that cannot be predicted from conventional data. Mintz et al. [29] reported osteosarcoma chemoresistance was associated with osteoclastogenesis and bone resorption based on decreased expression of osteoclastogenesis-inhibitory factors in tumors showing a poor response to chemotherapy. We report here 10 protein spots associated with the chemosensitivity (necrosis rate) of osteosarcoma to preoperative chemotherapy. Although the 10 spots are currently under investigation, further studies may lead to new diagnostic or prognostic markers for osteosarcoma and new therapeutic targets.

Proteomic analysis using 2D-DIGE can provide important, novel clues for understanding the biology of bone and soft tissue sarcomas and for revealing candidate tumor markers and therapeutic targets.

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JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Distinct Gene Expression–Defined Classes of Gastrointestinal Stromal Tumor

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A B S T R A C T

Purpose

The majority of gastrointestinal stromal tumors (GIST) can be cured by surgery alone, but relapse occurs in 20% to 40% of cases. GISTs are considered to invariably arise through gain of function *KIT* or *PDGFA* mutation of the interstitial cells of Cajal (ICC). However, the genetic basis of the malignant progression of GISTs are poorly understood.

Patients and Methods

The expression levels of 54,613 probe sets in 32 surgical samples of untreated GISTs of the stomach and small intestine were analyzed with oligonucleotide microarrays. The representative GeneChip data were validated by real-time reverse transcriptase polymerase chain reaction and immunohistochemistry.

Results

Unbiased hierarchical clustering consistently separated the 32 cases of GIST into two major classes according to tumor site. The two major classes were further separated into novel subclasses, which were significantly correlated with various pathological prognostic parameters, the frequency of metastasis ($P < .05$), and clinical outcome. Immunohistochemical analysis of 152 independent patients with gastric GISTs revealed that the expression of dipeptidyl peptidase IV (T-cell activation antigen CD26) protein was significantly associated with poorer overall and disease-free survival ($P < .00001$).

Conclusion

CD26 appears to be a reliable biomarker of malignant GISTs of the stomach. The postoperative recurrence rate of CD26-negative cases was as low as 2.0% (two of 102). Therefore, postoperative follow-up of such patients might be made less intensive. CD26 may play an important role in the malignant progression of gastric GISTs and serve as a therapeutic target.

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INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are an established human tumor entity characterized by distinct clinical, genetic, and histopathological features.[1-3] The overall frequency of GISTs are estimated to be no more than 10 to 20 cases per million in Western countries,[1] but GISTs comprise the majority of primary mesenchymal tumors of the gastrointestinal tract. Approximately 60% to 70% of GISTs arise in the stomach, 20% to 30% in the small intestine, and 5% in the colon and rectum.[1,3] On the basis of similarities in immunohistochemical and ultrastructural features, it is considered that GISTs arise from interstitial cells of Cajal (ICC) or their precursor cells.[4] More than 80% of GISTs have gain of function mutations of the *KIT* proto-oncogene that encodes the c-Kit (CD117) receptor tyrosine

kinase,[5] and one third of GISTs without *KIT* mutation carry reciprocal mutations in the *PDGFRA* gene that encodes platelet-derived growth factor receptor α (PDGFRA) tyrosine kinase.[6,7]

GISTs show a wide spectrum of clinical courses. The majority of cases can be cured by surgical resection alone, but 20% to 40% of cases relapse during the postsurgical follow-up.[8-10] Distant metastasis to the liver is the most common manifestation of recurrence,[10] and our previous experience indicates that the 5-year and 10-year survival rates after grossly curative surgery are 81.7% and 67.4%, respectively.[8] Many pathological criteria based on tumor site, size, cell type, degree of necrosis,[8,10-12] mitotic rate, Ki-67 immunoreactivity (MIB1 labeling) as well as their combinations have been proposed for predicting the outcome of patients with GISTs. The National Institutes of Health convened a
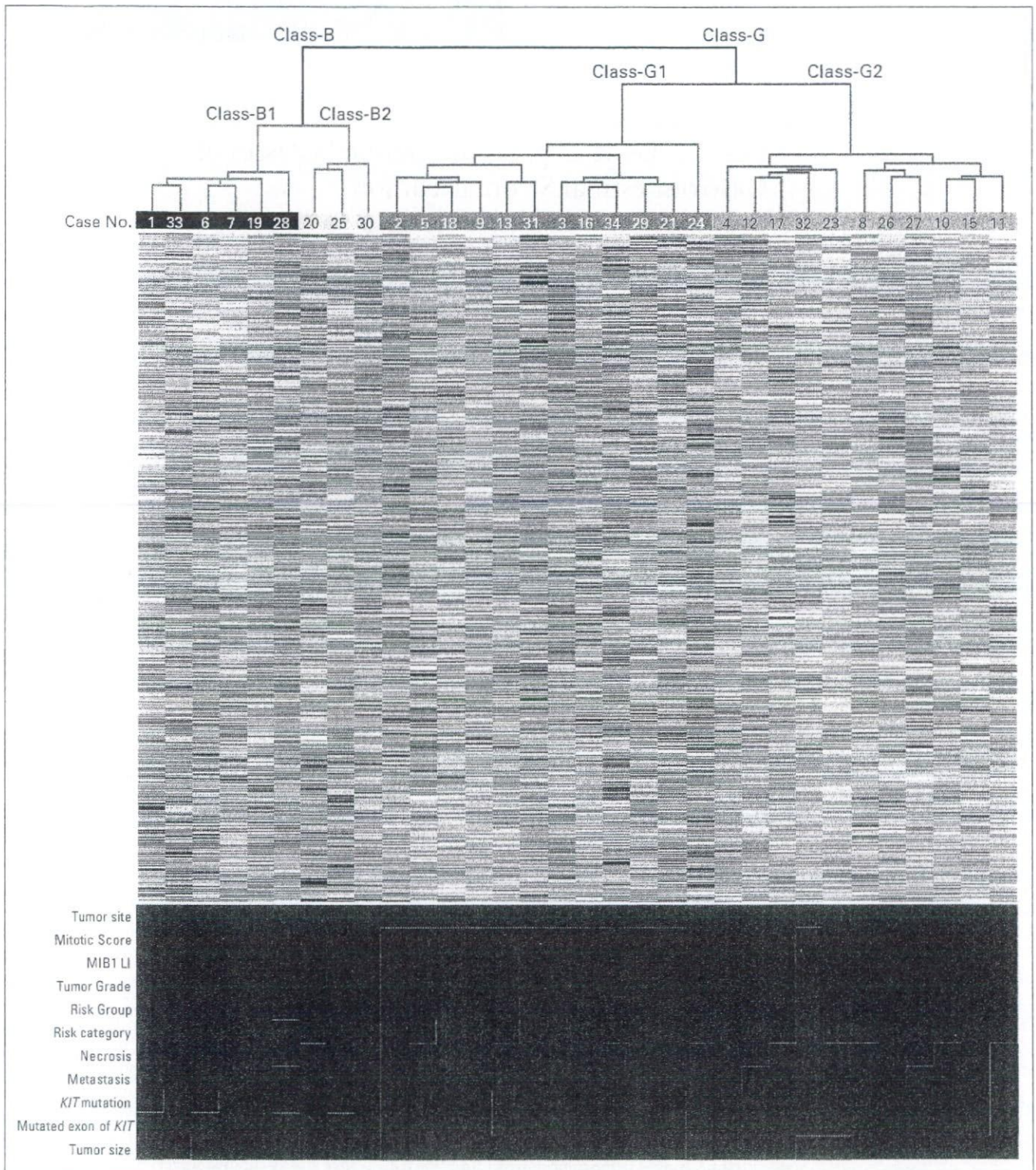
Fig 1. Four distinct gene expression–defined subclasses of gastrointestinal stromal tumors. Unsupervised hierarchical clustering separated 32 GIST cases into four subclasses based on the expression levels of the 21,214 probe sets of the GeneChip Human Genome U133 Plus 2.0 array (Affymetrix, Santa Clara, CA). Case numbers correspond to those of Appendix Table A1. The clinicopathological characteristics of the 32 cases in the four subclasses are indicated by red and green rows as follows: tumor site (red, small intestine/green, stomach), mitotic score (red, score 1/green, score 2), MIB1 labeling index (red, index 1/green index 2), tumor grade (red, grade 1/green, grade 2), risk group (red, low grade/green, high grade), risk category (red, low and intermediate risk/green, high risk), necrosis (red, absent/green, present), metastasis (red, absent/green, present), KIT mutation (red, absent/green, present), mutated exon of KIT (red, other than exon 11/green, exon 11), and tumor size (red, < 5 cm/green, ≥ 5.0 cm).

workshop in 2001, and a consensus (risk category) was proposed to estimate the relative risk of GISTs based on tumor size and mitotic count.[11] However, the cutoff values for these criteria have been determined empirically, and subjective assessments by skilled pathologists are inevitable. Therefore, it is necessary to identify an objective biomarker for recurrence of GISTs with a high positive or negative predictive value.

Imatinib mesylate (STI-571/Gleevec; Novartis Pharma, Basel, Switzerland), which selectively inhibits a group of tyrosine kinase receptors including *KIT* and *PDGFRA*, has been proven to be effective for the management of recurrent and unresectable GISTs.[13,14] However, the effect of imatinib mesylate varies depending on the domains of *KIT* and *PDGFRA* affected by the mutations.[15] Imatinib treatment is generally safe, but serious events such as gastrointestinal and intra-abdominal hemorrhage have been reported.[16,17] Furthermore, drug-refractory tumor cells develop due to second mutations of *KIT* during continuous therapy.[18] Although several clinical studies are currently underway to investigate the efficacy of emerging kinase inhibitors,[19,20] it is necessary to identify a new target molecule other than *KIT* or *PDGFR*.

In this study, we analyzed a well-characterized cohort of GIST cases in order to clarify the genomic alterations associated with the malignant progression of this tumor and to identify a biomarker that might be applicable to the prediction of outcome in patients with GISTs.

PATIENTS AND METHODS

Tumor Samples

All of the samples were obtained surgically at the National Cancer Center Hospital (Tokyo, Japan) between July 1972 and November 2005. Fresh frozen tumor specimens of 32 cases of GISTs of the stomach and small intestine were used for GeneChip (Affymetrix, Santa Clara, CA) analysis, and formalin-fixed paraffin-embedded tissue sections of 152 other cases of gastric GIST cases were used for independent validation. The study protocol for collection of tumor samples and clinical information was approved by the institutional review board, and patients provided written informed consent authorizing the collection and use of the tumor samples for research purposes.

Clinicopathological Assessment and Mutation Analysis

Immunohistochemistry for c-Kit, CD34, and Ki-67 was performed as described previously.[21,22] Mitotic score was determined by counting the number of mitotic figures in 10 consecutive high-power fields (HPF; ×400). Score 1 was ≤ 5 per 10 HPF, and score 2 was > 5 per 10 HPF. MIB1 labeling index (LI) was assigned as index 1 (< 10% MIB1-positive cells) and index 2 (≥ 10% MIB1-positive cells). Tumor grade was defined as grade 1 (index 1 and no tumor necrosis) and grade 2 (index 2 or tumor necrosis). Risk group was
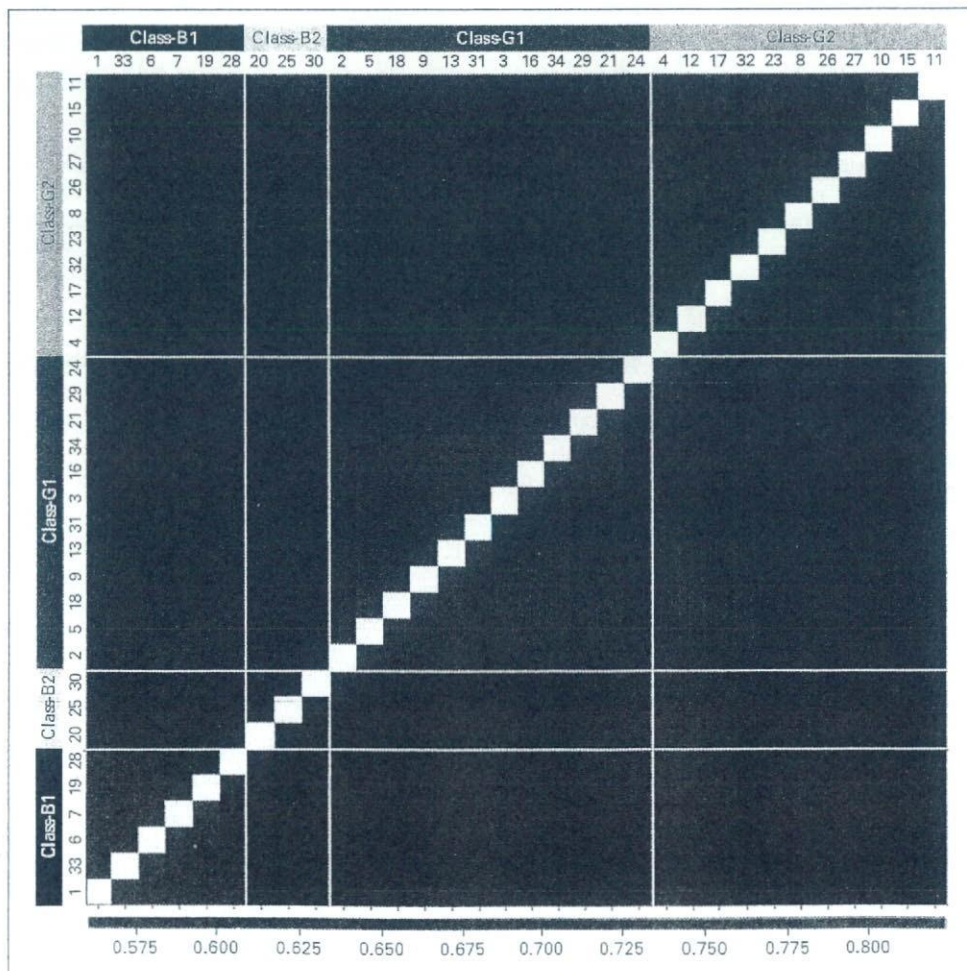

Fig 2. Heterogeneous gene expression of malignant gastrointestinal stromal tumors. The correlation coefficient value for each pair is shown in pseudo color according to the scale at the bottom. Red indicates higher correlation, and blue indicates lower correlation.

defined as low-risk group (grade 1 and tumor size of < 5.0 cm) and high-risk group (grade 1 and tumor size of ≥ 5.0 cm or any grade 2). Risk category was defined as described previously.[11] The mutational status of the *KIT* and *PDGFRA* genes was determined as described previously.[23]

GeneChip Analysis

Total RNA was extracted with IsoGen lysis buffer (Nippon Gene, Toyama, Japan) and purified with a RNeasy Mini kit (Qiagen, Hilden, Germany). We used GeneChip Human Genome U133 Plus 2.0 arrays (Affymetrix) to analyze the mRNA expression levels of 54,613 probe sets corresponding to more than 38,000 human UniGene Clusters in accordance with the manufacturer's protocols. The background correction, probe summarization, and normalization of all the GeneChip data were performed with the Microarray Analysis Suite 5 algorithm, and the processed values of all probe sets were then log-transformed for subsequent analyses, using the ArrayAssist 4.0 software package (Stratagene, La Jolla, CA).

Hierarchical clustering analysis was performed with centered values of the Pearson's correlation coefficient and Ward's linkage method. Clustering analysis was performed by biostatisticians (A.S., T.S., H.K.) who were blinded to the clinicopathological data.

Real-Time Reverse-Transcriptase Polymerase Chain Reaction

For cDNA synthesis, 5 μg of total RNA was reverse transcribed by random priming with Superscript II reverse transcriptase (Invitrogen). The gene-specific TaqMan primers and probes were designed by Applied Biosystems (Foster City, CA). Real-time reverse-transcriptase polymerase chain reaction (RT-PCR) was carried out using the ABI Prism 7000 Sequence Detection System (Applied Biosystems). The comparative C_t values were normalized to that of glyceraldehyde 3-phosphate dehydrogenase.[24]

Immunohistochemistry of CD26

Goat antihuman CD26 antibody (AF1180) was purchased from R&D Systems (Minneapolis, MN). Immunoperoxidase staining of formalin-fixed and paraffin-embedded tissue sections using the avidin-biotin complex was performed as described previously.[25] Immunohistochemical results were judged by three investigators (T.Y., K.H., U.Y.) without awareness of the clinical information. Endothelial cells of blood vessels served as internal positive controls. Tumors that showed any degree of CD26 staining were classified as positive.

Statistical Analysis

Estimates of overall and disease-free survival were computed using the Kaplan-Meier method using the StatFlex statistical software package version 5.0 (Artec, Osaka, Japan). Overall survival was calculated from the day of diagnosis until death or until the end of follow-up. Disease-free survival was calculated from the day of diagnosis until the day of relapse or death as a result of disease, whichever came first. Differences between survival curves were assessed for statistical significance with the log-rank test. Other statistical tests were performed using tools available in the R statistical package (version 2.0.1; http://www.r-project.org/).

RESULTS

Classification of GISTs Into Four Subclasses Based on Global Gene Expression

The clinicopathological, immunohistochemical, and genetic characteristics of the 32 cases of GIST used in the GeneChip analysis are presented in Appendix Table A1, online only.

To grasp the overall gene expression pattern, we first performed unsupervised analysis of all 54,613 probe sets. Hierarchical clustering separated the 32 GISTs into two principal classes, each of which was further divided into two subclasses (Appendix Fig A1, online only). To eliminate probes that had little or no variation across samples (probes that were not working well), we next selected a set of 21,214 probes showing intensity differences of more than 2^3-fold between the max-

imum and minimum signals across the 32 samples and repeated the same unsupervised analysis. Hierarchical clustering separated the 32 samples into the same four subclasses except for one sample (case 28; Fig 1). We further confirmed the stability of this gene expression–defined clustering by eliminating probe sets with intensity differences of less than 2^4-fold (6,231 probe sets), 2^5-fold (2,907 probe sets), and 2^6-fold (1,380 probe sets; data not shown).

Clinicopathological Significance of the Gene-Expression–Defined Subclasses

We named the two principal classes separated by unsupervised analysis of the 21,214 probe sets as class B (for bowel) and class G (for gastric), because all tumors of the small intestine were clustered into class B, and all tumors of the stomach were clustered into class G (Fig 1). The four subclasses were designated as class B1, class B2, class G1, and class G2 (from left to right in Fig 1). The subclasses were found to be associated with the known prognosis-relevant clinicopathological variables (Fig 1). Fisher's exact test showed that there were significant differences between class B1 and class B2 as well as between class G1 and class G2 in the frequency of mitotic score, MIB1 LI, tumor grade, risk group, and metastasis ($P < .05$; Appendix Table A2, online only). There was no significant difference in the presence of *KIT* mutation, mutated exon of *KIT*, tumor size, cell type, sex, or expression of c-Kit or CD34 (Table A2 and data not shown). Mitotic score, MIB1 LI, tumor grade, risk group, and metastasis did not remain significantly different ($P < .05$) between class B1 and class B2, when Holm's adjustment of P values was applied for dealing with the multiple testing situation.[26]

Appendix Figure A2A and A2B shows the Kaplan-Meier plots for disease-free survival of patients in the subclasses. The gene expression–defined clusters clearly separated the patients into those with good outcome (class B1 and class G1) and those with poor outcome (class B2 and class G2; $P < .005$). Remarkably, none of the patients in class B1 or class G1 died during follow-up period of 108 months.

Heterogeneous Gene Expression of Malignant GISTs

The correlation coefficient values of 21,214 probe sets between all the combinations of the 32 GIST cases were calculated, and are presented as a pseudocolored heat map in Figure 2. There were high similarities of overall gene expression within cases of class B1 and

Table 1. Heterogeneous Gene Expression of Malignant Gastrointestinal Stromal Tumors

Class	No. of Pairs	Average Correlation Coefficient	CI	t Value	df	P
B1	15	0.78	0.77 to 0.79	5.72	3.11	.0096*
B2	3	0.69	0.64 to 0.74			
G1	66	0.74	0.73 to 0.75	3.69	95.93	.0004†
G2	55	0.71	0.71 to 0.72			

NOTE. Pearson's product-moment correlation coefficients were calculated among cases belonging to the same subclass, and were then Z-transformed to correct estimated errors to yield a normal distribution. The averages of these transformed values were compared between class B1 and class B2 as well as between class G1 and class G2 (Welch's t-test).
* < .01.
† < .001.

within cases of class G1, but not within cases of class B2 or within cases of class G2 (Fig 2). The average correlation coefficient values were significantly different between class B1 and class B2 ($P < .01$, Welch's t-test) as well as between class G1 and class G2 ($P < .001$; Table 1). These findings suggest that genomic diversity increases significantly during the malignant progression of GISTs.

Gene Expression Changes Associated With Malignant Progression of GISTs

There were 122 probe sets whose expression was increased in class B1 compared with class B2, and 400 probe sets whose expression was increased in class G1 compared with class G2 (Appendix Fig A3A, online only). There were 97 probe sets whose expression was increased in class B2 compared with class B1, and 321 probe sets whose expression was increased in class G2 compared with class G1 (Fig A3B). Only eight probe sets (eight UniGene clusters) were commonly increased in class B1 and class G1 relative to each respective counterpart (Fig A3A and Appendix Table A3), and 12 probe sets (12 UniGene clusters) were commonly increased in class B2 and class G2 relative to each respective counterpart (Fig A3B and Appendix Table A4), suggesting

that the genomic alterations promoting malignant progression differ between small intestinal GISTs and gastric GISTs.

We conducted real-time RT-PCR analysis of 20 representative genes differentially expressed between class G1 and class G2 to validate the results of the GeneChip analysis. Appendix Figure A4 represents 10 of these 20 genes.

CD26 Is a Significant Prognostic Factor of Gastric GISTs

Among the 400 probe sets whose expression was significantly increased in class G2 compared with class G1, we noticed that the *DPP4* (dipeptidyl peptidase IV) gene (which encodes the CD26 protein) was ranked in the first, second, third, and fifth places (Appendix Table A8, online only). Immunohistochemistry of 21 gastric GIST cases for which specimens were available revealed there were 12 CD26-positive (Fig 3A, 3B, 3D, and 3E) and nine CD26-negative cases (Fig 3C and 3F). The expression of CD26 protein appeared to be correlated well with gene expression–defined classes except for one case (case 2; Fig 3G). The disease-free and overall survival of patients with CD26-positive GISTs was worse than that of patients with CD26-negative GISTs ($P < .05$; Appendix Fig A5, online only). Appendix
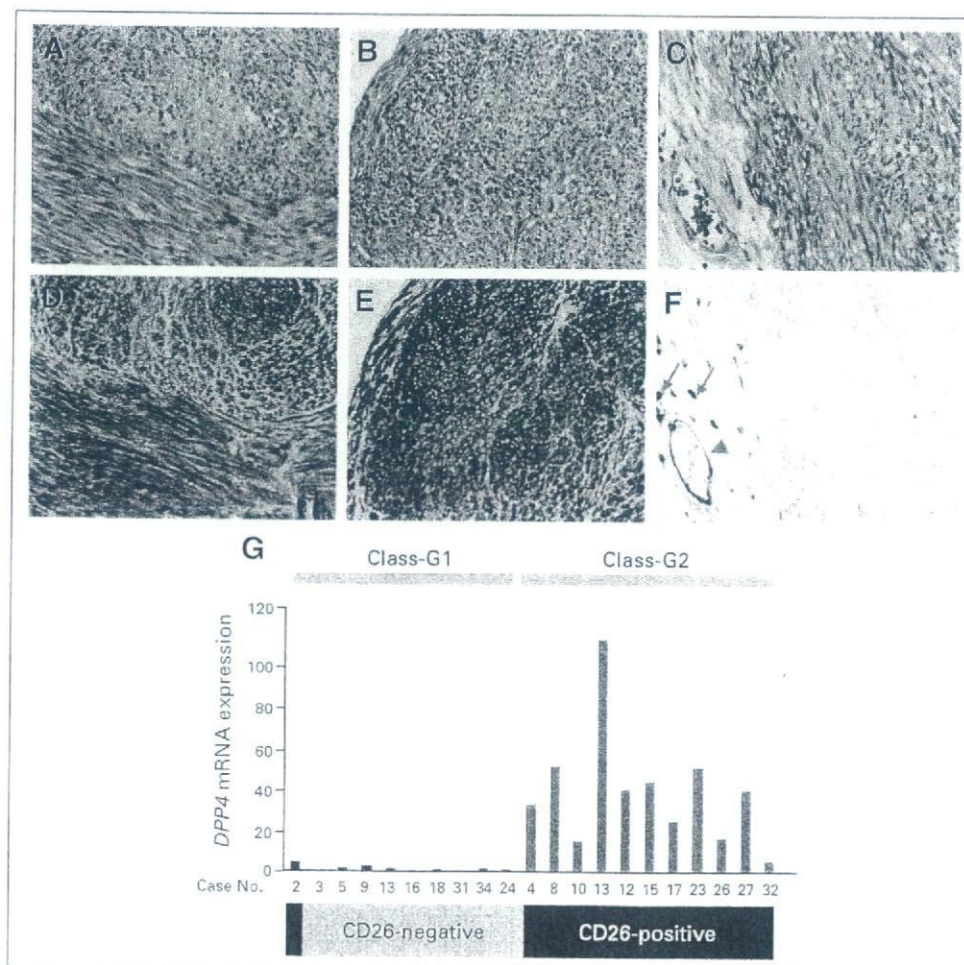

Fig 3. Correlation of dipeptidyl peptidase IV (*DPP4*) mRNA with CD26 protein expression. (A-C) Hematoxylin and eosin and (D-F) immunoperoxidase staining of (A, B, D, E) CD26-positive and (C, F) CD26-negative gastric gastrointestinal stromal tumors. The arrowhead indicates vascular endothelium, and arrows indicate CD26-positive inflammatory cells. (G) Relative *DPP4* mRNA expression level was determined by real-time reverse transcriptase polymerase chain reaction (in arbitrary units). The bar at the bottom indicates CD26-positive (black) and CD26-negative (gray) cases.

Table A5 (online only) presents the relationship between CD26 expression and gene expression–defined subclasses of small intestinal and gastric GISTs.

We then examined the clinical significance of CD26 protein expression in an independent validation cohort consisting of 152 gastric GISTs. The patients comprised 83 males (54.6%) and 69 females (45.4%). The average age at diagnosis was 59 years (range, 28 to 83 years), and the duration of follow-up ranged from 4 to 352 months (mean, 117 months). Follow-up computed tomography (CT) imaging was performed every 3 to 6 months. Of the 152 patients, 22 (14.5%) developed distant metastasis (14 to liver, four to peritoneum, two to bone, one to lung, and one to lymph node), seven of them were treated with imatinib mesylate. Immunohistochemically, 149 cases were positive for c-Kit, and 148 cases were positive for CD34.

Of the 152 gastric GISTs, 50 were CD26 positive (32.9%), and the remaining 102 were CD26 negative (67.1%). CD26 positivity was significantly ($P < .05$, Fisher's exact test) associated with tumor size, necrosis, mitotic score, MIB1 LI, tumor grade, risk group, risk category, and metastasis (Table 2). CD26 positivity was significantly associated with poor overall and disease-free survival ($P < .00001$; Fig 4A and 4B). The estimated overall survival rate at 10 years after surgery was 97.4% in CD26-negative patients and 69.9% in CD26-positive patients.

Almost all the CD26-negative cases were MIB1 LI index 1 (100 of 102), but the CD26-positive cases comprised a mixture of index 1 (29 of 50) and index 2 (21 of 50; Table 2). MIB1 LI is known to represent cell proliferation activity. We hypothesized that the CD26-positive cases might be further stratified by MIB1 LI. As shown in Figures 4C and 4D, the 152 gastric GIST cases were divided into three groups: CD26 negative, CD26 positive and index 1, and CD26 positive and index 2. There were significant differences in disease-free survival among these three groups ($P < .01$).

DISCUSSION

Several microarray analyses using smaller numbers of GIST cases had been conducted before this study.[27-31] GISTs show gene expression profiles different from those of other mesenchymal tumors.[27,31] The status of KIT/PDGFRA mutation has been reported to affect the global gene expression profile of GISTs.[29,30] However, none of these studies investigated the clinicopathological significance of the gene expression profiles, probably because long-term follow-up (for 5 to 10 years or more) is necessary for assessing the clinical outcome of this generally low-grade malignant tumor.[10]

Unsupervised hierarchical clustering is a well-established statistical method that separates cases based on similarities and dissimilarities of overall gene expression.[32] GISTs are considered to invariably arise through gain of function KIT or PDGFRA mutation of ICC. Most GISTs are composed of a fairly uniform population of spindle cells.[3,11] Allander et al[27] reported marked homogeneity in the gene expression of GISTs with KIT mutation. We assumed that low-grade GISTs constitute a uniform population and could be separated from high-grade GISTs by simple unsupervised clustering. The most principal determinant that separated the 32 GIST cases in this study was the site of tumor origin: the small intestine (class B) or stomach (class G; Fig 1), similarly to findings reported previously.[29] The second most principle determinant, however, was exactly as anticipated. Low-grade GISTs constituted a population with homogeneous gene expression profiles (classes B1 and G1; Fig 2) and was separated from high-grade GISTs, which constituted a heterogeneous population (classes B2 and G2, Fig 2).

In order to apply the observations obtained using GeneChip analysis to clinical practice, we selected the DPP4 gene, because its expression showed the greatest significant differences between class G1 and class G2. We further validated the clinical significance of the DPP4 gene product, CD26, in a large independent cohort of gastric GIST cases (Fig 4 and Table 2). Because the postoperative recurrence rate of CD26-negative cases was as low as 2.0% (two of 102) even in this cohort, the postoperative follow-up of these patients could have been significantly less intensive. Objective assessment of CD26 expression is possible using formalin-fixed paraffin-embedded tissue specimens (Figs 3D to 3F) and can be readily incorporated into routine pathological diagnosis along with c-Kit and CD34. For these reasons, CD26 is considered to be a biomarker superior to other known prognostic parameters.

CD26 is not only a biomarker of malignant GISTs, but may also play an important role in malignant progression. CD26 is a 110-kDa cell membrane glycoprotein that belongs to the serine protease family (EC 3.4.14.5).[33] It is expressed on a wide variety of cell lineages including T lymphocytes, endothelial and epithelial

Table 2. Correlations Between Clinicopathological Characteristics and CD26 Expression in 152 Cases of Gastric Gastrointestinal Stromal Tumors

| | CD26 Expression | | | | | |
| | Negative | | Positive | | | Holm's |
Characteristic	No.	%	No.	%	P*	Method P
Tumor size, cm						
< 5.0	72	73.5	26	26.5	.0307	.0316
≥ 5.0	30	55.6	24	44.4		
Necrosis						
No	100	69.4	44	30.6	.0158	.0316
Yes	2	25.0	6	75.0		
Mitotic score						
1	101	79.5	26	20.5	4.46×10^{-13}	3.57×10^{-12}
2	1	4.0	24	96.0		
MIB1 labeling index						
Index 1	100	77.5	29	22.5	3.49×10^{-10}	2.44×10^{-9}
Index 2	2	8.7	21	91.3		
Tumor grade						
1	98	77.2	29	22.8	1.08×10^{-8}	5.38×10^{-8}
2	4	16.0	21	84.0		
Risk group						
Low grade	95	77.2	28	22.8	1.47×10^{-7}	$5.88 > 10^{-7}$
High grade	7	24.1	22	75.9		
Risk category						
Very low	8	100.0	0	0.0	2.99×10^{-6}	8.96×10^{-6}
Low	60	78.0	17	22.0		
Intermediate	25	64.1	14	35.9		
High	9	32.1	19	67.9		
Metastasis						
No	100	76.9	30	23.1	1.38×10^{-9}	8.31×10^{-9}
Yes	2	9.1	20	90.9		

NOTE. Differences at $P < .05$ were considered significant.
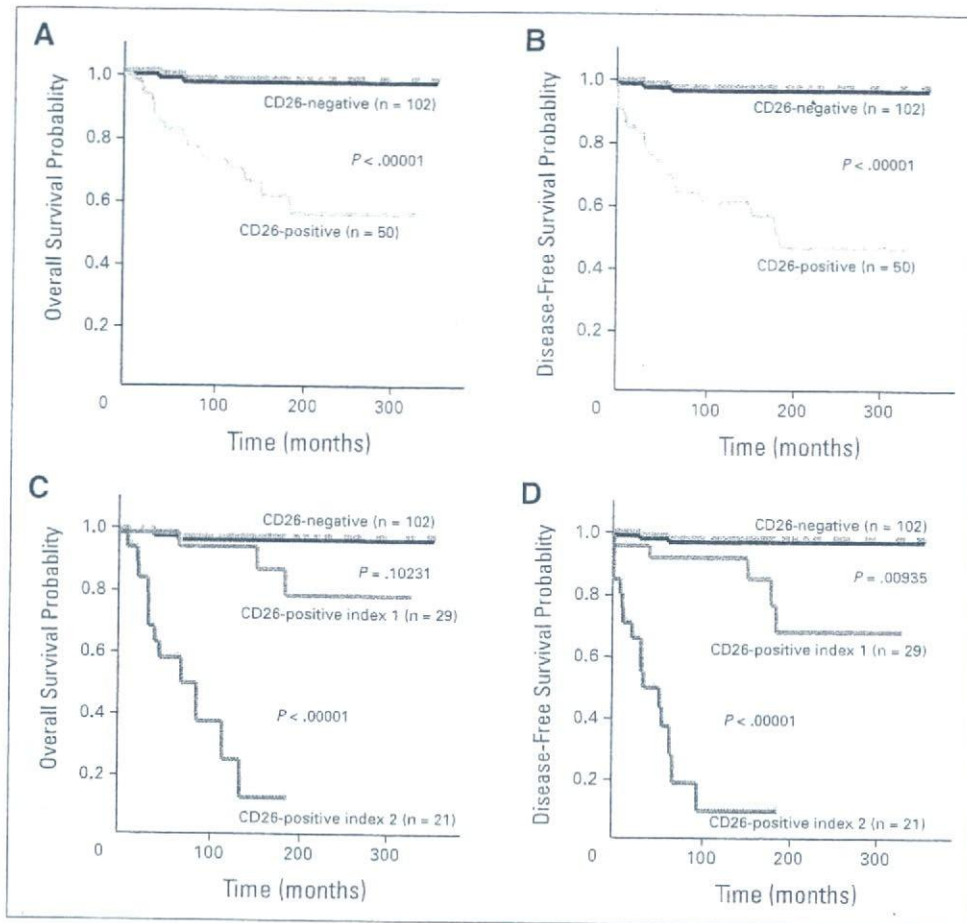*Fisher's exact test.

Fig 4. Correlation of CD26 expression with patient outcome in a validation cohort. (A) Kaplan-Meier analysis of overall survival of patients with CD26-positive (yellow) and CD26-negative (blue) gastric gastrointestinal stromal tumors (GISTs). (B) Kaplan-Meier analysis of disease-free survival of patients with CD26-positive (yellow) and CD26-negative (blue) gastric GIST. (C) Kaplan-Meier analysis of overall survival of patients with CD26-negative (blue), CD-26 positive and index 1 (gray), and CD-26 positive and index 2 (red) gastric GIST. (D) Kaplan-Meier analysis of disease-free survival of patients with CD26-negative (blue), CD-26 positive and index 1 (gray), and CD-26 positive and index 2 (red) gastric GIST.

cells. CD26 selectively cleaves the *N*-terminal dipeptide from cytokines and chemokines, and modulates their function. Although the role of CD26 in tumor development is still controversial,[33] an intriguing observation has been reported in a series of publications by Kotani and colleagues.[34,35] Differential diagnosis of follicular carcinoma of the thyroid from follicular adenoma has been one of the most difficult tasks for surgical pathologists. CD26 expression is highly specific to carcinoma and is able to predict distant metastasis of apparently benign thyroid tumors.[35] Unfortunately, CD26 expression was not associated with the outcome of small intestinal GISTs (data not shown), indicating that the molecular mechanisms behind the malignant progression of small intestinal GISTs differ from those of gastric GISTs. Further studies using cell culture and animal models are required to determine the exact biologic consequences of CD26 in GIST cells.

CD26 may serve as a therapeutic target molecule. Anti-CD26 monoclonal antibody has been shown to inhibit the growth of anaplastic large cell T-cell lymphoma both in vitro and in vivo.[36] Several orally active CD26 enzyme inhibitors have been developed as a new class of antidiabetic drugs. These inhibitors are generally safe and well tolerated, and no serious adverse effect has been noticed even in elderly patients.[37,38] These characteristics of CD26 inhibitors may make them suitable for long-term preventive administration to postoperative patients with GISTs.

At present, the precise molecular mechanism that induces the expression of CD26 remains to be clarified. CD26 may not be the cause of malignant progression of gastric GISTs, but its clear-cut association with the increased risk of postoperative recurrence warrants diagnostic application. It will certainly be necessary to validate our results in an independent study.

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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®).

Glossary Terms

Dipeptidyl peptidase IV (DPP4): A cell membrane serine exopeptidase that cleaves dipeptides from the N terminus of proteins. DPP4 is involved in the metabolic inactivation of glucagon-like peptide-1 (GLP1).

Hierarchical clustering: An analytical tool used to find the closest associations among gene profiles and specimens under evaluation.

c-kit: A member of the PDGFR family, c-kit is a tyrosine kinase receptor that dimerizes following ligand binding and is autophosphorylated on intracellular tyrosine residues.

PDGFRA (platelet-derived growth factor alpha): The receptor for PDGF exists distinctly as the dimeric $\alpha\alpha$ or $\beta\beta$ form. All dimer combinations of PDGF A and B signal through PDGFR-$\alpha\alpha$; PDGF BB signals through PDGFR-$\beta\beta$; PDGF CC signals through the $\alpha\alpha$ and $\alpha\beta$ receptors; and PDGF DD signals through the $\beta\beta$ and $\alpha\beta$ receptors.

Ki67: A marker of proliferation, Ki67 is a protein that is expressed in the nucleus of proliferating cells. Absent only in resting cells, cells in the G1, S, G2, and M phase of the cell cycle express this marker.

論　策

わが国の小児造血器腫瘍診療施設の実態

国立病院機構名古屋医療センター臨床研究センター[1]，茨城県立こども病院[2]，愛知医科大学小児科[3]，
京都大学大学院小児発達医学[4]，日本小児白血病リンパ腫研究グループ[5]

堀部　敬三[1][5]　土田　昌宏[2][5]　鶴澤　正仁[3][5]　中畑　龍俊[4][5]

要　旨

　小児造血器腫瘍の標準的治療法の確立と質の高い臨床試験を行うために2003年にわが国の全
ての小児白血病研究グループが結集して日本小児白血病リンパ腫研究グループ（JPLSG）が設立さ
れた．この結果，わが国のほぼ全ての小児造血器腫瘍診療施設がJPLSGに参加していると考えら
れる．今回，JPLSG参加施設の基本情報把握のため施設調査を行い，わが国の小児造血器腫瘍の
診療実態と今後の研究基盤および診療体制の整備について検討した．方法は，調査票を郵送にて
送付回収した．回収率は100％で186施設について検討した．主な結果は，都道府県別の施設数
は，2施設以下27県，10施設以上3都府県，小児血液腫瘍担当医師数が2名以下96施設，施設
責任者もしくは実務担当者が血液専門医でない施設78施設，小児外科腫瘍を診療している施設
108施設，2005年度に造血幹細胞移植を実施した施設111施設，小児血液専任のデータ管理者が
いる施設10施設．小児造血器腫瘍の診療は，少ないスタッフで固形腫瘍や移植医療とともに行わ
れている実態が明らかとなった．施設間格差はまだ大きく，大都市圏での施設の集約化，地方施
設の診療スタッフ確保，さらに専門医療の教育研修システムの構築が急がれる．また，臨床試験
を円滑に行うには意識改革とともにスタッフの負担軽減に繋がる支援体制の強化が必要と思われ
た．

キーワード：小児造血器腫瘍，小児白血病，診療体制

はじめに

　急性リンパ性白血病をはじめとする小児造血器腫瘍
は，化学療法，支持療法，造血幹細胞移植療法，さら
には診断技術の向上に基づいたリスク層別法の発達に
より80％以上の長期生存が可能となってきた[1]．これ
らの治療法の多くは欧米の研究グループで行われた臨
床試験によって開発されたが，我が国でも1970年代か
ら自主的に組織された治療研究グループによって治療
研究が推進され，小児造血器腫瘍のほとんどの症例が
いずれかの研究グループの治療法で治療されてきた．
現在では，小児病白血病研究グループ（CCLSG），小児
白血病研究会（JACLS），九州山口小児がん研究グルー
プ（KYCCSG），東京小児がん研究グループ（TCCSG）
の4つの研究グループに集約されており，ALLの治療
研究が独自に行われている[2]．一方，稀少な難治性疾患
については，単一グループでは十分な症例数が得られ
ないため治療開発が困難であったことから90年代に
なって厚生省研究班による全国規模の多施設共同研究

が推進され，乳児白血病，急性骨髄性白血病の治療法
開発が行われてきた[3][4]．しかし，これまでは各研究グルー
プおよび参加施設が臨床試験としての認識に乏しかっ
たため，治療研究は倫理審査が行われないまま簡素な
治療計画書のみによって行われ，研究的治療も症例登
録基準があいまいなこと，治療変更が各施設の自由裁
量であったこと，症例報告書の提出・内容確認が不十
分なこと，有害事象の報告義務がないことなど，必ず
しも質の高い研究体制の下で行われていたとはいえな
かった．そこで，臨床研究基盤整備と質の高い臨床試
験の推進のために2002年に小児造血器腫瘍の標準的
治療法の確立のための研究班がスタートした[5]．これを
期に，2003年に我が国のすべての小児白血病研究グ
ループが結集して日本小児白血病リンパ腫研究グルー
プ（JPLSG）が設立され，グループ間共同研究として全
国共同治療研究が開始された．これまでにJPLSGとし
て10の臨床試験が開始されており，乳児ALL，非ホジ
キンリンパ腫，急性骨髄性白血病（AML）は，全国統
一の治療研究が行われている[5]．その結果，すべての患
者さんに同じ治療法，臨床試験を受ける機会が与えら
れるようになった．

　がん治療は，毒性の強い治療法を組み合わせて行う
ことから，専門的知識と経験が要求される．本来は，

表1 JPLSG参加施設の施設母体

		CCLSG	JACLS	KYCCSG	TCCSG
大学病院：	91 施設	13	36	6	36
小児病院：	15 施設	2	6	0	7
がんセンター	4 施設	3	0	1	0
国公立総合病院（NHOを含む）	41 施設	6	28	3	4
日赤病院	15 施設	1	12	0	2
その他	20 施設	2	14	1	3
合計	186 施設	27	96	11	52

CCLSG：小児癌白血病研究グループ，JACLS：小児白血病研究会，KYCCSG：九州山口小児がん研究グループ，TCCSG：東京小児がん研究グループ，NHO：国立病院機構

表2 都道府県別参加施設数

施設数	1	2	3	4	6	7	8	9	>10
都道府県数	9	18	8	1	2	2	2	2	3

>10：14，17，22 施設

小児がん専門医が当たるべきであるが，我が国には専門医制度は未だ確立されておらず，個々の医師・医療機関の経験をもとに診療が行われている．小児がんは稀少な病型が多いため，多くの病型に十分な診療経験を持った医師の育成には，短期間に多数例を経験できる施設が必要である．また，ほとんどの症例が臨床研究に参加して治療されることから，診療施設は，臨床試験を実施しうる体制が求められる．JPLSGでは，質の高い医療と臨床試験を担保するために，以下の施設基準を設けている．(1)日本小児血液学会会員がいる．(2)包括医療ができる小児がん治療チームがある．(3)機関審査委員会（IRB）または倫理審査委員会がある．(4)施設監査が受け入れられる．また，わが国のほぼ全ての小児造血器腫瘍診療施設がJPLSGに参加していると考えられることから，JPLSG参加施設が我が国の小児血液がんの診療の担い手であるともいえる．今回，施設の基本情報の把握のために行ったJPLSG参加施設の調査結果をもとにわが国の小児造血器腫瘍の診療実態と今後の研究基盤および診療体制の整備について検討したので報告する．

方　法

平成18年7月1日時点のJPLSG参加施設の187施設に調査票を郵送して回収し集計した．調査票の回収率は100％（一部未記入を含む）であった．今回，その後に退会した1施設を除いた186施設（表8参照）について検討した．グループ別施設数の内訳は，CCLSG 27施設，JACLS 96施設，TCCSG 52施設，KYCCSG 11施設であった．

調査票にある項目は，以下の通りである．施設研究責任者氏名，実務担当者氏名，施設病床数，小児科病床数，小児血液腫瘍病床数，病棟形態，専門医研修施設認定状況，後期研修受け入れ状況，小児科常勤医数，小児血液腫瘍担当医数，学会入会・専門医取得状況，放射線治療医・小児外科医・麻酔科医の有無，診療対象腫瘍性疾患分野，メソトレキサート（MTX）血中濃度測定・全身放射線照射・無菌室管理の可否，造血幹細胞移植実施件数，病名告知実施状況，患者支援設備・スタッフの有無，研究審査状況，研究支援体制の有無．

結　果

参加施設の組織母体の内訳をグループ別に示す（表1）．大学病院の占める割合が37.5％〜69.2％と較差が見られ，グループ間で施設背景に差異がみられた．また，都道府県別の参加施設数は，1〜2施設の県が27施設と過半数を占めたが，大都市圏（3都府県）では，14〜22施設と多かった（表2）．施設病床数は500床以上の大病院が80％を占めた（図1a）．小児科病床数も30床以上の施設が70％以上を占めた（図1b）．そのうち小児科単独病棟を持つ施設は105施設で，81施設は混合病棟であった．小児血液腫瘍病床数は，6床以上確保されている施設は35％に過ぎず，約半数は不定の回答であった（図1c）．専門医研修施設認定状況は，日本小児科学会専門医研修施設が174施設（93.5％），日本血液学会専門医研修施設が154施設（82.8％）であった．

小児科医師数については，小児科常勤医師数が10名以上の施設が60％を占めるものの，4名以下の施設が32施設あった（図2a）．また，小児血液腫瘍を担当する医師数は2名以下が96施設と過半数を占めた（図
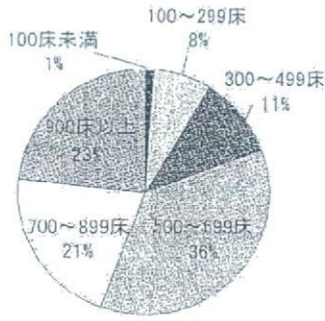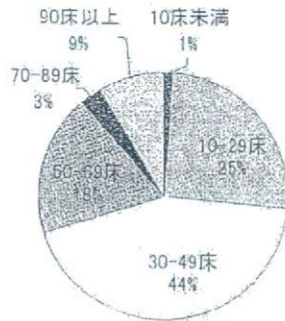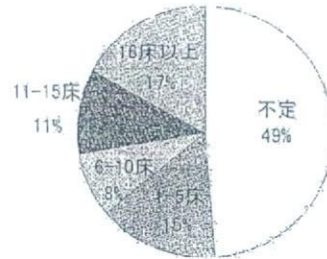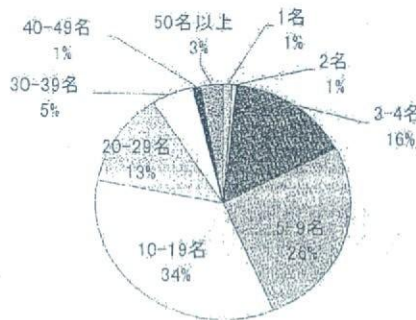
a. 施設病床数　　　　b. 小児科病床数　　　　c. 小児血液腫瘍病床数
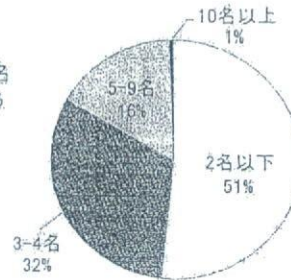
図1　施設規模

a. 小児科常勤医数　　　　b. 小児血液腫瘍担当医数

図2　医師数

表3　施設責任者もしくは実務担当者の学会入会・専門医取得状況

（施設数）	有	無
日本小児血液学会会員	185	1
日本小児がん学会会員	153	15
日本血液学会会員	156	14
小児科専門医	179	7
日本血液学会血液専門医	100	78

表4　放射線治療医・小児外科医・麻酔科医の有無

	常勤	非常勤	無し
放射線治療医	153 施設	19 施設	8 施設
小児外科医	134 施設	15 施設	37 施設
麻酔科医	183 施設	2 施設	1 施設

2b). 医師のうち, 施設研究責任者もしくは実務担当者の学会入会・専門医取得状況を調査したところ, いずれもが日本小児血液学会会員でない施設が1施設, いずれもが小児科専門医でない施設が7施設, いずれもが血液専門医でない施設が78施設であった（表3）. また, 関連診療科として放射線治療医, 小児外科医, 麻酔医の有無について尋ねたところ, それぞれ8施設, 37施設, 1施設で常勤または非常勤医師いずれもが不在であった（表4）.

小児がんの診療分野としては, 造血器腫瘍のほか, 小児外科腫瘍が108施設（58.1％）, 眼腫瘍が50施設（26.9％）, 骨軟部腫瘍が80施設（43.0％）, 脳腫瘍が88施設（47.3％）で診療されていた. MTX血中濃度測定

が自施設で可能な施設は136施設（73.1％）に留まっていた.

造血幹細胞移植のための設備と実施状況は, 無菌室が159施設（85.5％）, 全身放射線照射装置は145施設（87.0％）で設置されており, 2005年度においては, 111施設（59.7％）で自家移植, 血縁移植, 非血縁骨髄移植, 臍帯血移植のいずれかが実施されていた（表5）.

患者支援設備・スタッフについては, 145施設（78.0％）に院内学級が設置されていたが, 患者支援の設備やスタッフは, 26.9～46.2％に留まっていた（表6）.

IRBまたは倫理審査委員会の設置は, IRBは175施設（94.1％）に, 倫理審査委員会は2施設を除くすべての施設に設置されていた. しかし, プロトコールの倫理審査実施については, 常にと回答した施設は154施設（82.8％）に留まり, 時に回答した施設が4施設認められた. また, 小児血液腫瘍専任のデータマネジャー

表5　2005年度造血幹細胞移植実施状況

	自家	同種			移植の実施
		血縁*	非血縁	臍帯血	
有	82施設	80施設	52施設	49施設	111施設
無	104施設	106施設	134施設	137施設	75施設

表6　患者支援設備・スタッフの有無

	院内学級	家族用宿泊施設	患者支援ボランティアグループ	親の会	保育士・CLS	小児心理士
有	145施設	50施設	67施設	60施設	77施設	86施設
無	41施設	136施設	119施設	126施設	100施設	91施設

CLS : Child Life Specialist

表7　主要国の小児造血器腫瘍診療施設規模の比較

国名		日本	アメリカ合衆国	ドイツ	フランス	イギリス
小児人口 (0〜14歳) (統計年)[10]		1,752万人 (2005年)	6,076万人 (2004年)	1,204万人 (2004年)	1,116万人 (2003年)	1,089万人 (2004年)
施設数 (グループ, 調査年)		186 (JPLSG, 1997〜2001)[3]	231 (COG, 2003〜2005)[10]	92 (GPOH, 2002〜2006)[7]	33 (SFCE, 2006)[8]	22 (MRC-CLWP, -)[8]
	集計対象疾患	造血器腫瘍	造血器腫瘍+固形腫瘍	造血器腫瘍+固形腫瘍	造血器腫瘍+固形腫瘍	造血器腫瘍+固形腫瘍
年間臨床試験登録数 (ただし、ドイツとイギリスは年間疾患登録数) 別施設数	登録数 50〜	0	9	9	8	22
	40〜49	0	6	6	3	0
	30〜39	0	16	6	8	0
	20〜29	2	28	12	7	0
	10〜19	11	61	19	5	0
	5〜9	64	68	11	1	0
	1〜4	90	37	19	1	0
	<1	19	6	10	0	0
年間登録数20例以上の施設 (JPLSGは10例以上)で占める登録数の割合		21%	58%	77%	94%	100%

JPLSG: Japanese Pediatric Leukemia/Lymphoma Study Group. COG: Children's Oncology Group. GPOH: Gesellschaft für Pädiatrische Onkologie und Hämatologie. SFCE: La Société Française de Lutte contre les Cancers et Leucémies de l'Enfant et de l'Adolescent. MRC-CLWP: Medical Research Council-Childhood Leukemia Working Party

のいる施設はわずか10施設 (5.4%) であった.

考　察

　日本小児白血病リンパ腫研究グループ(JPLSG)参加施設の実態調査結果を報告した。わが国の小児造血器腫瘍診療施設のほとんどがJPLSGに参加していることから、今回の調査結果は、我が国の小児血液腫瘍の診療の実態を表している。参加施設の多くが日本小児科学会専門医研修施設かつ日本血液学会専門医研修施設であることから、教育機能のある施設で小児血液腫瘍の診療が行われているといえる一方で、参加施設の過半数が、少数の入院患者を2名以下の専門スタッフで診療している実態がうかがわれた。これは、厚生労働省研究班で調査された5年間の小児白血病リンパ腫の臨床研究登録数を調査した際に年間登録数が10例以上の施設はわずか16施設にすぎず、過半数が年間登録数2例以下の施設であったことと合致する結果である[9]。欧米では、造血器腫瘍を始め、稀少で濃厚な治療を要する小児がんの診療は、主に大規模診療施設で治療されている。実際、イギリス[8]、フランス[1]、ドイツ[6]、アメリカ[9]では、それぞれ100%, 94%, 77%, 58%の患者が年間20例以上の小児がん登録数のある施設で診療されている(表7)。とりわけ、イギリスでは、小児がん診療センターが22施設しかなく、施設条件として4〜5名のコンサルタントと血液分野と固形分野に精通した医師がそれぞれ2名以上いる体制で年間80例以上の新患を診療することが推奨されている[8]。一方、わが国では、白血病リンパ腫の年間登録数が10

表8　JPLSG参加施設一覧　2007.3.31現在

CCLSG	JACLS	KYCCSG
国立病院機構北海道がんセンター	大阪府立母子保健総合医療センター	国立病院機構九州がんセンター
中通総合病院	近畿大学医学部附属病院	九州大学病院
新潟大学医歯学総合病院	和歌山県立医科大学附属病院	大分県立病院
新潟県立がんセンター新潟病院	兵庫医科大学附属病院	浜の町病院
福島県立医科大学附属病院	神戸大学医学部附属病院	福岡大学病院
日本大学医学部附属板橋病院	兵庫県立こども病院	久留米大学医学部附属病院
国立国際医療センター	大阪市立大学医学部附属病院	鹿児島市立病院
静岡県立静岡がんセンター	中野こども病院	山口大学医学部附属病院
静岡県立こども病院	市立吹田市民病院	宮崎大学医学部附属病院
愛知医科大学病院	姫路赤十字病院	北九州市立医療センター
金沢大学医学部附属病院	近畿大学医学部附属堺病院	鹿児島大学病院
富山大学医学部附属病院	川崎医科大学附属病院	**TCCSG**
富山市民病院	岡山大学医学部・歯学部附属病院	茨城県立こども病院
金沢医科大学附属病院	国立病院機構岡山医療センター	神奈川県立こども医療センター
滋賀医科大学附属病院	岡山赤十字病院	熊本大学医学部附属病院
大阪医科大学	岡山済生会総合病院	群馬大学医学部附属病院
鳥取大学医学部附属病院	倉敷中央病院	慶應義塾大学病院
国立病院機構香川小児病院	広島大学医学部附属病院	国立病院機構熊本医療センター
徳島大学医学部附属病院	広島赤十字・原爆病院	国立成育医療センター
長崎大学医学部・歯学部附属病院	国立病院機構呉医療センター	埼玉医科大学病院
秋田大学医学部附属病院	香川大学医学部附属病院	埼玉県立小児医療センター
市立秋田総合病院	高知大学医学部附属病院	東京慈恵会医科大学附属病院
大阪労災病院	高知医療センター	自治医科大学附属病院
鳥取県立中央病院	愛媛大学医学部附属病院	順天堂大学医学部附属順天堂病院
石川県立中央病院	松山赤十字病院	昭和大学藤が丘病院
高知赤十字病院	愛媛県立中央病院	信州大学医学部附属病院
沖縄県立南部医療センター	島根大学医学部附属病院	聖マリアンナ医科大学附属病院
JACLS	大分大学医学部附属病院	聖路加国際病院
旭川赤十字病院	佐賀大学医学部附属病院	千葉大学医学部附属病院
札幌医科大学附属病院	産業医科大学附属病院	千葉県こども病院
北海道大学医学部附属病院	北九州市立八幡病院	帝京大学医学部附属病院
KKR札幌医療センター	琉球大学医学部附属病院	東海大学医学部附属病院
旭川医科大学附属病院	京都大学医学部附属病院	東京医科歯科大学附属病院
北海道立小児総合保健センター	国立病院機構京都医療センター	東京医科大学附属病院
市立函館病院	京都桂病院	東京大学医学部附属病院
特定医療法人北楡会札幌北楡病院	神戸市立中央市民病院	東京女子医科大学東医療センター
浜松医科大学附属病院	西神戸医療センター	東邦大学医療センター大森病院
聖隷浜松病院	天理よろづ相談所病院	獨協医科大学附属病院
豊橋市民病院	日本赤十字和歌山医療センター	都立清瀬小児病院
安城更生病院	滋賀県立小児保健医療センター	都立駒込病院
藤田保健衛生大学附属病院	大津赤十字病院	日本医科大学附属病院
名古屋市立大学医学部附属病院	島根県立中央病院	山梨大学医学部附属病院
名古屋大学医学部附属病院	松江赤十字病院	横浜市立大学医学部附属病院
名古屋第一赤十字病院	福井大学医学部附属病院	東京大学医科学研究所
名古屋第二赤十字病院	市立岸和田市民病院	北里大学医学部附属病院
国立病院機構名古屋医療センター	市立島田市民病院	筑波大学附属病院
一宮市立市民病院	財団法人田附興風会北野病院	群馬県立小児医療センター
小牧市民病院	国立病院機構舞鶴医療センター	杏林大学医学部付属病院
岐阜大学医学部附属病院	京都第一赤十字病院	長野県立こども病院
岐阜市民病院	京都市立病院	東京慈恵会医科大学柏病院
三重大学医学部附属病院	明石市立市民病院	東京慈恵会医科大学附属第三病院
県西部浜松医療センター	松下記念病院	成田赤十字病院
岡崎市民病院	社会保険神戸中央病院	松戸市立病院
名鉄病院	京都府立医科大学附属病院	帝京大学ちば総合医療センター
名古屋市立東市民病院	弘前大学医学部附属病院	東京歯科大学市川総合病院
奈良県立医科大学附属病院	青森県立中央病院	足利赤十字病院
関西医科大学附属枚方病院	岩手医科大学附属病院	東邦大学医療センター大橋病院
大阪大学医学部附属病院	岩手県立北上病院	埼玉医科大学総合医療センター
大阪市立総合医療センター	東北大学病院	聖マリアンナ医科大学横浜市西部病院
大阪赤十字病院	山形大学医学部附属病院	帝京大学医学部附属溝口病院
国立病院機構大阪医療センター	いわき市立総合磐城共立病院	昭和大学病院
大阪府立急性期・総合医療センター	宮城県立こども病院	済生会横浜市南部病院
		東京西徳洲会病院
		防衛医科大学校附属病院

例以上の施設において全体のわずか21.4%を診療しているに過ぎず[2]，如何に小規模診療施設に依存した診療体制にあるかがわかる．さらに，参加施設の60%近い施設が同じスタッフで固形腫瘍の診療や移植医療も行っており，欧米ではすでに分業化が確立した診療分野を，わが国では少ないスタッフで手広く診療している実態が浮き彫りとなった．また，都道府県別では，2施設以下が27県あるものの，6〜9施設が8道府県あり，3都府県では，参加施設数がそれぞれ，22，17，14と多い．年間900例足らずの小児造血器腫瘍の新規患者に対してこれら186施設で診療が行われているが，診療体制の格差が大きく，また，専門性の高い施設が限られている．専門医の育成のためには短期で十分な診療経験を持たせる必要があることからも診療規模の大きな施設が求められる．とりわけ，大都市圏では，症例の集約化とそれを受け入れる施設の整備とマンパワーの確保（集約化）が必要である．一方，症例の少ない地方地域では，診療スタッフの確保が重要課題であるとともにセンター病院とサテライト病院の連携システムを構築して患者の利便性に配慮した診療システムの構築が望ましいと思われる．

施設責任者もしくは実務担当者自身が小児科専門医でない施設が7施設，血液専門医でない施設は78施設に及ぶ．小児造血器腫瘍の臨床試験を行うJPLSGの参加施設として医療の質の確保するためには，小児がん専門医制度がない現段階では，血液専門医の存在が小児血液疾患診療の質の担保の目安と考えられる．JPLSGでは，2年後を目途に参加施設基準に血液専門医がいることを加える予定である．

臨床試験の実施の条件としてプロトコールの倫理審査は必須であるが，未だプロトコールの倫理審査の完全実施率は82.8%に留まっていた．臨床研究に対する倫理的配慮の意識の一層の徹底が必要である．また，診療現場で臨床試験をサポートするスタッフを置いている施設はわずか10施設であり，今後，臨床研究の質の確保と医師の負担軽減のためには施設への支援の充実が必要であろう．さらに，多くの施設が同一スタッフで固形腫瘍の診療も行っていることから臨床研究基盤の共有化が効率的で，かつ施設負担の軽減に繋がるかもしれない．

小児造血器腫瘍の医療の質の確保には療養環境の整備も不可欠である．78%の施設に院内学級が設置されているものの，患者支援の設備やスタッフは，50%未満に留まっており，トータルケアの充実も求められる．

結　語

小児造血器腫瘍の診療は，固形腫瘍や造血幹細胞移植など欧米ではすでに分業化が確立した診療分野と合わせて少ないスタッフで診療が行われている実態が明らかとなった．施設間格差は未だ大きく，大都市圏での施設の集約化，地方施設の診療スタッフ確保，および専門医療の教育研修システムの構築が急がれる．また，このような状況下で臨床試験を円滑に行うには意識改革とともにスタッフの負担軽減に繋がる支援体制の強化が必要と思われた．

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The Realities of the Medical System for Pediatric Hematologic Malignancies in Japan

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Gathering all of the pediatric leukemia groups in Japan, the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) was organized to undertake high quality clinical trials to establish the standard therapy for pediatric hematologic malignancies in 2003. In this study, the realities of the medical system for pediatric hematologic malignancies in Japan were revealed by the questionnaire to the hospitals participating in JPLSG. Replies were obtained from all 186 hospitals and were analyzed. There were 96 hospitals with less than 3 staff, 78 hospitals with no staff on the hematologic board, 108 and 111 hospitals with clinical service for solid tumors and hematopoietic stem cell transplantation (HSCT), respectively. A clinical research coordinator working for pediatric malignancies was found only in 10 hospitals. The study revealed that clinical services for hematologic malignancies, solid tumors and HSCT were all provided by the small number of staff, and that the service quality varied among the hospitals. In conclusion, intensified service systems in metropolitan areas, the securing of staff in local areas, and on education system for raising specialists will be needed in the near future. Supporting system for local staff to relieve the burden will be also required to carry out high quality clinical trials.

Retrospective Analysis of Relapsed or Primary Refractory Childhood Lymphoblastic Lymphoma in Japan

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Background and Procedure. To assess the clinical course with response to second-line treatment and to evaluate the role of hematopoietic stem cell transplantation (SCT) in children with relapsed or primary refractory lymphoblastic lymphoma (LBL), we analyzed data of 48 patients with relapsed/primary refractory diseases among 260 LBL patients identified in a national survey of 1996–2004. **Results.** Twenty-six patients achieved second complete remission; 9 achieved partial remission. Of 13 patients who showed progression despite first and second line therapy, only one patient was alive on the second relapse after unrelated cord blood transplantation. Among 40 relapsed patients, the median time between initial diagnosis and relapse was 12.5 months (range 3–56 months). The sites of relapse were isolated BM (n = 9), primary local site with BM (9), primary local site (6), isolated CNS (4), local site with mediastinum (4), primary local site with other site (4), and others (4). Of all 48 patients, 3 were alive after chemotherapy alone. Of the 33 patients, 14 were alive after high dose chemotherapy (HDC)/SCT. With a 27.5-month median follow up period, the 3-year OS rate was 43.2 ± 7.4% (estimate ± SE). Univariate analysis identified two features (relapse within 12 months, T cell phenotype) as significant variables that predicted poor survival. Multivariate analysis showed novel statistically significant variables including relapse within 12 months from initial diagnosis (Hazard ratio 3.60) and absence of HDC/SCT (2.64). **Conclusion.** Outcomes of patients with relapsed/primary refractory LBL were poor, but HDC/SCT for these patients was associated with good results. Pediatr Blood Cancer 2009;52:591–595. © 2009 Wiley-Liss, Inc.

Key words: children; lymphoblastic lymphoma; recurrence; refractory

INTRODUCTION

Malignant lymphoma is the fourth most frequent of all Japanese childhood cancers. It represents 5% of all new cases. Lymphoblastic lymphoma (LBL) is a major histology of childhood NHL, accounting for about 30%. Excellent outcomes for children with LBL have been reported with protocols closely modeled on therapy designed for acute lymphoblastic leukemia (ALL) [1]. However, 20–40% of patients develop relapse or primary refractory disease. They have poor prognoses [2,3]. The clinical courses and outcomes of these relapsed or primary refractory LBL of children have not been well documented [2,4].

To determine the response to second-line treatment and the outcomes of children with a relapsed or primary refractory LBL, and to evaluate the role of high dose chemotherapy and stem cell transplantation (HDC/SCT) in these patients, we performed a retrospective nationwide analysis of LBL patients in Japan.

PATIENTS AND METHODS

Among 260 patients with LBL registered in a national survey during 1996–2004, 48 patients (18.5%) from 39 institutions with primary refractory or relapsed diseases were found, including 8 primary refractory diseases and 40 relapses. Their medical records were reviewed. Relapse was defined as appearance of new lesions, re-growth of original masses and obvious enlargement of the mediastinal mass as revealed by imaging study with pathological examination in principle, and appearance of tumor cells in bone marrow and cerebrospinal fluid. Among 40 relapsed patients, 25 were confirmed relapse by histological/cytological examinations, 9 were defined with only clinical courses and imaging studies, and the rest of 6 were unknown about precise information. Among five patients recurred with mediastinal masses, four were confirmed by histological/cytological study, and one was determined by only imaging studies. Clinical data including treatment and follow-up information were gathered from a review of relapsed or primary

refractory patient charts through the Japanese Pediatric Leukemia Lymphoma Study Group (JPLSG). The JPLSG comprises four children's hematology/oncology study groups: Japan Association of Childhood Leukemia Study, Tokyo Children's Cancer Study Group, Japan Children's Cancer and Leukemia Study Group, and Kyushu-Yamaguchi Children's Cancer Study Group. First line treatments differed among groups. The most frequently used treatment regimens were based on the framework of the LSA2-L2 protocol or the BFM group strategy [5,6]. After 4–6 weeks of ALL-therapy-like induction, some courses of consolidation and intensification were done for first line therapy followed by maintenance consisting of multi-agent block therapy or oral 6-MP with weekly MTX. Actual drugs and dose during consolidation, intensification and maintenance varied among groups. Total durations of therapy were of two types: 18 and 24 months.

Second line treatment also varied. Among 41 patients for whom descriptions of second line chemotherapy regimen were available, 11 received their own first line protocol similar to high risk ALL

induction, 12 received therapy of other high risk ALL induction, 5 received AML type therapy, 3 underwent an ifosfamide, carboplatin, etoposide (ICE) [7] regimen, 6 received myeloablative stem cell transplantation as a re-induction therapy, and 4 received other therapies. Because of the lack of uniformity in the therapeutic regimens for refractory or recurrent cases, we mainly examined these patients' characteristics with the prognostic significance of the variables on overall survival.

Using Kaplan–Meier estimates, curves were calculated for the probability of overall survival together with standard error (SE). Univariate analyses of the association of various clinical factors were done with overall survival. The curves were compared using a double-sided log rank test. $P < 0.05$ at both sides was considered significant. The overall survival (OS) rate was calculated from the time of initial diagnosis to death. Progression free survival (PFS) was calculated from the time of relapse or refractory phase to disease progression. Multivariate analyses were performed using the Cox proportional-hazard model. Variables with P-values ≤ 0.1 in prior univariate testing were included.

RESULTS

Table I portrays representative characteristics of primary refractory or relapsed patients. Male patients were 66.7%, which is similar to the 70% males among all NHL patients. Of the patients, 81% showed greater than clinical stage III at initial diagnosis. Among 48 patients, 2 eventually revealed an NK type immunophenotype after initiation of first line LBL type therapy. Both achieved complete remission (CR) with first line therapy, but recurred. One was refractory to second line therapy; the patient received unrelated cord blood transplantation (UCBSCT) and died of graft failure. Another patient achieved partial remission (PR) with second line therapy, received allogeneic bone marrow transplantation (BMT), and entered into continuous CR.

Sites of relapse were the primary local site (12.5%), and the primary site with another site (35.4%) (Table II). Of 40 relapsed patients, 33 exhibited recurrence during first line chemotherapy and 7 after it (3–56 months after diagnosis, median 12.5 months). The patients' clinical courses and outcomes are shown in Figures 1 and 2. Of all primary refractory/relapsed patients, 26 patients achieved CR; and 9 patients achieved PR after second line chemotherapy. Among 13 patients who progressed in spite of first and second line chemotherapy, 1 patient was alive at the analysis on second relapse after UCBSCT, 8 patients died of therapy related toxicity, and 4 died of disease progression. Among the eight primary refractory patients, only one patient who had CNS local disease was alive after

TABLE I. Patient Characteristics Initial Diagnosis (n = 48)

Age at diagnosis (years), median (range)	9 (1–15)
Male sex (%)	32 (66.7%)
Stage (Murphy's classification)	
I	2
II	7
III	26
IV	13
Histological Immunophenotype	
Precursor B	9
Precursor T	32
Others (not determined 4, NK 2,T, B mix 1)	7

TABLE II. Site of Relapse

Primary site only	6
BM	9
CNS	4
BM and CNS	1
Mediastinum	1
Others	2
Primary site + α*	17

BM denotes bone marrow; CNS, central nervous system; * → α BM, 9; Mediastinum, 4; CNS, 1; Others, 3.

chemotherapy with radiation without HDC/SCT. HDC/SCT was done for five patients. Two patients were alive; one survived for 50 months after auto BMT for local mediastinal disease; the other was PR for 5 months after UCBSCT. Among 40 relapsed patients, 2 were alive under chemotherapy alone and gained CR after second line chemotherapy, 1 was alive for 55 months after BM relapse, and 1 was alive for 57 months with radiation after CNS local disease. Among 28 patients who had HDC/SCT after relapse, 12 patients were alive; 7 had had advanced disease and 5 had had local disease.

With a median follow-up period of 27.5 months, the 3-year OS rate was $43.2 \pm 7.4\%$ (estimate $\pm$ SE) (Fig. 3). Univariate analysis identified two features that were significant (Table III) as variables that were predictive of OS: relapse within 12 months and T cell phenotype. The presence of HDC/SCT was not significant. Regarding the total duration of first line therapy, we found no significant difference between 18 months and 24 months ($P = 0.90$). The 3-year progression free survival rate was $37.0 \pm 7.3\%$. Univariate analysis for PFS with the same variables for OS showed a significant difference only in the presence of HDC/SCT (3-year PFS $36.9 \pm 9.1\%$ vs. 21.4 ± 11.0, $P = 0.03$).

The OS rates for 25 patients who underwent HDC/SCT during CR or PR, and for 8 patients who received chemotherapy without HDC/SCT after achieving CR or PR were $61.5 \pm 10.3\%$ and $37.5 \pm 17.1\%$, respectively; they were not significantly different ($P = 0.06$). Regarding patients who underwent HDC/SCT during CR or PR, 6 among 19 patients who underwent allogeneic SCT had relapsed; 4 among 6 patients who had undergone autologous SCT had relapsed. Of those 19 allogeneic SCT recipients, 10 survived without further progression (median 22 months after transplantation), although only 2 of 6 autologous recipients survived (median 40 months). Regarding transplantation-related toxicity, three allogeneic recipients died of toxicity, although none had died with autologous transplantation. Among all transplanted patients, the median times to transplantation from the refractory/relapse phase were 5 months for allogeneic (n = 26) and 4 for autologous (n = 7). BM involvement appeared respectively in 10 cases and 1. The OS rates between these were, respectively $54.0 \pm 10.4\%$ and $28.6 \pm 17.1\%$. No significant difference was observed ($P = 0.42$), although a higher OS rate was observed in the allogeneic group. The donor type, whether related or unrelated, also showed no significant difference ($P = 0.86$) among allogeneic transplantation cases. Regarding the transplant preparative regimen, except for one patient who could not undergo the myeloablative regimen, all preparative regimens were myeloablative. Additive chemotherapy varied among patients, for example (ara-C, ara-C + VP-16, VP16 + CY, ara-C + VP-16 + CY, CY + TT, BUS + L-PAM, L-PAM + FDA); no significant difference was found between TBI (n = 22) and non-TBI regimens (9) ($P = 0.73$).