for intermediate-grade NHL.[8] High levels of acute GVHD had a deleterious effect on OS but were associated with an improved relapse rate for LBL.[8] Thus, our study confirmed that greater effort is required to reduce GVHD-related complications after myeloablative allo-HSCT.

We confirmed that chemoresistance before allo-HSCT and prior autograft were significant risk factors for both OS and TRM. RIST or a less organ-toxic myeloablative allo-HSCT using a combination of fludarabine plus intravenous busulfan may be applied more safely in this population to reduce TRM.[19-21,33,34] However, further studies are needed to determine whether reduced-intensity conditioning could control activity of chemoresistant disease. In contrast to previous studies, we showed that prior radiotherapy was associated with a significantly worse OS, which may be related to the fact that 44 (54%) of the 81 patients who had a history of local radiotherapy had refractory disease at transplantation. Hence, it might be that prior radiotherapy was a marker of survival for more advanced and refractory disease.

In conclusion, we confirmed that myeloablative allo-HSCT is a curative therapeutic option in a subset of patients with NHL, but it carries a high risk of toxicities and TRM. Chemoresistant disease and a history of previous autograft are risk factors for both OS and TRM. Whether the introduction of a reduced-intensity transplantation procedure results in reduction of TRM should be evaluated, and more effective GVHD prophylaxis while maintaining a GVL effect should be developed.

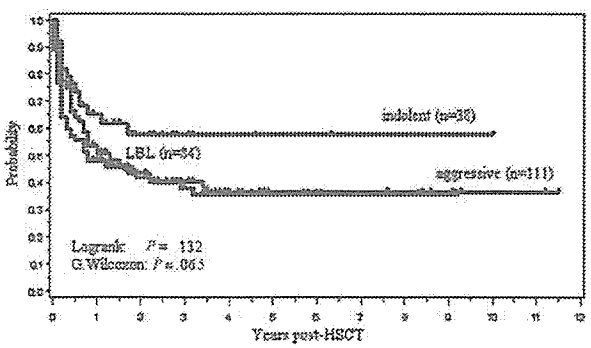

Figure 3. Overall survival stratified according to the clinical subtype. Indolent lymphoma included all grades of FL and extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue. Aggressive lymphoma included all lymphomas except for indolent and lymphoblastic lymphoma (LBL).

Table 4. Univariate and multivariate analyses of overall survival

Variable	No.	Univariate analysis Hazard ratio (95% CI)	Univariate analysis P	Multivariate analysis Hazard ratio (95% CI)	Multivariate analysis P
Age at transplant			.134	—	—
Younger than 40 y	158	1.00		—	—
40 y or older	75	1.32 (0.92-1.90)		—	—
Clinical subtype			.126	—	—
Indolent	38	1.00		—	—
Lymphoblastic	84	1.57 (0.88-2.80)		—	—
Clinical subtype			.045	—	—
Indolent	38	1.00		—	—
Aggressive	111	1.77 (1.01-3.11)		—	—
Aggressive lymphoma			.004	—	—
PTCL	22	1.00		—	—
Non-PTCL	89	3.45 (1.47-7.69)		—	—
Response to chemotherapy			< .001	—	—
Sensitive	128	1.00		—	—
Resistant	105	3.31 (2.30-4.76)		3.12 (2.16-4.51)	< .001
Prior autograft			< .001	—	—
No	193	1.00		—	—
Yes	40	2.59 (1.73-3.87)		2.18 (1.43-3.30)	< .001
Prior radiotherapy			< .001	—	—
No	152	1.00		—	—
Yes	81	1.99 (1.41-2.83)		1.47 (1.02-2.11)	.037
Years of transplantation			.932	—	—
1996-2001	187	1.00		—	—
1990-1995	46	1.02 (0.67-1.54)		—	—
Donor			.076	—	—
HLA-matched	197	1.00		—	—
HLA-mismatched	36	1.50 (0.96-2.33)		—	—
HLA-matched donor			.769	—	—
Related	154	1.00		—	—
Unrelated	43	0.93 (0.58-1.50)		—	—
Source of stem cells*			.095	—	—
BM	159	1.00		—	—
PBSCs	70	1.37 (0.95-2.00)		—	—
Conditioning regimen			.107	—	—
TBI-containing	193	1.00		—	—
Others	40	1.42 (0.93-2.17)		—	—
GVHD prophylaxis†			.227	—	—
Cyclosporin + methotrexate	204	1.00		—	—
Tacrolimus + methotrexate	22	1.40 (0.81-2.40)		—	—
Acute GVHD-time‡	—	1.25 (0.85-1.84)	.264	1.28 (0.87-1.90)	.213

CI indicates confidence interval; PTCL, peripheral T-cell lymphoma; HLA, human leukocyte antigen; BM, bone marrow; GVHD, graft-versus-host disease; and —, not applicable.

*Those who received cord blood (n = 2) or BM + PBSCs (n = 2) were excluded because of the small number of patients.

†Seven patients using other GVHD prophylaxis were excluded.

‡Acute GVHD was treated as time-dependent variable.

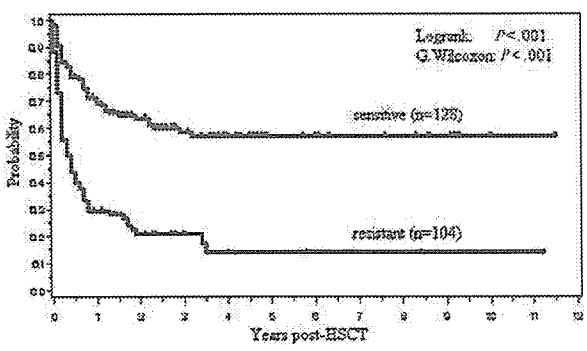

Figure 5. The relation between overall survival and response to chemotherapy.

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Appendix

The following institutions contributed data to this study: Asahikawa Medical College Hospital, Hokkaido University Hospital, Sapporo Hokuyu Hospital, Akita University Hospital, Gunma Saiseikai Maebashi Hospital, Jichi Medical School Hospital, Suifu Hospital, Saitama Cancer Center Hospital, Jikei University Kashiwa Hospital, Chiba Aoba Municipal

Hospital, Tokyo Metropolitan Komagome Hospital, National Cancer Center Hospital, Keio University Hospital, Toranomon Hospital, Yokohama City University Hospital, Kanagawa Cancer Center, Tokai University Hospital, Kurobe City Hospital, Kanazawa University Hospital, Ishikawa Prefectural Central Hospital, Nagoya City University Hospital, Japanese Red Cross Nagoya First Hospital, Nagoya Daini Red Cross Hospital, Meitetsu Hospital, JA Aichi Showa Hospital, Kyoto University Hospital, Kyoto Prefectural University of Medicine Hospital, Osaka University Hospital, Osaka City University Hospital, Kansai Medical University Hospital, Kinki University Hospital, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka City General Hospital, Rinku General Medical Center Izumisano Hospital, Hyogo College of Medicine Hospital, Hyogo Medical Center for Adults, Okayama University Hospital, Okayama Medical Center, Shimane Prefectural Central Hospital, Takamatsu Red Cross Hospital, Ehime Prefectural Central Hospital, University of Occupational and Environmental Health Hospital, Kitakyushu Municipal Medical Center, Kyushu Cancer Center, Kokura Memorial Hospital, Fukuoka University Hospital, Hamanomachi Hospital, Harasanshin Hospital, Saga Prefectural Hospital Koseikan, Sasebo Municipal General Hospital, Miyazaki Prefectural Miyazaki Hospital, Imamura Bun-in Hospital, and Ryukyu University Hospital.

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Nutritional Support for Patients Suffering From Intestinal Graft-versus-Host Disease After Allogeneic Hematopoietic Stem Cell Transplantation

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Background: Patients who exhibit gastrointestinal (GI) involvement due to graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (SCT) are often recommended to withhold oral intake (NPO) to avoid further damage to the GI mucosa. However, it is possible that continuing oral intake could be beneficial in many patients compared to total parenteral nutrition (TPN).

Objective: The primary objective of this prospective study was to evaluate whether programmed step-ladder oral dieting (enteral nutrition; EN) is feasible and beneficial for these patients.

Methods: A total of 18 patients who exhibited GI-acute GVHD (stage I to III gut GVHD) after SCT received an EN dieting program, and changes in clinical and laboratory parameters were compared to those in a control cohort of 17 patients who were placed on NPO with TPN. Patients with GVHD were included prospectively and those with intestinal bleeding/obstruction, severe pancreatitis, and cytomegalovirus enterocolitis were excluded.

Results: None of the patients in the EN group experienced significant adverse events, including exacerbation of GI symptoms. Although there was no statistically significant difference in the volume or frequency of diarrhea or the time to complete dietary recovery, parameters including body weight and serum levels of total protein and albumin tended to improve faster in the EN group.

Conclusion: The EN diet is safely applicable to patients suffering from GI involvement by GVHD. Am. J. Hematol. 81:747–752, 2006.

Key words: graft-versus-host disease (GVHD); enteral nutrition; immunonutrition

INTRODUCTION

Graft-versus-host disease (GVHD) is a major complication of allogeneic hematopoietic stem cell transplantation (SCT) that influences the ultimate prognosis of patients [1]. Gut involvement due to GVHD particularly impairs the host nutritional status and QOL due to long-lasting diarrhea and anorexia. Hence, effective supportive care of patients suffering from GVHD should include attention to intense nutritional support and bone mineral retention, since many receive concomitant steroid therapy. Additionally, normal intestinal architecture and functions are required to prevent biliary stasis, retarded bowel movement, bacterial translocation, and resultant systemic infection [2,3]. With the development of gut GVHD, patients are often recommended to withhold oral intake (NPO, "bowel rest") to avoid further damage to the gastrointestinal (GI) mucosa. However, this raises a serious concern since NPO care can induce atrophic deficit of the GI mucosa and resultant dysfunction

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TABLE I. Grade of Programmed EN Dieting

Step	Staple food (form of rice)	Side dishes (approved foods and cuisines)	Nutritive value
0	Liquid	Juice (without grain, without oranges), electrolytic supplement solution	500–2000 ml
1	Liquid	Water gruel, starch gruel, clear soup, consomme, juice, miso soup	Calories 300–350 kcal Protein 5–7 g Fat 1.5–2 g Dietary fiber 1.5 g
2	Mush	Potato, vegetables, canned fruits, vegetable juices, noodles, tofu, whitefish	Calories 600–650 kcal Protein 20–25 g Fat 5–8 g Dietary fiber 1.5–8 g
3	Rice gruel	Eggs, breads, banana, apple	Calories 900–1000 kcal Protein 30–35 g Fat 10–13 g Dietary fiber 8–9 g
4	Boiled rice	Blue-skinned fish, oil (~3 g/day)	Calories 1200–1300 kcal Protein 40–45 g Fat 15–20 g Dietary fiber 9–10 g
5	Boiled rice	Chicken (low fat), yogurt, oil (~8 g/day)	Calories 1500–1600 kcal Protein 60–65 g Fat 30–35 g Dietary fiber 12–13 g

Note: A patient-oriented stepped-up dieting program was gradually applied over six steps that varied with regard to the solidity, intensity, and acceptability by the patient.

of the GI system. Moreover, it has recently been reported that enteral nutrition (EN) was more effective than parenteral nutrition for the nutritional support of patients with an injured intestine due to trauma or an invasive operation [4,5]. Taken together, these findings suggest that the current patient management procedure that includes the interruption of oral feeding to enforce "bowel rest" in SCT patients suffering from GVHD should be critically reevaluated. Furthermore, EN, if tolerable, may be a preferred route for maintaining digestive and absorptive function as intact as possible.

In those suffering from GI involvement of GVHD, such evaluation becomes more complex since diarrhea is very often multifactorial and includes secretory dysfunction, osmotic factors, and rapid passage. Hence, the establishment of a standard care procedure remains very difficult. To address these concerns, we conducted a controlled cohort study to evaluate the benefit of different nutritional support measures for patients suffering from acute gut GVHD after SCT. Our clinical hypothesis was that a programmed and controlled scheduled oral nutritional support with EN is beneficial for patients who have mild to moderately progressing acute symptoms of gut GVHD.

PATIENTS AND METHODS

Patients

Seventy patients who were treated at the National Cancer Center Hospital from January 2001 to December 2003 and who developed GI symptoms by GVHD were involved in this prospective study. Forty among those eligible patients met the following inclusion criteria: (i) pathologically diagnosed GVHD with biopsied specimens, (ii) presented symptoms within 100 days after SCT, and (iii) clinically diagnosed as stage I to III gut GVHD and grade II to III acute GVHD according to the clinical grading criteria [6,7]. Patients who had intestinal tract bleeding, intestinal obstruction, or severe pancreatitis were excluded from this analysis, since these pathophysiologies are considered contraindications for EN. Additionally, patients with pathologically diagnosed cytomegalovirus enterocolitis were also excluded, and thus a total of 35 patients were left for this study.

Methods

In the study periods, two different nutritional intervention procedures were applied; patients who developed gut GVHD before July 2002 ($n = 17$) were treated with NPO and total parenteral nutrition (TPN) (C group), while the remaining patients who developed gut GVHD after July 2002 ($n = 18$) were treated by programmed GVHD dieting intervention (EN group). The patients were consecutively registered to our database at National Cancer Center Hospital, and this prospective study was approved by the IRB. The programmed EN dieting consisted of six steps with regard to solidity, intensity, and acceptability for intestinal digestion, as shown in Table I. Each food and nutrient was made more solid and dense

in a step-up manner, after the confirmation of stable symptoms that lasted for a minimum of 3 days. Each step of programmed EN dieting was suitably stepped down when intolerance or exacerbation of gut GVHD symptoms developed. Patients were made NPO with the appearance of significant abdominal symptoms (nausea, vomiting, and abdominal pain). Patients in the EN group only received oral intake without enteral tube feeding. On the other hand, the patients in group C were adequately allowed to eat according to their symptoms with TPN.

We evaluated "time to complete dietary recovery," which was defined as the duration from the start of nutritional management (stopping oral intake or start of programmed EN dieting) to the restoration of a normal diet with the recovery of nutritional parameters. Nutritional parameters evaluated in this study included (1) clinical symptoms, including volume and frequency of diarrhea, and body weight and (2) laboratory data, including total serum protein and albumin. Body mass index (BMI) was calculated as BMI = {height (m)}2/body wt (kg).

Statistical Analysis

Our clinical hypothesis was that a programmed and controlled schedule of nutritional support with oral intake (EN dieting) could be effective in the support of patients suffering from acute gut GVHD with mild to moderately progressing symptoms. We evaluated "the time to complete dietary recovery," which was defined as the duration from the start of nutritional management (stopping oral intake or start of EN dieting) to the recovery to normal diet, various enteral symptoms, and nutritional parameters. The time to complete dietary recovery is shown with a time–event cumulative curve, and the log-rank test was used to compare groups C and EN. Nutritional parameters are given as the mean of each group by time course, and the data in groups C and EN were compared by an analysis of variance (ANOVA). A P value of less than 0.05 was considered significant.

RESULTS

Patients' Characteristics

The patients' clinical backgrounds are summarized in Table II, which shows that there are no essential differences between groups C and EN. Older patients tended to receive a reduced-intensity regimen more often than a conventional regimen.

Safety of Programmed EN Dieting

Throughout the study, no severe adverse events associated with nutritional intervention were observed, indicating that our procedure with gradual stepped-up or -down dieting was safe. No severe infectious episodes were observed in each group. EN dieting had to be terminated early in 2 of 18 cases due to prolonged GI symptoms and exacerbation of an underlying malignant disorder. There were 4 censored cases in group C, mainly due to recurrence of the basic malignant disorder.

TABLE II. Patients' Characteristics

	EN group (N = 18)	C group (N = 17)
Age median (range)	53 (22–64)	53 (23–69)
Sex male/female	12/6	14/3
Disease		
AML	6	8
MDS	3	2
ALL	4	2
CML	3	1
NHL	1	2
ATL	1	1
Solid tumors	0	1
Transplantation source		
BM	1	3
PBSC	17	14
Transplantation regimen		
Conventional	5	7
Reduced intensity	13	10
Donor HLA typing		
Full match	14	14
1 locus mismatch	4	0
2 loci mismatch	0	3
GVHD prophylaxis		
CSP alone	10	8
CSP + MTX	6	4
CSP + ATG	2	2
Others	0	3
Gut GVHD stage		
1	5	9
2	7	3
3	6	5
GVHD grade		
II	6	8
III	12	9
Onset day of gut GVHD (mean of day)	74	68

Note: Patients who underwent SCT and developed gut GVHD were enrolled in this study. Patients who developed gut GVHD before July 2002 (n = 17) were treated with no oral intake (C group), while the EN group (n = 18) was treated by programmed GVHD dieting. AMI, acute myelogeneous leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; CML, chronic myelogeneous leukemia; NHL, non-Hodgkin lymphoma; ATL, adult T-cell leukemia; BM, bonemarrow; PBSC, peripheral blood stem cell; CSP, cyclosporine; MTX, methotrexate; ATG, anti-thymocyte globulin.

Efficacy of Programmed EN Dieting

Although there was a wide variation in each patient in diarrhea volume and frequency of diarrhea, we adapted ANOVA to evaluate whether there is a statistically significant difference between the two groups

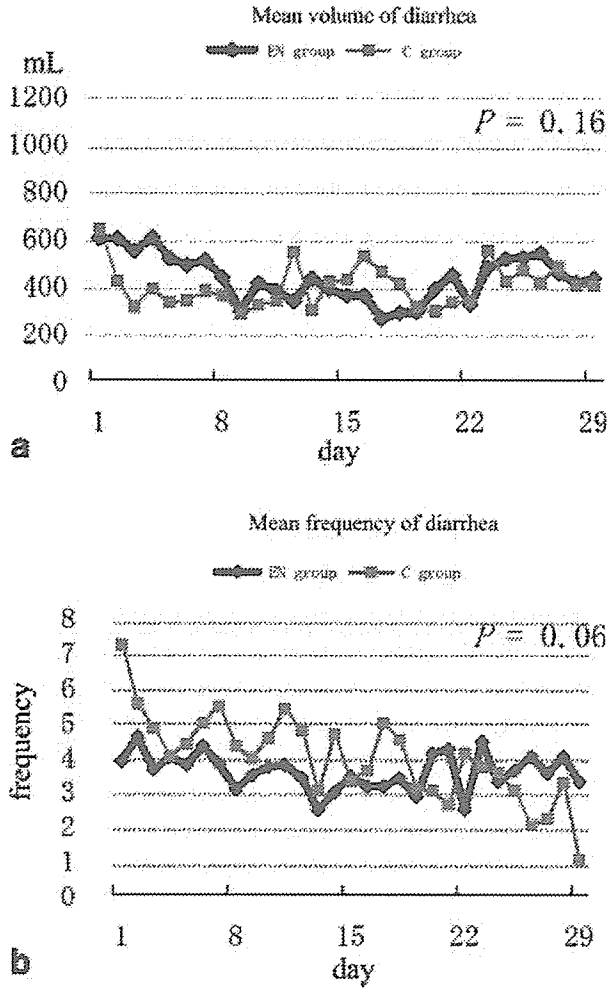

Fig. 1. Changes in mean volume and frequency of diarrhea. No difference was observed between the C and EN groups in the time-course of diarrhea as evaluated by volume ($P = 0.16$) (a) and frequency ($P = 0.06$) (b).

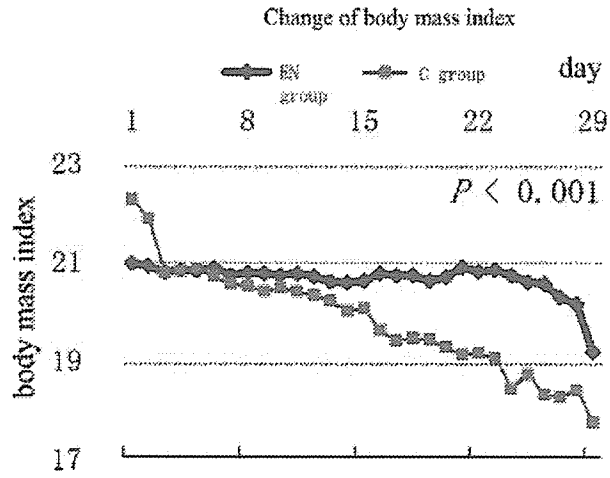

Fig. 2. Changes in BMI. The mean changes in BMI, with the first evaluation as a control, were compared between the two groups. A slower decrease in body weight tended to be observed in the EN group, while patients retained their BMI significantly better in the EN group than in the C group ($P < 0.001$). BMI was calculated as BMI = {height (m)}^{2}/body wt (kg).

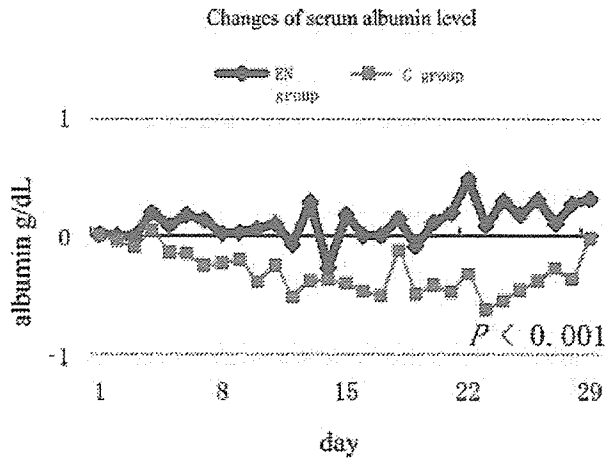

Fig. 3. Changes in albumin as nutritional parameter. One of the nutritional parameters, albumin (Alb), was evaluated between the C and EN groups. In the EN group, patients maintained significantly more stable levels of Alb ($P < 0.001$).

($P = 0.16$ and 0.06, respectively, Figure 1a and b). The mean body weight values in each group were compared by considering the absolute changes after adjusting by the value at the initial evaluation. In comparing the two groups, the decrease in body weight after the start of nutritional management was more obvious in group C than in group EN but this difference was not statistically significant ($P = 0.09$), since there was a wide interpatient variation. On the other hand, the change in BMI was significantly different between the two groups (Figure 2, $P < 0.001$).

Nutritional status was also estimated by laboratory parameters, including serum levels of total protein and albumin (Alb), which were determined as absolute changes by adjusting by the value at the first evaluation at the starting point of nutritional management, and a significantly slower decrease was noted in the EN group ($P < 0.001$) (Figure 3). These nutritional parameters remained higher in group EN than in group C. During the study period, no patient actually met with stopping rules mentioned above and consequently, the total number of days for NPO was not evaluated. The time to complete dietary recovery was compared between the two groups. While 38 days were required for the

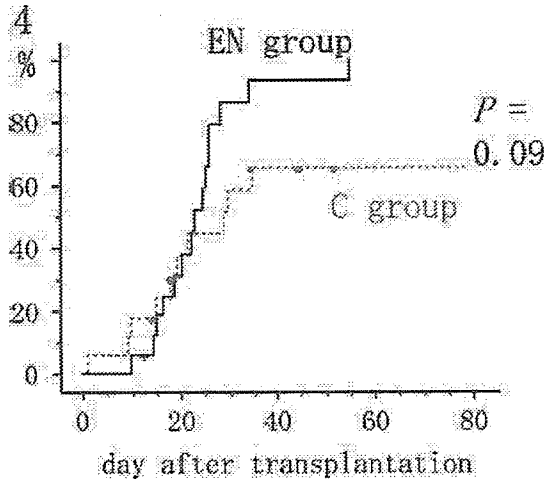

Fig. 4. Time to complete dietary recovery. The number of days required for return to a normal diet was 38 days in group C, while it was 31 days in group EN, with no statistically significant difference (P = 0.09).

recovery to a normal diet in group C, 31 days were required in group EN (Figure 4).

DISCUSSION

Since Weisdorf et al. reported that central venous parenteral nutritional support improved long-term survival in patients who underwent bone marrow transplantation (BMT) [8], intravenous TPN has been widely used in SCT. However, it has not yet been confirmed which procedure, enteral or parenteral nutrition, can provide more effective and safer nutritional support. In this study, we considered that the patients in the EN group may have preserved nutritional parameters better than the other group and ate sooner, although no differences were found in the time to complete dietary recovery. A clinical study group at Johns Hopkins University randomized BMT patients into two groups to receive different types of nutritional support, TPN or EN, and they did not observe any differences in nutritional parameters between the two groups [9]. In their study, patients who received TPN were allowed to eat anything they liked, while those with EN had few chances to receive TPN treatment. Moreover, those who had been receiving TPN were allowed to take oral intake and thus were not on strict NPO. Additionally, in our study, the two groups of patients were evaluated in different study periods, and there was a significant difference in the modality of the supportive measures. These points make a direct and strict comparison between the TPN and EN groups very difficult and unreliable. These biases, which are inherent to studies in this field, also existed in our study, which might explain why we failed to detect significant differences in clinical benefits.

We used to routinely advise patients to stop oral intake with the development of gut GVHD. Thereafter, they were encouraged to drink or eat gradually, since it has been suggested that inadequate nutritional support further deteriorates gut GVHD symptoms. To establish clearly defined subjective guidelines, we conducted this interventional cohort study. We found that both controlled and uncontrolled EN can be administered safely. Since the time to complete dietary recovery was almost comparable in the two groups, the results suggest that any EN program is acceptable and does not harm or degrade the QOL of patients suffering from GVHD. If this is confirmed, a restricted diet would not be necessary for those with moderately symptomatic gut GVHD. Nevertheless, the evaluation of nutritional parameters in this study suggested that controlled EN did a better job of maintaining body weight and serum nutritional status, compared to the results in the NPO group. The random administration of food intake may be inadequate compared to scheduled dieting, which attempts a gradual build-up of intestinal mucosa by the comprehensive supply of nutrients including glucose, protein, fat, fiber, etc. This may have a secondary advantage of keeping the mucosal barrier intact and preventing bacterial translocation through the GI tract.

Nevertheless, since the cause of diarrhea is multifactorial, it is inherently difficult to assess the effectiveness of and standardize nutritional intervention procedures. In the literature, four pathologies have been reported to be contraindications for EN since they cause undesirable bowel movement, i.e., presence of gastrointestinal bleeding, intestinal obstruction, severe pancreatitis, and intestinal perforation. The pathophysiology of diarrhea associated with gut GVHD includes osmotic and secretory diarrhea. Hypertonic EN is considered to further deteriorate symptoms of diarrhea. Hence, it is reasonable to suggest that dietary foods in EN adequately maintain an isotonic status as well as nutritional status to improve immunologic function. An intact GI system is vital for maintaining normal immune functions, and a novel concept of nutrition support, "immunonutrition," has been introduced, which focuses on the maintenance of the comprehensive biological protection system against external pathogens to maintain normal immune function [10]. Clinical benefits of immunonutrition, including improvement of nutritional parameters, decreased risk of infection, and shorter duration of hospitalization, have been reported in patients in the perioperation period and in those who required care in the ICU [11,12]. However, currently a precise evaluation

of the efficacy of each component of immunonutritional agents is difficult [13], and controversy still exists regarding the value of immunonutrition after SCT. This study did not evaluate this proposed immunonutrition, and to accomplish this in SCT practice, prospective monitoring of immune parameters would be required.

The serum level of albumin can be significantly affected by many variables including diarrhea associated with GVHD and, hence, would not be a very good marker for the evaluation of protein status in the HSCT population. However, in our experience, serum albumin decreased after SCT to suggest the possibility of the use in the estimation of patient's nutrition status at least for a short period of follow-up, when referring to the general description in the guideline by American Society for Parenteral and Enteral Nutrition, i.e., "low serum levels indicate which hospitalized patients are at increased risk of morbidity and mortality" [14].

In conclusion, the current study is hampered by preexisting biases including a small number of studied patients, a cohort analysis in different periods, and a lack of adequate measures for data evaluation. Nevertheless, it appears that patients supported by programmed EN experienced no exacerbation of gut GVHD symptoms, with a suggested benefit of enhanced maintenance of nutrition status. Further study is warranted to prospectively evaluate the value of various nutrients including arginine, o-3 fatty acid, and nucleic acid [13] and various clinical outcomes including the cost, complications, and QOL in an attempt to improve the nutritional and immune status of transplanted patients.

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