Standard Japanese Edition, version 1.1; Scientific Consulting, Apex, NC, USA).

Statistical methods

The ORR, percentage CR, and their 95% confidence intervals (CIs) were calculated with per protocol sets (PPS) of data for all eligible patients and full analysis sets (FAS) of data for all enrolled patients under the F-distribution. The median PFS time, time to CR (TTCR) and time to response (TTR), and their 95% CIs were estimated for all eligible and evaluative patients using the method of Kaplan and Meier, and were compared using the log–rank test. In addition, pretreatment factors affecting the ORR and PFS were analyzed for all eligible and evaluative patients by univariate and multivariate analyses using Fisher's exact test, Wilcoxon's rank sum test, the log–rank test, the logistic regression model or Cox's proportional hazard regression model.

All statistical analyses were performed using SAS software (version 6.12; SAS Institute, Cary, NC, USA). Data used for theses analyses were finally confirmed on March 31, 2004.

Results

Patient characteristics

A total of 69 patients were enrolled from 21 institutions (see Appendix I); 34 patients were allocated to Arm C and 35 patients to Arm S. Patient characteristics at study entry are summarized in Table 1. The median age was 52 years (range, 26–69 years). The major characteristics of the two arms were very similar in both the enrolled and eligible patients. Retrospectively, we analyzed the Follicular Lymphoma International Prognostic Index (FLIPI) in all patients.[29] FLIPI was equally distributed between the two arms. Twenty-eight patients (82%) in Arm C and 30 patients (86%) in Arm S were judged

Table 1. Patient characteristics

Factor	Enrolled (n = 69)			Eligible (n = 66)		
	Arm C	Arm S	Total	Arm C	Arm S	Total
Sex						
Female	18	18	36	17	18	35
Male	16	17	33	15	16	31
Age (years)						
Median	53	50	52	54.5	49.5	52.5
Range	36–65	26–69	26–69	36–65	26–69	26–69
Performance status (ECOG)						
0	29	30	59	28	29	57
1	5	5	10	4	5	9
Histopathology (REAL)†						
Follicular, grade 1	12	11	23	11	11	22
Follicular, grade 2	21	19	40	20	19	39
Follicular, grade 3	0	2	2	0	2	2
Marginal zone B-cell	1	0	1	1	0	1
Low grade B-NHL, NOS‡	0	2	2	0	2	2
No specimen submitted§	0	1	1	0	0	0
Clinical stage (Ann Arbor)						
III	14	15	29	13	14	27
IV	20	20	40	19	20	39
B-symptoms						
Absent	30	33	63	29	32	61
Present	4	2	6	3	2	5
LDH						
Normal	32	31	63	31	30	61
Elevated	2	4	6	1	4	5
No. of extranodal sites						
0–1	25	26	51	24	25	49
≤2	9	9	18	8	9	17
International Prognostic Index						
Low	21	21	42	21	20	41
Low-intermediate	12	12	24	10	12	22
High-intermediate	1	1	2	1	1	2
High	0	1	1	0	1	1
Follicular Lymphoma International Prognostic Index						
Low	16	15	31	16	15	31
Intermediate	12	15	27	10	14	25
High	6	5	11	5	5	10

†According to the diagnosis by the central pathology review. ‡Low-grade B-cell non-Hodgkin lymphoma (NHL) not otherwise specified. §Specimen was not submitted to the central pathology review. LDH, lactic dehydrogenase.

Table 2. Response to therapy

Arm		n	No. of patients achieving response						Response rate (95% CI)	
			CR	CRu	PR	SD	PD	NE	%CR	ORR
Arm C	Eligible	32	19 21 30	2	9	1	0	1	66% (47–81%)	94% (79–99%)
	Enrolled	34	21 23 33	2	10	1	0	0	68% (50–83%)	97% (85–100%)
Arm S	Eligible	34	22 23 33	1	10	0	0	1	68% (50–83%)	97% (85–100%)
	Enrolled	35	21 23 33	1	10	0	0	2	66% (44–81%)	94% (81–99%)

Response to each therapy was evaluated according to the International Workshop Criteria for Non-Hodgkin's Lymphoma. CI, confidence interval; CR, complete response; CRu, complete response/unconfirmed; NE, not evaluative due to insufficient follow-up; ORR, overall response rate; PD, progressive disease; PR, partial response; SD, stable disease.

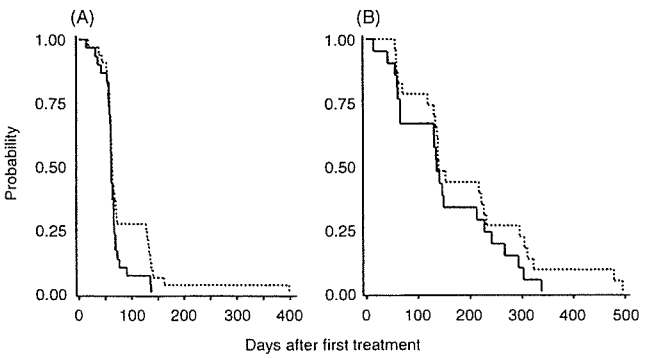

Fig. 1. (A) Time to response (TTR) and (B) time to complete response (TTCR). Medians were estimated by the Kaplan-Meier method. A total of 63 patients (Arm C [-], 30; Arm S [---], 33) were analyzed for TTR, and 44 patients (Arm C, 21; Arm S, 23) for TTCR with per protocol sets of data. Median TTRs in Arm C and Arm S were 61 days (95% confidence interval [CI] 59 to 65 days) and 62 days (95% CI 60–70 days), respectively. The 75th percentile TTRs in Arm C and Arm S were 66 days (95% CI 63 to 76 days) and 140 days (95% CI 66–135 days), respectively ($P = 0.0994$, log–rank test). Median TTCRs in Arm C and Arm S were 136 days (95% CI 65 to 213 days) and 140 days (95% CI 134–227 days), respectively. The 75th percentile TTCRs in Arm C and Arm S were 228 days (95% CI 141 to 293 days) and 295 days (95% CI 153–323 days), respectively ($P = 0.2201$, log–rank test).

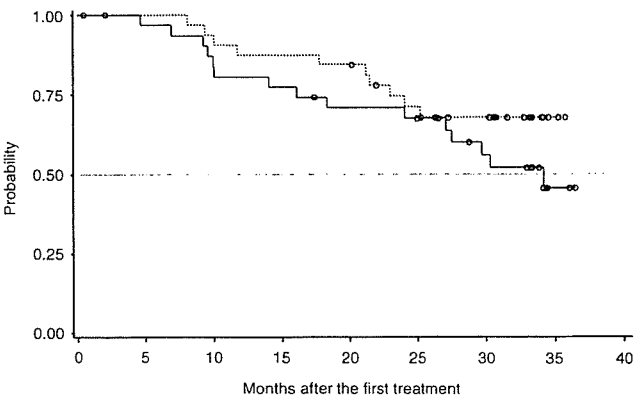

Fig. 2. Progression-free survival (PFS). Medians were estimated by the Kaplan-Meier method. The upper limit of the 95% confidence interval (CI) for Arm C has not yet been determined. A total of 65 patients (Arm C, 32; Arm S, 33) were analyzed with per protocol sets of data. The median PFS time for patients in Arm C (-) was 34.2 months (95%CI, 27.1 months, inestimable), whereas that for patients in Arm S (...) had not yet been reached, with a median follow-up time of 28.2 months. Log–rank test, $P = 0.220$. (o) Censored.

to belong to the low, or low-intermediate risk group categorized by FLIPI. Three patients were judged ineligible by an extramural review committee, because two of them had concomitant active cancer and one had a history of prior chemotherapy, including doxorubicin for the treatment of breast cancer. Sixty-five patients (94%) were confirmed to have FL in the central pathology review.

Response to treatment and survival

Sixty-six eligible patients (Arm C, 32 patients; Arm S, 34 patients) were evaluated with PPSs of data, and 69 patients (Arm C, 34 patients; Arm S, 35 patients) with FASs of data. One patient allocated to Arm C could not be evaluated for response because the first cycle of chemotherapy given was not CHOP (doxorubicin in the CHOP regimen was erroneously replaced with daunorubicin). Two patients (one patient eligible and one ineligible) allocated to Arm S could not be evaluated because they had withdrawn from the study before starting treatment.

As shown in Table 2, similar results of the ORRs and the percentage CRs were obtained in Arm C and Arm S. The ORRs and percentage CRs calculated with PPSs and FASs were similar. Kaplan-Meier curves of TTR and TTCR were plotted for eligible and evaluative patients in each arm, as shown in Fig. 1. Although the median TTRs for patients in Arm C and Arm S were not different (61 days *versus* 62 days, respectively), the 75th percentile TTRs for patients were shorter in Arm C (66 days) than Arm S (127 days), with no statistical difference ($P = 0.0994$, log–rank test). The median TTCRs were similar in Arm C and Arm S (136 days and 140 days, respectively). As shown in Fig. 2, the median PFS time for patients in Arm C ($n = 32$) was 34.2 months

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Table 3. Hematological toxicity

Toxicity	Arm	*n*	Grade 0–2	Grade 3	Grade 4
Any hematological toxicity	Arm C	34	2 (6%)	3 (9%) 32 (94%)	29 (85%)
	Arm S	33	0 (0%)	10 (30%) 33 (100%)	23 (70%)
Leukopenia	Arm C	34	5 (15%)	16 (47%) 29 (85%)	13 (38%)
	Arm S	33	3 (9%)	23 (70%) 30 (91%)	7 (21%)
Neutropenia	Arm C	34	2 (6%)	3 (9%) 32 (94%)	29 (85%)
	Arm S	33	1 (3%)	9 (27%) 32 (97%)	23 (70%)
Thrombocytopenia	Arm C	34	32 (94%)	1 (3%) 2 (6%)	1 (3%)
	Arm S	33	33 (100%)	0 (0%) 0 (0%)	0 (0%)
Anemia	Arm C	34	31 (91%)	3 (9%)	–
	Arm S	33	31 (94%)	2 (6%)	–

Hematological toxicity was evaluated according to the JCOG Toxicity Criteria, an expanded version of the NCI-CTC version 1.0. All hematological toxicities (possibly related to rituximab, or unknown relationship to rituximab) observed during the treatment and follow-up period (for 6 months after the last cycle of CHOP for Arm C, and for 4 months after the last rituximab infusion for Arm S) are listed.

(95%CI, 27.1 months – inestimable), whereas that for patients in Arm S ($n = 33$) had not yet been reached, with a median follow-up time of 28.2 months. One patient (#38) in Arm S died of tumor progression 730 days after the first treatment. No other patients died within approximately 3 years of observation.

Adverse events

Information about AEs was available for 67 patients (Arm C, 34 patients; Arm S, 33 patients) who received protocol treatment. Hematological toxicity was documented at its highest grade throughout the study period. As shown in Table 3, major hematological toxicity was neutropenia; grade 3 or greater neutropenia was observed in 32 patients (94%) in Arm C and in 33 patients (100%) in Arm S; grade 4 neutropenia was seen in 29 patients (85%) in Arm C and in 23 patients (70%) in Arm S. All hematological toxicities were controllable and reversible, although some patients required hematopoietic cytokines.

Grade 3 or greater non-hematological AEs observed during treatment and initial follow-up periods are listed in Table 4. A total of 11 patients (Arm C, seven patients, 21%; Arm S, four patients, 12%) developed 14 events of grade 3 or greater non-hematological adverse events. All non-hematological toxicities were reversible. There was no therapy-related death.

Prognostic factors

Pretreatment factors affecting ORR and PFS were analyzed. Because the sample size of each arm was small, analyses were not performed separately for the two arms, but results were pooled ($n = 64$). There were two factors affecting ORR when analyzed by the Wilcoxon's rank sum test. Patients with PS 0 (41CR, 13PR, 1NC, 0 PD) demonstrated a superior response to those with PS 1 (3CR, 6PR, 0NC, 0PD) ($P = 0.0182$, Wilcoxon's rank-sum). Patients with a tumor size <5 cm (32CR, 6PR, 1NC, 0 PD) had a superior response to those with tumors equal to 5 cm (12CR, 13PR, 0NC, 0 PD) ($P = 0.0066$, Wilcoxon's rank-sum).

However, no factor significantly affected PFS. Multivariate analyses were also performed using the same factors, excluding IPI. There was no factor that independently affected ORR and PFS.

HACA and pharmacokinetics of rituximab

Out of 67 patients who received rituximab, HACA assays were performed for 65 patients (Arm C, 33; Arm S, 32) at 8 months after treatment, and for 64 patients (Arm C, 33; Arm S, 31) at 10 months after treatment. No patient developed HACA. For all 27 patients (Arm C, 14; Arm S 13) who received four rituximab infusions and whose planned monitoring of

Table 4. Grade 3 or greater-non-hematological adverse events

Arm	Patient	Serious adverse event†	Grade‡	Onset timing	Relating drug (causative)
Arm C	#04	Hyperglycemia	3	6th cycle (day 4)	CHOP (diabetes)
(*n* = 32)	#07	Hyperglycemia	3	4th cycle (day 2)	CHOP, rituximab
	#13	Hypertension	3	1st cycle (day 3)	CHOP, rituximab
	#21	Total bilirubin elevation	3	2nd cycle (day 5)	– (constitutional)
	#23	Abdominal pain	3	1st cycle (day 9)	CHOP, rituximab
	#58	Acute cholangitis with elevated AST and ALT	3	3rd cycle (day 10)	CHOP, rituximab
	#59	Hyperglycemia, hypertension	3	5th cycle (day 6)	CHOP, rituximab
Arm S	#25	Total bilirubin elevation	3	6th cycle (day 132)	– (constitutional)
(*n* = 33)	#56	Diarrhea	4	1st cycle (day 13)	– (alimentary)
		Febrile neutropenia	3	3rd cycle (day 12)	CHOP
		Interstitial pneumonia	3	3rd cycle (day 15)	CHOP
	#62	Total bilirubin elevation	3	4th cycle (day 7)	CHOP
	#69	AST and ALT elevation	3	1st cycle (day 10)	CHOP
				2nd cycle (day 8)	CHOP
				6th cycle (day 29)	CHOP

†Grade 3 or greater adverse events other than hematological toxicities that were observed during the treatment and follow-up period (for 6 months after the last cycle of CHOP for Arm C, and for 4 months after the last rituximab infusion for Arm S). ‡JCOG Toxicity Criteria, an expanded version of the NCI-CTC, version 1.0.

Table 5. Pharmacokinetic parameters of rituximab

Arm		Dose (mg/day)	AUC (μg. h/mL)	Cmax† (μg/mL)	$T_{1/2}$ (h)	Clearance‡ (litter/h)	MRT (h)	Vd (litter)
Arm C	Mean	593.9	372 498.9	262.5	232.3	0.0259	335.1	4.49
(n = 14)	SD	51.1	111 660.4	73.2	113.8	0.0301	164.2	0.66
Arm S	Mean	596.4	418 901.3	433.5	356.9	0.0128	514.9	5.57
(n = 13)	SD	82.6	107 002.6	134.9	163.4	0.0077	235.9	1.95

†Actual measured value. ‡Calculated under the one-compartment model. Time points for serum collection were as follows; Arm C: before, and 10 min and 2 days after each rituximab infusion, and 1 week, 1, 4 and 6 months after the sixth rituximab infusion. Arm S: before, 10 min after each rituximab infusion and 2 days, 1 and 2 weeks, and 1 and 4 months after the sixth rituximab infusion. AUC, area under the curve; Cmax, maximum concentration; $T_{1/2}$, elimination half-life; MRT, mean residence time; Vd, volume of distribution.

serum rituximab levels were completed, pharmacokinetic parameters were calculated throughout the four infusions. As shown in Table 5, Arm S showed higher values for the parameters of area under the curve (AUC), maximum concentration (Cmax), elimination half-life (T1/2), mean residence time (MRT), and volume of distribution (Vd).

Discussion

In this randomized phase II trial, we have demonstrated that the combined use of rituximab and CHOP yielded an ORR of 94% and 97%, and a percentage CR of 66% and 68% in the concurrent arm and the sequential arm, respectively. These ORRs and percentage CRs are superior to those reported for combination chemotherapy regimens containing anthracycline without rituximab, which were conducted after stringent clinical staging with CT. The percentage CR obtained by six to eight cycles of CHOP chemotherapy in untreated patients (n = 83) with FL was reported to be 36% (90%CI, 27–46%).[30] The ORR and percentage CR of CHOP chemotherapy obtained by Kimby *et al.* in their randomized study comparing chlorambucil plus prednisone *versus* CHOP in symptomatic low-grade NHL (n = 127), were 60% and 18%, respectively.[31]

Data of the present study was comparable to the preceding study on CHOP combined with rituximab in patients with indolent B-NHL regarding efficacy and tolerability. Although the precise schedule of the administration of rituximab in the first phase II study of R-CHOP reported by Czuczman *et al.* was not the same as that of the present study, the concept of concurrent use is identical between their trial and Arm C in the present study.[14] However, the percentage CR of Arm C is less than that of Czuczman *et al.*'s trial, and the median PFS of Arm C appears to be shorter in the present study, although more than 82% of all enrolled patients in our study were in the low or low-intermediate risk group by FLIPI. In Czuczman *et al.*'s trial, as the last two infusions of rituximab were administered 1 month after the sixth CHOP cycle, like in our sequential arm, the design of Czuczman *et al.*'s trial had characteristics of both the concurrent arm and the sequential arm. So it is possible that the higher percentage CR and longer PFS in Czuczman *et al.*'s trial compared to our concurrent arm were partly due to the mixed design of the administration schedule of rituximab, in addition to the possible selection bias in phase II studies.

The South-west Oncology Group (SWOG) in the USA studied six cycles of CHOP followed by four weekly infusions of rituximab in newly diagnosed patients with FL at advanced stages (31% with bulky disease and 30% with B-symptoms). Sixteen (19%) of the 84 evaluative patients had an improved tumor response after rituximab treatment, with an ORR of 72%, including 54% with a CR or CRu. The PFS was 76% at the median follow-up of 2.7 years.[32] The PFS data of the sequential arm in our trial is similar to that of the SWOG trial.

Cancer and Leukemia Group B (CALGB) conducted a randomized phase II study to explore a more suitable administration schedule of rituximab with fludarabine in previously untreated chronic lymphocytic leukemia (CLL) patients.[33] Patients randomly received either six monthly courses of fludarabine concurrently with rituximab followed 2 months later by four weekly doses of rituximab for consolidation therapy, or fludarabine alone followed 2 months later by rituximab consolidation therapy. The ORR with the concurrent regimen was 90% compared to 77% with the sequential regimen. With a median follow-up time of 23 months, the number of relapsed patients was 18 (35%) in the concurrent regimen and 15 (28%) in the sequential regimen. Although PFS and survival appeared to be somewhat longer with the sequential treatment, CALGB concluded that the concurrent use of rituximab and fludarabine was superior. Our randomized phase II study for indolent B-cell NHLs showed similar percentage ORRs and percentage CRs between the two arms, and a seemingly longer PFS in the sequential arm. Because patients in the concurrent arm in the CALGB study received consolidated administration of rituximab after induction therapy, the concurrent arm in the CALGB study had characteristics of the concurrent arm and sequential arm of our present study.

In a randomized phase III study that compared eight cycles of R-CVP to CVP for previously untreated patients with advanced FL, a significantly prolonged TTP of R-CVP was reported (median 32 months *versus* 15 months for CVP; $P < 0.0001$).[18] The median TTP of R-CVP was similar to the median PFS of Arm C in our study. As the toxicity is stronger in CHOP than CVP, it is worthwhile to conduct a randomized phase III trial to compare R-CHOP to R-CVP.

The maintenance use of rituximab after first-line rituximab therapy was also reported to prolong PFS or event-free survival (EFS).[34,35] Future trials to explore the role of maintenance use of rituximab after first-line rituximab containing chemotherapy like Arm C are warranted.

About 25% of patients in Arm S did not achieve a response (PR or higher) before the initiation of rituximab treatment, despite the completion of six cycles of CHOP. In Arm C, more than 90% of patients showed a response after the six cycles of CHOP plus rituximab. The same tendency was also shown in the TTCR, as shown in Fig. 1B. The TTCR of each patient in Arm C was relatively shorter than that in Arm S.

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While grade 3 or greater non-hematological AEs were observed in 11 patients (Arm C, seven patients, 21%; Arm S, four patients, 13%), both arms were well tolerated. Two patients were withdrawn from the study before completion of the planned treatment by AE. One patient in Arm C developed acute cholangitis after the third cycle of CHOP plus rituximab. The other patient in Arm S developed interstitial pneumonia after the third cycle of CHOP. Both patients fully recovered. Hematological toxicities were observed in all treated patients; grade 4 neutropenia was frequent and was observed in 85% of patients in Arm C and in 70% in Arm S. However, these hematological toxicities were manageable with or without supportive care using hematopoietic growth factor. No patient was withdrawn from the study due to hematological toxicity. Grade 3 or greater thrombocytopenia was rare in Arm C and absent in Arm S. Although hematological and non-hematological toxicities were slightly more frequent in Arm C, toxicities were clinically acceptable in both arms.

In conclusion, CHOP combined with rituximab was highly effective in untreated patients with indolent B-NHL, especially FL, either in a concurrent or sequential combination, with acceptable toxicities. Although the time to achieve a response was more rapid with the concurrent combination than the sequential combination, PFS appeared to be slightly longer with the sequential combination, although the difference was not statistically significant. We conclude that both combination schedules deserve further investigation. Considering the promising results of rituximab maintenance therapy reported by other investigators, it would be worthwhile to conduct future trials to establish the role of rituximab maintenance after concurrent and sequential combinations of rituximab plus CHOP therapy.

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Appendix

Participating institutions and principal investigators of the IDEC-C2B8 Study Group included: Sapporo National Hospital (K. Aikawa, M. Nakata), Sapporo Hokuyu Hospital (M. Kasai, Y. Kiyama), Tochigi Cancer Center (Y. Kano, M. Akutsu), International Medical Center of Japan (A. Miwa, N. Takesako), National Cancer Center Hospital East (K. Itoh, T. Igarashi, K. Ishizawa), National Cancer Center Hospital (K. Tobinai, Y. Kobayashi, T. Watanabe), Tokyo Medical University (K. Ohyashiki, T. Tauchi), Tokai University School of Medicine (T. Hotta, T. Sasao), Hamamatsu University School of Medicine (K. Ohnishi), Aichi Cancer Center Hospital (Y. Morishima, M. Ogura, Y. Kagami), Nagoya University School of Medicine (T. Kinoshita, T. Murate, H. Nagai), Nagoya National Hospital (K. Tsushita, H. Ohashi), Mie University School of Medicine (S. Kageyama, M. Yamaguchi), Kyoto Prefectural University of Medicine (M. Taniwaki), Kyoto University School of Medicine (H. Ohno, T. Ishikawa), Shiga Medical Center for Adults (T. Suzuki), Center for Cardiovascular Diseases and Cancer, Osaka (A. Hiraoka, T. Karasuno), Hyogo Medical Center for Adults (T. Murayama), Hiroshima University School of Medicine (A. Sakai), National Kyushu Cancer Center (N. Uike), Nagasaki University School of Medicine (T. Maeda, K. Tsukasaki).

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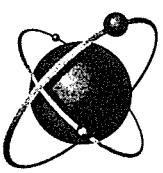
4. Antibody Therapy for Malignant Lymphoma

Kensei Tobinai



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INTERNAL MEDICINE

□ SYMPOSIUM II □

4. Antibody Therapy for Malignant Lymphoma

Kensei Tobinai

Abstract

Rituximab, a genetically engineered, chimeric anti-CD20 monoclonal antibody, induces the apoptosis of B-lymphoma cells, in addition to the lyses by complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC), as shown in Fig. 1 (1). A Japanese phase I study of rituximab in relapsed or refractory patients with B-cell non-Hodgkin's lymphoma (B-NHL) showed an overall response rate (ORR) of 64% (7/11) with minimal toxicities. Elimination half-life ($T_{1/2}$) of serum rituximab was 445± 361 hours, and the serum rituximab was detectable at three months. In the subsequent phase II study, 90 relapsed or refractory patients with indolent B-NHL or mantle cell lymphoma (MCL) were treated with rituximab at 375 mg/m^2×4 weekly infusions. ORRs in indolent B-NHL and MCL were 61% (37/61) and 46% (6/13), respectively. In this presentation, the results of clinical trials of antibody therapy of malignant lymphoma are summarized, focusing on the two recent Japanese multicenter trials of rituximab and a Japanese feasibility study of anti-CD20 radioimmunotherapy with yttrium-90-lableled ibritumomab tiuxetan (2).

Key words: antibody therapy, malignant lymphoma, CD20, rituximab, radioimmunotherapy

(DOI: 10.2169/internaimedicine.46.1782)

1. Chimeric Anti-CD20 Antibody, Rituximab

1) Phase II study of rituximab in relapsed or refractory aggressive B-NHL

To evaluate the efficacy and feasibility of rituximab monotherapy in Japanese patients with relapsed or refractory aggressive B-NHL, a multicenter phase II study was conducted (3). Sixty-eight patients were enrolled and treated with rituximab at 375 mg/m^2 by eight consecutive weekly infusions. The ORRs of the 68 enrolled patients and of the 57 eligible patients were 35% and 37%, respectively. The median progression-free survival (PFS) of the 53 evaluable patients was 52 days, whereas the time to progression of the 21 eligible responders was 245 days. Elevated serum lactate dehydrogenase (LDH) and refractoriness to prior chemotherapy unfavorably affected ORR and PFS ($P<0.01$). Serum trough levels of rituximab and the area-under-the-curve (AUC) for responders were higher than for non-responders ($P<0.05$). In conclusion, treatment with eight weekly infusions of rituximab has significant anti-lymphoma activity for relapsed or refractory aggressive B-NHL (3).

2) Randomized phase II study of rituximab plus CHOP (R-CHOP) in untreated indolent B-NHL

To explore the more promising administration schedule of R-CHOP for indolent B-NHL for further investigations, this randomized phase II study was conducted (4). Untreated patients with advanced, indolent B-NHL were randomized to receive either six courses of CHOP concurrently with rituximab (Arm C) or sequential six courses of CHOP followed by six courses of weekly rituximab (Arm S). The primary endpoint was ORR. Sixty-nine patients were randomized to Arm C (n=34) and Arm S (n=35). ORR with Arm C was 94% (95% confidence interval [CI], 79-99%) including 66% of complete response (CR) compared with 97% (95% CI, 85-100%) including 68% of CR with Arm S. Patients with Arm C experienced more grade 4 hematologic toxicities (85% vs. 70%) and grade 3 or 4 non-hematologic toxicities (15% vs. 9%) as compared with Arm S. Both arms were well tolerated. With a median follow-up time of 28.2 months, the median PFS time was 1,026 days in Arm C, and has not been reached in Arm S ($P=0.227$). IgH/Bcl-2 copy numbers, especially in peripheral blood, decreased more rapidly in Arm C than in Arm S. In conclusion, R-CHOP is highly effective for untreated indolent B-NHL either by concurrent or sequential combination. The time to

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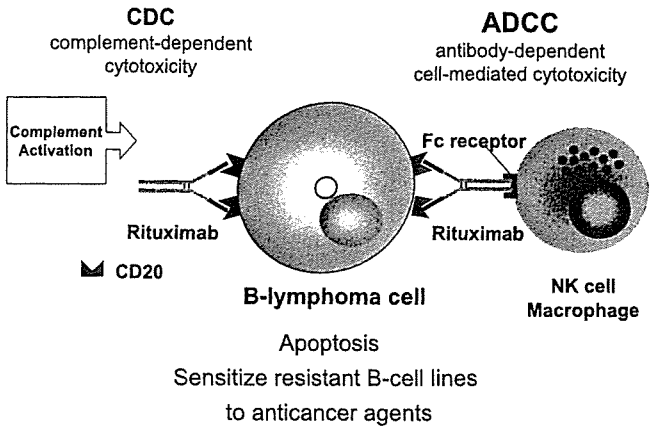

Figure 1. Putative Mechanism of Action of Rituximab.

response was more prompt with the concurrent combination, whereas PFS appears to be longer with the sequential combination. Minimal residual disease (MRD) can be effectively eradicated either by the concurrent or sequential combination;however, rapid clearance of MRD by the concurrent combination may not lead to the prolongation of PFS.

2. Radioimmunotherapy of B-cell NHL with Yttrium-90-labeled, Murine Anti-CD20 Antibody, Ibritumomab Tiuxetan

Ibritumomab is a murine anti-CD20 monoclonal antibody that was engineered to form rituximab. Tiuxetan is a MX-DTPA linker chelator that is attached to ibritumomab to form ibritumomab tiuxetan (Zevalin™). The ibritumomab tiuxetan is radiolabeled with either ^{111}In (^{111}In-Zevalin™) for imaging or dosimetry studies or with ^{90}Y (^{90}Y-Zevalin™) for therapy of B-NHL (5). Between 2002 and 2003, a phase I and feasibility study of ibritumomab tiuxetan was conducted for Japanese patients with B-NHL at the National Cancer Center. In this study, ten patients had relapsed or refractory B-NHL, including nine with follicular lymphoma and one with mantle cell lymphoma, and eight of them had been treated with rituximab. The encountered toxicities were primarily hematologic. None of the three patients in the 0.3 mCi/kg cohort developed critical toxicities, whereas two of the six patients in the 0.4 mCi/kg cohort developed critical toxicities, including one patient who developed long-lasting neutropenia and thrombocytopenia. Among the ten enrolled patients, seven showed objective responses, including five patients achieving CR and two patients achieving partial response (PR). Based on these results, we concluded that yttrium-90-labeled, murine anti-CD20 antibody, ibritumomab tiuxetan is highly effective for relapsed or refractory patients with indolent B-NHL with acceptable toxicities, and that the recommended phase II dose was 0.4 mCi/kg. Subsequently, we conducted a pivotal multicenter phase II study of ibritumomab tiuxetan for relapsed or refractory indolent B-NHL.

Summary

Rituximab, a chimeric anti-CD20 antibody, was approved for indolent B-NHL in Japan in 2001, and was approved for aggressive B-NHL in 2003. French, US and German phase III studies indicated that rituximab plus CHOP is a new standard therapy for aggressive B-NHL. A Japanese phase I study of an anti-CD20 radioimmunoconjugate, ibritumomab tiuxetan, showed high efficacy with acceptable toxicities, and a pivotal phase II study was completed. Monoclonal antibody therapies will have significant roles in the treatment of malignant lymphoma.

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ORIGINAL REPORT

Prognostic Significance of T-Cell or Cytotoxic Molecules Phenotype in Classical Hodgkin's Lymphoma: A Clinicopathologic Study

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ABSTRACT

Purpose
Classical Hodgkin's lymphoma (CHL) is characterized by Hodgkin's and Reed-Sternberg (H-RS) cells, most of which are derived from germinal-center B cells. Nevertheless, one or more markers for T cells and follicular dendritic cells (FDC) may be expressed in a minority of H-RS cells in some CHL patients, although the clinical significance of this remains controversial. The aim of this study was to clarify the association between phenotypic expression and clinical outcome in CHL.

Patients and Methods
Participants were 324 consecutive CHL patients, comprising 132 patients with nodular sclerosis (NS), 35 patients with NS grade 2 (NS2), and 157 patients with mixed cellularity (MC). We evaluated the presenting features and prognosis of patients on categorization into four phenotypically defined groups: B-cell ($CD20^+$ and/or $CD79a^+$; n = 63), T-cell and/or cytotoxic molecules ($CD3^+$, $CD4^+$, $CD8^+$, $CD45RO^+$, $TIA\text{-}1^+$, and/or granzyme B^+; n = 27), FDC ($CD21^+$ without B-cell marker; n = 22), and null-cell types (n = 212). Other potential prognostic factors were examined.

Results
The T-cell and/or cytotoxic molecules group showed a significantly poorer prognosis than the other three groups ($P < .0001$). This finding was seen consistently in multivariate analyses. Morphologic subtyping (NS/NS2/MC) and Epstein-Barr virus positivity were not identified as independent prognostic factors.

Conclusion
The presence of T-cell and/or cytotoxic antigens in H-RS cells may represent a poor prognostic factor in CHL, even if their expression is not regarded as lineage specific. Examination of T-cell and/or cytotoxic molecules phenotype in CHL patients is recommended as a routine pathologic practice.

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INTRODUCTION

The recent availability of a large number of monoclonal antibodies for leukocyte surface markers has provided further evidence for the B-cell origin of Hodgkin's and Reed-Sternberg (H-RS) cells in many but not all patients.[1,2] The application of molecular methods, single H-RS cell analysis,[3] and comparative genome expression analysis[4] has provided additional definitive evidence that H-RS cells of classical Hodgkin's lymphoma (CHL) are derived from germinal-center B cells.[5-7] Nevertheless, a small number of patients with CHL are immunoreactive for T-cell antigens,[8,9] and rare occurrences of CHL are even derived genotypically from T cells.[10,11] Adding to this complexity, we reported previously nine patients with CHL with a follicular dendritic cell (FDC) phenotype without other B-cell or T-cell markers.[12] These phenotypic analyses were interpreted variously to suggest the distinct cellular origin (B cells, T cells, or FDCs) of H-RS cells, notwithstanding that the expression of these cell-associated antigens was found to lack clear lineage specificity. Of note, the association between the expression of these markers and clinical outcome in CHL has been controversial.

In this study, we investigated comprehensively 324 patients with CHL to clarify their clinicopathologic features and survival, with special reference to phenotypic properties (four phenotypes: B cell, T cell and/or cytotoxic molecules

[T/CM], FDC, and null cell) and positivity for Epstein-Barr virus (EBV) on H-RS cells.

PATIENTS AND METHODS

Patient Samples

A total of 324 consecutive patients with CHL diagnosed between April 1982 and March 2005 at Aichi Cancer Center Hospital (Nagoya, Japan) were selected from patient records. Approval for the study was provided by the Institutional Review Board of Aichi Cancer Center.

For the diagnosis of CHL, all patients in this study were negative for human T-cell leukemia virus type 1 antibody in sera. The tumor cells showed no sinusoidal spread and grew separately from each other in all areas of the biopsies to exclude Hodgkin's-like anaplastic large cell lymphoma (ALCL) under the Revised European-American Lymphoma classification.[13] Patients with nodular lymphocyte-predominant Hodgkin's lymphoma, which is now termed B-cell neoplasm, also were excluded.

Each patient case was reviewed independently by two pathologists (N.A. and S.N.), who used a combination of morphologic review and immunostaining to assign each patient case to one of the categories of the modified WHO classification scheme.[14] Controversial determinations were reassessed jointly by the two pathologists until a consensus was reached. Morphologically related entities, such as Hodgkin's-like ALCL and peripheral T-cell lymphoma with Reed-Sternberg–like cells, were ruled out by three external lymphoma experts (T. Yoshino, Okayama, Japan; K. Ohshima, Kurume, Japan; and Y. Matsuno, Tokyo, Japan), who were blinded to the phenotype and clinical course of the patients.

Tissue Specimens and Histology

Tissue samples were fixed in 10% formalin and embedded in paraffin, then sectioned at 5-μm intervals and stained with hematoxylin and eosin. Imprint smears of surgically rejected specimens were stained with May-Grünwald-Giemsa stain.

Immunohistochemistry

Formalin-fixed paraffin sections were subjected to immunoperoxidase studies using the avidin-biotin peroxidase complex method. Monoclonal

Table 1. Clinical and Phenotypic Characteristics According to Histology (NS *v* NS2 *v* MC)

Characteristic	NS		NS2		MC		*P**
	No.	%	No.	%	No.	%	
Total No. of patients	132		35		157		
Sex							.001
Male	76		26		121		
Female	56		9		36		
Ratio	1.36		2.89		3.36		
Age, years							.0001
Median	31		50		57		
Range	12-84		5-88		4-89		
> 45	46	35	21	60	112	71	< .0001
> 60	32	24	12	34	65	41	.009
PS > 1	22	17	10	29	21	13	.089
Clinical stage III/IV	54	41	22	63	59	38	.023
Presence of "B" symptoms	42	34	16	55	45	37	.11
Bulky mass	26	21	6	20	13	10	.056
Mediastinal mass	71	58	11	39	30	24	< .0001
Extranodal > 1 site	14	12	8	29	15	12	.060
WBC > 15,000/μL	20	19	4	17	5	6	.026
Hb < 10.5 g/dL	26	25	11	48	18	21	.031
Serum albumin < 4.0 g/dL	48	53	9	69	38	51	.46
LDH > normal	36	43	14	61	30	42	.27
Survival, months							.54
Median	27.1		26.8		24.1		
Range	4.5-163+		2.0-171+		1.2-254+		
Immunophenotype†							
CD20	18 of 122	15	4 of 35	11	32 of 147	22	.19
CD21	13 of 111	12	5 of 25	20	12 of 92	13	.54
cyCD3	2 of 66	3	1 of 17	6	1 of 83	1	.47
CD4	4 of 35	11	0 of 9	0	0 of 36	0	.067
CD8	2 of 35	6	0 of 8	0	0 of 36	0	.28
CD15	90 of 131	69	28 of 34	82	84 of 154	55	.002
CD30	118 of 131	90	32 of 35	91	142 of 155	92	.90
CD45RO	5 of 104	5	1 of 29	4	1 of 113	1	.22
CD79a	3 of 34	9	1 of 8	13	8 of 43	19	.47
TIA-1	9 of 132	7	1 of 35	3	2 of 156	1	.045
Granzyme B	9 of 132	7	1 of 35	3	6 of 157	4	.42
EBV	16 of 126	13	18 of 34	53	115 of 154	75	< .0001

Abbreviations: NS, nodular sclerosis; NS2, nodular sclerosis grade 2; MC, mixed cellularity; PS, performance status; Hb, hemoglobin; LDH, lactate dehydrogenase; cyCD3, cytoplasmic CD3; EBV, Epstein Barr virus.

*χ^2 test for independence, or Fisher's exact probability test, NS *v* NS2 *v* MC.

†No. positive of No. tested patients.

antibodies used were CD3, CD8, UCHL-1/CD45RO, L26/CD20, 1F8/CD21, Ber-H2/CD30, CD79a, and ALK1 (DAKO, Glostrup, Denmark); CD4 (Novocastra Laboratories, Newcastle, United Kingdom); LeuM1/CD15 (Becton Dickinson, Sunnyvale, CA); TIA-1 (Coulter Immunology, Hialeah, FL); and granzyme B (Monosan, Uden, the Netherlands). All antibodies were first heated in a microwave, then the antibodies were used. Reaction for the reagents was considered positive when more than 5% of the H-RS cells stained, although in practice many of the positive samples showed marking in more than 10% of cells.

In Situ Hybridization Study

The presence of EBV small RNAs was determined by in situ hybridization using EBV-encoded small nuclear early-region oligonucleotides on formalin-fixed, paraffin-embedded sections as described previously.[15]

Statistical Analysis

Differences in characteristics between the two groups were examined by the χ^2 test, Fisher's exact test, Student's t test, and Mann-Whitney U test as appropriate. Patient survival data were analyzed by the Kaplan-Meier method. Differences in survival were tested by the log-rank test. Survival for this study was evaluated in terms of disease-specific survival (DSS), measured from the date of diagnosis until the date of death as a result of a lymphoma-related cause. In DSS analysis, patients were censored at the time of death if this was from a cause unrelated to lymphoma, and deaths from treatment-related causes were classified as death from lymphoma. Univariate and multivariate analyses were performed with Cox proportional hazards regression models. Results are expressed as hazard ratios (HRs) and 95% CIs. All data were analyzed with the aid of STATA software (version 9.0, STATA Corp, College Station, TX).

RESULTS

Clinicopathologic Characteristics

Patient characteristics are summarized in Table 1. There were 223 male and 101 female patients with a median age of 48 years (range, 4 to 89). Histopathologically, they included 132 patients with nodular sclerosis (NS; median age, 31 years; range, 12 to 84 years, male-to-female ratio, 1.36), 35 with NS grade 2[16] (NS2; median age, 50 years; range, 5 to 88 years; male-to-female ratio, 2.89), and 157 with mixed cellularity (MC; median age, 57 years; range, 4 to 89 years, male-to-female ratio, 3.36). On comparison, patients with NS showed a significantly younger age at onset ($P = .0001$) and a higher ratio of females ($P = .001$). Patients with NS2 were associated significantly with several aggressive clinical parameters, namely advanced clinical stage in 22 patients (63%; $P = .023$) and anemia (hemoglobin < 10.5 g/dL) in 11 patients (48%; $P = .031$).

Immunophenotypic Characteristics

Phenotypic features are summarized in Table 1. There were significant differences in the results of positivity or negativity of H-RS cells for TIA-1, CD15, and EBV among NS, NS2, and MC patients. NS patients showed significantly higher rates for TIA-1 expression than those with NS2 or MC ($P = .045$), whereas MC patients showed significantly lower CD15 positivity ($P = .002$). Furthermore, EBV was harbored in 75% of MC patients, which is significantly higher than the ratios for NS and NS2 (13% and 53%, respectively; $P < .0001$).

Phenotypic Distribution of CHL

Based on the immunohistochemically recognizable features of the H-RS cell, the present series of CHL patients were delineated into four phenotypic groups, as summarized in Table 2. The first group included 63 patients with the B-cell phenotype with expression of CD20 or CD79a. The second group included 27 patients with the T/CM phenotype with expression of CD3, CD4, CD8, CD45RO, and/or CMs such as TIA-1 and granzyme B (Fig 1), but not CD20, CD79a. The third group included 22 patients with the FDC phenotype with expression of CD21, but not any of the other B- or T-cell markers. The fourth group included 212 patients with the null-cell phenotype without expression of the B-cell, T-cell, or FDC-related markers. In the T/CM group, the expression of CMs was found in 20 patients, five of whom lacked the other T-cell markers. All patients in this T/CM group were also negative for ALK1 by additional immunohistochemical staining.

Clinicopathologic characteristics of these four immunophenotypic groups are summarized in Table 3. On comparison, patients in the T/CM group had a younger onset (median age, 44 years; $P = .048$), higher ratio of females (male- to-female ratio, 1.25), and lower ratio of EBV on H-RS cells (35%; $P = .025$).

Moreover, the present series of CHL patients could be categorized into two phenotypic groups, $CD15^+$ and $CD15^-$, with CD15 expression identified in 202 (63%) of the 319 patients examined.

Table 2. Phenotypic Distribution of Classical Hodgkin's Lymphoma

	B-Cell Group		T/CM Group		FDC Group		Null-Cell Group	
Characteristic	No.	%	No.	%	No.	%	No.	%
Total No. of patients	63	20	27	8	22	7	212	65
Immunophenotype*								
CD3	0	—	4 of 21	19	0	—	0	—
CD4	0	—	4 of 16	25	0	—	0	—
CD8	0	—	2 of 16	13	0	—	0	—
CD45RO	0	—	6 of 22	27	0	—	0	—
TIA-1	0	—	12 of 26	46	0	—	0	—
Granzyme B	0	—	16 of 27	59	0	—	0	—
CD20	54 of 63	86	0	—	0	—	0	—
CD79a	12 of 20	60	0	—	0	—	0	—
CD21	0	—	0	—	22 of 22	100	0	—

Abbreviations: T/CM, T-cell and/or cytotoxic molecules; FDC, follicular dendritic cell.
*No. positive of No. tested patients.

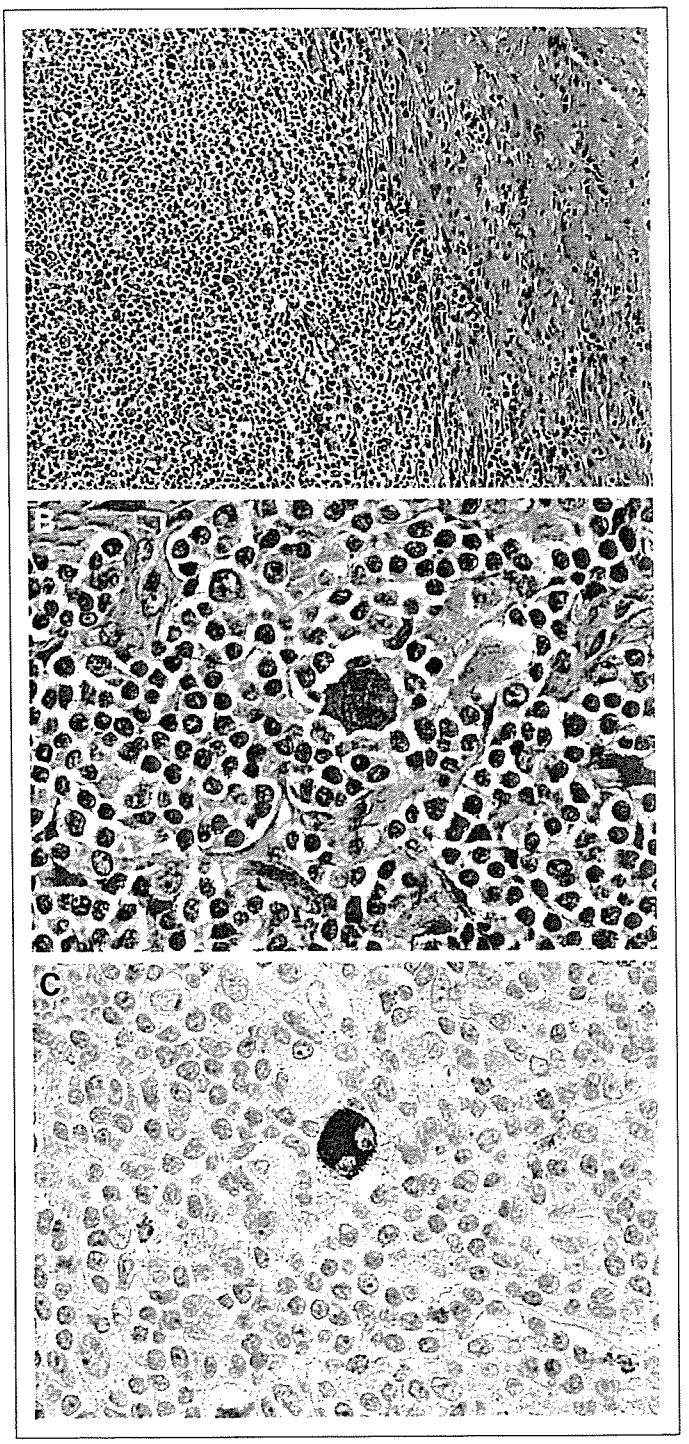

Fig 1. Classical Hodgkin's lymphoma (CHL) with T-cell and/or cytotoxic molecule expression. (A) T-cell and/or cytotoxic molecule–positive CHL patient sample shows fibrous collagen bands dividing the lymph node into nodules and is categorized as nodular sclerosis (original magnification ×40). (B) Reed-Sternberg cells are present (original magnification ×400) and (C) are immunoreactive for granzyme B (original magnification ×400).

Comparison of these patients revealed no clinical differences between them (data not shown). Seven patients showing the $CD15^-$ and $CD30^-$ phenotype were diagnosed on the basis of the morphology, and immunophenotype of the absence of B- or T-cell markers and positivity of Fascin.

EBV Distribution in CHL

EBV was detected in 149 of 314 (47%) patients, with no association seen with histopathologic group. The EBV-positive group was characterized by a higher ratio of males and an older age of onset than the EBV-negative group. CD20 expression was more frequently detected in the EBV-positive group ($P = .025$).

Therapeutic Response

A total of 183 patients received combination chemotherapy consisting of first-line treatment regimens as follows: doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD; 146 patients); cyclophosphamide, vincristine, procarbazine, and prednisone (15 patients); bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone (six patients); and cyclophosphamide, doxorubicin, vincristine, and prednisone (16 patients; Table 3). Ninety-four patients received radiation therapy, and 88 received both chemotherapy and radiation. In 106 patients with stage I/II disease, 78 patients (74%) received ABVD-based chemotherapy and six underwent radiation therapy only. No significant differences in treatment types were seen among phenotypic subgroups. In total, 77% patients (134 of 174) with CHL achieved a complete response with the initial therapy. Notably, the T/CM group showed a lower complete response rate (58%) and a higher no response rate (16%) than the other three groups.

Survival

DSS curves of the NS, NS2, and MC patients showed no significant differences among them. In Figure 2A, however, the DSS curves of the four phenotypic groups based on immunohistochemical evaluation revealed a significant difference ($P = .0041$). In the 139 patients who received ABVD-based chemotherapy, the survival rate of the T/CM-positive CHL patients was significantly poorer than that of the others ($P < .0001$; Fig 2B), and five patients showed an aggressive clinical course within 24 months of diagnosis. Median survival of stage I and II patients was 55 and 27 months, respectively. Two patients with stage I/II disease expressing the T/CM phenotype died within 12 months. Survival of the B-cell group tended to be relatively inferior to that of the null-cell group, but without statistical significance (data not shown). Finally, patients with EBV-positive CHL showed a tendency to poor prognosis compared with EBV-negative patients, but without significance by the log-rank test ($P = .11$).

Prognostic Factors

Univariate analysis identified 13 prognostic factors for the 288 patients of the entire series of CHL patients: phenotype (T/CM type; $P = .001$), serum albumin less than 4.0 g/dL ($P = .001$), performance status more than 1 ($P = .001$), and advanced clinical stage (III/IV; $P = .021$). The International Prognostic Factor Project (IPFP) score (≥ 5) also showed prognostic significance ($P = .003$). Hemoglobin level less than 10.5 g/dL, age older than 45 years, and lymphocyte count less than 600/μL showed marginal significance, whereas histologic profile (NS2) was not significant (Table 4).

Multivariate analysis with individual factors showed phenotype (T/CM type: HR, 3.97; 95% CI, 1.85 to 8.48; $P < .0001$) and age older than 45 years (HR, 2.55; 95% CI, 1.23 to 5.29; $P = .012$) to be significant and independent prognostic factors in the 228 CHL patients. In the 139 patients who received ABVD-based chemotherapy, T/CM phenotype was a significant and independent prognostic factor. Moreover, T/CM phenotype also influenced survival significantly in advanced CHL patients, independent of IPFP score (Table 4).

Table 3. Clinical Characteristics According to Phenotype

Characteristic	B-Cell Group (n = 63) No.	%	T/CM Group (n = 27) No.	%	FDC Group (n = 22) No.	%	Null-Cell Group (n = 212) No.	%	P*
Sex									.35
Male	42		15		17		149		
Female	21		12		5		63		
Ratio	2.0		1.25		3.4		2.37		
Age, years									.048
Median	57		44		55		46		
Range	9-89		13-84		16-82		9-88		
> 50	38	60	11	41	14	64	88	42	.019
PS > 1	7	11	8	30	5	23	33	16	.14
Clinical stage III/IV	21	33	12	44	12	55	90	43	.33
B symptoms	16	31	10	40	10	53	67	37	.43
Bulky mass	5	10	4	15	5	25	31	17	.43
Extranodal > 1 site	6	13	6	24	1	5	24	14	.34
WBC > 15,000/μL	1	3	4	19	4	25	20	14	.11
Hb < 10.5 g/dL	5	14	6	29	5	33	39	28	.33
Serum albumin < 4.0 g/dL	13	39	13	65	6	55	63	55	.28
LDH > normal	9	32	6	29	4	40	61	52	.094
Treatment									
Type of chemotherapy									.15
ABVD	23	66	9	41	9	64	77	58	
ABVD/C-MOPP	3	8	3	14	5	36	17	13	
C-MOPP	1	3	3	14	0	0	11	8	
BEACOPP	0	0	1	5	0	0	5	4	
CHOP	6	17	3	13	0	0	7	5	
Other	2	6	3	13	0	0	16	12	
Chemotherapy only	22	61	11	48	9	56	74	51	
Chemotherapy and RT	13	36	11	48	5	31	59	41	
RT only	0	0	0	0	0	0	6	4	
Observation	1	3	1	4	2	13	5	4	
Response to combination chemotherapy†									.22
CR	26	81	11	58	11	85	86	78	
PR	6	19	5	26	2	15	17	16	
NR	0	0	3	16	0	0	7	6	
Relapse/progressive disease	8	23	13	59	5	38	54	40	.054
Survival, months									.0041
Median	21.9		15.4		56.0		28.3		
Range	1.2-142+		4.5-145		7.5-163+		2.0-254+		

Abbreviations: T/CM, T-cell and/or cytotoxic molecules; FDC, follicular dendritic cell; PS, performance status; Hb, hemoglobin; LDH, lactate dehydrogenase; ABVD, doxorubicin, bleomycin, vinblastine, and dacarbazine; C-MOPP, cyclophosphamide, vincristine, procarbazine, and prednisone; BEACOPP, bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; RT, radiation therapy; CR, complete response; PR, partial response; NR, no response.

*X^2 test for independence, or Fisher's exact probability test, B *v* T/CM *v* FDC *v* null.

†ABVD, ABVD/C-MOPP, C-MOPP, BEACOPP, or CHOP.

DISCUSSION

Our study in 324 consecutive patients with Hodgkin's lymphoma had three major findings. First, among the four phenotypic subclassifications (B-cell, T/CM, FDC, and null-cell groups), the T/CM group had a significantly poorer prognosis in uni- and multivariate analyses. To our knowledge, this is the first study to report the prognostic significance of this factor. Second, among the histopathologic groups (NS, NS2, and MC) of CHL, no significant differences were found in clinical features, except age at onset and sex ratio. Finally, EBV positivity was more prevalent in MC, occurred mostly in older men, and was not identified as an independent prognostic factor.

T-cell marker and/or CM expression has been demonstrated immunohistochemically on H-RS cells in approximately 5% to 20% of CHL patients, although there is little information in the literature regarding the clinicopathologic significance of their expression. In our series, T/CM marker expression was detected in 27 (8%) of 324 CHL patients, and was significantly associated with an adverse prognosis.

Genotypic evidence from several groups has indicated that the expression of T-cell phenotype on H-RS cells is aberrant.[10,17] Consistent findings regarding T-cell marker positivity and its prognostic significance have been reported.[17] In one report, however, the proportion of T-cell marker expression was low.[10] Conversely, CM positivity was reported in 10% to 18% of CHL patients.[18,19] Our relatively lower percentage (6%) of cytotoxic phenotype in CHL patients might have been influenced by the exclusion of borderline cases, which posed a problem in differential diagnosis from Hodgkin's-like ALCL under the Revised European-American Lymphoma classification.[13]

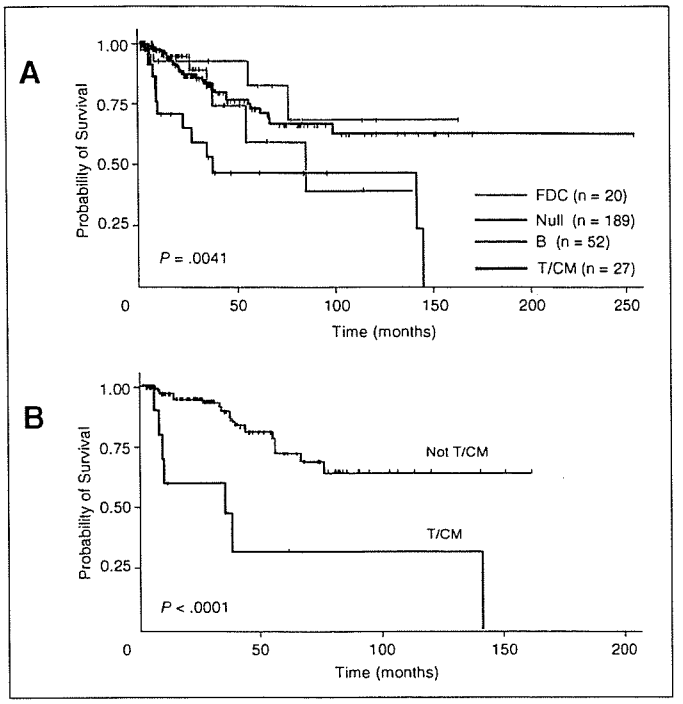

Fig 2. Survival data for four subgroups by phenotypic differentiation (B cell, T cell and/or cytotoxic molecules [T/CM], null cell, follicular dendritic cells [FDC]) in classical Hodgkin's lymphoma. (A) Disease-specific survival according to four phenotypic groups. (B) Prognosis of patients with the T/CM phenotype (———) is significantly poorer than that of those without this phenotype (– – – –) in classical Hodgkin's lymphoma patients who received chemotherapy with doxorubicin, bleomycin, vinblastine, and dacarbazine.

We reported previously that CM expression has an independent prognostic impact associated with unfavorable survival in nodal peripheral T-cell lymphoma, unspecified.[15] Moreover, TIA-1 and/or granzyme B expression on Hodgkin's-like ALCL was significantly associated with an adverse prognosis (Asano et al, submitted for publication). These data suggest that the expression of CMs may be predictive of the overall survival of CHL patients. The case of a CHL patient with evidence of clonal T-cell receptor γ (TCR-γ) gene rearrangement who had considerably shorter disease-specific survival has been reported.[17] Studies of TCR-γ rearrangement in H-RS cells have been technically challenging. A clonal TCR-γ chain gene was undetected in any of the patients with successful amplification of DNA by polymerase chain reaction analysis. This finding indicates that few patients with the T/CM phenotype have CHL of possible T-cell origin, although problems may have existed in the sensitivity of TCR-γ gene detection. The biologic significance of T/CM expression in CHL without genetic evidence of T-cell origin remains to be elucidated. These issues warrant additional investigation.

According to the WHO classification, histopathologic grouping in CHL is made in consideration of background inflammatory cells, including lymphocytes, plasmacytes, histiocytes, and eosinophils. In this study, we compared these morphologic groups (NS, NS2, and MC) in terms of clinical characteristics and survival, but found no significant differences among them, except for a younger age at onset and higher ratio of females in NS. As reported previously,[14] the present MC group was characterized by a higher ratio of positivity for EBV compared with the NS group.

The clinicopathologic significance of EBV as a prognosticator in CHL patients is still controversial.[20-26] Several recent studies have documented a marked survival disadvantage in older EBV-positive CHL patients compared with EBV-negative patients.[21,22] In our study, however, no significant survival difference was seen between EBV-positive and -negative patients. These results conflict with those reported by others, but the clinical features of our EBV-positive patients were compatible with those reported previously.[20,23,24]

The prognostic significance of B-cell or FDC marker in CHL is also controversial.[27] In this study, the expression of B-cell and FDC markers was detected in 20% and 7% of CHL cases, respectively. The B-cell group showed a relatively unfavorable clinical course compared with the null-cell group, whereas that of the FDC group was relatively favorable. These results may be in keeping with a recent report which identified the FDC marker as an independent favorable prognostic factor for overall survival in patients with diffuse large B-cell lymphoma.[28]

Clinical prognostic factors for CHL have been studied by Hasenclever et al.[29] They showed that the IPFP score is useful in

Table 4. Cox Proportional Hazards Model HR and 95% CI Estimates for Death As a Result of Lymphoma-Related Causes in Patients With CHL

		Univariate			Multivariate Total CHL			Multivariate ABVD Therapy Group			Multivariate Advanced CHL		
Variables	Unfavorable Factors	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Phenotype	T/CM	3.07	1.61 to 5.86	.001	3.97	1.85 to 8.48	< .0001	9.23	3.17 to 20.9	< .0001	2.62	1.05 to 6.50	.038
Serum albumin	< 4.0 g/dL	3.83	1.69 to 8.68	.001	2.32	0.95 to 5.70	.066	2.31	0.73 to 7.26	.15		—	
Performance status	> 1	2.64	1.46 to 4.78	.001	1.57	0.76 to 3.27	.22	2.31	0.88 to 6.08	.09		—	
Stage	III/IV	1.94	1.10 to 3.41	.021	1.37	0.64 to 2.94	.42	1.84	0.68 to 4.97	.23		—	
Hemoglobin	< 10.5 g/dL	1.79	0.99 to 3.21	.052	1.25	0.60 to 2.61	.56	1.08	0.40 to 2.88	.88		—	
Age	> 45 years	1.71	0.98 to 2.96	.058	2.55	1.23 to 5.29	.012	1.72	0.65 to 4.55	.28		—	
Lymphocyte count	< 600/μL	2.24	0.94 to 5.32	.068	1.45	0.58 to 3.60	.43	1.25	0.27 to 5.93	.78		—	
EBV	Positive	1.59	0.90 to 2.78	.11		—			—			—	
WBC	> 15,000/μL	1.76	0.69 to 4.47	.23		—			—			—	
Histology	NS2	1.49	0.73 to 3.06	.27		—			—			—	
CD15	Negative	1.38	0.78 to 2.45	.28		—			—			—	
Sex	Male	1.11	0.61 to 2.03	.72		—			—			—	
IPFP score	5 or more	3.18	1.48 to 6.85	.003		—			—		2.73	1.19 to 6.24	.018

Abbreviations: HR, hazard ratio; CI, confidence interval; CHL, classical Hodgkin's lymphoma; ABVD, doxorubicin, bleomycin, vinblastine, and dacarbazine; T/CM, T-cell and/or cytotoxic molecules; EBV, Epstein-Barr virus; NS2, nodular sclerosis grade 2; IPFP, International Prognostic Factor Project.

determining the prognosis of advanced CHL, and in clinical decision making for individual patients. In the present study, and consistent with other findings,[30] the IPFP score was found to have prognostic significance in CHL. Moreover, among patients with early-stage (I/II) CHL, those with an IPFP score of 3/4 showed a poorer prognosis than those with low-risk score (< 3), although there were no patients with a high IPFP score (5 or more) in the stage I/II patients (data not shown). One notable consideration is that T-cell or cytotoxic phenotype remained a significant prognostic factor even after adjustment for IPFP score.

Compared with Western CHL reports, the patients in this study were characterized by a low NS rate, low CD15 positivity, and poor prognosis.[14,27,31] According to these findings, the patients may have included far fewer NS cases with a favorable prognosis and $CD15^+$ $CD30^+$ phenotype than in these Western studies. However, the T/CM phenotypic appearance of H-RS cells is present in Western as well as Japanese patients,[10,17-19] possibly indicating that the T/CM phenotype in CHL carries a poor prognosis in both Western and Asian patients.

In conclusion, we demonstrated that patients with CHL with the T/CM phenotype have a significantly poorer prognosis than those with the other phenotypic groups. Examination of T-cell markers in CHL patients is recommended as a routine pathologic practice.

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Appendix

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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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