Table 2. Prognostic factors affecting overall survival of total entry series

Variables	Unfavorable factors	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P
Comparison with risk factors					
EBV	Positive	3.5 (2.3-5.5)	<0.0001	2.5 (1.5-4.1)	0.001
B symptom	Present	3.2 (2.0-5.1)	<0.0001	2.0 (1.2-3.5)	0.008
LDH	>normal	2.6 (1.6-4.1)	<0.0001	2.0 (1.2-3.4)	0.011
PS	2-4	2.4 (1.6-3.8)	<0.0001	—	—
Age	>60 y	2.0 (1.2-3.1)	0.006	—	—
Stage	III/IV	1.8 (1.1-2.8)	0.010	—	—
Extranodal disease	>1 site	1.5 (0.9-2.3)	0.083	—	—
Comparison with IPI category					
IPI	HI/H	2.1 (1.4-3.3)	0.001	2.0 (1.3-3.1)	0.003
EBV	Positive			3.3 (2.1-5.3)	<0.0001

Abbreviations: CI, confidential interval; LDH, lactate dehydrogenase; PS, performance status; IPI, International Prognostic Index.

years (P = 0.0008), the presence of B symptoms (P = 0.0058), and LDH level equal to or more than normal value (P = 0.040). Clinical stage, PS, and extranodal involvement of more than one site were nonsignificant factors. In multivariate analysis, the factors that turned out to correlate significantly with survival were B symptom (P = 0.0026) and age (P = 0.0045). Because the relative risk associated with each of the two factors was comparable, we constructed a prognostic model by combining these prognostic variables in the following way: patients with a score of 0 (n = 18), no adverse factors; patients with a score of 1 (n = 39), one factor; and patients with a score of 2, two factors (n = 21). This prognostic model for age-related EBV+ B-cell LPDs was able to efficiently identify three groups of patients with different outcomes (Fig. 3*B*; P < 0.0001). For the patients with scores of 0, 1, and 2, the median overall survival times were 56.3, 25.2, and 8.5 months, respectively.

Discussion

We recently have documented 22 cases named as senile EBV-associated B-cell LPDs arising in elderly patients aged ≥60 years without predisposing immunodeficiencies, suggesting that this disease has a relationship with an immunologic deterioration derived from the aging process (6). Among 1,792 large B-cell LPD cases examined by EBERs *in situ* hybridization, 156 cases harbored EBV without underlying immunodeficiency-related diseases. This larger series revealed that 149 (96%) of these patients are more than 40 years of age, the increasing positive percentages of which were observed in parallel with the elder patient populations (≥40 years) for all cases examined and reached the highest peak at ages ≥90 years. These data provided additional evidence that EBV-positive B-cell LPDs without predisposing immunodeficiency mainly occur in elderly patients, although seven patients were found to be <40 years of age. Considering these rare cases, the term of "age related" may be more appropriate than that of senile for further understanding the overall age distribution of EBV-positive B-cell LPDs without predisposing immunodeficiency.

This study was predominantly a comparison of clinical features in age-related EBV+ B-cell LPDs and EBV-negative DLBCLs. An analysis of 96 patients with age-related EBV-positive B-cell LPDs, in which the clinical data were available, highlighted the clinical features of this disease—high age at onset, frequent association with poor prognostic components of IPI, and aggressive clinical course. These features were significantly different from those of EBV-negative DLBCL besides more frequent involvement of the skin, supporting the concept that age-related EBV-associated B-cell LPDs constitute a distinct disease with a broad spectrum. However, it could not be definitively concluded whether this disease

Table 3. Prognostic factors affecting overall survival of age-related EBV-positive B-cell LPDs

Variables	Unfavorable factors	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P
B symptoms	Present	2.3 (1.3-4.3)	0.0058	2.6 (1.4-4.8)	0.0026
Age	>70 y	2.4 (1.4-4.3)	0.0008	2.5 (1.3-4.8)	0.0045
LDH	>normal	1.9 (1.0-3.4)	0.040	—	—
Stage	III/IV	1.8 (1.0-3.2)	0.062	—	—
PS	2-4	1.2 (0.7-2.1)	0.57	—	—
Extranodal disease	>1 site	1.3 (0.7-2.3)	0.38	—	—
IPI category	HI/H	1.8 (1.0-3.2)	0.064	—	—

Abbreviations: LPDs, lymphoproliferative disorders; CI, confidence interval; EBV, Epstein-Barr virus; LDH, lactate dehydrogenase; PS, performance status; IPI, International Prognostic Index.

represented a heterogeneous group of disorders including several lymphoma subtypes.

The morphologic spectrum of age-related EBV+ B-cell LPDs seems to be broader than has been previously realized (data not shown). This disease comprised a spectrum ranging from polymorphic proliferation, sometimes suggestive of a reactive process, to large-cell lymphomas mostly consisting of transformed cells and, therefore, was subdivided into two subtypes, i.e., polymorphic and large-cell lymphomas, based on morphology and conventional immunophenotyping in our previous report (6). However, in the present study, we failed to show any statistical difference in the clinical profiles between these two subgroups. Indeed, several cases had areas that seem more monomorphic in the same or other tissues, thus indicating a continuous spectrum between polymorphic and large-cell lymphoma subtypes. The results that we found in the histologic subgrouping of age-related EBV+ B-cell LPDs seemed to parallel those of the post-transplant LPDs, in which current classification schemes are not fully predictive of prognosis (15, 21). Further investigation should be done to refine the distinction of age-related EBV+ B-cell LPDs into more homogeneous categories with prognostic relevance.

The prognosis of age-related EBV+ B-cell LPDs was significantly poorer than that of EBV-negative tumors. One possible explanation is that the EBV association as a biological marker seemed to be closely associated with the higher IPI index because 35% of patients with this disease were categorized in the high-risk IPI group, which is higher than 15% of the present series of EBV-negative DLBCL or 19% of DLBCL reported by the Non-Hodgkin's Lymphoma Classification project (22, 23). The other is the age distribution and performance status of the patients (Table 1). Due to higher age or poorer PS, many patients with age-related EBV-positive B-cell LPDs might not maintain the intensity of chemotherapy. However, subgroup analyses by age or the IPI also showed that age-related EBV-positive B-LPDs had lower CR rate and inferior overall survival compared with EBV-negative DLBCLs. Multivariate analysis in all cases further identified EBV association and IPI category as an independent prognostic factor. These findings emphasized that age-related EBV-positive B-cell LPDs merits separate consideration because of the diagnostic and therapeutic problems it poses.

Indeed, in multivariate analysis, two host-related factors, i.e., age older than 70 years and the presence of B symptoms, were prognostically significant. In the present series of age-related EBV+ B-cell LPDs, the IPI scoring system did not seem to work with the same efficacy as in DLBCLs for identifying subsets of patients with different prognoses. However, the extension of the disease (clinical stage and extranodal involvement of more than one site) and the biology or cell turnover of the tumor (LDH level) were no longer significant. These findings further supported our assertion that this disease is distinct from DLBCLs and significantly influenced by the host immune status in outcome of patients. Our prognostic model based on the two simple clinical variables of age older than 70 years and the presence of B symptoms also seemed to better define the clinical outcome of age-related EBV+ B-cell LPDs categorized as a single group with an overall superior predictive capacity as compared with IPI (log-rank, 0.0002 versus 0.1). Of course, an external validation study should be done on the larger series of cases in the future.

It is presumed that the pathogenesis of age-related EBV-positive B-cell LPDs has a close relation with an immunologic deterioration or senescence in immunity derived from the aging process because this disease seemed analogous in many respects to that immunodeficiency-associated LPDs, such as EBV association, waxing and waning of disease, and polymorphic proliferation of large bizarre B cells (16). Aging in humans is known to be associated with impaired immune status such as increased infections, the more global phenomenon termed "immune senescence" (24). Indeed, in the present series, 28% of the age-related EBV+ B-cell LPD cases examined were immunohistochemically positive for EBNA2, indicating the reduced immunity to EBV, i.e., type III latency which is believed to occur only in the setting of profound immunodeficiency (25). EBV DNA in peripheral blood mononuclear cells was more frequently detected in healthy individuals older than 70 years of age (8 of 9, 89%) than in ones <70 years (1 of 11, 9%) using real-time PCR (26). Yanagi et al. also showed that EBNA-2 IgG antibodies evoked in young children by asymptomatic primary EBV infections remain elevated throughout life using sera, suggesting the intervention of reactivation of latent and/or exogenous EBV superinfection (27). These data provided additional support on the speculation that age-related decline in immunity may be contributing to the pathogenesis of age-related EBV+ B-cell LPDs.

Biological interfaces may be assumed between age-related EBV+ B-cell LPDs and other EBV-associated B-cell neoplasms such as lymphomatoid granulomatosis and plasmablastic lymphoma, the distinction of which is currently based on the constellation of clinical, morphologic, and immunophenotypic features (28, 29). In our series, nine cases showed pulmonary involvement and four ones had gingival lesions at presentation, posing the differential diagnostic problems from lymphomatoid granulomatosis and plasmablastic lymphoma, respectively, although they were not prototypic in morphology as the latter two. Classic Hodgkin lymphoma (CHL) is also well known to have EBV harboring in 30% to 50% of the cases with achieving a general consensus of the B-cell derivation of the H-RS cells in most (30, 31). Interestingly, three population-based studies of Clarke et al. (32), Stark et al. (33), and more recently, Jarrett et al. (34), without selection bias documented that a marked survival disadvantage in older EBV-positive CHL patients as compared with EBV-negative CHL cases, which was contrasted with no effect of EBV status on the clinical outcome of HL patients selectively enrolled in clinical trails, with a tendency of their relatively younger age distribution (35, 36). As the interpretation for this age-related influence of EBV on clinical outcome of CHL patients, Gandhi et al. (37) and Jarrett et al. (34) clearly indicated that a decline in cellular immunity to EBV with age may contribute to the pathogenesis of EBV+ CHL in older patients. This standpoint is tempting to speculate that EBV+ CHL and age-related EBV+ B-cell LPDs may constitute a continuous spectrum. Our study may also raise an even more fundamental question: whether biological properties, such as an interaction or balance between latent EBV infection and host immunity, precede the morphologic and immunophenotypic evaluation for further understanding the overall clinicopathologic profiles of EBV-associated B-cell LPDs and/or lymphomas. Much still needs to be learned about the detailed clinicopathologic

features, the immunology, and the molecular biology of these diseases in a further study.

Innovative therapeutic strategies such as immunotherapy against EBV should be explored for age-related EBV+ B-cell LPD patients (38, 39), because conventional combination chemotherapy had only a limited effect in an analysis of this larger series. For poor risk patients with aggressive lymphomas such as DLBCL, the superiority of high dose chemotherapy with stem cell support over conventional method is now under confirmation (40–42). This therapeutic approach may not, however, be suitable for age-related EBV+ B-cell LPDs because the older age distribution of the patients, many (70%) of which were more than 65 years old, made the application of high-dose chemotherapy difficult enough. Rituximab is a non-cytotoxic drug that showed efficacy when adding to cyclophosphamide-Adriamycin-vincristine-prednisone (CHOP) on elderly patients with DLBCL (43). In our present series, only one case was documented to have received chemotherapy combined with rituximab for an initial treatment, preliminarily providing a good efficacy of this agent on age-related EBV+ B-cell LPD. Now, we are conducting prospective clinical trials to test the efficacy of chemotherapy with rituximab as a multi-institutional study on age-related EBV+ B-cell LPD patients.

In conclusion, the current study elucidates that age-related EBV-associated B-cell LPDs constitute a distinct clinicopathologic group in contrast with EBV-negative DLBCLs, in which conventional chemotherapy has a limited efficacy for this disease. A study to test the efficacy of rituximab with chemotherapy for age-related EBV+ is now ongoing. In the future, less toxic treatment strategy such as a cell therapy for EBV-specific viral antigens will be needed and should be evaluated in clinical trials.

Acknowledgments

The authors are grateful to Dr. Masao Seto for his scientific discussion and encouragement to prepare this manuscript.

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VOLUME 24 · NUMBER 28 · OCTOBER 1 2006

JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

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

Naoko Asano, Aya Oshiro, Keitaro Matsuo, Yoshitoyo Kagami, Fumihiro Ishida, Ritsuro Suzuki, Tomohiro Kinoshita, Yoshie Shimoyama, Jun-Ichi Tamaru, Tadashi Yoshino, Kunio Kitamura, Hisashi Fukutani, Yasuo Morishima, and Shigeo Nakamura

From the Department of Pathology and Molecular Diagnostics, Division of Epidemiology and Prevention, and Department of Hematology and Chemotherapy, Aichi Cancer Center; Department of HSCT Data Management, Department of Hematology, and Department of Pathology and Clinical Laboratories, Nagoya University, Nagoya; Department of Internal Medicine, Shinshu University School of Medicine, Matsumoto; Department of Pathology, Saitama Medical Center, Saitama Medical School, Kawagoe; Department of Pathology, Faculty of Health Sciences, Okayama University, Okayama; Department of Internal Medicine, Ichinomiya Municipal Hospital, Ichinomiya; and Department of Internal Medicine, Aichi Hospital, Aichi Cancer Center, Okazaki, Japan.

Submitted March 9, 2006; accepted July 27, 2006; published online ahead of print at www.jco.org on September 5, 2006.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Address reprint requests to Naoko Asano, MD, Department of Pathology and Molecular Diagnostics, Aichi Cancer Center, 1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan; e-mail: nasano@aichi-cc.jp.

0732-183X/06/2428-4626/$20.00

DOI: 10.1200/JCO.2006.06.5342

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.

J Clin Oncol 24:4626-4633. © 2006 by American Society of Clinical Oncology

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.

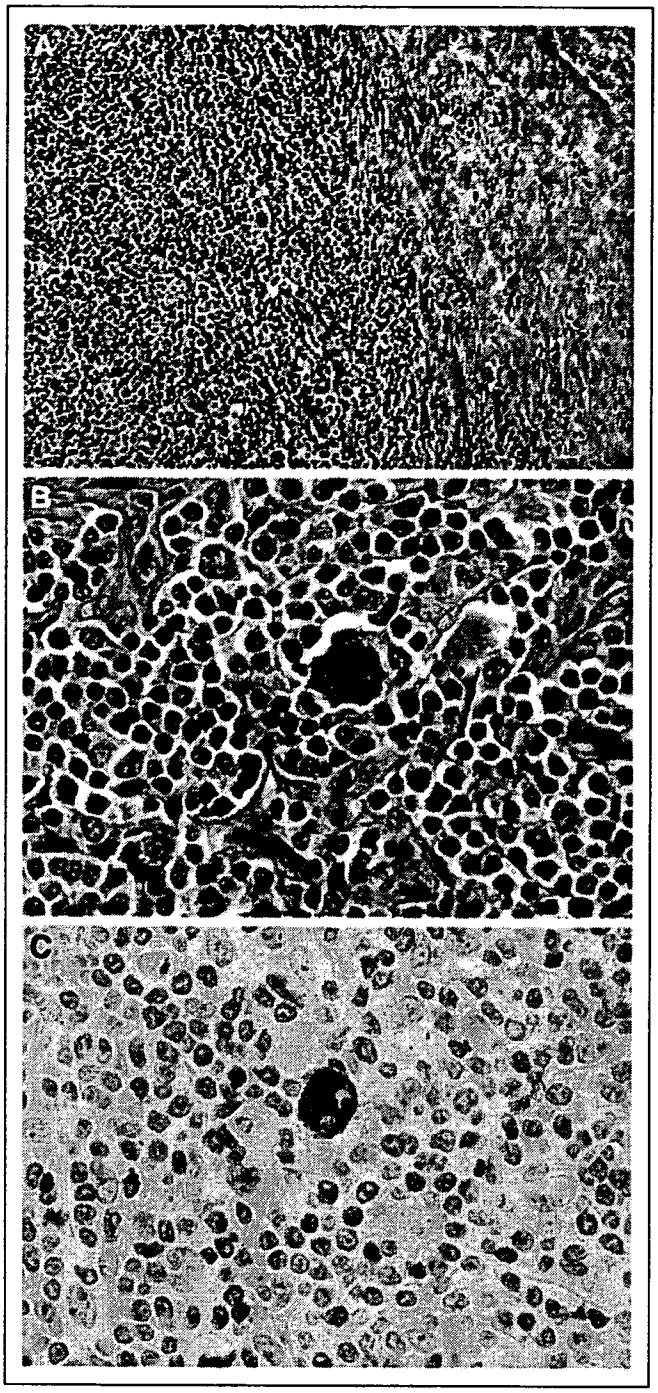

Fig 1. Classical Hodgkin's lymphoma (CHL) with T-cell and/or cytotoxic molecule expression. (A) T-cell and/or cytotoxic molecule–positive CHL patient sample shows fibrous collagen bands dividing the lymph node into nodules and is categorized as nodular sclerosis (original magnification ×40). (B) Reed-Sternberg 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)		T/CM Group (n = 27)		FDC Group (n = 22)		Null-Cell Group (n = 212)		P*
	No.	%	No.	%	No.	%	No.	%	
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.

*χ^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]

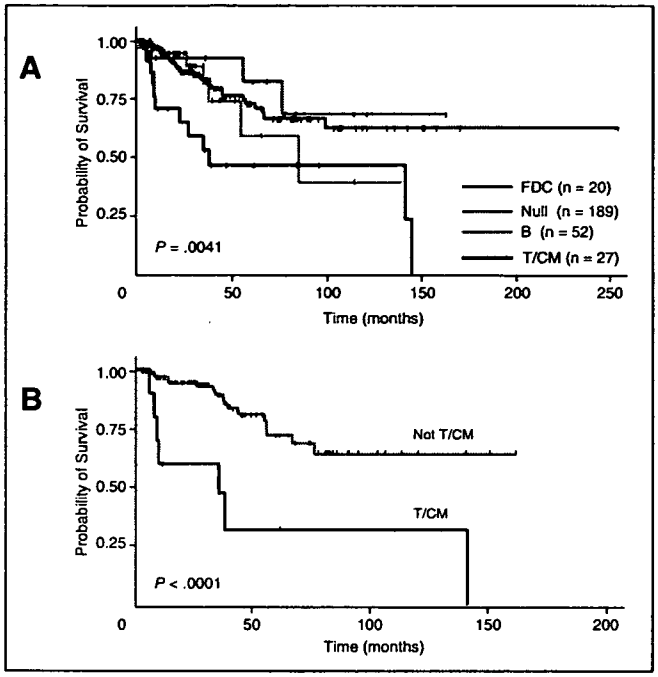

Fig 2. Survival data for four subgroups by phenotypic differentiation (B cell, T cell and/or cytotoxic molecules [T/CM], null cell, follicular dendritic cells [FDC]) in classical Hodgkin's lymphoma. (A) Disease-specific survival according to four phenotypic groups. (B) Prognosis of patients with the 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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Acknowledgment

We thank H. Ishida for technical assistance.

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.

Author Contributions

Conception and design: Naoko Asano, Shigeo Nakamura
Administrative support: Yasuo Morishima, Shigeo Nakamura
Provision of study materials or patients: Naoko Asano, Aya Oshiro, Yoshitoyo Kagami, Tomohiro Kinoshita, Yoshie Shimoyama, Kunio Kitamura, Hisashi Fukutani, Yasuo Morishima, Shigeo Nakamura
Collection and assembly of data: Naoko Asano, Aya Oshiro, Jun-Ichi Tamaru, Tadashi Yoshino, Shigeo Nakamura
Data analysis and interpretation: Naoko Asano, Keitaro Matsuo, Fumihiro Ishida, Ritsuro Suzuki, Shigeo Nakamura
Manuscript writing: Naoko Asano, Keitaro Matsuo, Shigeo Nakamura
Final approval of manuscript: Naoko Asano, Aya Oshiro, Keitaro Matsuo, Yoshitoyo Kagami, Fumihiro Ishida, Ritsuro Suzuki, Tomohiro Kinoshita, Yoshie Shimoyama, Jun-Ichi Tamaru, Tadashi Yoshino, Kunio Kitamura, Hisashi Fukutani, Yasuo Morishima, Shigeo Nakamura

VOLUME 25 · NUMBER 34 · DECEMBER 1 2007

JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

VCAP-AMP-VECP Compared With Biweekly CHOP for Adult T-Cell Leukemia-Lymphoma: Japan Clinical Oncology Group Study JCOG9801

Kunihiro Tsukasaki, Atae Utsunomiya, Haruhiko Fukuda, Taro Shibata, Takuya Fukushima, Yoshifusa Takatsuka, Shuichi Ikeda, Masato Masuda, Haruhisa Nagoshi, Ryuzo Ueda, Kazuo Tamura, Masayuki Sano, Saburo Momita, Kazunari Yamaguchi, Fumio Kawano, Shuichi Hanada, Kensei Tobinai, Masanori Shimoyama, Tomomitsu Hotta, and Masao Tomonaga

From the Nagasaki University, Nagasaki; Imamura Bun-in Hospital; Kagoshima University, Kagoshima; National Cancer Center Research Institute; National Cancer Center Hospital, Tokyo; Sasebo City General Hospital, Sasebo; Ryukyu University, Nishihara; St Marianna University, Yokohama; Nagoya City University; National Hospital Organization Nagoya Medical Center, Nagoya; Fukuoka University, Fukuoka; Saga University, Saga; National Hospital Organization Nagasaki Medical Center, Ohmura; Kumamoto University; and National Hospital Organization Kumamoto Medical Center, Kumamoto, Japan.

Submitted April 20, 2007; accepted September 6, 2007; published online ahead of print at www.jco.org on October 29, 2007.

Supported by Grants-in-Aid No. 2S-1, 5S-1, 8S-1, 11S-1, 14S-1, 17S-1, 1-1, 4-5, 7-29, and 9-10 from the Cancer Research from the Ministry of Health, Labor and Welfare of Japan (1990 to present), for the Second-Term Ten-Year Strategy for Cancer Control from the Ministry of Health and Welfare (1994 to 2004) and for Basic Research from the Science and Technology Agency (1991 to 1993).

Presented in part at the 47th Annual Meeting of the American Society of Hematology, December 10-13, 2005, Atlanta, GA.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Address reprint requests to Kunihiro Tsukasaki, MD, PhD, Nagasaki University Graduate School of Biomedical Science, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan; e-mail: tsukasak@net.nagasaki-u.ac.jp.

0732-183X/07/2534-5458/$20.00

DOI: 10.1200/JCO.2007.11.9958

ABSTRACT

Purpose
Our previous phase II trial for treating human T-lymphotropic virus type I–associated adult T-cell leukemia-lymphoma (ATLL) with vincristine, cyclophosphamide, doxorubicin, and prednisone (VCAP), doxorubicin, ranimustine, and prednisone (AMP), and vindesine, etoposide, carboplatin, and prednisone (VECP) showed promising results. To test the superiority of VCAP-AMP-VECP over biweekly cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP), we conducted a randomized controlled trial exclusively for ATLL.

Patients and Methods
Previously untreated patients with aggressive ATLL were assigned to receive either six courses of VCAP-AMP-VECP every 4 weeks or eight courses of biweekly CHOP. Both treatments were supported with granulocyte colony-stimulating factor and intrathecal prophylaxis.

Results
A total of 118 patients were enrolled. The complete response (CR) rate was higher in the VCAP-AMP-VECP arm than in biweekly CHOP arm (40% *v* 25%, respectively; $P = .020$). Progression-free survival rate at 1 year was 28% in the VCAP-AMP-VECP arm compared with 16% in the CHOP arm ($P = .100$, two-sided $P = .200$). Overall survival (OS) at 3 years was 24% in the VCAP-AMP-VECP arm and 13% in the CHOP arm ($P = .085$, two-sided $P = .169$). For VCAP-AMP-VECP versus biweekly CHOP, grade 4 neutropenia, grade 4 thrombocytopenia, and grade 3 or 4 infection rates were 98% *v* 83%, 74% *v* 17%, and 32% *v* 15%, respectively. There were three toxic deaths in the VCAP-AMP-VECP arm.

Conclusion
The longer OS at 3 years and higher CR rate with VCAP-AMP-VECP compared with biweekly CHOP suggest that VCAP-AMP-VECP might be a more effective regimen at the expense of higher toxicities, providing the basis for future investigations in the treatment of ATLL.

J Clin Oncol 25:5458-5464. © 2007 by American Society of Clinical Oncology

INTRODUCTION

Adult T-cell leukemia-lymphoma (ATLL) is a distinct peripheral T-lymphocytic malignancy associated with human T-cell lymphotropic virus type I.[1-3] The diverse clinical features of this disease have led to its classification.[4] Aggressive ATLL (ie, acute or lymphoma type) has usually been treated as a subtype of aggressive non-Hodgkin's lymphoma (NHL), whereas indolent ATLL (ie, chronic or smoldering type) has been managed as a subtype of chronic lymphoid leukemia.[5-7] Aggressive ATLL generally has a poor prognosis compared with aggressive B-cell lymphoma and peripheral T-cell lymphoma excluding ATLL.[7-9] Median survival time (MST) of patients with aggressive ATLL is approximately 8 months because of the multidrug resistance (MDR) phenotype of malignant cells, rapid proliferation of the cells, a large tumor burden with multiorgan failure, hypercalcemia, and/or frequent infectious complications.[4-7,10]

In the two previous multicenter trials for advanced NHL, Japan Clinical Oncology Group (JCOG) 8101 (1981 to 1983) and JCOG8701 (1987 to 1991) evaluating the efficacy of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) –like regimens and a multiagent regimen of the second generation, respectively, a significantly

shorter survival time was demonstrated for ATLL patients than for other NHL patients.[11,12] Thus, the first trial exclusively applied to aggressive ATLL, JCOG9109, was started (1991 to 1993). The chemotherapy protocol involved the use of deoxycoformycin, an inhibitor of adenosine deaminase, which was found to be effective against refractory ATLL.[13] However, there were no improvements in overall response rate (ORR) or survival time compared with the previous trials.[14]

The next phase II study (JCOG9303, 1994 to 1996), with the chemotherapy protocol LSG15 against aggressive ATLL consisting of a dose-intensified multiagent chemotherapy with vincristine, cyclophosphamide, doxorubicin, and prednisone (VCAP), doxorubicin, ranimustine, and prednisone (AMP), and vindesine, etoposide, carboplatin, and prednisone (VECP) with granulocyte colony-stimulating factor (G-CSF) and intrathecal prophylaxis, showed promising results with complete response (CR) and partial response rates of 36% and 45%, respectively, and an MST of 13 months at the expense of toxicities.[15] Ranimustine, an alkylating agent crossing the blood-brain barrier, and intrathecal prophylaxis with methotrexate and prednisone were incorporated because ATLL frequently involves the CNS.[4,16] Carboplatin and ranimustine were incorporated because the activity of these agents is not affected by the expression of P-glycoprotein, a product of the MDR gene *MDR1*, which is frequently expressed on ATLL cells.[17] The promising results of JCOG9303 prompted us to conduct a randomized controlled trial comparing the LSG15 regimen with biweekly CHOP against aggressive ATLL. Dose intensification of CHOP with prophylactic use of G-CSF was expected to improve survival among patients with aggressive NHL, and our randomized phase II study (JCOG9505) comparing biweekly CHOP with dose-escalated CHOP to treat aggressive NHL excluding ATLL revealed biweekly CHOP to be more promising.[18] Therefore, we regarded biweekly CHOP as a standard treatment for NHL including aggressive ATLL at the time of designing this phase III study.

PATIENTS AND METHODS

Patients

Previously untreated patients with aggressive ATLL (ie, acute-, lymphoma-, or unfavorable chronic-type ATLL) were eligible. Unfavorable chronic-type ATLL (defined by at least one of the following three factors: low serum albumin, high lactate dehydrogenase, or high blood urea nitrogen concentration) had an unfavorable prognosis similar to acute- or lymphoma-type ATLL.[6] The diagnosis of ATLL was made based on seropositivity for human T-cell lymphotropic virus type I and histologically and/or cytologically proven peripheral T-cell malignancy.

Eligibility criteria, which were identical to those for the previous studies JCOG9109 and JCOG9303, included no prior chemotherapy, age 15 to 69 years, preserved organ functions, and performance status (PS) of 0 to 3 or 4 as a result of hypercalcemia caused by ATLL.[14,15] The study protocol and the informed consent document were approved by both the JCOG Protocol Review Committee and the institutional review board of each institution.

Registration

Registration involved a telephone call or facsimile from the participating physicians to the JCOG Data Center, National Cancer Center, Tokyo, Japan. After an evaluation of eligibility, the patient was assigned to receive either modified (m) LSG15 or mLSG19 with a minimization method for balancing PS (0 or 1 *v* 2, 3, or 4) and institution.

Treatment

mLSG15 in JCOG9801 was a modified version of LSG15 in JCOG9303, consisting of the following three regimens: VCAP (vincristine 1 mg/m^2, maximum, 2 mg; cyclophosphamide 350 mg/m^2; doxorubicin 40 mg/m^2; and prednisone 40 mg/m^2) on day 1, AMP (doxorubicin 30 mg/m^2, ranimustine 60 mg/m^2, prednisone 40 mg/m^2) on day 8, and VECP (vindesine 2.4 mg/m^2 on day 15, etoposide 100 mg/m^2 on days 15 to 17, carboplatin 250 mg/m^2 on day 15, and prednisone 40 mg/m^2 on days 15 to 17) on days 15 to 17; the next course was started on day 29.[15] The modifications to mLSG15 compared with LSG15 were as follows: the total number of cycles was reduced from seven to six because of progressive cytopenia, especially thrombocytopenia, after repeating the VCAP-AMP-VECP therapy; and cytarabine 40 mg was used with methotrexate 15 mg and prednisone 10 mg for prophylactic intrathecal administration at the recovery phases of courses 1, 3, and 5 after confirmation of a platelet recovery of more than 70 × 10^9/L within 2 days before the next systemic chemotherapy because of the high frequency of CNS relapse in the JCOG9303 study.

mLSG19, a modified version of LSG19, consisted of eight cycles of CHOP (cyclophosphamide 750 mg/m^2; doxorubicin 50 mg/m^2; vincristine 1.4 mg/m^2, with a maximum of 2 mg, on day 1; and prednisone 100 mg on days 1 to 5) every 2 weeks.[18] The modification was an intrathecal administration identical to that in mLSG15.

Neutrophil count was checked twice a week for G-CSF use during the protocol treatment. When a serious infection occurred as a result of severe neutropenia, the doses of cyclophosphamide, doxorubicin, ranimustine, vindesine, etoposide, and carboplatin were decreased to 75% thereafter. If a second infection occurred, treatment was stopped.

Supportive Therapy

Supportive therapy for opportunistic infections was administered as in JCOG9303.[15] When the neutrophil count decreased to less than 1 × 10^9/L, G-CSF was administered subcutaneously every day until recovery to more than 5 × 10^9/L was achieved. Each course of mLSG19, VCAP in mLSG15, or AMP/VECP in mLSG15 was started after confirmation of a neutrophil count of more than 1.2 × 10^9/L, more than 1.0 × 10^9/L, or more than 0.5 × 10^9/L, respectively. Administration of G-CSF was discontinued on the day of chemotherapy and the day before. In cases when the hemoglobin level was less than 8 g/dL or platelet count was less than 20 × 10^9/L, an RBC or platelet transfusion was administered, respectively. Erythropoietin was not recommended for supportive care.

Response and Toxicity Evaluation

Response was judged using our own criteria for ATLL as described.[14,15] Toxicity was graded according to the JCOG toxicity criteria, an expanded version of the National Cancer Institute Common Toxicity Criteria version 1.0.[19]

Statistical Analysis

This trial was designed as a multicenter prospective randomized controlled trial. All analyses were performed on an intent-to-treat basis. The primary end point was overall survival (OS), and the secondary end points were progression-free survival (PFS), CR rate, and toxicity. The planned duration of accrual was 3 years, and the planned follow-up time was 2 years. The study was designed as a superiority trial, with the one-sided hypothesis according that the superiority of the control arm to the mLSG15 arm was out of concern a priori. This is because mLSG15 was expected to be associated with frequent and severe toxicities compared with the control arm.[15,18] The required sample size was 114 eligible patients in total, for 80% power to detect a hazard ratio of 0.6 under the assumption that survival times were exponentially distributed (corresponding to a 15% difference in the 3-year survival rate when the rate in the mLSG19 arm is 10%) with a one-sided type I error of 0.05. The planned sample size was 130 randomly assigned patients, with the expectation that 10% would be ineligible. The duration of accrual and the follow-up time were amended to 5 years and 1 year, respectively, in 2001 because of slow accrual.

OS was defined as the time from random assignment until death from any cause or until the last follow-up for patients who were alive. PFS was defined as the time from random assignment until death from any cause,

relapse, or progressive disease or until the last follow-up for patients who were alive. The CR rate and ORR were defined as the proportion of patients with CR and with CR or partial response, respectively, of all randomly assigned patients. Survival estimates were calculated using the Kaplan-Meier method and compared by stratified log-rank test for all randomly assigned patients, with PS as a stratification factor. An analysis of adverse events was conducted for all patients who received the protocol treatment, whether partially or completely. As a sensitivity analysis, the Cox regression was carried out. In accordance with the hypothesis, all of the *P* values are presented as one sided, except for when explicitly stated as two sided. All analyses were performed with SAS software Release 8.2 (SAS Institute, Cary, NC).

The JCOG Data Center collected and managed case report forms. In-house interim monitoring for quality control was performed at the center, and the monitoring reports were semiannually submitted to and reviewed by the JCOG Data and Safety Monitoring Committee. One interim analysis was planned after half of the planned number of patients had been off treatment with an adjustment for multiplicity by the alpha-spending function of O'Brien-Fleming.[20]

RESULTS

Patient Characteristics

Between July 1998 and October 2003, 118 patients were enrolled from 27 participating institutions (Fig 1). In June 2001, an interim analysis was performed according to the protocol and did not meet the prespecified stopping criteria ($\alpha = .00022$), and the study was continued. The final analyses were performed in February 2005 based on the follow-up data from December 2004 ($\alpha = .04992$). Fifty-seven patients were assigned to the mLSG15 arm (VCAP-AMP-VECP), and the remaining 61 patients were assigned to the mLSG19 arm (biweekly CHOP). The characteristics of the 118 patients are listed in Table 1. Two patients, one from each arm, were deemed ineligible after random assignment because they were judged to have organ dysfunctions not caused by the invasion of ATLL cells by the case report form review. Age, sex, and subtypes of ATLL were well balanced between the arms. However, there were some imbalances in prognostic factors. Although patients were stratified by PS of 0 or 1 versus 2, 3, or 4 at random assignment, there was an imbalance between PS 0 and 1. PS 0 was more frequent in the biweekly CHOP arm than the VCAP-AMP-VECP arm. Also, bulky mass (> 5 cm in diameter) was less frequent in the biweekly CHOP arm. In contrast, "B" symptoms were more frequent in the biweekly CHOP arm.

Table 1. Characteristics of Randomly Assigned Patients

Characteristic	VCAP-AMP-VECP (n = 57)* No. of Patients	%	Biweekly CHOP (n = 61)* No. of Patients	%
Age, years				
Median	56		58	
Range	36-69		33-69	
Sex				
Male	27	47	34	56
Female	30	53	27	44
Subtypes of ATLL				
Acute	40	70	41	67
Lymphoma	12	21	14	23
Unfavorable chronic	5	9	6	10
PS				
0	19	33	30	49
1	27	47	19	31
2	8	14	10	16
3	2	4	2	3
4	1	2	0	0
B symptoms				
Absent	39	68	34	56
Present	18	32	27	44
Bulky mass, cm				
< 5	36	63	49	80
≥ 5	17	30	9	15
≥ 10	4	7	3	5

Abbreviations: VCAP, vincristine, cyclophosphamide, doxorubicin, and prednisone; AMP, doxorubicin, ranimustine, and prednisone; VECP, vindesine, etoposide, carboplatin, and prednisone; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; ATLL, adult T-cell leukemia-lymphoma; PS, performance status.
*Two patients, one in each arm, were ineligible because of organ dysfunction.

Response and Survival

Responses in all randomly assigned patients are listed in Table 2. The CR rate, including uncertified CR, was higher in the VCAP-AMP-

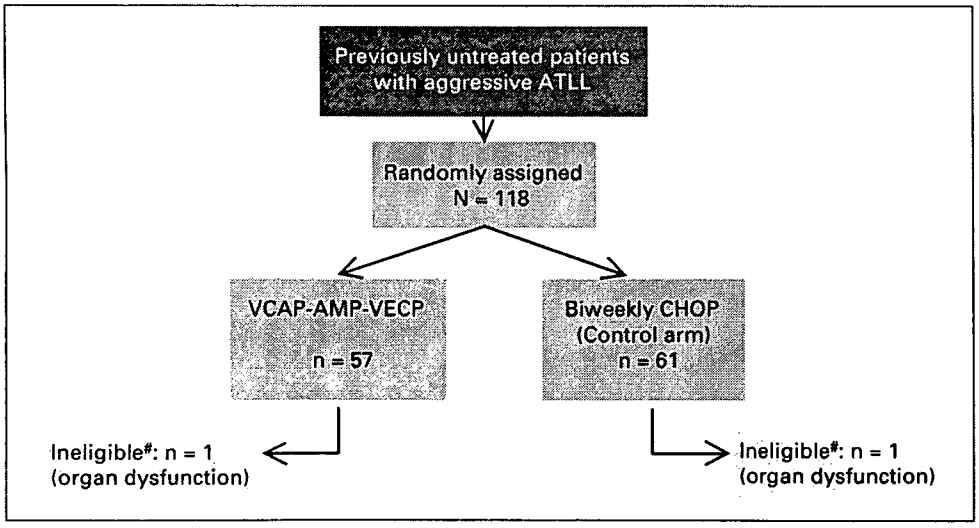

Fig 1. Enrollment and treatment of patients. (#) Included in the analysis. ATLL, adult T-cell leukemia-lymphoma; VCAP, vincristine, cyclophosphamide, doxorubicin, and prednisone; AMP, doxorubicin, ranimustine, and prednisone; VECP, vindesine, etoposide, carboplatin, and prednisone; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone.

Table 2. Response to Treatment

Response	% of Patients: VCAP-AMP-VECP (n = 57)	Biweekly CHOP (n = 61)	P
CR	40	21	
CRu	0	3	
PR	32	41	
NR	9	16	
PD	18	16	
Not assessable	2	2	
CR + CRu	40	25	.020*
95% CI	27.6 to 54.2	14.5 to 37.3	
CR + CRu + PR	72	66	NS*
95% CI	58.5 to 83.0	52.3 to 77.3	

Abbreviations: VCAP, vincristine, cyclophosphamide, doxorubicin, and prednisone; AMP, doxorubicin, ranimustine, and prednisone; VECP, vindesine, etoposide, carboplatin, and prednisone; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; CR, complete response; CRu, unconfirmed complete response; PR, partial response; NR, no response; PD, progressive disease; NS, not significant.
*Fisher's exact test (one sided).

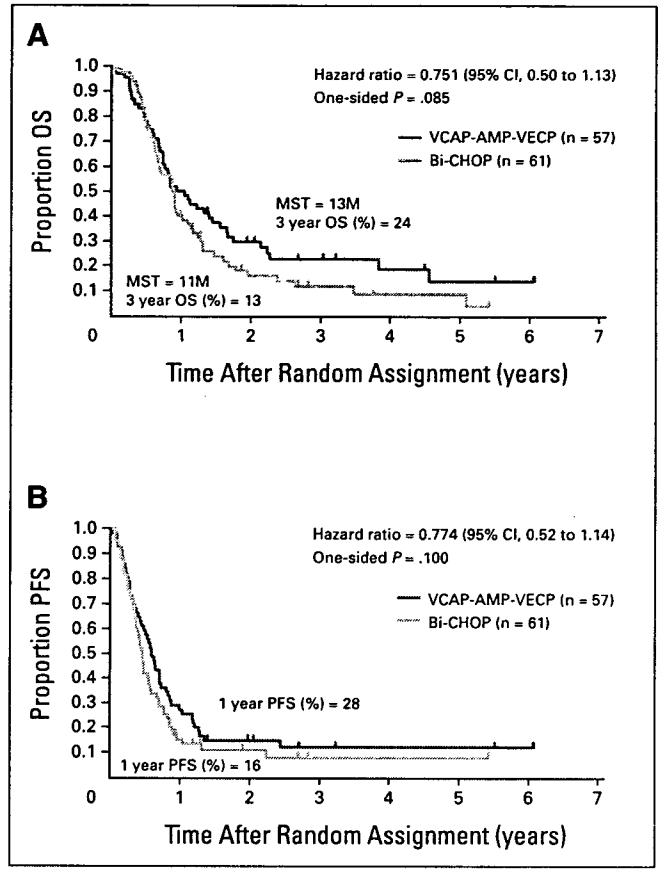

Fig 2. (A) Kaplan-Meier estimate of overall survival (OS) for all randomly assigned patients. (B) Kaplan-Meier estimate of progression-free survival (PFS) for all randomly assigned patients. VCAP, vincristine, cyclophosphamide, doxorubicin, and prednisone; AMP, doxorubicin, ranimustine, and prednisone; VECP, vindesine, etoposide, carboplatin, and prednisone; Bi-CHOP, biweekly cyclophosphamide, doxorubicin, vincristine, and prednisone.

VECP arm (40%) than the biweekly CHOP arm (25%; $P = .020$), and ORRs were similar (72% *v* 66%, respectively) between the arms.

Median follow-up time for all randomly assigned patients was 10.9 months. The MST and OS rate at 3 years without censoring the transplantation patients were 12.7 months and 24%, respectively, in the VCAP-AMP-VECP arm and 10.9 months and 13%, respectively, in the biweekly CHOP arm (Fig 2A). For OS, the preplanned, one-sided, log-rank $P = .085$ (two-sided $P = .169$), and the hazard ratio was 0.75 (95% CI, 0.50 to 1.13). A Cox regression analysis with PS (0 *v* 1 *v* 2 to 4) as stratum for baseline hazard functions was performed to evaluate the effect on OS of the factors of age, B symptoms, subtypes of ATLL, lactate dehydrogenase, blood urea nitrogen, bulky mass, and treatment arms. According to this analysis, the hazard ratio and *P* value for the treatment arms were 0.62 (95% CI, 0.38 to 1.01) and $P = .028$ (two-sided $P = .056$), respectively. The difference between the crude analysis and this result was because of unbalanced prognostic factors, such as PS 0 versus 1, and the presence or absence of bulky lesions between the treatment arms. The median PFS time and PFS rate at 1 year were 7.0 months and 28% in the VCAP-AMP-VECP arm and 5.4 months and 16% in the biweekly-CHOP arm, respectively ($P = .100$, two-sided $P = .200$; hazard ratio = 0.77; 95% CI, 0.52 to 1.14; Fig 2B).

The rate of completion of the planned treatment was 32% in the VCAP-AMP-VECP arm and 49% in the biweekly CHOP arm. Progressive disease or relapse, as a reason for discontinuation of treatment, was observed in 40% of patients in the VCAP-AMP-VECP arm and 31% in the CHOP arm. These results seem to be associated with the periods of treatment (ie, 24 weeks in the VCAP-AMP-VECP arm and 16 weeks in the biweekly CHOP arm) because OS and PFS were better in the VCAP-AMP-VECP arm. Reasons for going off treatment, such as toxicity, were relatively numerous in the VCAP-AMP-VECP arm.

The period needed to complete each course of chemotherapy and proceed to the next course was stable in the biweekly CHOP arm (median, 15 days in courses 1 to 2 and 14 days in courses 7 to 8). In contrast, the more advanced the therapy, the more time that was required, especially after course 3 as a result of bone marrow suppression, mainly neutropenia, despite using G-CSF, in the VCAP-AMP-VECP arm (median, 30 days in courses 1 to 2 and 42 days in courses 5 to 6). The average duration of G-CSF use was 12.9 days and 5.4 days per course in the VCAP-AMP-VECP and biweekly CHOP arms, respectively.

Toxicities

Excluding one patient in the biweekly CHOP arm who refused protocol chemotherapy, 117 patients were assessable for toxicity (Table 3). The major toxicities in both arms were cytopenia and infection. In general, toxicity was more severe in the VCAP-AMP-VECP arm. In the VCAP-AMP-VECP arm versus biweekly CHOP arm, rates of grade 4 neutropenia, grade 4 thrombocytopenia, and grade 3 or 4 infection were 98% *v* 83%, 74% *v* 17%, and 32% *v* 15%, respectively. Three treatment-related deaths, two from sepsis and one from interstitial pneumonitis related to neutropenia, were reported in the VCAP-AMP-VECP arm. Two cases of myelodysplastic syndrome were reported, one each in both arms.

Subgroup Analysis

As shown in Figure 3, there was interaction between the treatment arms and PS, which suggests that the intensive

Table 3. Hematologic and Nonhematologic Toxicities in 117 Treated Patients

Toxicity	Grade	% of Patients: VCAP-AMP-VECP (n = 57)	% of Patients: Biweekly CHOP (n = 60)
Neutropenia	4	98	83
Thrombocytopenia	4	74	17
T-bilirubin	3 + 4	5	2
ALT	3 + 4	11	5
Hyperglycemia	3 + 4	13	4
Hyponatremia	3 + 4	5	5
Hypokalemia	3 + 4	12	2
Stomatitis	3 + 4	7	2
Dyspnea	3 + 4	7	5
Infection	3 + 4	32	15
Neuropathy	3 + 4	2	7
Treatment-related deaths, No.	—	3*	0

Abbreviations: VCAP, vincristine, cyclophosphamide, doxorubicin, and prednisone; AMP, doxorubicin, ranimustine, and prednisone; VECP, vindesine, etoposide, carboplatin, and prednisone; CHOP, cyclonhosphamide, doxorubicin, vincristine, and prednisone.
*Two patients died of sepsis, and one died of interstitial pneumonitis.

VCAP-AMP-VECP regimen is more likely to benefit patients with a poor PS, possibly reflecting a more advanced stage of ATLL. Similarly, the younger population was more likely to benefit from VCAP-AMP-VECP (Fig 4). In contrast, no such interaction was observed in the analysis concerning disease type and bulky tumor (data not shown). Fourteen patients, seven in each group, received allogeneic hematopoietic stem-cell transplantation (alloHSCT). Four and three patients received the transplantation before progressive disease in the VCAP-AMP-VECP arm and biweekly CHOP arm, respectively. The estimated OS rates at 2 years for patients receiving alloHSCT were similar (43% for both arms).

DISCUSSION

To our knowledge, this trial, JCOG9801, comparing the efficacy and safety of VCAP-AMP-VECP and biweekly CHOP, is the first phase III trial exclusively conducted for ATLL in the world. We found a better OS in patients with aggressive ATLL treated with VCAP-AMP-VECP compared with biweekly CHOP, as well as a higher CR rate and longer PFS. Although the primary analysis of OS failed to show statistical significance (hazard ratio = 0.75, $P = .085$), a sensitivity analysis demonstrated the consistent result even after an adjustment of imbalance in baseline prognostic factors (hazard ratio = 0.62, $P = .028$). We consider the longer OS at 3 years and higher CR rate of VCAP-AMP-VECP than biweekly CHOP in this trial to be clinically meaningful and the former to be recommended as the first choice for patients with this disease despite higher toxicities.

Hematologic toxicity and infections were more frequent in the VCAP-AMP-VECP arm than the biweekly CHOP arm, which are similar findings to the previous JCOG9303 study with the original VCAP-AMP-VECP regimen.[15] Although both regimens were supported with G-CSF, four more drugs were incorporated in VCAP-AMP-VECP compared with biweekly CHOP, with a dose-dense and long period of chemotherapy. Three treatment-related deaths, which were related to severe neutropenia despite using G-CSF, were reported in the VCAP-AMP-VECP arm. Although VCAP-AMP-VECP caused remarkable thrombocytopenia, no serious hemorrhagic events were documented, possibly because platelet transfusion was encouraged without modifying the schedule of chemotherapy based on the decrease in the platelet count. ATLL patients treated with VCAP-AMP-VECP should be carefully monitored for complications, especially cytopenia and infections,

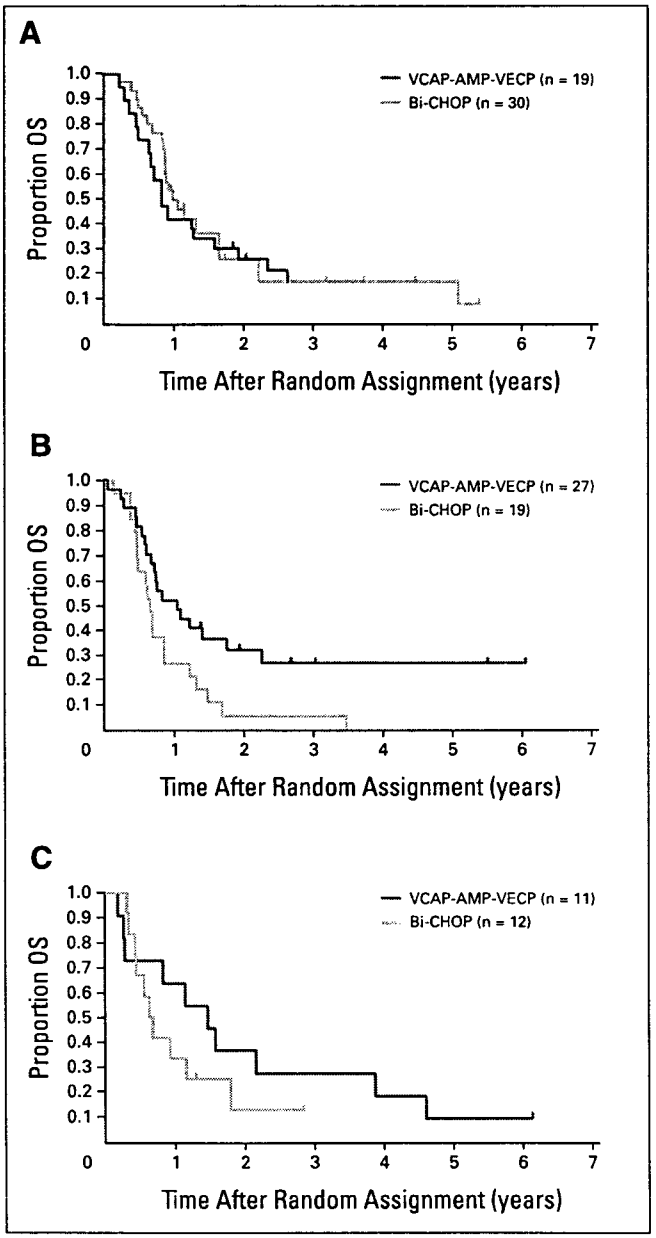

Fig 3. Kaplan-Meier estimate of overall survival (OS) for all randomly assigned patients according to performance status (PS) at diagnosis: (A) PS of 0; (B) PS of 1; and (C) PS of 2, 3, or 4. VCAP, vincristine, cyclophosphamide, doxorubicin, and prednisone; AMP, doxorubicin, ranimustine, and prednisone; VECP, vindesine, etoposide, carboplatin, and prednisone; Bi-CHOP, biweekly cyclophosphamide, doxorubicin, vincristine, and prednisone.

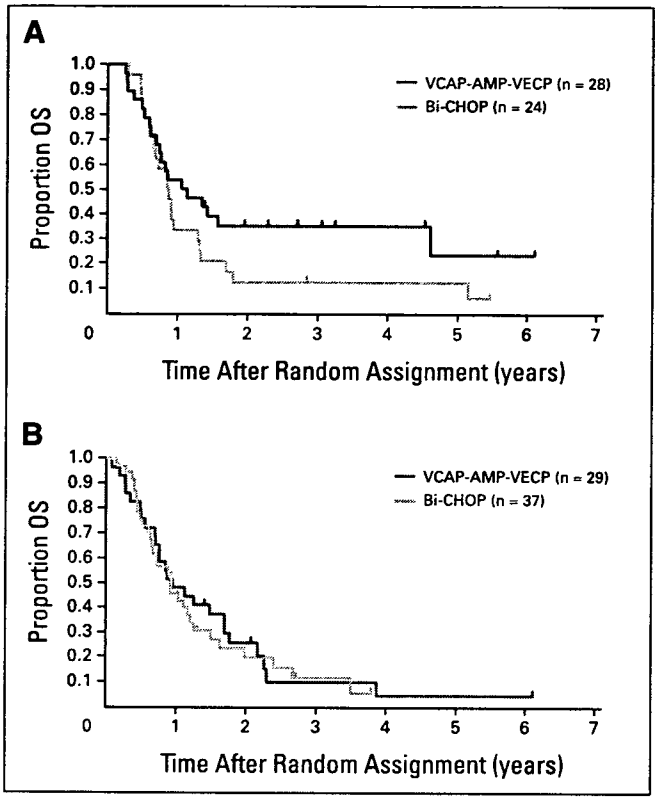

Fig 4. Kaplan-Meier estimate of overall survival (OS) for all randomly assigned patients according to age at diagnosis: (A) age < 56 years and (B) age ≥ 56 years. VCAP, vincristine, cyclophosphamide, doxorubicin, and prednisone; AMP, doxorubicin, ranimustine, and prednisone; VECP, vindesine, etoposide, carboplatin, and prednisone; Bi-CHOP, biweekly cyclophosphamide, doxorubicin, vincristine, and prednisone.

with supportive care such as platelet transfusion, use of G-CSF, and the prophylaxis of opportunistic infection.[4-7]

Because of the geographically limited distribution and rarity of this disease, large-scale trials for the treatment of ATLL have rarely been performed. There have been trials of intensive chemotherapy and a unique combination chemotherapy consisting of interferon alfa and zidovudine.[21,22] However, the results were inferior to those of VCAP-AMP-VECP.[23] The following factors might explain the reasons for the superiority of VCAP-AMP-VECP compared with biweekly CHOP against aggressive ATLL. Four more drugs were incorporated in the VCAP-AMP-VECP arm, compared with the biweekly CHOP arm, with a dose-dense and long period of chemotherapy. Furthermore, carboplatin and ranimustine were incorporated, and these agents are not affected by P-glycoprotein, which is frequently expressed in ATLL cells at onset.[17]

According to the results of subgroup analyses, VCAP-AMP-VECP may be more beneficial in patients with more advanced ATLL or in younger patients. However, because this study excluded patients with a PS of 4 not caused by hypercalcemia, the results would not be applicable to patients with a PS of 4 caused by an opportunistic infection or organ involvement by ATLL.

Our previous studies in advanced NHL revealed that CR rate and OS were poorer in ATLL than in other aggressive NHL.[10-12] In the recent two studies, biweekly CHOP was better than or not inferior to standard CHOP for aggressive NHL excluding ATLL.[24,25] VCAP-AMP-VECP was superior to biweekly CHOP for ATLL in this study. A G-CSF–supported dose-intensified regimen with carboplatin and ranimustine might have overcome the characteristics of ATLL cells, such as rapid proliferation and P-glycoprotein expression.

AlloHSCT is now considered as promising for the treatment of young patients with ATLL.[26] Thus, despite higher toxicities and poor completion rate of the planned course of therapy, VCAP-AMP-VECP, which provided a higher CR rate and a probable survival advantage, is promising as induction chemotherapy preceding upfront alloHSCT for aggressive ATLL. However, to prove the effectiveness of this strategy, further studies are needed.

In conclusion, the results of the present phase III study suggest that VCAP-AMP-VECP is a more effective chemotherapy regimen for patients with newly diagnosed aggressive ATLL even with higher toxicity profiles. However, the MST of 13 months is still not satisfactory. We are now planning a phase II study of myeloablative alloHSCT after induction therapy with VCAP-AMP-VECP for young patients with this disease.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Kunihiro Tsukasaki, Atae Utsunomiya, Haruhiko Fukuda, Takuya Fukushima, Masayuki Sano, Kensei Tobinai, Masanori Shimoyama, Masao Tomonaga
Administrative support: Tomomitsu Hotta
Provision of study materials or patients: Atae Utsunomiya, Takuya Fukushima, Yoshifusa Takatsuka, Shuichi Ikeda, Masato Masuda, Haruhisa Nagoshi, Ryuzo Ueda, Kazuo Tamura, Masayuki Sano, Saburo Momita, Kazunari Yamaguchi, Fumio Kawano, Shuichi Hanada, Kensei Tobinai, Tomomitsu Hotta
Collection and assembly of data: Haruhiko Fukuda, Takuya Fukushima, Yoshifusa Takatsuka, Shuichi Ikeda, Masato Masuda, Haruhisa Nagoshi, Ryuzo Ueda, Kazuo Tamura, Masayuki Sano, Saburo Momita, Kazunari Yamaguchi, Fumio Kawano, Shuichi Hanada, Kensei Tobinai, Tomomitsu Hotta
Data analysis and interpretation: Haruhiko Fukuda, Taro Shibata, Takuya Fukushima, Masao Tomonaga
Manuscript writing: Kunihiro Tsukasaki, Haruhiko Fukuda, Taro Shibata, Kensei Tobinai, Masanori Shimoyama, Tomomitsu Hotta, Masao Tomonaga
Final approval of manuscript: Kunihiro Tsukasaki, Haruhiko Fukuda, Takuya Fukushima, Tomomitsu Hotta, Masao Tomonaga

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Acknowledgment

The Acknowledgment 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®)

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®).