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

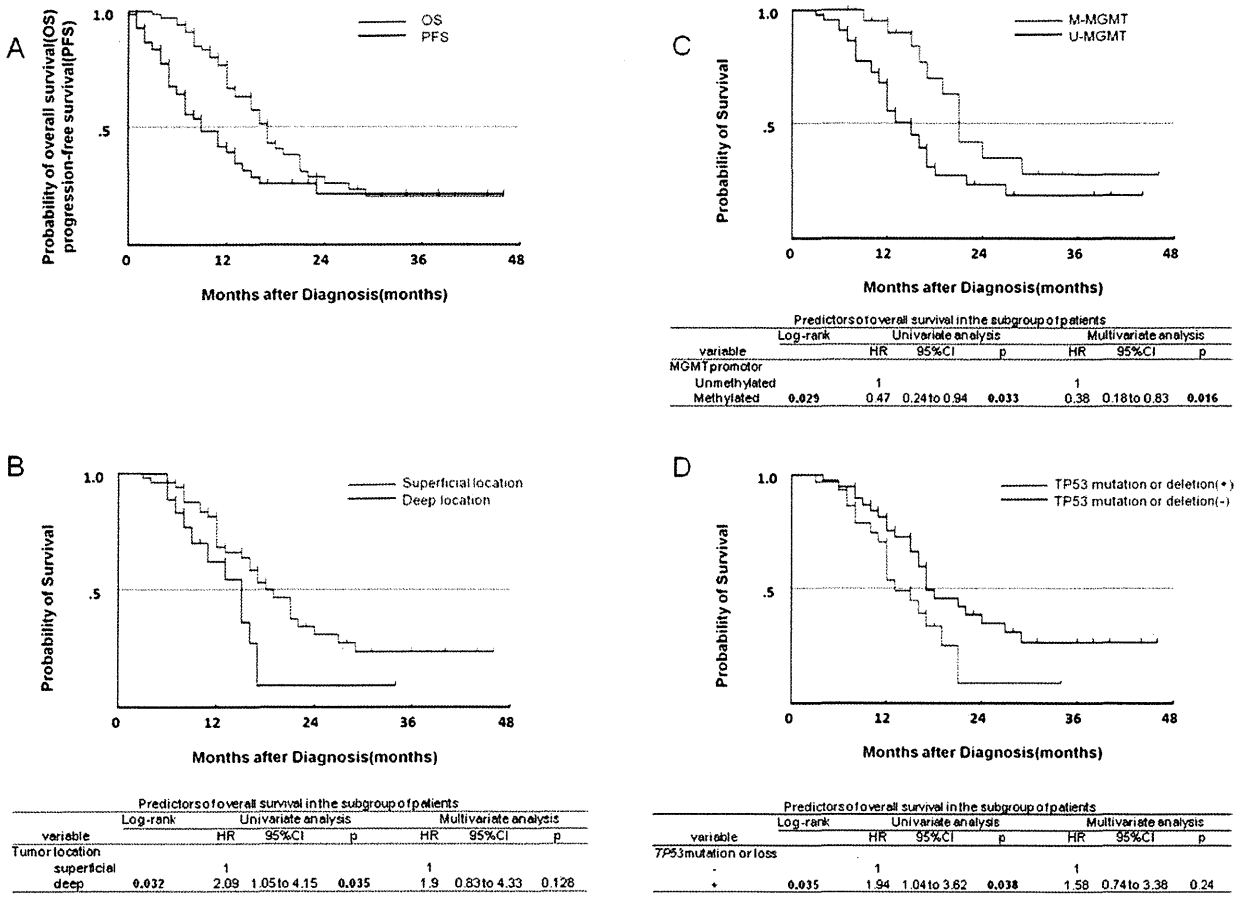
Next, we sought to determine the subpopulation that had benefited from the use of the IFN-β combination treatment. It is well known that patients with GBM containing the methylated MGMT promoter benefit from TMZ, but those with the unmethylated MGMT promoter show no such benefits.[1,2] Consistently, 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

variable	Log-rank	Univariate analysis HR	95%CI	p	Multivariate analysis 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

variable	Log-rank	Univariate analysis HR	95%CI	p	Multivariate analysis 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

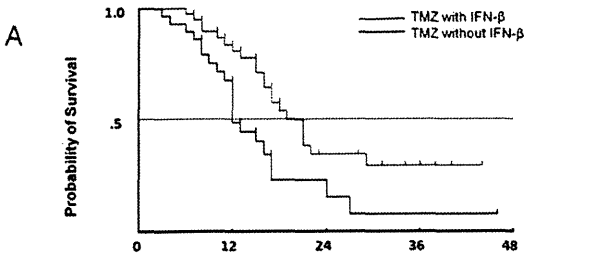
variable	Log-rank	Univariate analysis HR	95%CI	p	Multivariate analysis HR	95%CI	p
TP53 mutation or loss							
-		1			1		
+	0.035	1.94	1.04 to 3.62	0.038	1.58	0.74 to 3.38	0.24

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

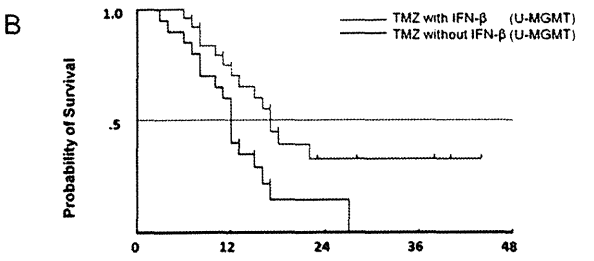
The current study demonstrated that the methylated MGMT promoter and the combination of IFN-β and TMZ were independent prognostic indicators of GBM patients on multivariate analysis. Epigenetic silencing by the MGMT promoter methylation correlates with improved survival in glioma patients treated with 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

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

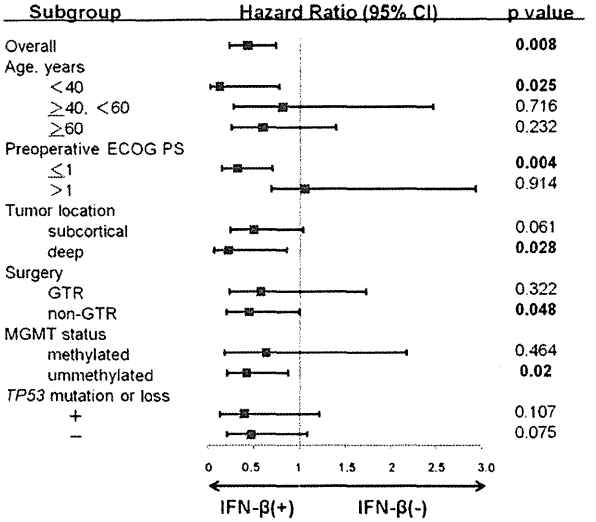


Figure 4. Estimated effect of temozolomide (TMZ) with interferon-β (IFN-β) versus TMZ without IFN-β on the hazard of overall survival (OS), according to baseline characteristics. The hazard ratio was computed using a proportional hazard model by selected factors. There were significant associations under 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 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

This study was presented in part at the 53rd Annual Meeting of the American Society for Radiation Oncology in Miami, October 2-6, 2011.

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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

conducted at the alpha level of 0.05 with a 95 % confidence interval. All the statistical analyses were performed by using SPSS Statistics version 17.0 (SAS Institute, Tokyo, Japan).

Results

Outcomes for the entire group

Median survival time (MST) for the entire patients from the start of WBRT was 5.7 months. The 6 months, 1- and 2-year survival rate were 43, 28 and 12 %, respectively. MST of the patients with RTOG's RPA Class 1 ($n = 5$), 2 ($n = 91$) and 3 ($n = 38$) were 10.3, 7.8 and 2.2 months, respectively (Fig. 1). Median intracranial progression-free survival (PFS) were 4.7 months, with 6 months, 1- and 2-year PFS of 35, 14 and 4 %, respectively. A total of 49 patients developed intracranial recurrence after WBRT. The sites of first recurrence after WBRT were as follows: local only (regrowth of preexisted tumors): 25 (51 %); new metastasis only: 10 (20 %); both of local and new metastasis: 12 (24 %); and leptomeningeal dissemination: 2 (4 %). Median local progression-free duration and median intracranial new metastasis-free duration for the entire patients were 9.7 and 18.0 months, respectively. At the time of analysis, 5 patients were alive with disease. The causes of death were identified in 118 patients. Of these, 38 patients (32 %) were due to intracranial tumor progression, whereas 76 patients (64 %) were due to systemic disease. Four patients (3 %) died from intercurrent disease. None had died directly from toxicity of WBRT.

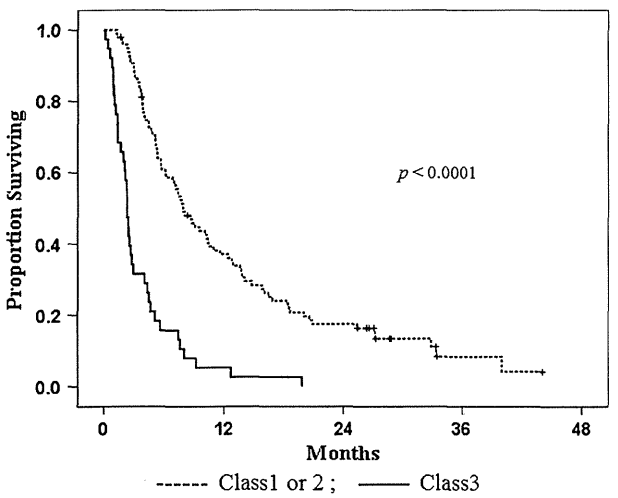

Fig. 1 Kaplan–Meier survival curve for overall survival by RPA criteria

Factors influencing survival after WBRT: univariate and multivariate analyses

Univariate analysis was performed on 12 different variables to evaluate their potential value on survival after WBRT. Univariate analyses identified 9 variables which significantly associated with good prognosis (Table 2).

Multivariate analysis was performed on 9 independent variables. Table 3 summarizes the result of the multivariate analysis for survival after WBRT. Multivariate analysis revealed that KPS (≥70 vs. 70, hazard rate (HR): 2.540, $p < 0.0001$), gender (female vs. male, HR: 2.293, $p < 0.0001$), activity of extracranial disease (absent/stable vs. progressive, HR: 2.134, $p = 0.015$), time to develop brain metastasis (<3 vs. ≥3 months, HR: 1.926, $p = 0.042$), and use of chemotherapy after WBRT (multiple vs. none/single regimens, HR: 3.406, $p < 0.0001$) were independent prognostic factors for overall survival.

Survivals depending on chemotherapy after WBRT

After WBRT, only two patients had no evidence of extracranial tumor. The two patients didn't receive further chemotherapy until disease progression. Another 132 patient had known extracranial tumor including primary, nodal or distant sites. They were indicated to start or continue chemotherapy when it was clinically applicable. A total of 64 patients with extracranial systemic disease underwent chemotherapy after WBRT. Thirty-one patients (23 %) received only a single chemotherapeutic regime, and 33 patients (25 %) received multiple regimens. Figure 2 shows the survival curve by the use of chemotherapy after WBRT. The MST of the patients who received none, single and multiple regimens after WBRT were 3.3, 7.5 and 16.4 months, respectively ($p < 0.0001$). The use of multiple chemotherapeutic regimens after WBRT was found to be associated with better survival after WBRT in multivariate analysis ($p < 0.0001$). Among 95 patients with pre-irradiation KPS ≥ 70, 59 patients (62 %) received chemotherapy, whereas 5 patients (13 %) with KPS < 70 received chemotherapy. Among patients with KPS ≥ 70, the MST of the patients who received none, single and multiple regimens after WBRT were 4.5, 7.9 and 16.4 months, respectively ($p < 0.0001$). Overall, 95 % of the patients included in this study received chemotherapy either before or after WBRT.

The effect of molecular-targeted therapy after WBRT

A total of 34 patients (25 %) received molecular-targeted therapy after WBRT for 1 month or more. Of these patients, the sites of primary disease were lung in 28, breast

Table 2 Results of univariate analyses for survival after WBRT

Parameters	*n*	Median survival time (months)	6-months survival (%)	1-year survival (%)	2-year survival (%)	*p* value
Overall patients	134	5.7	43	28	12	–
Age						
<65	87	7.4	54	31	13	
≥65	47	4.9	38	22	11	0.31
Gender						
Male	69	4.5	32	17	6	
Female	65	9.1	66	40	20	0.0009
Karnofsky performance status						
≥70	95	7.9	62	39	17	
<70	39	2.2	15	3	0	<0.0001
Neurologic status						
0–1	72	7.9	58	44	22	
2–4	62	4.5	36	1	0	<0.0001
RPA criteria						
Class 1–2	96	7.9	61	37	18	
Class 3	38	2.2	16	5	0	<0.0001
Site of primary tumor						
Lung	75	7.4	55	39	21	
Others	59	4.5	39	14	2	0.001
Activity of extracranical tumor						
Absent/stable	20	9.1	60	40	25	
Progressive	114	5.2	46	26	10	0.015
Time to develop brain metastasis						
<3 months	21	16.9	75	65	40	
≥3 months	113	5.2	43	21	7	0.002
Number of brain metastasis						
1–4	40	5.1	39	21	10	
≥5	94	6.2	52	31	13	0.53
Size of the largest lesion						
<20 mm	69	7.4	53	36	16	
≥20 mm	65	5.1	42	20	8	0.11
Chemotherapeutic regimens before WBRT						
None/single	50	7.2	52	42	20	
Multiple	84	5.2	46	19	8	0.019
Chemotherapeutic regimens after WBRT						
None/single	101	4.0	33	13	4	
Multiple	33	16.4	94	73	36	<0.0001

RPA recursive partitioning analysis, *WBRT* whole brain radiotherapy

in 5 and kidney in 1. All of the histological diagnoses of lung primary patients were adenocarcinoma. Twenty-seven lung primary patients received epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) for a median duration of 7 months. Figure 3 shows the survival curve by the use of molecular-targeted therapy after

Table 3 Results of multivariate analysis for survival after WBRT

Variables	Factors	Hazard rate (95 % CI)	*p* value
Karnofsky performance status	≥70 versus <70	2.540 (1.627–3.966)	<0.0001
Gender	Female versus male	2.293 (1.541–3.412)	<0.0001
Extracranial disease status	Absent/stable versus progressive	2.134 (1.160–3.928)	0.015
Time to develop brain metastasis	<3 versus ≥3 months	1.926 (1.025–3.620)	0.042
Number of chemotherapeutic regimens after WBRT	Multiple regimens versus none/single regimen	3.406 (2.013–5.761)	<0.0001

CI confidence interval, *WBRT* whole brain radiation therapy

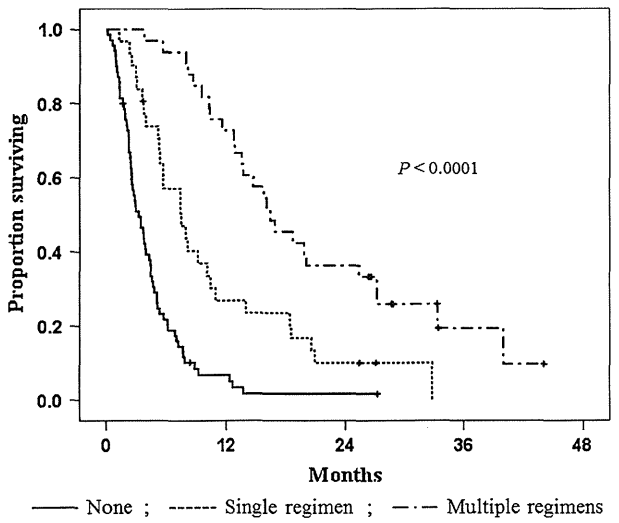

Fig. 2 Kaplan–Meier overall survival curve by the use of chemotherapeutic regimen after WBRT

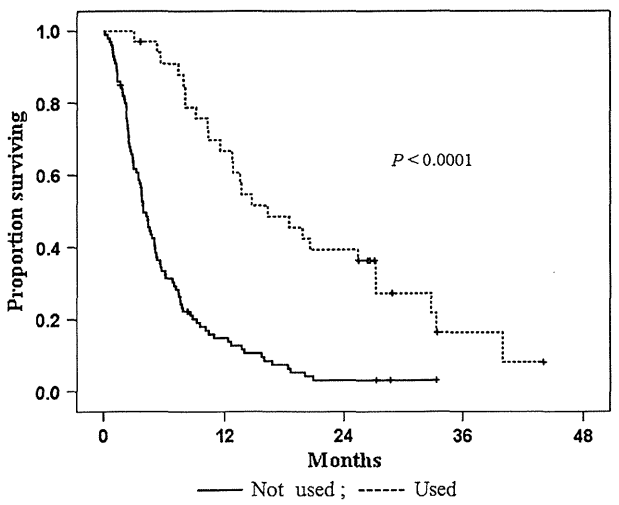

Fig. 3 Kaplan–Meier overall survival curve by the use of molecular-targeted therapy after WBRT

WBRT. The MST of the patients who received molecular-targeted therapy after WBRT was significantly longer than that of those who did not (16.4 vs. 4.0 months, $p < 0.0001$).

Discussion

Significant progress has been made over the last decades for a subset of patients with single or few brain metastases and well controlled systemic disease. In prospective randomized clinical trials, surgical resection or SRS combined with WBRT significantly prolonged survival in selected patients with single or few brain metastases (Patchell et al. 1990; Vecht et al. 1993; Andrews et al. 2004). MST of these patients who received combined therapy ranges 7–10 months. SRS alone in patients with one or few brain metastases was comparable to SRS combined with WBRT at least in terms of overall survival, with a MST of 8 months (Aoyama et al. 2006). Unfortunately, the patients who entered into these clinical trials represent only a small minority of patients with brain metastases. In clinical practice, it remains unclear whether these aggressive therapies have sufficient benefit for the majority of patients with uncontrolled systemic disease or numerous brain metastases. Currently, only WBRT is the standard treatment of choice for these patients. The indication of SRS for patients with brain metastases in clinical practice continues to be a matter of debate.

Various prospective and retrospective studies have shown that the treatment modality is the first most important prognostic factor on long-term survival, although the effect of patient selection bias is inevitable (Andrews et al. 2004; Lagerwaard et al. 1999; Patchell et al. 1990). To minimize the selection bias, we investigated only patients primarily treated with WBRT alone in this study. Numerous studies on prognostic factors in patients with brain metastases have been published previously. The results of this study re-confirmed the value of established prognostic factors reported in the literature. Multivariate analysis showed that good KPS, stable extracranial disease and female gender were independent predictors of better survival after WBRT, in line with previous literatures (Lagerwaard et al. 1999; Patchell et al. 1990; Aoyama et al. 2006; Gaspar et al. 1997; Swinson and William 2008). Dose these pretreatment characteristics fully determine the prognosis of patients with brain metastases?

Performance status is regarded as the second most important prognostic factor in patient's characteristics (Lagerwaard et al. 1999; Aoyama et al. 2006; Gaspar et al. 1997; Fleckenstein et al. 2004; 20). Generally, patients with low KPS are not indicated for aggressive therapy other than WBRT alone. In this study, the MST of the patients with KPS < 70 was only 2.2 months. The Performance status of the patients with brain metastases frequently deteriorated by extended intracranial disease. Additionally, patients with very low performance status were not indicated for further chemotherapy despite the existence of systemic disease. In this study, only 5 patients (13 %) with pre-treatment KPS < 70 received chemotherapy after WBRT. We conclude that poor survival time of the patients with low KPS is due to the systematic disease progression, as well as intracranial disease progression.

In line with our study, activity of extracranial primary disease is the third most important prognostic factor reported in the literature (Lagerwaard et al. 1999; Aoyama et al. 2006; Fleckenstein et al. 2004; 20). These finding suggests that survival of patients with brain metastases is in a large part, regulated by the extracranial status. Seventy-six patients (64 %) included in this study died due to systemic disease. This percentage is comparable to the reports of prospective clinical trials with SRS alone or SRS + WBRT for single or fewer numbers of brain metastases with well controlled systemic disease (Sneed et al. 1999; Andrews et al. 2004; Aoyama et al. 2006). This result highlights the modest effectiveness of WBRT on brain metastases. WBRT alone have adequate efficacy to avoid neurologic death for about two-thirds of patients with brain metastases. If we consider the high morbidity rate from systemic disease after WBRT, chemotherapy is the primary therapeutic approach for the control of extracranial disease. Therefore, 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.

The role of chemotherapy in brain metastasis itself has been limited. Although there is some breakdown of blood–brain barrier (BBB) around brain metastases, the concentrations of most of the chemotherapeutic agents are still very limited within the lesion (Gerstner and Fine 2007). However, some chemotherapeutic agents are known to have activity of crossing BBB. Temozolomide (TMZ) is a third generation alkylating agent, and it can cross the BBB because of its small size and lipophilic properties (Ostermann et al. 2004). Some clinical trials suggest that single agent TMZ has some activity in patients with recurrent brain metastases (Christodoulou et al. 2001; Siena et al. 2010). Several Phase II clinical trials of TMZ combined with WBRT were performed with promising results (Antonadou et al. 2002; Addeo et al. 2008). These trials proved improved response rate and neurologic function with addition of TMZ to WBRT. A phase III clinical trial of WBRT plus SRS with or without TMZ or Erlotinib in patients with brain metastases is now ongoing (ClinicalTrials.gov identifier: NCT00096265). Patients with 1–3 brain metastases from histologically confirmed non-small cell lung cancer, well circumscribed, maximum diameter of 4 cm or less, no metastasis within 10 mm of the optic apparatus, no metastasis in the brain stem and stable extracranial metastases are enrolled. Patients are randomized to three groups: Arm 1: WBRT + SRS, Arm 2: WBRT + SRS + TMZ, Arm 3: WBRT + SRS + erlotinib. Patients in Arm 2 and 3 begin TMZ or erlotinib on the first day of WBRT and continue up to 6 months. The primary endpoint is overall survival, and secondary endpoint includes time to CNS progression, performance status at 6 months, steroid dependence at 6 months, cause of death and effect of non-protocol chemotherapy.

Topotecan is a semi-synthetic analogue of the alkaloid camptothecin, which selectively inhibits topoisomerase I. Topotecan crosses the BBB, because of its low protein binding property (Baker et al. 1996). Single agent topotecan has positive activity in patients with brain metastases from small cell lung cancer (Korfel et al. 2002). A phase III multicentric clinical trial of topotecan and WBRT for patients with brain metastases form lung cancer was planned, however, was terminated because of low patient accrual (Neuhaus et al. 2009). This trial failed to show clear benefit of adding topotecan to WBRT. Another multicentric phase III clinical trial is ongoing (ClinicalTrials.gov identifier: NCT00390806). Patients with at least one brain metastasis form non-small cell lung cancer, who have received previous chemotherapy are enrolled. Patients are randomized to two groups: experimental arm: topotecan + WBRT, control arm: WBRT alone. The primary endpoint is overall survival, secondary endpoint includes response rate, time to response, time to progression, brain tumor symptom, safety and tolerability. We think that these clinical trials for brain metastasis should evaluate the effect of non-protocol chemotherapy on survival. In the next 5 years, the results of these phase III, multicentric clinical trials will become available to further define the role of these chemotherapeutic agents when combined with WBRT and SRS, or both.

Some investigators suggest that the permeability of BBB in brain tumors can alter during or ever after fractionated radiotherapy (Yuan et al. 2006; Wilson et al. 2009; Cao et al. 2005). After irradiation, the BBB may be partially disrupted so that some chemotherapeutic agents can reach a therapeutic level in the metastatic tumors. This is another explanation of the value of systemic chemotherapy after WBRT. In fact, subset analysis of this study showed that

the use of chemotherapy after WBRT was also an independent prognostic factor predicting longer local tumor progression-free duration (data not shown). We believe that some brain metastases become sensitive to chemotherapy after irradiation. Chemo-sensitivity of brain metastases can affect the survival of a part of patients with treated brain metastases. Therefore, systemic chemotherapy will be a treatment of choice for those who have systemic disease with irradiated brain metastases. If a patient have a plan of definitive chemotherapy for primary disease after the treatment of brain metastases, such patient can be a good candidate for more aggressive therapy for brain metastases.

Another topic of debate is whether molecular-targeted therapy has a significant role on brain metastasis or not. Some investigators advocated that EGFR-TKI has promising activity on previously untreated brain metastases from lung adenocarcinoma (Wu et al. 2007; Kim et al. 2009; Katayama et al. 2009). Another investigator reported activity of trastuzumab on brain metastasis from HER2-overexpressing breast cancer (Park et al. 2009). In this study, the MST of the patients who received molecular-targeted therapy after WBRT was significantly longer than that of those who did not. In the subset analysis of this study, use of molecular-targeted therapy after WBRT was also a significant predictor of longer local progression-free duration (data not shown). We believe that molecular-targeted therapy could have some activity on the local control of some brain metastases.

Patients with "synchronous" brain metastasis survived significantly longer than "metachronous" brain metastasis patients in this study. Short time to develop brain metastasis was marginally independent prognostic factor in multivariate analysis. This is in line with a literature of surgical removal or SRS for brain metastasis (Flannery et al. 2008; Bonnette et al. 2001; Hu et al. 2006). It is easy to assume that systematic disease of patients with "synchronous" brain metastasis would more likely to respond to the following chemotherapy. The "synchronous" brain metastasis may be more sensitive to radiotherapy, when compared to brain metastasis emerged after repeated chemotherapies. Also in agreement with some literature (Lagerwaard et al. 1999; Swinson and William 2008), female patients survived significantly longer than male patients. In particular, the prognosis of female patients with brain metastasis form lung primary has reported to be significantly better than that of male patients (Lagerwaard et al. 1999; Sánchez de Cos et al. 2009). We should further continue to investigate these clinical characteristics of brain metastases.

We acknowledge that the present study had certain limitations because of its retrospective nature. First, the results of this study might be highly influenced by patient's selection bias. Patients with brain metastases which well responded to WBRT may have more opportunity for receiving multiple chemotherapy after WBRT. Second, our cohort should deviate to patients with numerous brain metastases with uncontrolled systemic disease. Because we included only patients with brain metastases primarily treated by WBRT alone, patients with poor prognosis should be negatively selected for this study. Currently, we are investigating the patients with one or few brain metastases primarily treated by SRS alone, and it will be described in another report. Actual prognostic value of chemotherapy on survival after WBRT for brain metastases should be validated in future prospective clinical trials.

Conclusions

In addition to the confirmed prognostic factors previously reported in the literature, the use of multiple chemotherapeutic regimens after WBRT was associated with better survival. 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 future prospective clinical trials.

Conflict of interest None.

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Compliance with Good Clinical Practice in oncology registration trials in Japan

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Background: This study aimed to examine the quality in oncology registration trials for new drug application (NDA) or supplemental new drug application (sNDA) as extensions of the indications for use in Japan based on Good Clinical Practice (GCP) audit findings.

Materials and methods: We collected audit reports of on-site GCP inspections for registration trials in 383 NDAs or sNDAs that were reviewed by the Pharmaceuticals and Medical Devices Agency between the fiscal years 2004 and 2009.

Results: Among the 40 audits for oncology drug applications, the frequencies at which one or more deficiencies ascribed to institution, investigator, sponsor, and institutional review board were found to be 15 (37.5%), 13 (32.5%), 21 (52.5%), and 10 (25.0%), respectively. The exclusion of patients from the review objective due to serious violations of GCP in 40 audits for oncology drug applications was observed in 2 (5.0%) cases, whereas that in the remaining 343 audits for other drug applications was observed in 40 (11.7%) cases.

Conclusion: The overall compliance of GCP in oncology registration trials was moderately better than that in registration trials for other diseases, although there was no statistically significant difference between them.

Key words: audit, cancer, compliance, Good Clinical Practice, inspection, registration trial

introduction

Approval of new drug applications (NDA) or supplemental new drug applications (sNDA) for extension of the range of indication and/or posology as well as the method of administration is based on collecting evidential materials from registration trials that are strictly managed in terms of quality control and quality assurance. The registration trials for applications are conducted in conformity with Good Clinical Practice (GCP) that provides corroboration of both ethics and science. The purpose of GCP is to protect the human rights and safety of the subjects and is based on the World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subject in order to ensure accurate data and reliability in registration trials [1]. The Ministry of Health and Welfare [currently Ministry of Health, Labour and Welfare (MHLW)] of Japan had issued instructions regarding the old GCP guideline in October 1990, which was not legally binding [2]. In April 1997, a new GCP guideline was enforced in response to the implementation of the GCP released by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use for all Japanese registration trials that began from April 1998 onward [3, 4]. Major differences between the old and new GCP guidelines are related to the acquisition of written informed consent documents, intensification of the responsibility of the sponsor, clarification of the responsibility and role of the principal investigator, and improvements in the function of the institutional review board (IRB) and supports for registration trials [2, 3].

In Japan, the number of clinical trial protocol notifications for oncology drug applications is rapidly increasing with each passing year; oncology drug applications comprised ~15% of all clinical trial protocol notifications in the fiscal year 2007 [5]. The number of clinical trial protocol notifications among global registration trials has been increasing substantially; moreover, clinical trial protocol notifications for oncology drugs comprised 59% of global clinical trial protocol notifications, making it the largest field in drug applications in the fiscal year 2007 [6]. It appears that clinical development in the oncology drug field became both active and stable in Japan around this time. These conditions have also made it easier to carry oncology registration trials with sufficient quality according to GCP as compared with that in other drug fields.

Clinical trials for oncology drugs have many differentiating features as compared with those for other drugs. In oncology clinical trials, complicated inclusion/exclusion criteria, frequent dose modifications caused by toxic effects, numerous

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prohibited concomitant medications, tight schedules of clinical assessments, and long follow-up periods are required. In addition, since the pharmacological effects of oncology drugs generally influence cell proliferation or cell division, a large number of adverse events are frequently reported in oncology clinical trials as compared with clinical trials for other drugs. Thus, enormous effort and responsibility are imposed on trial participants, such as institutions, investigator, IRBs, and sponsors.

In this study, we examined GCP compliance in oncology registration trials in order to ensure high-quality clinical trials in Japan. The GCP compliance of the registration trials for NDA and sNDA was examined based on the Pharmaceuticals and Medical Devices Agency's (PMDA) judgment on recent overall results of on-site GCP audits. We have discussed the quality of oncology registration trials through a comparison of the deficiencies found in GCP inspections that were ascribed to the institution, investigator, sponsor, and IRB between 40 oncology drugs applications and 343 drug applications for other diseases.

materials and methods

GCP inspection of PMDA in Japan

The Office of Conformity Audit of PMDA carried out GCP inspections that consisted of document-based conformity audit at the PMDA along with on-site GCP audits [7]. The document-based conformity audit exhaustively inspects the consistency between application materials attached to the application form for approval and all evidential materials of all institutions retained by study sponsors (e.g. case report forms, monitoring records, etc.) from the viewpoint of Good Laboratory Practice, GCP, and conformity criteria of the application materials. The on-site GCP audit inspects the consistency between raw data (e.g. medical records, examination slips, and patient diaries) as evidential materials of surveyed medical institutions and evidential documents of surveyed institutions held by study sponsors (e.g., case report forms). In addition, the on-site GCP audit inspects the general institutional structure for registration trials at the institution (e.g. administration of the medical institution, IRB, maintenance of essential archives, and investigational drug accountability of the pharmacy). The objectives of on-site GCP audits in trial applications have been previously defined [8]. On-site GCP audits are generally carried out for four institutions in NDA and two institutions in sNDA. An institution in Japan or another country enrolling many patients into a pivotal registration trial of application is selected for on-site GCP audit. The PMDA finally judges GCP compliance as follows: conformation, conformation with proviso, or nonconformation. The results are sent to both the sponsor and the institution.

Conformation indicates complete compliance with the GCP in the registration trial for the application. Conformation with proviso means that the PMDA imposes the exclusion of patients from the review objective due to serious violations of the GCP and evaluates the registration trial comprising the remaining patients. If a critical GCP violation concerning ethics and/or science in the registration trial is found, the PMDA judges that all the materials in the registration trial related to GCP nonconformation should be deleted from the application for NDA or sNDA. In this case, the PMDA generally concludes in favor of rejection of the application. It should be noted that when the PMDA's judgment is nonconformation, these results are not publicly released; therefore, the frequency of nonconformations is not investigated.

data sources

In Japan, for each application, on-site GCP inspection for the registration trials—including trials conducted in Japan and overseas for the drugs—are conducted, and their comprehensive audit results are publicly released with exposures of the deficiencies found in GCP inspections that are ascribed to the institution, investigator, sponsor, and IRB [9]. In this study, 344 audits, which were reviewed by the PMDA and approved by the MHLW of Japan between April 2004 and March 2010 (fiscal years 2004 to 2009), were examined, excluding public domain approvals and audits without on-site GCP inspections [10]. For each audit, the following data were collected: medicinal classification of the approved drug, approval year, the PMDA's judgment on GCP compliance (conformation with/without proviso), the number of patients excluded due to serious violations of GCP, GCP deficiencies, and responsible participants of deficiencies (institution, investigator, sponsor, and IRB).

Fisher's exact test was used to compare the frequency distributions with respect to the deficiencies between the audits for anticancer drugs and those for other diseases. A two-sided $P \leq 0.05$ was considered to be statistically significant. All the analyses were carried out using the SAS software (version 9.1; SAS Institute Inc., Cary, NC).

results

conformation with/without proviso

The approval years and medicinal classifications for 383 audits are shown in Table 1. The audits for oncology drug applications comprised 40 (10.4%) of the 383 audits.

Table 2 shows the proportions of conformation with/without proviso overall and for each medicinal classification. Overall, 89.6% of conformation and 10.4% of conformation with proviso were observed. Among the 42 audits judged as conformation with proviso, the frequencies of audits with ≥1 deficiencies ascribed to the institution, investigator, IRB, and sponsor were 34 (81.0%), 23 (54.8%), 12 (28.6%), and 25 (59.5%), respectively. Additionally, the frequencies of audits in each deficiency ascribed to each responsible participant are shown in Table 3.

Conformation with proviso in 40 audits for anticancer drug applications were observed in 2 (5.0%) cases, whereas that in the remaining 343 audits for the other disease applications was observed in 40 (11.7%) ($P = 0.286$). The proportion of conformation with proviso in cancer registration trials tended to be smaller than that in the registration trials for other disease applications, although the number of audits varied depending upon the medicinal classification. Furthermore, although the number of excluded patients was unknown in 9 audits, among the 42 audits judged as conformation with proviso, the median number of excluded patients was 3 (range 1–182) in the remaining 33 audits.

responsible participants due to deficiencies

Table 4 shows the distributions of audits in which one or more deficiencies were ascribed to the responsible participants overall and in each medicinal classification. The proportion of approvals with ≥1 deficiencies ascribed to the institution, investigator, IRB, and sponsor were 15 (37.5%), 13 (32.5%), 10 (25.0%), and 21 (52.5%) in 40 audits, respectively, for oncology drug applications and 168 (49.0%), 145 (42.3%), 78 (22.7%), and 169 (49.3%),

Table 1. Summary of 383 registration trial approvals [*n* (%)]

Medicinal classification	Approval year, fiscal year						Total
	2004	2005	2006	2007	2008	2009	
Neurological	1 (4.2)	3 (7.3)	8 (12.3)	10 (10.9)	8 (11.0)	17 (19.3)	47 (12.3)
Metabolic	1 (4.2)	6 (14.6)	12 (18.5)	15 (16.3)	18 (24.7)	17 (19.3)	69 (18.0)
Oncology	2 (8.3)	7 (17.1)	6 (9.2)	8 (8.7)	9 (12.3)	8 (9.1)	40 (10.4)
Cardiovascular	3 (12.5)	3 (7.3)	4 (6.2)	7 (7.6)	10 (13.7)	9 (10.2)	36 (9.4)
Respiratory	1 (4.2)	1 (2.4)	1 (1.5)	2 (2.2)	0 (0.0)	5 (5.7)	10 (2.6)
Gastrointestinal	0 (0.0)	1 (2.4)	3 (4.6)	10 (10.9)	2 (2.7)	6 (6.8)	22 (5.7)
Hormonal	2 (8.3)	3 (7.3)	7 (10.8)	6 (6.5)	8 (11.0)	7 (8.0)	33 (8.6)
Urological	2 (8.3)	1 (2.4)	4 (6.2)	5 (5.4)	3 (4.1)	1 (1.1)	16 (4.2)
Antimicrobial	7 (29.2)	7 (17.1)	10 (15.4)	16 (17.4)	4 (5.5)	9 (10.2)	53 (13.8)
Biologics	2 (8.3)	4 (9.8)	5 (7.7)	6 86.5)	5 (6.8)	7 8.0)	29 (7.6)
Others	3 (12.5)	5 (12.1)	5 (7.7)	7 (7.6)	6 (8.2)	2 (2.3)	28 (7.3)
Total	24 (100)	41 (100)	65 (100)	92 (100)	73 (100)	88 (100)	383 (100)

Table 2. PMDA's judgment on GCP compliance in oncology and other drug audits [*n* (%)]

Judgments	Medicinal types		Total
	Oncology	Others	
Conformation (without proviso)	38 (95.0)	303 (88.3)	341 (89.6)
Conformation with proviso	2 (5.0)	40 (11.7)	42 (10.4)

Fisher's exact test for contingency table of judgments and medicinal types: $P = 0.286$.

GCP, Good Clinical Practice; PMDA, Pharmaceuticals and Medical Devices Agency.

respectively, in the remaining 343 audits for other drug applications. The deficiencies ascribed to the institution and investigator in the cancer registration trials tended to be lesser than those in the registration trials for other diseases ($P = 0.184$ for institution and $P = 0.309$ for investigator).

deficiencies ascribed to responsible participants

Table 5 shows the frequencies of audits in each deficiency ascribed to each responsible participant overall and in each medicinal classification. The deficiencies related to archives, eligibility criteria, and prohibited concomitant therapies in 40 audits for oncology drug applications were 1 (2.5%), 2 (5.0%), and 0 (0.0%), respectively, whereas those in the 308 other drug audits were 47 (13.7%), 43 (12.5%), and 28 (8.2%), respectively ($P = 0.043$ for archives, $P = 0.201$ for eligibility criteria, and $P = 0.099$ for prohibited concomitant therapies). On the other hand, the deficiency of 'insufficient review' by the IRB in 40 audits for oncology drug applications was higher than that in the 343 other drug audits (17.5% versus 5.5%, $P = 0.012$).

discussion

The results of the present study indicated that the overall compliance of GCP in oncology registration trials was passably better than that in registration trials for other diseases, although there was no statistically significant difference between them. According to Table 5, the problems related to archives in institutions were lesser but insufficient reviews by the IRB were more frequent in the oncology drug applications when compared with those for other diseases. Therefore, completeness of IRB reviews would enhance quality of drug applications in the oncology field.

Previous studies have analyzed a number of GCP deficiencies in registration trials for NDA or sNDA, approved by the MHLW of Japan, from the fiscal year 1997 to 2006 [11–18]. Since a white paper or annual report regarding the overall results of on-site GCP audit has not been officially published, these studies have repeatedly used the same data that were partly released by the PMDA, workshops, or symposiums. In addition, most of these studies examined GCP deficiencies immediately after the enforcement of the new GCP guidelines [11–15]. The examination of compliance with GCP in registration trials for NDA or sNDA in recent times is required.

Our study demonstrated 10.4% of conformations with proviso in registration trials overall in the past 5 years. Previous studies have reported that conformations with proviso comprised 17.6% of registration trials during the fiscal years 2001 and 2003 [16]. Based on the results of the present study and those of previous studies, compliance with GCP in Japanese registration trials has generally been improving [16, 17]. Furthermore, the present study revealed the overall GCP compliance of oncology registration trials tended to be better than that of registration trials for other drugs.

The present study revealed trial institution deviations, investigator deviations, and sponsor deviations in 40%–50% of the audits. The frequencies of deviations related to the trial institution or investigator were lower in the oncology registration trials as compared with those in the other drug registration trials. This may be because the development of oncology drugs is highly specialized; therefore, research sources—including the trial institution, investigator, and other health care professionals—for the registration trials of oncology drugs have much greater experience and can carry registration trials with greater compliance.

Table 3. Frequencies of audits in each deficiency ascribed to each responsible participant in 42 approvals judged as conformation with proviso

Responsible participants	Deficiencies	*n* (%)
Institution	Qualification requirements of hospitals were not met	6 (14.3)
	Lack of appropriate SOP	0 (0.0)
	All investigators were not identified in the contract	0 (0.0)
	Inappropriate contract	6 (14.3)
	Inappropriate informed consent	11 (26.2)
	CRFs filled incorrectly/and or insufficiently	8 (19.1)
	Problems related to archives	19 (45.2)
	Delay in communication of safety information	3 (7.1)
	Others	6 (14.3)
Investigator	Eligibility criteria were not met	13 (31.0)
	Prohibited concomitant therapies	7 (16.7)
	Laboratory tests were not performed according to the defined protocol	9 (21.4)
	Nonobservance of dose and/or schedule provided by the protocol	8 (19.1)
	Others	8 (19.1)
Sponsor	Inappropriate monitoring	24 (57.1)
	Delay in communication of safety information to institution	4 (9.5)
	Others	2 (4.8)
IRB	Qualification requirements of IRB were not met	2 (4.8)
	Lack of appropriate SOP	1 (2.4)
	Insufficient review	4 (9.5)
	Insufficient minutes of meetings	2 (4.8)
	Others	7 (16.7)

IRB, institutional review board; SOP, standard operational procedure; CRFs, case report forms.

Table 4. Frequencies of audits in which one or more deficiencies ascribed to the responsible participants were found by GCP inspection in oncology and other registered trials [*n* (%)]

Responsible participants	Medicinal type		Total	*P* value[a]
	Oncology	Others		
Institution				0.184
Yes	15 (37.5)	168 (49.0)	183 (47.8)	
No	25 (62.5)	175 (51.0)	200 (52.2)	
Investigator				0.309
Yes	13 (32.5)	145 (42.3)	158 (41.3)	
No	27 (67.5)	198 (57.7)	225 (58.8)	
IRB				0.696
Yes	10 (25.0)	78 (22.7)	88 (23.0)	
No	30 (75.0)	265 (77.3)	295 (77.0)	
Sponsor				0.740
Yes	21 (52.5)	169 (49.3)	190 (49.6)	
No	19 (47.5)	174 (50.7)	193 (50.4)	

[a]Fisher's exact test for contingency table of the presence of deficiencies ascribed to each responsible participant and medicinal types.
GCP, Good Clinical Practice; IRB, institutional review board.

Drug development generally takes considerably long due to the on-site GCP audit in response to a trial application. However, problems related to archives would essentially relate to the reliability of the registration trial regarding the existing subjects, ethics, and science. We noted no problems related to archives in the oncology drug registration trials; the frequency of this deficiency was clearly lower for oncology drugs as compared with other drugs. Thus, the compliance with GCP regarding archives was satisfactory in oncology drug registration trials.

The frequency of protocol deviation in oncology fields is lower than that for other medicinal classifications; however, protocol deviations for eligibility criteria or use of prohibited concomitant therapies would influence subject safety in registration trials. Therefore, investigators, clinical research coordinators (CRC), and other health care professionals who support registration trials should make an effort to have sufficient knowledge regarding the target disease and treatment and keep track of details regarding the protocol and GCP. The incidence of deficiencies at domestic investigational sites with CRC was 21% ($N = 270/1260$), which was lower than that of deficiencies at domestic investigational sites without CRC, i.e. 58% ($N = 188/325$) [7, 18]. Therefore, an effective approach for reducing deficiencies associated with protocol deviation would entail the careful selection of trial institutions with sufficient numbers of well-trained CRCs and suitable conditions for carrying out monitoring.

In the present study, deficiencies in monitoring were most frequent both overall and in sponsor deviations. Monitoring of the medical institution by the sponsor is enforced by GCP in order to ensure appropriate operation of the registration trial according to trial protocol and GCP. A previous study indicated that typical monitoring issues associated with sponsors in the fiscal year 2005 were as follows: operation of monitoring associated with standard operation procedure and source document verification (41%), timing of monitoring (9.5%), taking appropriate precautions to prevent deviation by monitoring report (8.5%), submission of monitoring report (5.5%), and other (35.5%) [18]. Appropriate monitoring for registration trial by a monitor who has been specifically trained and possesses scientific and clinical knowledge is important for ensuring quality control and quality assurance of registration trials. For further improvement in reducing deficiencies in monitoring, the monitor in the sponsor organization or contract research organization (CRO) should be sufficiently familiar with the protocol and GCP. Improved performance of various parties in the registration trial would not only facilitate operation of the registration trial by the sponsor but also the operation of investigator-initiated registration-directed clinical trials by the investigator, according to the revised GCP enforced from July 2003 [19].

Another major item of deficiency related to the sponsor is a delay in communicating information regarding adverse drug reactions; this is related to subject safety, ethics, and operation of the registration trial. A seamless communication system for delivering critical information is important for ensuring subject safety and appropriate operation of the registration trial. In

Table 5. Frequencies of audits in which each deficiency was found by GCP inspection in oncology drug and other drug applications [*n* (%)]

Responsible participants	Deficiencies	Oncology	Others	Total	*P* value[a]
Institution	Qualification requirements of hospitals were not met	1 (2.5)	6 (1.8)	7 (1.8)	0.541
	Lack of appropriate SOP	0 (0.0)	0 (0.0)	0 (0.0)	–
	All investigators were not identified in the contract	0 (0.0)	3 (0.9)	3 (0.8)	1.000
	Inappropriate contract	2 (5.0)	17 (5.0)	19 (5.0)	1.000
	Inappropriate informed consent	3 (7.5)	26 (7.6)	29 (7.6)	1.000
	CRFs filled incorrectly/and or insufficiently	8 (20.0)	81 (23.6)	89 (23.2)	0.696
	Problems related to archives	1 (2.5)	47 (13.7)	48 (12.5)	0.043
	Delay in communication of safety information	2 (5.0)	21 (6.1)	23 (6.0)	1.000
	Others	4 (10.0)	36 (10.5)	40 (10.4)	1.000
Investigator	Eligibility criteria were not met	2 (5.0)	43 (12.5)	45 (11.8)	0.201
	Prohibited concomitant therapies	0 (0.0)	28 (8.2)	28 (7.3)	0.099
	Laboratory tests were not carried out according to the defined protocol	6 (15.0)	59 (17.2)	65 (17.0)	0.823
	Nonobservance of dose and/or schedule provided by the protocol	5 (12.5)	23 (6.7)	28 (7.3)	0.195
	Others	5 (12.5)	48 (14.0)	53 (13.8)	1.000
IRB	Qualification requirements of IRB were not met	1 (2.5)	5 (1.5)	6 (1.6)	0.487
	Lack of appropriate SOP	0 (0.0)	2 (0.6)	2 (0.5)	1.000
	Insufficient review	7 (17.5)	19 (5.5)	26 (6.8)	0.012
	Insufficient minutes of meetings	0 (0.0)	12 (3.5)	12 (3.1)	0.623
	Others	2 (5.0)	49 (14.3)	51 (13.3)	0.138
Sponsor	Inappropriate monitoring	19 (47.5)	136 (39.7)	155 (40.5)	0.395
	Delay in communication of safety information to institution	5 (12.5)	50 (14.6)	55 (14.4)	1.000
	Others	1 (2.5)	13 (3.8)	14 (3.7)	1.000

[a]Fisher's exact test for contingency table of the presence of each deficiency and medicinal types.
GCP, Good Clinical Practice; IRB, institutional review board; SOP, standard operational procedure.

recent drug development protocols, registration trials such as randomized clinical trials are carried out globally in various trial institutions; in such a scenario, worldwide regional offices of the sponsor would be ideal for improving communication systems and ensuring smooth and timely communication.

There have been various approaches for improving social and scientific infrastructure for clinical research in Japan by academia, industry, and the government. In 2003, the MHLW drew up and published the nationwide 3-year clinical trial activation plan, under which it promoted various measures, including the creation of clinical trial networks and fostering of CRC. Subsequently, the MHLW created the office of clinical trial promotion, research, and development and launched the new 5 yearly clinical trial activation plan in 2007, which was expected to reinforce clinical research infrastructure to ensure patient safety and to secure access to new drugs and devices [20]. Furthermore, the MHLW science research grants 'research on clinical trials infrastructure development' were inaugurated to support framework development for promoting clinical trials (comprising grants to 10 leading academic medical centers). Thus, a study on 'the development of individual health care institution infrastructure models aimed at equally sharing cancer research infrastructure development' was started, and it became possible to pursue favorable institutional infrastructure development and human resources training concerning the ethical aspects of clinical research and methods of new drug development in the National Cancer Center Hospital [21, 22]. Furthermore, the Japanese Ministry of Education, Culture, Sports, Science and Technology provided grants to five universities and a clinical research organization named 'Coordination, Support and Training Program for Translational Research' in 2007 and onward [22, 23]. These various approaches promoted the establishment of a clinical trial infrastructure; we believe that an adequate infrastructure would be the optimal influence for ensuring compliance with GCP in registration trials.

Our study had certain limitations. We were not able to use the full data of on-site GCP audits for a number of trial institutions—such as the trial institution background, i.e. scale (university hospital, national hospital, private hospital, and clinic), region (Japan or other countries), number of subjects under on-site GCP audit, presence of supporting system for registered trial (CRC, site management organization, CRO, etc.)—because the PMDA review reports for on-site GCP audit are the only available data source and these do not have detailed data. Therefore, it is difficult to directly compare the results of the present study with those of previous studies. Because there

are few reports of on-site GCP audits by regulatory agencies, the present study described differences in deficiencies from on-site GCP audits between Japan and other countries. For further improving global compliance with GCP, we consider that each regulatory agency should disclose detailed results of on-site GCP audits on a regular basis.

GCP inspections have indicated certain deficiencies in the data of registration trials and the operation systems of registration trials; these were evaluated in the regulatory reviews of NDA or sNDA. However, the most important purpose of GCP inspection is to prevent a recurrence of GCP deficiencies for establishing higher quality in drug development. In 2009, the European Medicines Agency (EMEA) and the Food and Drug Administration (FDA), USA, initiated the EMEA–FDA GCP initiative that focuses upon enhanced and systematic GCP-related information exchanges between the EMEA and FDA combined with collaboration in the conduct of GCP inspections of registration trials [24]. The results of the present study suggest that the principle of compliance with GCP for registration trials has reached Japanese investigators and trial institutions, and high-quality GCP inspections are thereby being carried out by the PMDA. The clinical development of medicines is a global undertaking. Therefore, in the future, we consider it important that all regulatory agencies work in a collaborative and synergistic manner in order to achieve a system for the optimal use of GCP inspection resources and results and implement information exchanges.

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disclosure

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