Table 1. Clinical Characteristics[a]{TC}

Parameter	No. of Patients	%
	n=68	
Age(y)		
Median	55.0	
Range	12-84	
<40	12	17.6
≥40, <60	24	35.3
≥60	32	47.1
Sex		
Male	41	60.3
Female	27	39.7
Preoperative ECOG performance status		
Median	1	
Range	0-3	
Preoperative ECOG performance status		
≤1	45	66.2
>1	23	33.8
Tumor location		
Superficial	50	73.5
Deep	18	26.5
Surgery		
GTR	24	35.3
Non-GTR	44	64.7
Chemotherapy		
TMZ only	29	42.6
TMZ+ IFN-β	39	57.4

ECOG indicates Eastern Cooperative Oncology Group; PS, performance status; GTR, macroscopic (gross) total removal; TMZ, temozolomide.

Tumor samples and DNA Extraction

All patients provided their written informed consent for molecular studies of their tumor, and the protocol was approved by the ethics committee at each center. Sixty-eight brain tumor specimens were obtained at the time of first surgical resection.

Tumor tissue samples were immediately frozen and stored at −80°C until the extraction of genomic DNA. DNA was prepared using the QIAmp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Placental DNA was used as the normal control. The amount of DNA obtained from the tumor was sufficient for the subsequent genomic and epigenomic analyses.

Multiplex Ligation-Dependent Probe Amplification

Multiplex ligation-dependent probe amplification (MLPA) was used for the determination of allelic losses and gains of the gene in the tumor samples. The analysis was performed using the SALSA MLPA KIT P088-B1 and P105-C1 in accordance with the manufacturer's protocol (MRC Holland, Amsterdam, Netherland).[12-15] Information regarding the probe sequences and ligation sites can be found at www.mlpa.com. Amplification products were separated on an ABI® 3130 × I Genetic Analyzer (Applied Biosystems, Foster City, CA) and quantified with Genemapper 4.0 software (Applied Biosystems). Duplicate experiments were performed to obtain accurate MLPA values. Data analysis was performed with an original Excel-based program based on MRC-Holland's procedures. Normalization for sample data was first performed on control probes, and each tumor sample was then normalized using the data on 2 control samples, using peripheral blood DNA. Single regression for control and tumor data slope correction was performed. Abnormal/normal ratio limits were set at 0.65 and 1.3. Statistical analysis was performed using the same Coffalyser software.

Pyrosequencing

Tumor DNA was modified with bisulfate using the EpiTect bisulfite kit (Qiagen, Courtaboeuf Cedex, France). Pyrosequencing technology was used to determine the methylation status of the CpG island region of MGMT as described previously.[16,17] We used the touchdown PCR method. The primer sequences used were the MGMT forward primer, 5′-TTGGTAAATTAAGGTATAGAGTTTT-3′, and the MGMT biotinylated reverse primer, 5′-AAA CAATCTACGCATCCT-3′. PCR included a denaturation step at 95°C for 30 s, followed by annealing at various temperatures for 45 s, and extension at 72°C for 45 s. After PCR, the biotinylated PCR product was purified as recommended by the manufacturer. In brief, the PCR product was bound to Streptavidin Sepharose HP (Amersham Biosciences, Uppsala, Sweden), and the Sepharose beads containing the immobilized PCR product were purified, washed, and denatured using 0.2 N NaOH solution and washed again. Next, 0.3 mM pyrosequencing primer was annealed to the purified single-stranded PCR product, and pyrosequencing was performed using the PSQ HS 96 Pyrosequencing System (Pyrosequencing, Westborough, MA). The pyrosequencing primer was 5′-GGAAGTTGGGAAGG-3′. Methylation quantification was performed using the provided software.

TP53 and IDH1/IDH2 Sequencing

Direct sequencing of the *TP53* exons 5 to 8 and *IDH1/IDH2* was performed as previously described.[7,18,19] The primer sequences are listed in Table 2.

Table 2. List of Primer Sequences for Direct DNA Sequencing{TC}

Gene name	Exon		Sequence
TP53	Exon 5	F	5′-TTATCTGTTCACTTGTGCCC-3′
		R	5′-ACCCTGGGCAACCAGCCCTG-3′
	Exon 6	F	5′-ACGACAGGGCTGGTTGCCCA-3′,
		R	5′-CTCCCAGAGACCCCAGTTGC-3′
	Exon 7	F	5′-GGCCTCATCTTGGGCCTGTG-3′
		R	5′-CAGTGTGCAGGGTGGCAAGT-3′
	Exon 8	F	5′-CTGCCTCTTGCTTCTCTTTT-3′
		R	5′-TCTCCTCCACCGCTTCTTGT-3′.
IDH1		F	5′-CGGTCTTCAGAGAAGCCATT-3′
		R	5′-GCAAAATCACATTATTGCCAAC-3′
IDH2		F	5′-AGCCCATCATCTGCAAAAAC-3′
		R	5′-CTAGGCGAGGAGCTCCAGT-3′.

F indicates forward primer; R, reverse primer;.

For IDH sequencing, a fragment 129 bp in length, spanning the sequence encoding the catalytic domain of *IDH1*, including codon 132, and a fragment 150 bp in length spanning the sequence encoding the catalytic domain of *IDH2*, including codon 172, were amplified. We applied touchdown PCR, using the standard buffer conditions: it comprised 5 ng of DNA and AmpliTaq Gold DNA Polymerase (Applied Biosystems) run for 16 cycles with denaturation at 95°C for 30 s, annealing at 65 to 57°C (decreasing by 0.5°C per cycle) for 30 s, and extension at 72°C for 60 s in a total volume of 12.5 μl and add 30 cycles with denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 60 s, ending with at 72°C for 7 min to complete extension.

Direct sequencing was performed using BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems). The reactions were carried out using an ABI 3100 Genetic Analyzer (Applied Biosystems).

Statistical analysis

Statistical analysis was performed using the statistical software SPSS for Windows, version 17.0 (SPSS Inc, Chicago, Ill). The Mann-Whitney U test, χ^2 test, and Fisher exact test were used to test for association of clinical variables and molecular markers. Survival was estimated by using the Kaplan-Meier method, and survival curves were compared by using the log-rank test. Progression-free survival (PFS) was calculated from the day of first surgery until tumor progression, death, or end of follow up. Overall survival (OS) was calculated from the day of first surgery until death or the end of follow up. Univariate and multivariate analyses were performed to test the potential influence of baseline characteristics on survival. The effect of each single molecular marker on PFS and OS was investigated using the Cox proportional hazards model, adjusting for the major clinical prognostic factors, including age at diagnosis (<40 vs ≥40, <60 vs ≥60 years), ECOG performance status score (ECOG PS; ≤1 vs >1), extent of resection (macroscopic [gross] total resection [GTR] vs non-GTR), tumor location (superficial vs deep), MGMT promoter methylation status, chromosome 1p loss of heterozygosity (LOH), 19qLOH, *PTEN* loss, *CDKN2A* loss, *TP53* loss and mutation, *ERBB2* amplification, *EGFR* amplification, *IDH1* and *IDH2* mutation, and adjuvant therapy (with IFN-β vs without IFN-β). Factors with no significant association with survival, at a level of more than 0.05 in the multivariate analysis, were eliminated. The remaining factors in the multivariate proportional hazard model (P <.05) were considered to be independent predictors of survival.

To assess for the treatment effects of TMZ with IFN-β versus TMZ without IFN-β for overall survival (OS), the hazard ratio was computed using a proportional hazard model by baseline characteristics in stratified analysis.

RESULTS

Clinical parameters

Between May 2006 and June 2010, 68 consecutive patients newly diagnosed with primary GBM were registered in this study. Their clinical characteristics are summarized in Table 1. This study group comprised 41 men and 27 women aged 12-84 years (median, 55). The median preoperative ECOG PS score at diagnosis was 1 (range, 0-3); the preoperative ECOG PS score was <1 in 45 patients (66.2%). All tumors were located in the supratentorial region: 50 tumors were located in the superficial area (cortical or subcortical area), and 18 were located in deep anatomical structures such as the basal ganglia and corpus callosum. No tumor was noted in the optic nerve, olfactory nerve, and pituitary gland on pretreatment MRI. No tumor dissemination was detected by MRI. Surgical GTR was achieved in 24 patients (35.3%), and 44 patients underwent non-GTR (64.7%). None of the patients had concurrent active malignancy, and the baseline organ function before chemotherapy was as follows: absolute WBC ≥3000/mm^3 or neutrophil count ≥1,500/mm^3, platelet count ≥100,000/mm^3, hemoglobin ≥8.0 g/dl, AST less than 2.5 × the upper limit of normal (ULN), total bilirubin 2 × ULN, and creatinine 2 × ULN, and electrocardiogram showing no serious

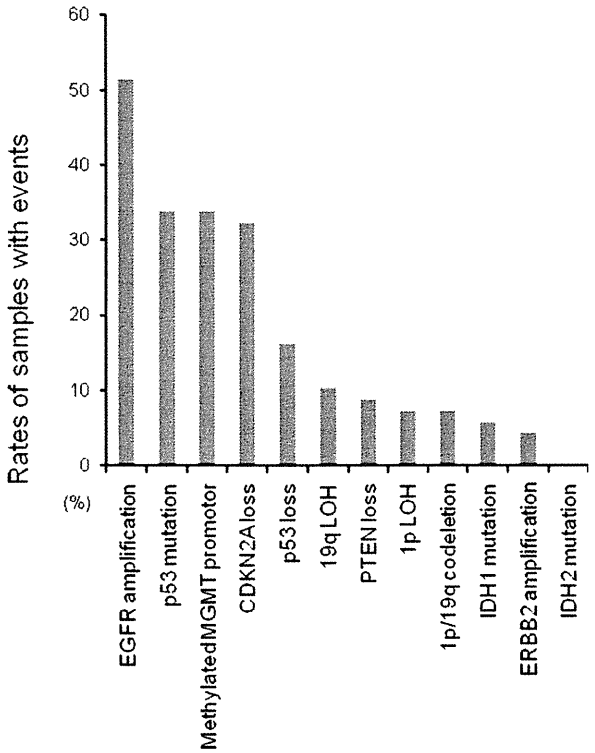


Figure 1. Frequency and pattern of genetic and epigenetic alterations in newly diagnosed primary glioblastoma multiforme (GBM).

arrhythmia and no serious ischemic heart disease. All patients received the standard Stupp regimen,[1] and among these, 39 patients were received combination treatment with IFN-β, as described in the method section.

Frequency of Genetic and Epigenetic Alterations

Of 68 cases, we could obtain sufficient genetic and epigenetic information in all cases. We used direct sequencing for *TP53* and *IDH1/2*. We employed MLPA for the analysis of 1p/19q LOH, loss of *TP53*, *PTEN* and *CDKN2A*, and amplification of *ERBB2* and *EGFR*. MLPA is a multiplex PCR method that detects abnormal copy numbers of up to 50 different genomic DNA sequences simultaneously. When comparing MLPA to FISH, MLPA not only has the advantage of being a multiplex technique but also one in which very small (50-70 nt) sequences are targeted, enabling MLPA to identify the frequent, single gene aberrations that are very small to be detected by FISH. Furthermore, for the detection of *EGFR* amplification, MLPA can examine exons 1-8, 13, 16, and 22, while previously reported real-time PCR covers only exons 2, 17, and 25. In our preliminary experiments, MLPA was found to be approximately 80% consistent with the real-time PCR method (data not shown). Notably, the methylation status of the MGMT promoter was analyzed by quantitative pyrosequencing technology. Although methylation-specific PCR analysis of MGMT promoter methylation is a widely applicable biomarker for the clinical setting, it is non quantitative and bears a risk of false-positive or false-negative results, especially when the DNA quality and/or quantity is low. Recent attempts to remedy some of these deficiencies have led to the development of an alternative sequence-based approach for methylation analysis, known as pyrosequencing. Pyrosequencing yields continuous methylation values ranging from 0-100%. Based on our comparisons with standard methylation-specific PCR and immunohistochemical study using the anti-MGMT antibody, we determined 14% as the threshold distinguishing unmethylation and methylation of the MGMT promoter in a given tumor.

As indicated in Figure 1 and Table 3, the alterations frequently observed were *EGFR* amplification (51.5%), *TP53* mutation (33.8%), *CDKN2A* loss (32.4%), *TP53* loss (16.2%), methylation of the MGMT promoter (33.8%), and *IDH1* mutation (5.9%). These findings were consistent with those in previous reports.[3,9,20,21]

Clinical, Genetic, and Epigenetic Parameters Associated With Survival in GBM Patients

The median follow-up time was 16.7 months (range, 3.4-46.7 months). The median PFS for all patients was 9.2 months (95% confidence interval [CI], 5.7-12.7). The median OS of all patients was 17.1 months (95% CI, 15.5-18.7) (Figure 2A). The log-rank tests demonstrated that tumor localization ($P = .032$), the MGMT methylation status ($P = .029$), and *TP53* mutation or loss ($P = .035$) were associated with the OS of patients with GBM (Figure 2B-D). These findings were similar to univariate analysis, where deep location ($P = .035$), unmethylated MGMT promoter ($P = .033$) and *TP53* mutation or loss ($P = .038$) were identified as candidate variables for poorer OS (Figure 2). In contrast, well-established prognostic factors such as age, ECOG PS, and the extent of tumor resection did not influence the outcome in this clinical setting. Next, we established multivariate survival models for OS. The model was designed to consider each of these factors without considering the interaction terms. The independent prognostic factors for OS were methylated MGMT promoter ($P = .016$).

Table 3. Relation Between Genetic and Epigenetic Parameters and Overall Survival

Parameter	No.	Months of OS	Log-rank test: *P*
1p LOH			
+	5	16.9	.27
–	63	21.9	
19q LOH			
+	7	17.1	.46
–	61	21.9	
1p/19q codeletion			
+	5	16.9	.27
–	63	21.9	
***PTEN* loss**			
+	6	21.4	.40
–	62	16.9	
***CDKN2A* loss**			
+	22	16.3	.64
–	46	17.4	
***TP53* loss**			
+	11	11.7	**.08**
–	57	17.4	
***ERBB2* amplification**			
+	3	13.9	.77
–	65	17.1	
***EGFR* amplification**			
+	35	17.4	.91
–	33	17.1	
***TP53* mutation**			
+	23	15.7	.128
–	45	17.6	
***TP53* mutation or loss**			
+	29	13.9	**.035**
–	39	17.6	
MGMT promotor			
Unmethylated	45	15.1	**.029**
Methylated	23	21.4	
***IDH1* mutation**			
+	4	19.9	.96
–	64	16.9	
***IDH2* mutation**			
+	0	NA	NA
–	68	NA	

OS indicates overall survival; NA, not available

Combination of IFN-β With TMZ Prolonged Survival

We analyzed whether the use of IFN-β affected the survival of consecutive GBM patients treated with TMZ-based chemotherapy. Of the total 68 patients, 39 (57.4%) received IFN-β in combination of TMZ. Interestingly, the median OS of the combination group was significantly greater with 19.9 months (95% CI, 15.3-24.5) as compared to the TMZ alone group, which was 12.7 months (95% CI, 10.5 to 14.9) (Figure 3A). The 12-month-survival rate was 67.6% for the standard TMZ-treated cohort, whereas it was 83.6% for the combination group. The 24-month survival rates were 22.1% and 34.5%, respectively, for the 2 groups. The difference was statistically significant as determined by the log-rank test and univariate and multivariate analyses.

Benefits of IFN-β for GBM Patients With the Unmethylated MGMT Promoter

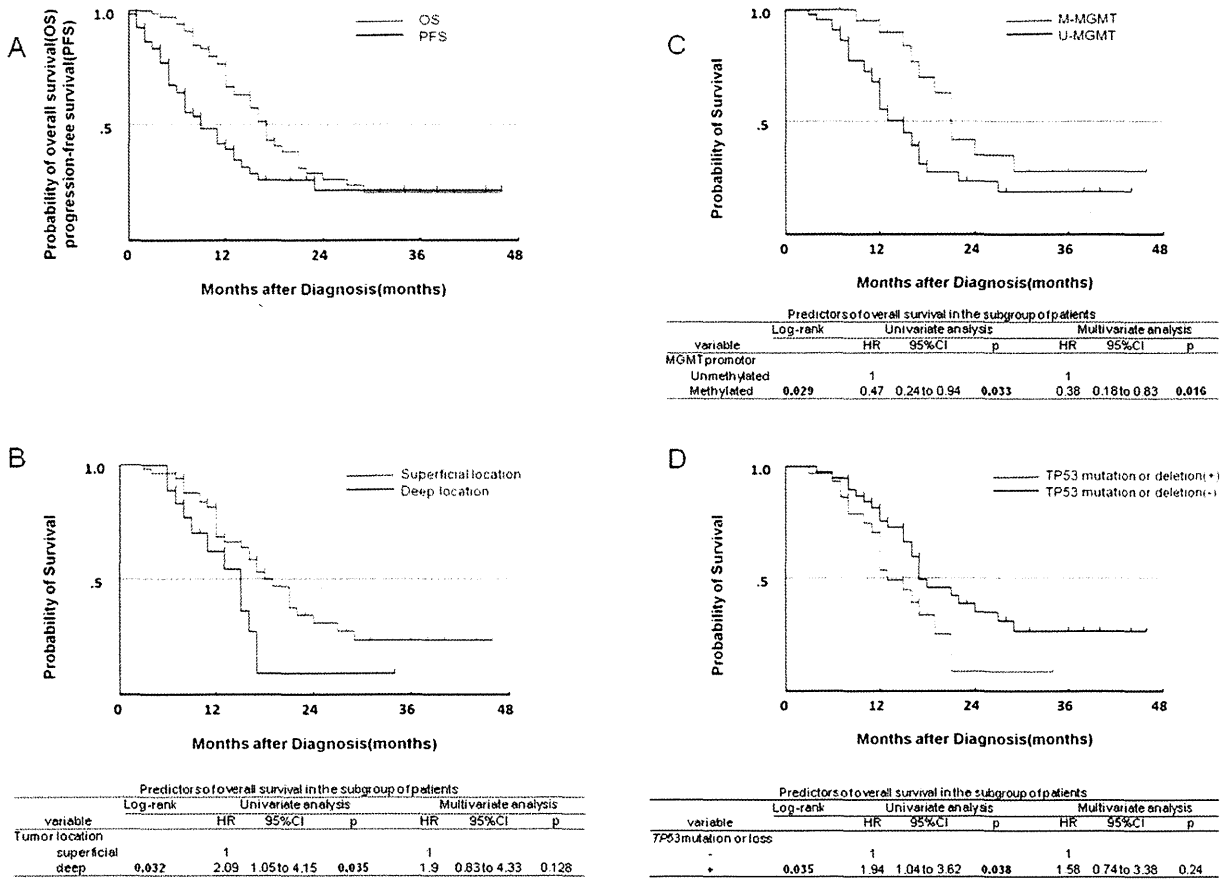
Next, we sought to determine the subpopulation that had benefited from the use of the IFN-β combination treatment. It is well known that patients with GBM containing the methylated MGMT promoter benefit from TMZ, but those with the unmethylated MGMT promoter show no such benefits.[1,2] Consistently, the median OS of 45 patients with the unmethylated MGMT status was significantly lesser than that of the patients with the methylated promoter (median OS = 15.1 months; 95% CI, 11.3-18.9). Notably, even in patients whose tumors had the unmethylated MGMT promoter, the median OS was prolonged to 17.2 months (95% CI, 13.9-20.6) when receiving TMZ with IFN-β as compared to the 12.5 months (95% CI, 11.3-13.7) in those receiving TMZ without IFN-β ($P = .017$) (Figure 3B).

Various associations of these clinical and molecular parameters were evaluated. A complete overview of the pairwise associations between these parameters and chemotherapy with or without IFN-β is provided in Figure 4. The relative hazards of OS between TMZ with or without IFN-β groups according to 6 baseline covariates, calculated by means of multivariate analysis, are shown. There were significant associations among patients under 40 years of age ($P = .025$), with ECOG PS ≤ 1 ($P = .004$), deep tumor location ($P = .028$), non-GTR ($P = .048$), and ummethylated MGMT status ($P = .02$) (Figure 4).

DISCUSSION

Genomic Analysis in Newly Diagnosed GBMs

In this study, we analyzed the genomic abnormalities in 68 consecutive newly diagnosed patients with GBM who were treated with TMZ-based chemotherapy. We observed *TP53* mutation (33.8%), *TP53* loss (16.2%), *EGFR* amplification (51.5%), *CDKN2A* loss (32.4%),



Predictors of overall survival in the subgroup of patients

	Log-rank	Univariate analysis			Multivariate analysis		
variable		HR	95%CI	p	HR	95%CI	p
MGMT promotor							
Unmethylated		1			1		
Methylated	0.029	0.47	0.24 to 0.94	0.033	0.38	0.18 to 0.83	0.016

Predictors of overall survival in the subgroup of patients

	Log-rank	Univariate analysis			Multivariate analysis		
variable		HR	95%CI	p	HR	95%CI	p
Tumor location							
superficial		1			1		
deep	0.032	2.09	1.05 to 4.15	0.035	1.9	0.83 to 4.33	0.128

Predictors of overall survival in the subgroup of patients

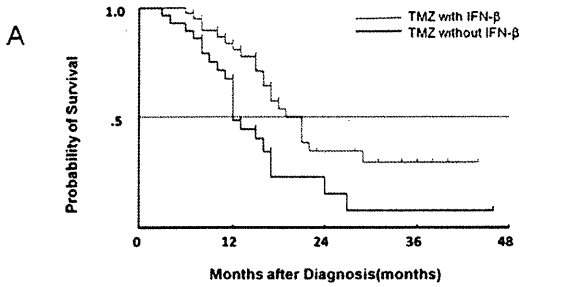
	Log-rank	Univariate analysis			Multivariate analysis		
variable		HR	95%CI	p	HR	95%CI	p
TP53 mutation or loss							
-		1			1		
+	0.035	1.94	1.04 to 3.62	0.038	1.58	0.74 to 3.38	0.24

Figure 2. Kaplan-Meier curves showing overall survival (OS) and progression-free survival (PFS) for the entire cohort (A), and OS according to (B) tumor location ($P = .032$), (C) MGMT promoter methylation status ($P = .029$), and (D) *TP53* mutation or loss ($P = .035$) (D). Predictors of overall survival in the subgroups of patients by univariate and multivariate analyses were shown (B-D). The hazards ratio (HR) was adjusted for the factors; age, Eastern Cooperative Oncology Group performance status (ECOG PS), the extent of tumor resection, MGMT promoter methylation status, *TP53* mutation or loss and TMZ with or without interferon-β (IFN-β) in the multivariate analysis.

and methylation of the MGMT promoter (33.8%). Recent large-scale efforts to characterize the GBM genome have identified additional alterations in genes not previously implicated in glioma, such as *ERBB2* and *IDH1/IDH2* mutation in primary and secondary GBM, respectively, and a significant incidence of mutation and genomic loss of *NF1*.[3,4,6] The TCGA study also noted *TP53* mutations and losses in 35% of the cases, which is a surprisingly higher frequency than that reported previously.[3,20,21] Furthermore, this study also revealed *EGFR* amplification (45%), *CDKN2A* loss (52.0%), and methylation of the MGMT promoter (20.9%). These results were consistent with our data. *IDH1* mutations have recently been identified in gliomas, which are a strong predictor of a more favorable prognosis.[6] Our study supported the finding that within the group of primary GBM, *IDH1* mutations are rare and tend to define a prognostically favorable outcome.

Factors for Prognosis and Prediction of Response to Therapy

The current study demonstrated that the methylated MGMT promoter and the combination of IFN-β and TMZ were independent prognostic indicators of GBM patients on multivariate analysis. Epigenetic silencing by the MGMT promoter methylation correlates with improved survival in glioma patients treated with TMZ.[2,22-25] The prognostic significance of MGMT promoter methylation has been shown in several clinical trials. In these studies, MGMT promoter methylation was an independent favorable prognostic factor and patients whose tumor contained a methylated MGMT promoter

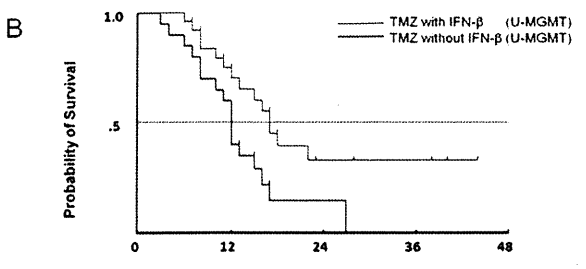


Predictors of overall survival in the subgroup of patients

	Log-rank	Univariate analysis			Multivariate analysis		
variable		HR	95%CI	p	HR	95%CI	p
Chemotherapy							
TMZ without IFN-β		1			1		
TMZ with IFN-β	**0.007**	0.43	0.23 to 0.81	**0.008**	0.46	0.23 to 0.92	**0.028**

Survival data

	All patients	TMZ with IFN-β	TMZ without IFN-β
Median OS (months)	17.1	19.9	12.7
12M-OS (%)	77	84	68
24M-OS (%)	29	35	22
Median PFS (months)	9.2	11.6	9.2
12M-PFS (%)	41	47	34



Survival data of patients with unmethylated MGMT

	All patients	TMZ with IFN-β	TMZ without IFN-β
Median OS (months)	15.1	17.2	12.5
12M-OS (%)	68	75	60
24M-OS (%)	23	32	14

Figure 3. Kaplan-Meier estimates of overall survival (OS) according to temozolomide (TMZ) with or without interferon-β (IFN-β) for all patients (A) ($P = .007$) and for patients with unmethylated MGMT promoter (U-MGMT) (B) ($P = .017$). The hazards ratio (HR) was adjusted for the factors; age, Eastern Cooperative Oncology Group performance status (ECOG PS), the extent of tumor resection, MGMT promoter methylation status, *TP53* mutation or loss, and TMZ with or without IFN-β in the multivariate analysis.

showed overall prolonged survival when treated with TMZ and radiotherapy. Our results demonstrated similarly that MGMT promoter hypermethylation determined by a novel pyrosequencing technology was significantly associated with better OS.

There are several contradicting reports on survival related to the prognostic value of *TP53* mutations in GBM, showing either no association or that the presence of *TP53* mutations was a favorable or an unfavorable prognostic factor.[9,20,21,26] On the other hand, our results

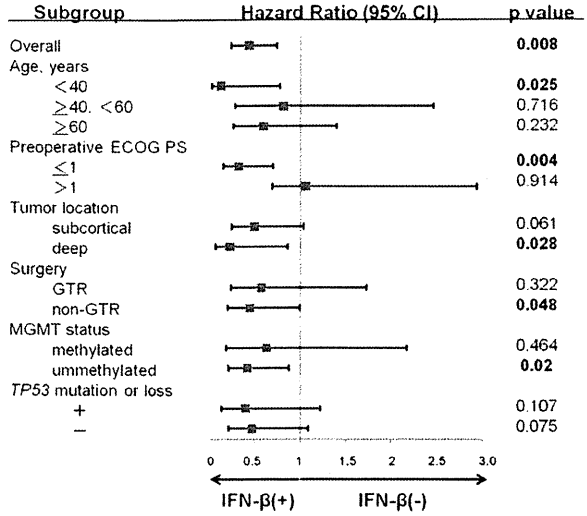


Figure 4. Estimated effect of temozolomide (TMZ) with interferon-β (IFN-β) versus TMZ without IFN-β on the hazard of overall survival (OS), according to baseline characteristics. The hazard ratio was computed using a proportional hazard model by selected factors. There were significant associations under 40 years of age (age, <40), with Eastern Cooperative Oncology Group performance status (ECOG PS) ≤1, deep tumor location, no macroscopic (gross) total resection (non-GTR), and ummethylated MGMT status.

demonstrated that *TP53* mutation or loss was significantly associated with poor OS only in univariate analysis, but not in multivariate analysis. These findings were not in conflict with recent evidence, which shows that *TP53* mutations not only disrupt its function but also possess gain-of-function and dominant-negative effects on the wild-type p53 protein, thus making the mutated *TP53* gene an oncogene.[27]

Benefits of IFN-β and TMZ combination treatment for GBM

The current study demonstrated that newly diagnosed primary GBM patients were associated with a favorable outcome on IFN-β and TMZ combination chemotherapy. The IFN-β and TMZ combination group achieved a median OS of 19.9 months (Figure 3A). This excellent result was almost equal to the median OS of only patients with the methylated MGMT promoter in the EORTC/NCIC trial.

IFN-β elicits pleiotropic biological effects such as antiproliferation, immunomodulation, and cell differentiation.[28] Furthermore, it has been widely used either alone or in combination with other antitumor agents in the treatment of malignant brain tumors and melanomas. In our previous studies, we showed that combination therapy with

IFN-β and nitrosourea has been particularly useful in the treatment of malignant gliomas in Japan.[10] IFN-β has multifaceted functions related to antitumor activity, such as cytostatic effects, participating in the differentiation of CTLs and potentiation of their antitumor immunological responses, and behavior as a drug sensitizer to enhance toxicity against various malignant neoplasms when administered in combination with nitrosourea.[10] Previously, in an in vitro study, we corroborated that IFN-β markedly enhanced chemosensitivity to TMZ[29]; this manifestation revealed that one of the major mechanisms by which IFN-β enhances chemosensitivity is the down-regulation of MGMT transcription. This effect was also confirmed in an experimental animal model.[30] A subanalysis in this study showed that patients whose tumor had an unmethylated promoter benefited from the addition of IFN-β, suggesting that the combination of IFN-β and TMZ might provide better clinical outcomes in patients with the unmethylated MGMT promoter (Figures 3B, 4). Although we discovered that the patients under 40 years of age at diagnosis and those who had an initial ECOG PS $\leq$1 seemed to receive the benefit from IFN-β and TMZ combination therapy, our phase I study revealed that the combination regimen of IFN-β and TMZ was safe and well tolerated even in patients with older age and worse PS (Figure 4; manuscript in submission). In addition, the benefit associated with IFN-β was shown in patients whose tumors were deep, who had undergone non-GTR (Figure 4). This finding suggests that IFN-β might be better for use in cases of complicated tumor removal, i.e., when the tumors were deep, all the tumors could not be removed because they were, for example, located in an eloquent area or around essential structures.

In summary, this study supported the hypothesis that in cases of newly diagnosed primary GBM, IFN-β and TMZ combination therapy was significantly associated with a favorable outcome. To our knowledge, this is the first study to associate the survival benefits derived from IFN-β and TMZ combination. These benefits were, in particular, well correlated in patients with an unmethylated MGMT promoter.

Our results are limited as opposed to a prospective clinical trial as retrospective studies might have been influenced by unrecognized biases. However, the subject group we used was a consecutive series of patients, and this study provides novel information on the treatment for GBM. Thus, accumulation of evidence for this treatment will help further improvement of this disease and hopefully become a novel therapy. We are planning a prospective randomized control trial to compare the clinical outcomes between TMZ alone and a combination of TMZ and IFN-β in newly diagnosed GBM patients.

CONFLICT OF INTEREST DISCLOSURES

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ORIGINAL ARTICLE

Primary CNS lymphoma treated with radiotherapy in Japan: a survey of patients treated in 2005–2009 and a comparison with those treated in 1985–2004

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Abstract
Background The aim of our study was to analyze changes over time in the characteristics, treatment, and outcome of patients with primary central nervous system lymphoma (PCNSL).
Methods Data on 315 patients with histologically proven PCNSL undergoing radiotherapy between 2005 and 2009 were collected from 20 Japanese institutions using a questionnaire. These data were then compared with data on 273 patients treated during the period 1995–2004 and those on 466 patients treated during the period 1985–1994.
Results In terms of patient and tumor characteristics, we found a significant increase in mean patient age in the 2005–2009 period compared to the 1985–2004 period (63 vs. 58–59 years, respectively) and in the percentage of patients with better performance status (PS) during the 2005–2009 period compared with the 1995–2004 period (World Health Organization PS 0–2: 73 vs. 65 %, respectively). Regarding treatment, relative to the 1995–2004 period, significant changes in the 2005–2009 period were (1) decreased rate of attempting tumor resection (23 vs. 44 %); (2) increased use of chemotherapy (78 vs. 68 %), and (3) increased use of methotrexate (MTX)-containing regimens (84 vs. 53 %). The 5-year overall survival rates were 15.3, 30.1, and 36.5 % for patients seen during the 1985–1994, 1995–2004, and 2005–2009 periods,

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respectively, but relapse-free survival did not improve between the 1995–2004 and 2005–2009 periods (26.7 vs. 25.7 % at 5 years, respectively). Patients receiving MTX-containing chemotherapy had 5-year survival rates of 19, 50, and 44 % during these three periods, respectively.
Conclusions Although patient backgrounds differed among the study periods, recent trends were a high patient age, better PS, avoidance of extensive tumor resection, more frequent use of chemotherapy, and improved survival. The recent improvement in survival may be due to improvements in second-line treatment and supportive care.

Keywords Lymphoma · Primary CNS lymphoma · Radiotherapy · Chemotherapy · Soluble interleukin-2 receptor

Introduction

Primary central nervous system lymphoma (PCNSL) is increasing in incidence and is currently one of the most important primary brain tumors. As a consequence, the clinical features of the disease as well as diagnostic procedures, recognition guidelines, and treatment policies have changed considerably. With the widespread recognition of the disease and improvement in diagnostic modalities, patient status, tumor characteristics, and treatment policy appear to be changing gradually [1–7]. Unfortunately, however, randomized studies on the treatment of PCNSL have been scarce, and uncertainties still remain regarding appropriate management [1–7].

In view of the relative rarity of PCNSL coupled with its increasing incidence and importance, we have been conducting nationwide surveys aimed at analyzing changes in the clinical features of the disease, treatment characteristics, and outcomes of the patients. The first study was conducted by Hayabuchi et al. [8] on patients seen between 1985 and 1994. The following two studies were conducted independently by the Japanese Society for Therapeutic Radiology and Oncology (JASTRO) Lymphoma Study Group (JLSG) and the Chubu Radiation Oncology Group (CROG) [9, 10] and included patients seen between 1995 and 1999. The fourth study was conducted by the JLSG and CROG and included those patients seen between 2000 and 2004 [11]. Data on a total of 739 patients were collected from the four previous studies. Given the time span of >5 years since the 2000–2004 survey, the Japan Radiation Oncology Study Group (JROSG) collected data on patients seen between 2005 and 2009. In the study reported here, we analyzed all of the patients in the previous and most recent surveys. Follow-up information was updated whenever possible for patients reported in the earlier studies.

Materials and methods

The study design was approved by the institutional review board (IRB) of Nagoya City University (Approval Number 506). Submission of the data was approved by the IRBs at each participating institution. Subjects of all of the surveys were patients with histologically proven PCNSL who had received radiation therapy. Patients who were suspected of having secondary CNS lymphoma were excluded from enrolling in the survey by each institution. Those patients who did not complete the planned radiotherapy were included. The clinical characteristics of the patients, their treatment, and the prognosis, shown in the Results, were obtained using a detailed questionnaire.

For our survey, we collected data on 315 patients from 20 Japanese medical institutions who started radiation therapy between 2005 and 2009. In the previous surveys, data on 466 patients from 62 institutions seen between 1985 and 1994 were collected [8], and for the period of 1995–1999, a total of 142 patients from 25 Japanese medical institutions were surveyed within the framework of the surveys conducted by JLSG and CROG, respectively

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[9, 10]. For the period of 2000–2004, 131 patients from 17 institutions were surveyed by the JLSG and CROG. The results of these previous surveys were published separately [8–11]. Since the number of patients included in the 1995–1999 and 2000–2004 surveys is relatively small compared to the preceding and current surveys, patient data for these two time periods were combined for this analysis ($n = 273$ for the period of 1995–2004). Thus, we compared data on 466, 273, and 315 patients receiving treatment for PCNSL in the periods 1985–1994, 1995–2004, and 2005–2009, respectively.

A total of 1,054 patients with histologically proven PCNSL therefore constituted the study population (subjects). Human immunodeficiency virus titer was negative in all patients who had received the test, and none of the other patients were considered to have acquired immunodeficiency syndrome-related PCNSL. Of the 20 institutions that participated in the most recent survey, eight (40 %) had also participated in the 2000–2004 survey; 76 % of the institutions which participated in the 2000–2004 survey had also participated in the 1995–1999 survey, and 68 % of the institutions participating in the 1995–1999 survey had also been included in the1985–1994 survey.

The extent of surgical resection had not been ascertained in the 1985–1994 survey, but it had been determined in the subsequent surveys. All other items were common to all surveys. Only one new item was added to the most recent survey: the soluble interleukin-2 receptor (sIL-2R) level before treatment. The performance status (PS) was scored using the World Health Organization (WHO) criteria, and the pre-surgery PS was used for this analysis. A number of items for which data were unclear in the previous surveys were included in the newest survey, and updated information was obtained. As is expected in such a survey, a number of items were unanswered by the investigators. Various chemotherapy regimens had been used, but for the convenience of analysis, these were categorized as either a high-dose ($\geq$1 g/m^2) methotrexate (MTX)-containing regimen, or others; about two-thirds of non-MTX-containing regimens were vincristine–cyclophosphamide–doxorubicin–prednisolone or similar regimens [12].

Differences in patient, tumor, and treatment characteristics between groups were examined using the Fisher's exact test. Survival rates were calculated from the date of the patient starting radiotherapy using the Kaplan–Meier method, and differences in pairs of survival curves were examined with the log-rank test. All statistical analyses were carried out using StatView ver. 5 (SAS institute, Cary, NC) and HALWIN (Gendaisuugakusha, Kyoto, Japan). The median length of follow-up for living patients was 33, 40.5, and 35 months for the 1985–1994, 1995–2004, and 2005–2009 periods, respectively.

Results

Table 1 shows patient and tumor characteristics in the three patient groups treated during the three survey periods. Several marked changes were noted. The mean patient age and proportion of patients with PS 0–2 have increased over time. The proportion of patients with multiple tumors was 52 % in the most recent series, while it was 38 and 47 % in the previous series. Other patient and tumor characteristics did not differ significantly between the pairs of groups, except that the proportion of T cell PCNSL was relatively higher in patients surveyed in the 1985–1994 study.

Table 2 shows the changes in treatment that occurred over time. As a surgical procedure, biopsy alone was performed in 77 % of the patients in the most recent series, whereas it had been performed in 56 % of the patients during 1995–2004. Over 90 % of the patients were treated with whole-brain irradiation with or without a focal boost throughout all study periods. The use of spinal irradiation decreased from 4.6 % during the 1995–2004 period to 1.6 % during the 2005–2009 survey. Mean total doses did not differ significantly among the three periods survey. Whole-brain doses were lower in 1995–2004 and 2005–2009 than in 1985–1994. In contrast, there were steady increases in the proportion of patients undergoing systemic chemotherapy over time. In particular, MTX-containing regimens steadily increased (in 84 % of patients undergoing chemotherapy in the most recent period).

Figure 1 shows the overall survival curves for the three groups. Patients treated between 1995 and 2004 and those treated between 2005 and 2009 showed significantly better survival rates than those treated between 1985 and 1994 (both $P < 0.0001$); the median survival time increased from 18 to 26 to 35 months, respectively. The 5-year survival was 15.3, 30.1 and 36.5 % for the 1985–1994, 1995–2004, and 2005–2009 periods, respectively. The P value between 1995–2004 and 2005–2009 was 0.062. Figure 2 shows the relapse-free survival curves for the patients with known data on recurrence in these three periods. Relapse-free survival of the patients was also better in the two more recent periods than in the period of 1985–1994 (both $P < 0.0001$). The median time to recurrence was 9, 20, and 21 months, and the 5-year relapse-free survival was 17.8, 26.7, and 25.7 % for 1985–1994, 1995–2004, and 2005–2009, respectively. There was no difference between the two most recent periods ($P = 0.62$).

Table 3 summarizes the survival data on the three groups according to patient- and tumor-related potential prognostic factors. In all study periods, patients aged <65 years and those with WHO PS of 0–2 had significantly higher survival rates. In one or two of the three series, patients without B symptoms, those with a normal lactate dehydrogenase (LDH) level, those with a single

Table 1 Patient and tumor characteristics

Characteristic	Survey period (years)			P^a
	1985–1994 (n = 466)	1995–2004 (n = 273)	2005–2009 (n = 315)	
Gender				
Male	276 (59)	163 (60)	191 (61)	0.90
				0.82
Age (years)				
Mean ± SD	58 ± 13	59 ± 11	62 ± 11	0.016
Median (range)	60 (5–86)	61 (15–93)	63 (17–85)	0.024
Performance status (PS)				
0–2	229/438 (52)	174/266 (65)	226/309 (73)	0.0006
				0.012
Lactate dehydrogenase				
High	103/267 (39)	74/234 (32)	99/305 (32)	0.11
				0.84
B symptoms[b]				
Yes	33/418 (7.9)	19/249 (7.6)	30/299 (10)	0.90
				0.33
Phenotype				
T cell	20/234 (8.5)	8/235 (3.4)	8/302 (2.6)	0.020
				0.61
Tumor number				
Multiple	175/460 (38)	128/271 (47)	163/315 (52)	0.015
				0.28
Tumor size at diagnosis (cm)				
Mean ± SD	3.8 ± 1.4	3.8 ± 1.4	2.7 ± 1.9	1.0
				0.30
CSF dissemination				
Yes	56/422 (13)	43/248 (17)	29/308 (9.4)	0.15
				0.83

Data are presented as the number of patients with the percentage given in parenthesis, unless indicated otherwise

CSF cerebrospinal fluid

[a] First and second P values are for comparison between the 1985–1994 and 1995–2004 surveys, and between the 1995–2004 and 2005–2009 surveys, respectively

[b] B symptoms: fever (>38 °C for 3 consecutive days), weight loss (>10 % in 6 months), and/or drenching night sweats

tumor, and those without CSF dissemination on diagnostic imaging had better prognoses, but the tumor size was not associated with the prognosis. Figure 3 shows survival curves according to the LDH and sIL-2R levels in the most recent series. Patients with an elevated sIL-2R level tended to have a poorer prognosis ($P = 0.054$). Regarding the association between LDH and sIL-2R levels, 51 % of patients with a high LDH level also had a high sIL-2R level, while the remaining 49 % had a normal sIL-2R level.

To analyze the influence of treatment-related factors on the outcome, patients who did not complete radiotherapy (receiving <30 Gy) and those who died soon after completing radiotherapy were excluded from the analysis. Table 4 shows survival data according to the treatment-related factors; no factors were found to be associated with an improved prognosis throughout all three periods. In the groups treated during 1995–2004 and 2005–2009, patients receiving systemic chemotherapy had better survival rates than those treated with radiation alone, and those who received MTX-containing chemotherapy had or tended to have a better prognosis than those who received other regimens. However, these phenomena were not observed in patients treated during the preceding decade. No radiotherapy-related factors were found to be associated with the prognosis, except that five patients receiving spinal irradiation had a poorer prognosis in the 2005–2009 series. Figure 4 shows the survival curves for patients treated with high-dose MTX-containing chemotherapy and radiation during the three survey periods; the patients seen during 1995–2004 and those seen during 2005–2009 had significantly better survival rates than those treated during 1985–1994 ($P = 0.0030$ and 0.0002, respectively), but there was no difference between the two most recent periods ($P = 0.95$).

Discussion

Given the increasing importance of PCNSL tumor in neuro-oncology, medical organizations in Japan consider it

Table 2 Treatment characteristics

Characteristic	Period (year)			P^a
	1985–1994 (n = 466)	1995–2004 (n = 273)	2005–2009 (n = 315)	
Surgery				
Biopsy	–	154/273 (56)	241/315 (77)	–
				0.000
Radiotherapy course				
Not completed	25/466 (5.4)	11/273 (4.0)	5/315 (1.6)	0.42
				0.070
Brain radiation field				
Partial brain	37/466 (7.9)	27/273 (9.9)	21/315 (6.7)	0.36
				0.16
Spinal radiation				
Yes	37/445 (8.3)	12/261 (4.6)	5/315 (1.6)	0.061
				0.034
Total dose (Gy)				
Mean ± SD	48.4 ± 11.2	47.9 ± 10.0	46.9 ± 8.6	0.61
				0.35
Whole-brain dose (Gy)				
Mean ± SD	35.6 ± 13.7	33.3 ± 13.0	33.9 ± 8.1	0.02
				0.57
Iv chemotherapy				
Yes	212/420 (50)	186/273 (68)	245/315 (78)	0.000
				0.008
MTX-containing regimen				
Yes	47/212 (22)	98/186 (53)	206/245 (84)	0.000
				0.000
It chemotherapy				
Yes	42/415 (10)	24/273 (8.8)	32/306 (11)	0.56
				0.50

Data are presented as the number of patients with the percentage given in parenthesis, unless indicated otherwise

Iv intravenous, *MTX* methotrexate, *It* intrathecal

[a] First and second *P* values are for comparison between the 1985–1994 and 1995–1999 surveys, and between the 1995–2004 and 2005–2009 surveys, respectively

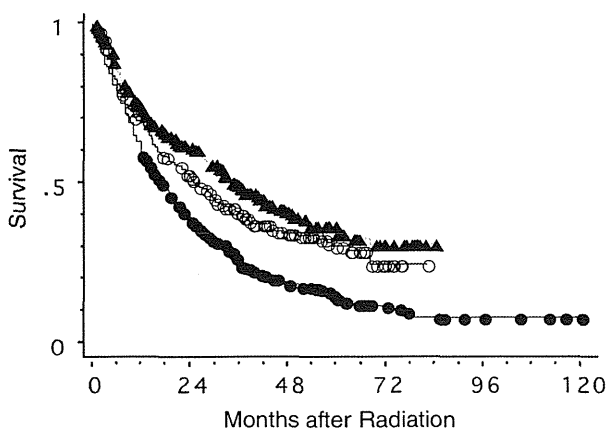

Fig. 1 Survival curves for patients with primary central nervous system lymphoma (PCNSL) seen in 1985–1994 (*filled circle*, n = 466), 1995–2004 (*open circle*, n = 273), and 2005–2009 (*filled diamond*, n = 315). Patients surveyed in 1995–2004 and 2005–2009 showed significantly better survival rates than those surveyed in 1985–1994 ($P < 0.0001$), but there was no difference between the 1995–2004 and 2005–2009 groups ($P = 0.062$)

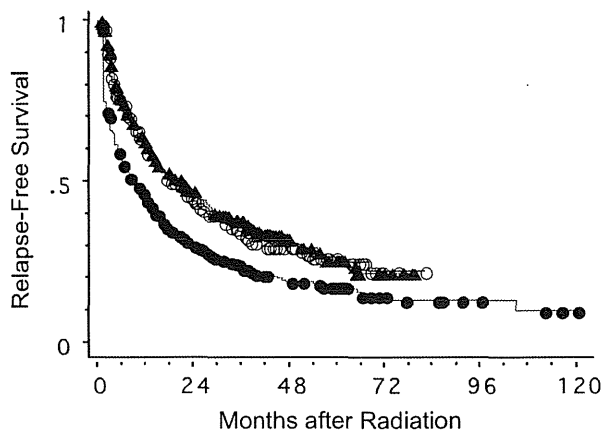

Fig. 2 Relapse-free survival curves for patients with PCNSL seen in 1985–1994 (*filled circle*, n = 408), 1995–2004 (*open circle*, n = 264), and 2005–2009 (*filled diamond*, n = 315). The patients surveyed in 1995–2004 and 2005–2009 showed significantly better relapse-free survival rates than those surveyed in 1985–1994 ($P < 0.0001$), but there was no difference between the 1995–2004 and 2005–2009 groups ($P = 0.62$)

Table 3 Survival data according to patient or tumor-related potential prognostic factors

Prognostic factor	1985–1994				1995–2004				2005–2009			
	n	MST	5-YSR (%)	*P*	*n*	MST	5-YSR (%)	*P*	*n*	MST	5-YSR (%)	*P*
Gender												
Male	276	17	17	0.92	163	26	30	0.76	191	37	38	0.31
Female	190	20	13		110	25	30		124	31	36	
Age (years)												
<65	294	20	21	0.0001	158	36	40	<0.0001	153	42	47	0.0009
≥65	172	14	5.4		115	17	15		162	29	23	
Performance status (PS)												
0–2	229	24	20	<0.0001	149	37	37	<0.0001	226	48.5	44	0.0001
3, 4	209	12	10		74	13	14		83	11.5	14	
B symptoms												
Yes	33	10	0	0.030	19	15	15	0.028	30	31	30	0.26
No	385	18	17		232	29	35		269	36	39	
Lactate dehydrogenase												
Normal	164	22	26	0.0007	160	35	37	0.0001	206	40	42	0.050
High	103	14	5.7		74	16	21		99	29	28	
Tumor number												
Single	285	22	18	0.0012	143	29	37	0.065	152	40	43	0.096
Multiple	175	12	11		128	23	23		163	31	31	
Tumor size (cm)[a]												
≤3.5	196	19	15	0.60	125	28	28	0.93	160	37	42	0.45
>3.5	197	17	18		137	26	34		131	33.5	29	
CSF dissemination												
Yes	56	10	14	0.039	43	43.5	36	0.45	29	15	26	0.022
No	366	19	16		205	26	32		279	37	39	

MST Median survival time in months, *5-YSR* 5-year survival rate

[a] Maximum tumor diameter at diagnosis

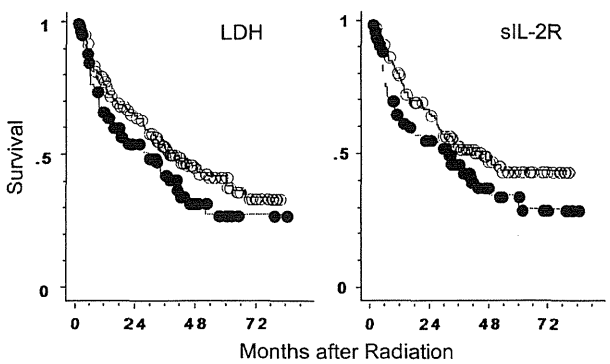

Fig. 3 Survival curves for patients treated between 2005 and 2009 according to the serum lactate dehydrogenase (*LDH*) and soluble interleukin-2 receptor (*sIL-2R*) levels. *Open circle* Normal level ($n = 206$ for LDH and 135 for sIL-2R), *filled circle* elevated level ($n = 99$ for LDH and 95 for sIL-2R). The *P* value was 0.050 for LDH and 0.054 for sIL-2R

meaningful to survey data on PCNSL every 5 years. To date, these surveys have been conducted by radiation oncology groups (JASTRO-JLSG, CROG, and JROSG) and, therefore, patients undergoing radiotherapy have been the subjects of these surveys. Consequently, data on patients treated with chemotherapy alone are unavailable, which is a limitation of our study. Although treatment with chemotherapy alone seems to be increasing in use in Western countries [13–15], such a treatment strategy was not popular in Japan before 2010—and was in fact exceptional. Therefore, we are confident that these survey data represent the status of PCNSL treatment up to and including 2009 in Japan. More recently, the strategy of primary chemotherapy with deferred radiotherapy appears to be gaining acceptance in Japan also, so these data might serve as a control for the evaluation of different treatment modalities in the future. Another limitation of our study is the long study period; patient backgrounds may considerably differ among the study periods, and comparison among patients in the different eras may be inappropriate for some items.

Various changes have been noted with regard to patient and tumor characteristics. The recent increase in aged patients may be related to the fact that subjects of these

Table 4 Survival data according to treatment-related factors

Prognostic factor	1985–1994				1995–2004				2005–2009			
	n	MST	5-YSR (%)	*P*	*n*	MST	5-YSR (%)	*P*	*n*	MST	5-YSR (%)	*P*
Surgical resection												
Extensive	–	–	–	–	53	24.5	30	0.66	40	40.5	12	0.63
Non-extensive	–	–	–		209	26	29		270	34	38	
Radiation field												
Whole brain	405	19	15	0.72	236	24.5	28	0.21	289	36	37	0.67
Partial brain	34	16	17		26	35	43		21	32	28	
Spinal radiation												
Yes	36	24	19	0.16	11	NR	55	0.30	5	5	–	0.0091
No	384	18	15		251	26	28		302	36	37	
Total dose (Gy)												
<50	134	18	17	0.97	80	28.5	34	0.98	141	42	41	0.38
≥50	305	8	16		182	25	28		169	32.5	31	
Whole-brain dose (Gy)												
<40	156	18	18	0.43	109	32	34	0.91	216	35.5	40	0.43
≥40	283	18	14		153	23	25		94	32	28	
Iv chemotherapy												
Yes	202	20	16	0.30	180	36	39	<0.0001	242	42	41	<0.0001
No	192	16	17		82	14	10		68	12.5	13	
Iv chemotherapy regimen												
MTX	46	20	19	0.66	92	55.5	50	0.061	203	45	44	0.0031
Other	156	21	15		88	29	30		39	27	23	
It chemotherapy												
Yes	39	16	20	0.78	22	NR	53	0.10	32	NR	59	0.097
No	350	19	16		232	24.5	26		269	34	34	

NR Not reached

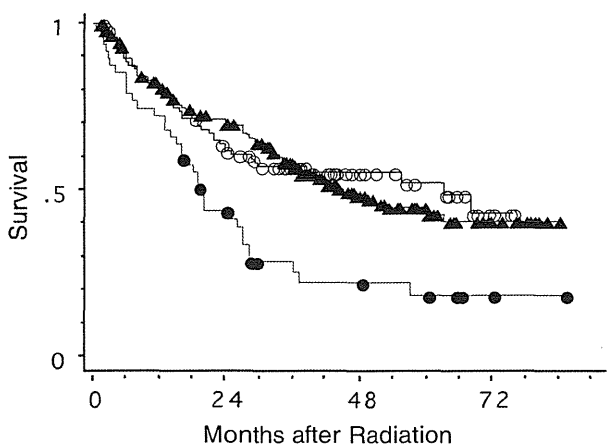

Fig. 4 Survival curves for patients treated with high-dose methotrexate-containing chemotherapy plus radiation in 1985–1994 (*filled circle*, $n = 46$), 1995–2004 (*open circle*, $n = 92$), and 2005–2009 (*filled diamond*, $n = 203$). The *P* value was 0.0030 for 1985–1994 vs. 1995–2004, 0.0002 for 1985–1994 vs. 2005–2009, and 0.95 for 1995–2004 vs. 2005–2009

surveys are histologically proven PCNSL patients. One possible explanation is the increasing acceptance in recent years of biopsy—even in aged patients—to confirm the diagnosis. The incidence of multiple tumors appears to be increasing, being 52 % in the most recent period compared to 38 and 47 % in the two earlier surveys, respectively; most previous reports suggest an incidence of between 30 and 40 % [16–19]. The improvement in imaging modalities and techniques, including the more frequent use of magnetic resonance imaging, may have contributed to the improved detection of small tumors. The proportion of T-cell lymphoma was high (8.5 %) in the 1985–1994 period, possibly reflecting the difficulty in determining the phenotype of lymphoma in that era.

In terms of treatment, attempts at tumor resection have decreased because it is now clear that surgical resection does not contribute to an improved prognosis [2, 11]. The results of our survey also supports this conclusion. However, Weller et al. [20] recently stated that resection of PCNSL might play a beneficial role provided that surgery is safely conducted. We noted no major changes in

radiotherapy between the different surveys. Shibamoto et al. [21] suggested the possible use of partial-brain radiation for solitary lesions, but such a policy has yet to spread nationwide. Reducing total as well as whole-brain radiation doses using chemotherapy has not become popular in Japan. The increased use of systemic chemotherapy and, in particular, MTX-based regimens appear to be a worldwide trend, as was also shown in our study.

The prognosis of PCNSL patients has improved recently. Improvement in supportive care may at least in part have contributed to these changes. The 5-year survival was 30.1 and 36.5 % in 1995–2004 and 2005–2009, respectively. However, relapse-free survival rates did not differ between these two periods, suggesting that although second-line treatment at recurrence has prolonged survival, the cure rate has not yet improved. This trend was also true for patients treated with high-dose MTX and radiation; no improvement was seen for the most recent period, suggesting that, in terms of cure, more than half of PCNSLs are resistant to currently available treatment. New treatments are therefore urgently needed.

Many prognostic factors of PCNSL, such as age, PS, and tumor multiplicity, have been reported [8, 11, 17, 19, 22], and the results of the univariate analyses we conducted in our study agree with previously published data. Consequently, we did not present the multivariate analysis data. In the most recent survey, we paid attention to sIL-2R as a prognostic marker and observed that patients with a high sIL-2R level tended to have a poorer prognosis. The prognostic value of sIL-2R has been reported for extracranial lymphoma [23, 24], but, to our knowledge, its role in PCNSL has not been reported. The serum sIL-2R level reflects the total amount of activated T lymphocytes and is correlated with disease activity [25]. It can also be elevated in cancers other than lymphoma, collagen disease, and infection [25, 26]. Since sIL-2R and LDH levels do not necessarily correlate with each other, sIL-2R may be another useful prognostic marker for PCNSL.

Very recently, a few Japanese groups have started to treat PCNSL patients with chemotherapy alone, following the trend set in Western countries. A randomized European study of chemotherapy alone versus chemotherapy + radiation indicated that chemotherapy alone was associated with a decreased progression-free survival, although overall survival was similar, partly due to the use of radiotherapy as a second-line treatment [27]. Since most studies are conducted in phase II settings, the data presented in our study may serve as a basis for studying the treatment and prognosis of PCNSL patients in Japan.

In conclusion, the results of our study reveal that recent trends in PCNSL are increased patient age, better PS, tumor multiplicity, avoidance of extensive tumor resection, more frequent use of high-dose MTX-containing chemotherapy, and improved survival, with no improvement in relapse-free survival. Newer strategies are therefore necessary to further improve the prognosis of PCNSL patients, and the present data may serve as a basis for designing new studies.

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Conflict of interest The authors declare that they have no conflict of interest.

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ORIGINAL PAPER

Effect of chemotherapy on survival after whole brain radiation therapy for brain metastases: a single-center retrospective analysis

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Abstract
Background and purpose Whether chemotherapy for systemic disease affects survival of patients with brain metastases or not has not been elucidated before. We performed comprehensive analysis of patients with newly-diagnosed brain metastases primarily treated with whole brain radiation therapy (WBRT) alone.
Materials and methods Data from 134 patients with newly-diagnosed brain metastases primarily treated with WBRT from 2007 to 2008 was retrospectively reviewed. Univariate and multivariate analyses were performed to identify significant prognostic factors.
Results Median survival time (MST) of this cohort from the start of WBRT was 5.7 months. MST of patients with RPA Class 1, 2 and 3 were 10.3, 7.8 and 2.2 months, respectively. Multivariate analysis revealed that karnofsky performance status ($\geq$70, $p < 0.0001$), gender (female, $p < 0.0001$), activity of extracranial disease (stable, $p = 0.015$), time to develop brain metastasis (<3 months, $p = 0.042$) and use of chemotherapy after WBRT (multiple regimens, $p < 0.0001$) were independent prognostic factors for better survival.
Conclusions Systemic chemotherapy for chemo-responsive cancer prolongs survival despite the presence of treated brain metastases. Irradiated brain metastases will lose their prognostic significance in a large number of patients. Systemic chemotherapy will be a treatment of choice for patients who have systemic disease after WBRT for brain metastases. These results should be validated in the future prospective clinical trials.

Keywords Brain metastasis · Brain metastases · Radiation therapy · Whole brain radiation therapy · Chemotherapy · Prognostic factors

Introduction

Brain metastasis affects 20–40 % of cancer patients (Soffietti et al. 2002). Brain metastasis is one of the major causes of morbidity in cancer patients. The prognosis of patients with brain metastasis is generally poor with a median survival time (MST) of 1–2 months with corticosteroids only (Weissman 1988; Lagerwaard et al. 1999).

The route of metastatic dissemination to the brain is often hematogeneous, therefore, the entire brain can be seeded with micrometastatic focus. Traditionally, whole brain radiation therapy (WBRT) has been regarded as the standard treatment for patients with brain metastasis. Overall survival of the patients after WBRT ranges 3–6 months (Lagerwaard et al. 1999; Gaspar et al. 2010; Tsao et al. 2005). Various dose/fractionation schedules of WBRT were tested in clinical studies, which resulted in no significant difference in median survival time after WBRT (Tsao et al. 2005; Gaspar et al. 2010).

Recently, significant progress has been made for a subset of patients with single or few brain metastases and well controlled systemic disease. Surgical resection or stereotactic radiosurgery (SRS) combined with WBRT significantly prolonged survival (Patchell et al. 1990; Vecht et al. 1993; Andrews et al. 2004). Median survival of

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patients who received these aggressive therapies ranges 7–10 months. Unfortunately, patients who entered into these clinical trials represent only a small minority of the patients with brain metastases. For the majority of patients with multiple brain metastases and uncontrolled systemic disease, only WBRT is the standard treatment of choice.

The role of chemotherapy in brain metastasis has been limited because of the concern about the activity of chemotherapeutic agent to cross the blood–brain barrier (BBB). Recently, the activity of chemotherapy in brain metastasis is highlighted (Robinet et al. 2001; Walbert and Gilbert 2009; Mehta et al. 2010). Concurrent chemoradiation therapies with BBB permeable agents, such as Temozolamide or topotecan are currently under investigation in prospective clinical trials. Some investigators suggested that the permeability of BBB can alter after fractionated radiotherapy for brain metastasis (Yuan et al. 2006; Wilson et al. 2009). However, whether the use of chemotherapy affects survival of the patients with brain metastasis or not has not been elucidated before.

The primary aim of this study was to perform comprehensive analysis of 134 consecutive patients with newly-diagnosed brain metastases primarily treated by WBRT alone in a single institution. The secondary aim was to define independent prognostic factors associated with longer survival after WBRT. The final aim was to investigate the prognostic value of chemotherapy on survival after WBRT in patients with brain metastases.

Materials and methods

Patient characteristics

The database of patients who underwent radiotherapy for brain metastases at our institution was reviewed. A total of 264 patients were treated with WBRT between 2007 and 2008. Of these, 23 patients received WBRT as a salvage therapy after SRS. Another 39 patients received WBRT as an adjuvant therapy after resection of metastatic brain tumor. Forty-seven patients were metastases from radiosensitive primary tumor such as leukemia, lymphoma or small cell carcinoma. Excluding these patients, we reviewed the medical records of 155 patients with newly diagnosed brain metastases treated with WBRT as a primary therapy. Of these, 19 patients presented with symptoms or radiographic findings of leptomeningeal metastasis. We excluded these patients with leptomeningeal metastasis because they are known to have extremely limited survival. Two patients were ineligible for evaluation because of allergy to contrast media. Finally, a group of 134 patients were subjected to extensive analysis. The clinical and image interpretation data from these patients were entered into database in December 2010. Distribution of baseline patient and tumor characteristics is shown in Table 1.

Table 1 Distribution of baseline patient and tumor characteristics

Parameters	*n*	%	Parameters	*n*	%
Median age (years)	60		Extracranial distant metastases		
Gender			Absent	11	8
Male	69	51	Stable	16	12
Female	65	49	Progressive	107	80
Karnofsky performance status (KPS)			Activity of extracranical tumor		
100–90	46	34	Absent/stable	20	15
80–70	49	37	Progressive	114	85
60–50	29	22	Time to diagnosis of brain metastasis		
40–0	10	7	<3 months	21	16
Neurologic status			3–12 months	33	25
0	45	34	1–2 years	22	16
1	27	20	≥2 years	58	43
2	34	25	Type of the diagnostic brain image		
3	21	16	MRI	106	79
4	7	5	CT	28	21
RPA criteria			Number of brain metastases		
Class 1	5	4	1–4	40	30
Class 2	91	68	5–10	39	29
Class 3	38	28	11–24	29	22
Site of primary tumor			≥25	26	19
Lung	75	56	Size of the largest lesion		
Breast	27	20	≤10	31	23
Upper gastrointestinal tract	11	8	11–20	46	34
Colorectum	10	8	21–30	34	25
Genitourinary tract	5	4	>30	23	17
Others	6	5	Chemotherapeutic regimens before WBRT		
Histological type			None	22	16
Adenocarcinoma	114	85	Single	28	21
Squamous cell carcinoma	9	7	Multiple	84	63
Others	11	8	Chemotherapeutic regimens after WBRT		
Primary tumor status			None	70	52
Absent	57	42	Single	31	23
Stable	25	19	Multiple	33	25
Progressive	52	39	Molecular targeted therapy after WBRT (>1 month)		
			No	100	74
			Yes	34	26

RPA recursive partitioning analysis, *MRI* magnetic resonance imaging, *CT* computed tomography, *WBRT* whole brain radiation therapy

Imaging studies

Diagnosis of brain metastases was performed mainly with magnetic resonance images (MRI). In our institute, all patients with lung cancer routinely undergo brain imaging for initial staging or scheduled follow-up. Patients with other solid tumors underwent brain imaging when brain metastasis is clinically suspected. In this study, initial diagnostic brain images included MRI in 106 patients (79 %) and CT in 28 patients (21 %). Radiological features assessed included number, maximum tumor diameter and location. For follow-up brain images, change in size of the tumors and presence of new metastases were recorded. At least 20 % increase in diameter of the each preexisted tumor before WBRT, taking as reference on the smallest diameter after WBRT, was defined as local progression.

Treatment strategy

Treatment strategy for brain metastasis at our institution was previously described elsewhere (Narita and Shibui 2009; Hashimoto et al. 2011). Patients who received WBRT alone as a primary treatment for brain metastases were subjected for this study. Patients with brain metastases generally have extracranial systemic disease. After WBRT, patients with known systemic disease were indicated to start or continue chemotherapy if they still had active chemotherapeutic regimen with sufficient organ function and with Karnofsky performance status (KPS) of 70 or more. Salvage SRS was considered for recurrent brain metastases after WBRT. Some patients with known chemo-sensitive tumor continued palliative chemotherapy for recurrent brain metastases.

Consent for the treatment was obtained from each patient after the sufficient explanation of potential risks of treatment. All the patients provided written informed consent. Our institutional review board has approved this study.

Whole brain radiation therapy

One hundred and thirty-four patients were intended to receive WBRT. Of these, 128 patients were delivered to a dose of 30 Gy in 10 fractions. Another 3 patients were delivered to 37.5 Gy in 15 fractions, whereas one patient was delivered to 20 Gy in 5 fractions. Two patients discontinued irradiation course because of the deterioration of general condition at a dose of 12 and 24 Gy, respectively.

Retrospective analysis

All the medical charts of the eligible patients were reviewed. Information on potential prognostic factors (age, gender, KPS, neurologic status, site of primary tumor, primary tumor status, activity of extracranial distant metastases, time to develop brain metastasis, number of brain metastases, size of the largest lesion, use of chemotherapy before or after WBRT) was collected.

Initial neurological function was classified into 4 categories (No symptoms: grade 0, Minor symptoms; fully active without assistance: grade 1, Moderate symptoms; fully active but requires assistance: grade 2, Moderate symptoms; less than fully active: grade 3, Severe symptoms; totally inactive: grade 4). Radiation Therapy Oncology Group's (RTOG) recursive partitioning analysis (RPA) classes were coded into 3 categories as follows: Class 1: Patients with KPS $\geq$ 70, <65 years of age with controlled primary and no extracranial metastases; Class 3: KPS $<$ 70; Class 2: all the others (Gaspar et al. 1997).

For the evaluation of extracranial disease status, if there were no evidence of residual tumor after therapy, the activity was coded as "absent". If any tumor existed and there is no increase in size of the tumor for more than 6 months, the activity was coded as "stable". A continuous use of same chemotherapeutic regimen didn't impair the coding of "stable". If any tumor existed with any situation other than "stable", the activity was coded as "progressive".

Patients whose brain metastases were detected at the same time or soon after the diagnosis of primary tumor (so-called "synchronous" brain metastasis) may have different prognosis. We defined "synchronous" brain metastasis as those detected at the same time or detected within 3 months of the initial diagnosis of primary tumor.

For the analysis of prognostic effect of chemotherapy before or after WBRT, three different cohorts were defined: none, single regimen and multiple regimens. If a patient received two or more different types of chemotherapeutic regimens, the status was coded as multiple regimens. Any type of hormonal therapy was regarded as a single regimen. The status of the use of molecular targeted therapy was defined as "yes", if a patient continued to receive a specific regimen for more than 1 month.

Statistical analysis

Overall survival from the start of WBRT was calculated with the Kaplan–Meier method. For univariate and multivariate analysis, all the variables were dichotomized according to the clinical relevance from previous literature. Univariate analyses were performed by using log-rank test. Possible confounded variables were excluded from multivariate analysis. A Cox's proportional hazards model was developed to identify significant factors influencing survival after WBRT. All the tests of hypotheses were