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CLINICAL INVESTIGATION **Brain**

COMPARISON OF CLINICAL OUTCOMES OF SURGERY FOLLOWED BY LOCAL BRAIN RADIOTHERAPY AND SURGERY FOLLOWED BY WHOLE BRAIN RADIOTHERAPY IN PATIENTS WITH SINGLE BRAIN METASTASIS: SINGLE-CENTER RETROSPECTIVE ANALYSIS

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Purpose: Data comparing the clinical outcomes of local brain radiotherapy (LBRT) and whole brain RT (WBRT) in patients with a single brain metastasis after tumor removal are limited.

Patients and Methods: A retrospective analysis was performed to compare the patterns of treatment failure, cause of death, progression-free survival, median survival time, and Karnofsky performance status for long-term survivors among patients who underwent surgery followed by either LBRT or WBRT between 1990 and 2008 at the National Cancer Center Hospital.

Results: A total of 130 consecutive patients were identified. The median progression-free survival period among the patients who received postoperative LBRT ($n = 64$) and WBRT ($n = 66$) was 9.7 and 11.5 months, respectively ($p = .75$). The local recurrence rates (LBRT, 9.4% vs. WBRT, 12.1%) and intracranial new metastasis rate (LBRT, 42.2% vs. WBRT, 33.3%) were similar in each arm. The incidence of leptomeningeal metastasis was also equivalent (LBRT, 9.4% vs. WBRT, 10.6%). The median survival time for the LBRT and WBRT patients was 13.9 and 16.7 months, respectively ($p = .88$). A neurologic cause of death was noted in 35.6% of the patients in the LBRT group and 36.7% of the WBRT group ($p = .99$). The Karnofsky performance status at 2 years was comparable between the two groups.

Conclusions: The clinical outcomes of LBRT and WBRT were similar. A prospective evaluation is warranted.

Local brain radiotherapy, Whole brain radiotherapy, Single brain metastasis, Clinical outcomes, Long-term result.

INTRODUCTION

Whole brain radiotherapy (WBRT) has served as the standard of care for patients with brain metastases worldwide (1, 2). In patients with a single brain metastasis, postoperative WBRT has demonstrated better intracranial tumor control for both surgical lesions and nonsurgical new lesions and a lower rate of a neurologic cause of death compared with surgery alone (3). However, the addition of WBRT did not result in a survival benefit or extend the duration of the interval that the patients remained functionally independent. Some prospective trials, with the exception of one, and pooled analyses have clarified that a survival benefit for surgery followed by WBRT does exist compared with WBRT alone (1, 4–7). Other studies have also revealed that surgery followed by WBRT increased the duration of neurocognitive functional independence, as well as intracranial tumor control (4–6, 8, 9). Accordingly, surgery followed by WBRT has been the standard of care for patients with a single brain metastasis.

The median survival time of patients with brain metastases is considered to be approximately 2–7 months; favorable and unfavorable subgroups can be classified using recursive partitioning analysis (RPA) (10). However, about 2–8% of patients with brain metastasis can achieve longer survival periods (11, 12). Delayed WBRT toxicity, hypopituitarism, dementia, and memory disturbances influencing cognitive function have also been discussed, although the primary brain lesion is mainly responsible for the deterioration of functional independence (11, 13, 14).

Because WBRT is widely believed to induce dementia in patients with brain metastases, local brain RT (LBRT) as a substitute for WBRT has been widely accepted in some

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Table 1. Patient characteristics (n = 130)

Characteristic	All patients	Range	LBRT (n = 64)	WBRT (n = 66)	p
Age (y)	58	24–87	58 (38–87)	58 (24–79)	.35
Karnofsky performance status	70	40–100	70 (40–100)	70 (40–100)	.35
RPA class	II	I–III	II (I–III)	II (I–III)	.78*
I	40	30.8	19	21	
II	55	42.3	26	29	
III	35	26.9	19	16	
Cancer type (%)					.96*
Lung cancer	55	42.3	29	26	
Non–small-cell lung cancer	54		29	25	
Small-cell lung cancer	1		0	1	
Breast cancer	18	13.8	9	9	
Colorectal cancer	14	10.8	6	8	
Skin cancer	6	4.6	3	3	
Other	37	28.5	17	20	
Diameter of brain tumor (mm)	38	10–65	38 (10–65)	38 (15–60)	.57
Removal status					.11
Gross total removal	124	95.4	59	65	
Partial removal	6	4.6	5	1	

Abbreviations: RPA = recursive partitioning analysis; WBRT = whole brain radiotherapy; LBRT = local brain radiotherapy,
Data presented as median, with range in parentheses.
* Chi-square test.

institutions in Japan (15). LBRT delivered by linear accelerator to the tumor bed with a margin determined using the two-field technique (opposing portal irradiation) according to a dose-fractionated schedule had been applied for the treatment of single brain metastasis after surgical removal at the National Cancer Center Hospital before September 2004. This was based on the ethics that we presumed we could treat intracranial relapse using stereotactic RT after LBRT. After discussion with neurosurgeons, radiooncologists, and medical oncologists, however, the treatment policy was changed. WBRT has been used for the treatment of all patients with single brain metastasis after tumor removal since October 2004. A Phase I-II clinical trial of postoperative LBRT was reported, and the investigators concluded that LBRT was not a suitable substitute for WBRT (16). However, that previous study included only 12 patients, and 7 of these patients died of intracranial tumor progression. The median survival time was 7.2 months, similar to that after WBRT. Another retrospective study implied that LBRT might have a similar benefit to that of WBRT in patients with a single brain metastasis (17). Bahl *et al.* (18) reported 7 cases of postoperative LBRT, of which 4 cases recurred at the same site. These studies included only a small number of patients, and any conclusions regarding the clinical outcome of postoperative LBRT, especially compared with that of postoperative WBRT, are thus difficult to make. In the present analysis, we retrospectively compared the clinical outcomes of patients with a single brain metastasis who received surgery followed by either WBRT or LBRT.

PATIENTS AND METHODS

Patient population

From the database of the neurosurgery division at the National Cancer Center Hospital, we identified patients who had undergone brain tumor removal followed by RT between 1990 and 2008. The patients were included in the present analysis if they met the following criteria: age ≥18 years, a single brain metastasis identified by magnetic resonance imaging, and tumor removal followed by either WBRT or LBRT. The exclusion criteria were as follows: extracranial malignant lymphoma or hematological tumor; brain biopsy only; previous brain RT; surgery followed by observation, with brain RT once progression was recognized; and postoperative gamma knife or linear accelerator-based radiosurgery. All the patients who received LBRT (n = 64) were treated before October 2004, and all the patients who received WBRT (n = 66) were treated after October 2004.

Data collection and definitions of terms

All the medical charts for the eligible patients were reviewed. To compare the clinical outcomes of postoperative WBRT and LBRT, we collected the following data:; preoperative magnetic resonance imaging; date of surgery and RT; RPA classification before surgery; Karnofsky performance status (KPS) at presentation; primary tumor site; date of recognition of local recurrence or intracranial new metastases; patterns of progression; leptomeningeal metastasis development; date of death; and neurologic cause of death. For the additional evaluation of long-term survivors (≥2 years after surgery), we also reviewed the KPS at 2 years after surgery.

Local recurrence was defined as recurrence at the surgical site. Intracranial new metastases included the detection of new brain metastases other than those occurring at the surgical site or the development of leptomeningeal metastases. Leptomeningeal metastases were diagnosed using a cytologic examination of cerebrospinal fluid.

Surgery and RT

The surgical indications for single brain metastasis were generally as follows: tumor diameter ≥30 mm or a tumor diameter of <30 mm with neurologic dysfunction.

Whole brain RT was administered through two lateral ports covering the brain and meninges to the foramen magnum. Normally, WBRT was delivered using a 4-MV or 6-MV linear accelerator at

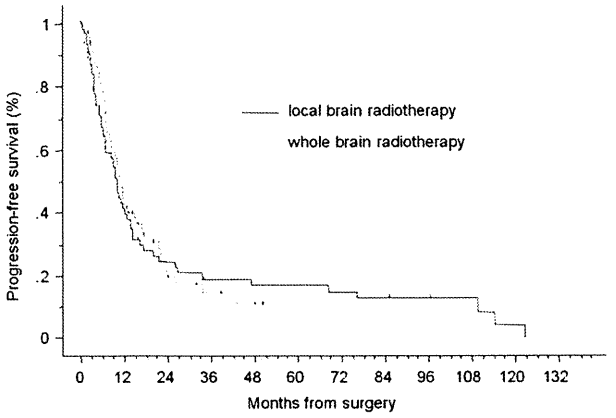

Fig. 1. Progression-free survival for patients with local brain radiotherapy (black line) and whole brain radiotherapy (dashed line).

a total dose of 30 Gy in 10 fractions or 37.5 Gy in 15 fractions. Patients who received LBRT underwent computed tomography simulation in the supine position. The clinical target volume consisted of the tumor cavity plus a 1.5-cm margin, and the planning target volume was created by expanding the clinical target volume by 0.5 cm. LBRT was administered using a 6-MV linear accelerator to the tumor bed using a two-field technique according to a dose-fractionated schedule. Normally, LBRT was delivered at a total dose of 50 Gy in 25 fractions.

Statistical analysis

Postoperative differences in local recurrence, intracranial new metastases, the development of leptomeningeal metastases, and neurologic cause of death were compared between the WBRT and LBRT groups using the Fisher exact test. Numeric data, including RPA, KPS, and age, were compared using the Mann-Whitney *U* test. Progression-free survival was defined as the interval between the date of surgery to the date of the recognition of local recurrence or intracranial new metastases. Death was treated as an event, and the absence of disease progression was treated as a censored observation on the last day of follow-up. Overall survival was defined as the interval from the date of surgery to the date of death. Patients who were lost to follow-up were treated as a censored observation on the last day of follow-up. Univariate and multivariate analyses using the Cox proportional hazard model were performed to identify relevant factors affecting survival. The numeric factors analyzed in the Cox analyses were dichotomized according to the median number. All statistical analyses were performed using StatView, version 5.0 (SAS Institute, Tokyo, Japan).

RESULTS

Of the 421 surgical cases, we identified 130 patients who met the eligibility criteria. The characteristics of these patients are listed in Table 1. Of the 130 patients, 66 had received postoperative WBRT and 64 had received postoperative LBRT. Of the 66 patients who had received WBRT, 34 (51.5%) were treated to a dose of 30 Gy delivered in 10 fractions, and 31 (47.0%) were treated to a dose of 37.5 Gy delivered in 15 fractions. Of the 64 patients who received LBRT, 57 (89.1%) were treated to a dose of 50 Gy in 25 fractions, and 7 were treated with a variety of dose-fractionation schedules (24 Gy in 12 fractions to 60 Gy in 30 fractions).

The median progression-free survival period for the patients who received postoperative LBRT and WBRT was 9.7 and 11.5 months, respectively ($p = .75$; Fig. 1). The patients who underwent LBRT and WBRT developed 33 and 30 recurrences, respectively. The local recurrence rates (9.4% vs. 12.1%) and intracranial new metastases rates (42.2% vs. 33.3%) were not significantly different between the LBRT and WBRT groups (Table 2). The incidence of leptomeningeal metastases in patients receiving LBRT and WBRT was 9.4% and 10.6%, respectively ($p = .99$).

The median survival time for patients who received postoperative LBRT and WBRT was 13.9 and 16.7 months, respectively ($p = .88$; Fig. 2). Of the 64 patients who received LBRT and the 66 patients who received and WBRT, 59 and 49 died, respectively. A neurologic cause of death was noted in 35.6% of the patients in the LBRT group and 36.7% of the patients in the WBRT group ($p = .99$; Table 2). Univariate analyses revealed that only the RPA classification correlated significantly with survival (hazard ratio [HR], 0.436; $p = .002$). In particular, RT (LBRT vs. WBRT) did not correlate with survival (HR, 1.031; $p = .88$; Table 3). Multivariate analyses revealed that RPA was the only significant factor associated with survival (HR, 0.399; $p = .001$). Neither LBRT nor WBRT was related to survival (HR, 0.933; $p = .74$; Table 4).

Table 2. Patterns of treatment failure in patients who received WBRT and LBRT

Variable	LBRT ($n = 64$)	WBRT ($n = 66$)	
Total recurrences identified *(n)*	33	30	*p*
Local recurrence	6 (18.2)	8 (26.7)	.61
Distant metastasis	27 (81.8)	22 (73.3)	.61
Development of leptomeningeal metastases *(n)*	6	7	.99
Total deaths identified *(n)*	59	49	
Neurologic cause of death	21 (35.6)	18 (36.7)	.98*
Other	21 (35.6)	17 (34.7)	
Unknown	17 (28.8)	15 (30.6)	

Abbreviations: WBRT = whole brain radiotherapy; LBRT = local brain radiotherapy.
Data in parentheses are percentages.
* Chi-square test.

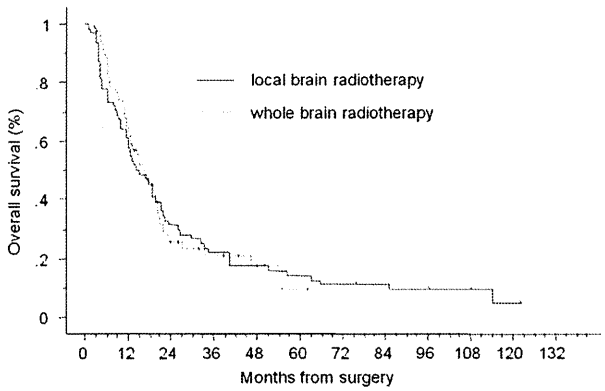

Fig. 2. Overall survival in patients with local brain radiotherapy (black line) and whole brain radiotherapy (dashed line).

We further analyzed the patterns of RT after recurrence in patients who received either postoperative LBRT or WBRT. Of the 33 patients who developed recurrences after postoperative LBRT, additional RT was performed in 15 (45.5%). Of the 15 patients, 6 underwent gamma knife or linear accelerator-based radiosurgery. LBRT was performed in 5 patients, and 4 received WBRT. Of the 30 patients who developed recurrences after postoperative WBRT, 16 (53.3%) received additional RT. Of the 16 patients, 13 received gamma knife or linear accelerator-based radiosurgery, and 3 received LBRT.

Among the patients who survived for >2 years, we compared the KPS at 2 years after surgery. A total of 20 patients who had received postoperative LBRT and 13 who had received postoperative WBRT were identified. The median KPS score at 2 years for these patients in the LBRT and WBRT groups was 80 (range, 60–100) and 80 (range, 60–100; $p = .99$), respectively. Of the 20 patients who had received LBRT, 9 experienced relapse in a local lesion, 2 had focal signs without relapse, which might have indicated radiation necrosis, and 7 had been well without relapse. For 2 other patients, this information was not available.

DISCUSSION

We have revealed the clinical outcomes of postoperative LBRT among patients with single metastasis and compared them with those of patients who underwent postoperative WBRT. The clinical outcomes, including progression-free survival, overall survival, local recurrence, intracranial new metastases, development of leptomeningeal metastases, and neurologic cause of death, were not significantly different between the two groups. In an analysis of relapse patterns, the patients treated with LBRT tended to have a lower probability of developing local recurrence (9.4% vs. 12.1%) and a greater probability of developing intracranial new metastases (42.2% vs. 33.3%), although these values were not significantly different. The probability of developing leptomeningeal metastases was also similar in each group (9.4% vs. 10.6%).

Previous reports have indicated that the addition of WBRT after tumor removal significantly reduces the local recurrence rate (3, 9). However, approximately 6–50% of patients develop relapses at new intracranial sites in the brain (5, 9, 19). Furthermore, about 20–30% of patients with brain metastasis die of neurologic causes even if a radiation boost has been added using stereotactic radiosurgery to increase local control, although the presence of extracranial lesions is the strongest factor for predicting survival (7, 20, 21). In our study, intracranial new metastases were predominant in both groups. The frequency of intracranial recurrence (new local and intracranial metastases) was somewhat greater than in previous series, although the rate of a neurologic cause of death was equivalent. Importantly, the patterns of treatment failure were similar in the LBRT and WBRT groups. Muacevic *et al.* (22) insisted that postoperative WBRT should be applied in patients with a single brain metastasis to destroy so-called micrometastases, based on the results of their randomized trial. They compared patients with a small single metastasis who received either surgery plus WBRT or gamma knife surgery alone. Their sample size was underpowered, although the risk of intracranial new metastases seemed to be lower in the WBRT cohort. To date, no randomized trials comparing the clinical outcomes of postoperative WBRT and postoperative gamma knife or linear accelerator-based radiosurgery, or LBRT have been reported.

We have demonstrated a similar efficacy for LBRT and WBRT. WBRT has problems in terms of delayed toxicity developing leukoencephalopathy, although the number of long-term survivors with brain metastasis seems to be somewhat low (11, 12). LBRT might be beneficial with regard to the protection of normal brain tissue. We compared the KPS

Table 3. Univariate analyses regarding survival

Variable	HR	95% CI	*p*
RT (LBRT vs. WBRT)	1.031	0.698–1.523	.88
RPA classification			
I vs. III	0.436	0.259–0.733	.002
II vs. III	0.808	0.514–0.127	.35
Removal status (gross total removal vs. partial removal)	0.948	0.385–2.334	.91
Tumor diameter (≥38 vs. <38 mm)	1.053	0.718–1.543	.79
Cancer type (lung cancer vs. other)	0.694	0.470–1.025	.062

Abbreviations: RT = radiotherapy; HR = hazard ratio; CI = confidence interval; other abbreviations as in Table 1.

Table 4. Multivariate analyses regarding survival

Variable	HR	95% CI	*p*
RT (LBRT vs. WBRT)	0.933	0.614–1.416	.743
RPA classification			
I vs. III	0.399	0.232–0.688	.001
II vs. III	0.736	0.455–1.191	.22
Removal status (gross total removal vs. partial removal)	0.622	0.239–1.615	.33
Tumor diameter (≥38 vs. <38 mm)	0.852	0.559–1.297	.45
Cancer type (lung cancer vs. other)	0.662	0.438–1.001	.05

Abbreviations as in Tables 1 and 3.

at 2 years to examine any delayed toxicity. Because of the nature of the present retrospective study, the detailed neurocognitive function or quality of life of the patients could not be identified. Among the long-term survivors, however, the KPS was preserved in both treatment groups. Thus, LBRT might be indicated for elderly patients at risk of developing dementia if LBRT has the same ability to control primary brain tumors, which is considered to be the main factor affecting neurocognitive function (14).

The present study had some limitations because of its retrospective nature. First, the radiation dose varied. About 90% of the LBRT patients received a dose of 50 Gy delivered in 25 fractions, and approximately 50% of the WBRT patients received a dose of 30 Gy delivered in 10 fractions; the others received a dose of 37.5 Gy delivered in 15 fractions. According to the summary by Tsao *et al.* (1), no differences in terms of survival or neurocognitive function were observed among the various dose-fraction schedules of WBRT. Second, the present study was a historical case-control study comparing LBRT and WBRT. Patients at risk of developing multiple metastases might have undergone WBRT during the period before 2004, when we started performing WBRT as the standard of care. Thus, the patients who were treated with LBRT might have had better general condition compared with the patients who were treated with WBRT. We compared the baseline characteristics of each treatment arm and used multivariate analyses to reduce any potential biases.

CONCLUSIONS

We have demonstrated the clinical efficacy of LBRT compared with WBRT on a large scale. The clinical outcomes, including progression-free survival, overall survival, patterns of treatment failure, development of leptomeningeal metastases, and a neurologic cause of death, were similar in both treatment groups. The KPS at 2 years was also similar when the two groups were compared. This result should be evaluated in a prospective manner.

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Reactivation of Hepatitis B Virus After Glioblastoma Treatment With Temozolomide

—Case Report—

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Abstract

A 61-year-old man with glioblastoma and positive for hepatitis B surface antigen (HBsAg) developed acute hepatitis due to hepatitis B virus (HBV) reactivation after concomitant postoperative treatment with temozolomide (75 mg/m^2/day) and radiation therapy (60 Gy in 30 fractions). Corticosteroids were not used during chemo-radiation therapy, and grade 4 lymphocytopenia was observed. The levels of liver function tests (LFTs), including levels of aspartate aminotransferase and alanine aminotransferase, increased 5 weeks after the completion of chemo-radiation therapy, and reached the maximum levels of 1,549 IU/l (normal 13 to 33 IU/l) and 1,653 IU/l (normal 8 to 42 IU/l), respectively, after 2 weeks. At this point, serum HBV-deoxyribonucleic acid (DNA) level had increased to 630-fold over the baseline, and therapy with the antivirus agent entecavir (0.5 mg daily) was started. Over the next 2 weeks, the levels of LFTs and HBV-DNA improved. The present and previous cases suggest that grade 3/4 lymphocytopenia or grade 2 lymphocytopenia with corticosteroid use might have a significant effect on HBV reactivation. To avoid this complication, HBsAg-positive patients with glioblastoma should consult a hepatologist for initiating antivirus therapy before temozolomide treatment.

Key words: hepatitis B virus, reactivation, glioblastoma, temozolomide, immunosuppression

Introduction

The reactivation of hepatitis B virus (HBV) is a well-recognized complication of cytotoxic chemotherapy for malignant disease. HBV reactivation usually occurs in patients with hematological malignancies, but is also known in patients with solid tumors, including breast cancer, gastrointestinal cancer, and lung cancer.[1,2]

Temozolomide is an alkylating agent that exerts cytotoxic activity by inducing deoxyribonucleic acid (DNA) damage and apoptosis of tumor cells,[7] and is part of the standard postoperative chemotherapy for the treatment of glioblastoma.[9] Temozolomide carries the risk of HBV reactivation,[1,2] but few cases of temozolomide-induced HBV reactivation have been reported, so the incidence and associated risk factors, and the optimal management of glioblastoma patients with chronic HBV infection remain unclear.

We treated a patient with glioblastoma who was positive for hepatitis B surface antigen (HBsAg) and developed acute hepatitis due to HBV reactivation during temozolomide treatment, and discuss the management of patients with glioblastoma who have chronic HBV infection.

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Case Report

A 61-year-old man presented with generalized convulsions. He had been informed of his HBV carrier status but had not received any treatment. On admission, he tested positive for HBsAg and hepatitis B envelope (HBe) antibody, and negative for HBe antigen, hepatitis C virus antibody, and human immunodeficiency virus. The blood HBV-DNA concentration was 10^3 copies/ml. Magnetic resonance imaging of the brain showed a tumor in the bilateral frontal lobes involving the corpus callosum (Fig. 1A). The patient presented with slight disorientation and left hemiparesis. Partial tumor removal was achieved through a right frontal craniotomy, and the histological diagnosis was glioblastoma (Fig. 1B).

Betamethasone 8 mg was intravenously administered for 3 days following the operation. Ten days after resection, local radiation therapy (60 Gy in 30 fractions over 6 weeks) and temozolomide chemotherapy (75 mg/m^2/day) were initiated. Before chemotherapy and radiotherapy, the liver function tests (LFTs) were normal: aspartate aminotransferase (AST) was 26 IU/l (normal 13 to 33 IU/l), and alanine aminotransferase (ALT) was 28 IU/l (normal 8 to 42 IU/l). During chemo-radiation therapy, the lowest measured white blood cell count was 2900/μl, absolute neutrophil count was 2240/μl, and absolute lymphocyte

count was 190/μl. Four weeks after the completion of chemo-radiation therapy, the levels of LFTs started to increase, and one week later continued to deteriorate. AST increased to 685 IU/l and ALT increased to 744 IU/l. At this time we consulted with a hepatologist to determine the cause of the LFT changes. Abdominal computed tomography (CT) with contrast medium revealed a mass lesion in the liver (Fig. 2). Alpha fetoprotein (AFP) and protein induced by vitamin K or antagonists-II (PIVKA-II) were elevated to 257.6 ng/ml (normal < 10.0 ng/ml) and 8,349 mAU/ml (normal < 40 mAU/ml), respectively.

Our diagnosis was hepatocellular carcinoma (HCC) that had possibly developed before temozolomide treatment. Further, since the HCC was not obstructing the bile duct, we thought that the HCC was not the cause of the LFT changes. The patient's medication regimen at initial presentation consisted of valproic acid, propranolol, and famotidine. After the LFT changes, we stopped administration of famotidine but continued valproic acid and propranolol. The HBV-DNA level increased to $10^{5.8}$ copies/ml. On the basis of the laboratory data and radiological findings, we determined that temozolomide-induced HBV reactivation was the main cause of the LFT changes and acute hepatitis, although the possibility of drug-induced hepatitis or HCC-related LFT change was not completely excluded. Accordingly, we started treatment with the antivirus agent entecavir (0.5 mg daily). For 5 days after the start of entecavir treatment, AST and ALT continued to increase to the maximum levels of 1,549 IU/l and 1,653 IU/l, respectively, but thereafter improved markedly. Two weeks after the start of entecavir treatment, the LFTs returned to almost normal levels. The HBV-DNA level also decreased to $10^{4.8}$ copies/ml (Fig. 3).

After normalization of the LFTs, we started treatment with adjuvant temozolomide at 150 mg/m^2 daily for 5 days/28 days while continuing entecavir therapy. The second cycle used 200 mg/m^2 daily for 5 days/28 days, and no further elevation of LFTs and HBV-DNA level was observed, even though the lowest lymphocyte count was 110/μl (Fig. 3). Four weeks after the onset of LFT changes, the levels of AFP and PIVKA-II were 405.2 ng/ml and 10,195 mAU/ml, respectively, and continued to exacerbate his condition. Transarterial embolization was performed for the treatment of HCC. Three weeks later, the patient's AFP and PIVKA-II levels improved, decreasing to 166.4 ng/ml and 182 mAU/ml, respectively. However, after sec-

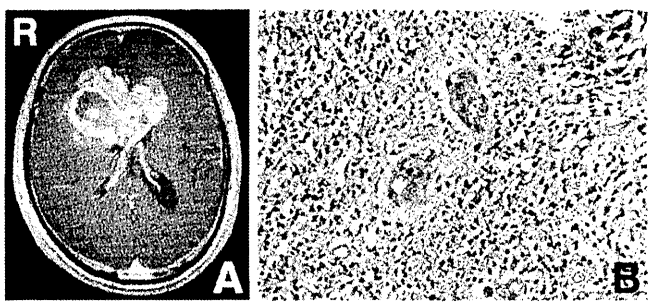

Fig. 1 A: Preoperative T_1-weighted magnetic resonance image with contrast medium showing a tumor in the bilateral frontal lobes involving the corpus callosum. B: Photomicrograph of the tumor specimen showing glioblastoma with cellular anaplasia and prominent microvascular proliferation. Hematoxylin and eosin stain, original magnification ×200.

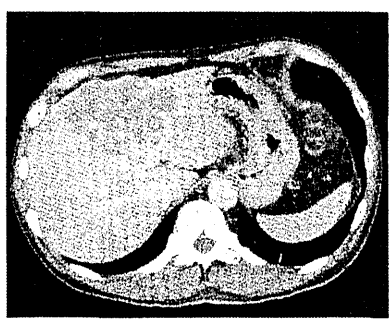

Fig. 2 Abdominal computed tomography scan with late phase contrast enhancement showing a low density mass lesion of 9-cm diameter in the liver.

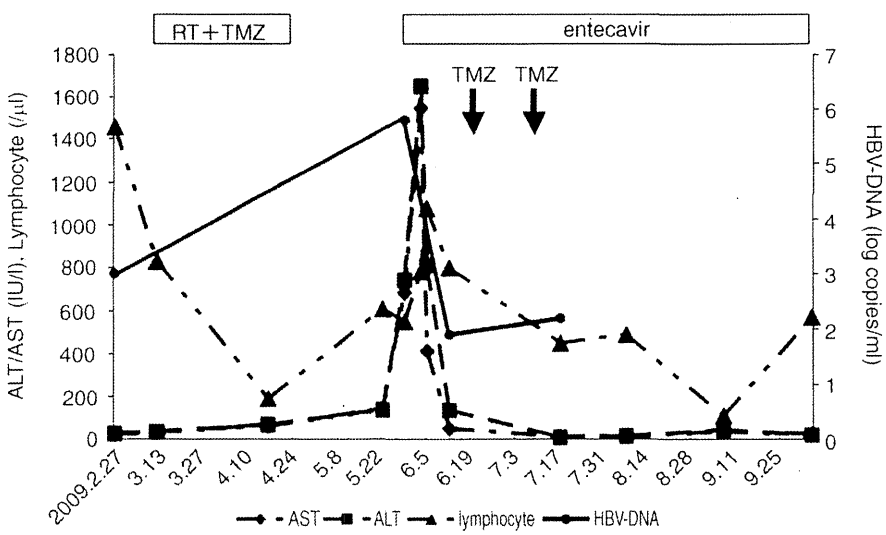

Fig. 3 Time courses of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), lymphocytes, and hepatitis B virus-deoxyribonucleic acid (HBV-DNA) levels. Four weeks after completion of concomitant temozolomide and radiation therapy (RT + TMZ), AST and ALT began to increase and continued to increase to a maximum of 1,549 IU/l and 1,653 IU/l, respectively. HBV-DNA level increased to $10^{5.8}$ copies/ml. After entecavir administration, ALT, AST, and HBV-DNA levels improved during the ensuing 2 weeks. After normalization of liver function, two cycles of adjuvant temozolomide (*arrows*) were initiated while continuing entecavir, without further elevation of AST and ALT even though the lowest lymphocyte count was 110/μl.

ond transarterial embolization, he developed conscious disturbance, and was transferred to a nursing hospital 6 months after the completion of chemo-radiation therapy. Twelve months after the neurosurgical operation, he died of glioblastoma progression.

Discussion

The reactivation of HBV by immunosuppressive agents is characterized by increased levels of serum HBV-DNA, abnormal LFTs, and clinical hepatitis of varying degrees of severity, which may result in death.[13] HBV reactivation occurs in 38–48% of HBsAg-positive patients with lymphoma or other hematological malignancies, who are undergoing conventional therapies, including cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP).[4] The risk factors for HBV reactivation include male sex, young age, steroid use, anthracycline use, high pre-chemotherapy HBV-DNA level, and diagnosis of lymphoma or breast cancer.[3,12,13] Two possible mechanisms may explain HBV reactivation during chemotherapy: immunosuppression enhances virus replication, leading to hepatic toxicity, or chemotherapy-induced T-cell depletion dampens the host response to viral antigens, which enables broader hepatocyte infection, and following the subsequent withdrawal of cytotoxic chemotherapy, a rebound immune response results in hepatocyte destruction.[12]

HBV reactivation-induced hepatitis has been defined as an increase in HBV-DNA level to 10-fold or more when compared with the baseline level, or as an absolute increase in HBV-DNA level to more than $1,000 \times 10^6$ genome equivalents/ml in the absence of other systemic infections.[11] In our patient, the HBV-DNA level increased by 630-fold over the baseline, when the LFTs were elevated, indicating HBV reactivation. However, we could not completely exclude the possibility of drug-induced hepatitis or HCC-related LFT elevation, because the patient had received medication (valproic acid, propranolol, and famotidine) during chemotherapy just before the LFT changes and had underlying HCC. However, the patient continued to receive valproic acid and propranolol even after the LFTs were elevated. Abdominal CT did not reveal bile duct stenosis due to HCC, and both the LFTs and HBV-DNA level improved shortly after entecavir treatment before HCC therapy. Therefore, we presume that the possibility of drug-induced hepatitis or HCC-related increase in LFTs is much lower than that of HBV reactivation, although famotidine-induced hepatitis remains much less likely. Famotidine is also known to induce agranulocytosis[5] and can cause immunosuppression. However, the lowest white blood cell count and absolute neutrophil count in our patient were 2900/μl and 2240/μl, respectively, so the possibility of famotidine-induced agranulocytosis leading to HBV reactivation was thought to be quite low.

HBV infection is one of the causative factors in the development of HCC, and the patient probably had HCC before temozolomide treatment. However, HCC was unlikely to be involved in the development of HBV reactivation after temozolomide treatment, because HCC was localized at the time of increases in LFTs and did not impair the patient's general condition, including the immune system.

Only two cases of HBV reactivation after temozolomide treatment for glioblastoma have been reported (Table 1).[1,2] A 65-year-old woman with glioblastoma presented with HBV reactivation on day 27 of cycle 3 of adjuvant temozolomide therapy and died 2 weeks after the onset.[2] She had a remote history of hepatitis B infection but did not undergo hepatitis examination before starting treatment. She did not receive steroid medication before the onset of HBV reactivation, and her lowest lymphocyte count was 450/μl. A 50-year-old HBsAg-positive man with glioblastoma presented with HBV reactivation 5 weeks after the completion of concomitant radiotherapy and temozolomide.[1] He was successfully treated with the antiviral agent lamivudine over the ensuing 7 weeks. He was treated with 4 mg of dexamethasone during radiation therapy and 2 mg just before the onset of HBV reactivation. His lowest lymphocyte count was 580/μl. Our patient developed the symptoms 4 weeks after completing concomitant radiotherapy and temozolomide, and was successfully treated with entecavir during the ensuing 2 weeks. He had no steroid exposure before the onset of HBV reactivation, and his lowest lymphocyte count was 190/μl. All these cases suggest that grade 3/4 lymphocytopenia or grade 2 lymphocytopenia with corticosteroid use might have a significant effect on the development of HBV reactivation. The guidelines issued in the Joint Report of the Intractable Liver Disease Study Group of Japan and the Japanese Study Group of the Standard Antiviral Therapy for Viral Hepatitis recommend that all patients should be screened for HBsAg, and anti-hepatitis B core and anti-HBs antibodies before chemotherapy is initiated. HBsAg-positive patients should be advised to consult a hepatologist for initiating antiviral therapy, such as entecavir before starting chemotherapy.[10]

HBV reactivation after chemotherapy with temozolomide may be a rare complication. However, temozolomide is associated with CD4$^+$ T-cell dysfunction and therefore may cause increased susceptibility to opportunistic infections by agents such as *Pneumocystis* pneumonia.[8] This characteristic immunosuppression may also induce HBV reactivation. In the Japanese population, 1.4% of individuals are positive for HBsAg,[6] so HBV reactivation during glioblastoma treatment with temozolomide may become a critical issue. To avoid this complication, patients with glioblastoma should be screened for hepatitis B, and HBsAg-positive patients should be referred to a hepatologist for initiating antivirus therapy before starting temozolomide treatment. Moreover, HBV reactivation should be included in the differential diagnosis of patients with elevated LFTs.

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Table 1 Summary of three cases of hepatitis B virus (HBV) reactivation induced by temozolomide (TMZ)

Author (Year)	Age (yrs)/ Sex	HBV status	Diagnosis	Onset	Nadir			Steroid	Status of hepatitis
					WBC (/μl)	Neutrophil (/μl)	Lymphocyte (/μl)		
Grewal et al. (2007)[2]	65/F	remote history of HBV infection, not determined by laboratory test	GBM	day 27 of cycle 3	N/A	1250	450	stop 1 week after operation	died of hepatitis 2 weeks after onset
Chheda et al. (2007)[11]	50/M	HBsAg (+)	GBM	5 weeks after completion of TMZ concomitant with RT	5300	3890	580	dexamethasone 4 mg 5 weeks before onset and 2 mg at onset	successfully treated with lamivudine over the next 7 weeks
Present case	61/M	HBsAg (+)	GBM	4 weeks after completion of TMZ concomitant with RT	2900	2240	190	stop 3 days after operation	successfully treated with entecavir over the next 2 weeks

F: female, GBM: glioblastoma, HBsAg: hepatitis B surface antigen, M: male, N/A: not available, RT: radiation therapy, WBC: white blood cell.

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JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Phase I Trial of a Personalized Peptide Vaccine for Patients Positive for Human Leukocyte Antigen–A24 With Recurrent or Progressive Glioblastoma Multiforme

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ABSTRACT

Purpose
Personalized selection of suitable peptides for patients could offer a novel approach to developing cancer vaccines that boost anticancer immunity. We present the results of a phase I trial of 14 kinds of vaccine candidates (ITK-1) in patients with recurrent or progressive glioblastoma multiforme (GBM).

Patients and Methods
From January 2006 to January 2008, 12 patients from eight Japanese hospitals who were positive for human leukocyte antigen–A24, including 10 patients refractory to temozolomide (TMZ), were enrolled. The dose escalation trial included three dose groups (1, 3, and 5 mg) to determine the safety and tolerability of ITK-1 peptides. Immunologic response was monitored by measuring levels of cytotoxic T-lymphocyte precursors and peptide-specific immunoglobulin G. In another ITK-1 phase I trial for advanced prostate cancer, the vaccination schedule was skipped or discontinued in all three patients receiving the highest dose (5 mg/peptide) because of injection site reactions. This trial was therefore ended without enrollment for the highest dose, and data were analyzed by intention to treat.

Results
No serious adverse drug reactions were encountered, and treatment was well tolerated. The vaccine induced dose-dependent immune boosting. The recommended dose of ITK-1 peptides is 3 mg/peptide.

Conclusion
Personalized vaccination with ITK-1 peptides could be recommended in further stages of clinical trials. The safety and increased frequency of immune boosting offers potential clinical benefits in cases of recurrent or progressive GBM, even in TMZ-refractory settings.

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INTRODUCTION

Glioblastomas are characterized by aggressive growth and diffuse infiltration of neighboring brain structures. Representing the largest group of primary CNS tumors, glioblastomas generally show poor prognosis.[1] The latest information in the Surveillance, Epidemiology and End Results (SEER) Program has demonstrated poor survival rates in patients with glioblastoma, with a rising incidence and no improvement in the overall survival (OS) rate over the last two decades.[2] The standard initial treatment for this tumor is surgical resection and postoperative adjuvant therapies, including temozolomide (TMZ) concomitant with 60 Gy of radiation therapy (RT), according to Stupp et al.[3] Regardless of initial treatment, however, most patients relapse and cannot be cured.[4,5]

Personalized selection of peptides suitable for each patient could represent a novel approach to cancer vaccinations to boost antitumor immunity in a majority of patients. We have previously shown the feasibility of vaccination with personalized peptides for advanced glioblastoma multiforme (GBM) patients in a translational clinical study in which peptides were selected by pre-existing cellular and humoral responses to candidate vaccine peptides.[6] In this study, we conducted a phase I trial of personalized vaccination with peptides selected from 14 kinds of human leukocyte antigen (HLA) -A24–restricted peptide candidates based on pre-existing humoral immunity in patients with recurrent or progressive GBM.

Table 1. Peptides and Sequences

Peptide	Sequence	Antigen Name	Amino Acid Position	Reference
EGFR-800	DYVREHKDNI	EGF-R	800-809	Shomura et al[13]
EZH2-735	KYVGIEREM	EZH2	735-743	Ogata et al [12]
Lck-208	HYTNASDGL	$p56^{lck}$	208-216	Harashima et al [8]
Lck-486	TFDYLRSVL		486-494	
Lck-488	DYLRSVLEDF		488-497	
MRP3-503	LYAWEPSFL	MRP3	503-511	Yamada et al [14]
MRP3-1293	NYSVRYRPGL		1293-1302	
PAP-213	LYCESVHNF	PAP	213-221	Inoue et al [9]
PSA-248	HYRKWIKDTI	PSA	248-257	Harada et al [7]
PSMA-624	TYSVSFDSL	PSMA	624-632	Kobayashi et al [10]
PTH-rP-102	RYLTQETNKV	PTH-rP	102-111	Yao et al [16]
SART2-93	DYSARWNEI	SART2	93-101	Nakao et al [11]
SART2-161	AYDFLYNYL		161-169	
SART3-109	VYDYNCHVDL	SART3	109-118	Yang et al [15]

PATIENTS AND METHODS

Patients

Patients were eligible for enrollment if they had recurrent or progressive supratentorial GBM that had been diagnosed histologically and had proven resistant to conventional RT and/or chemotherapies, including TMZ. Patients were required to show positive humoral responses to at least four of 14 HLA-A24–restricted candidate peptides (ITK-1), determined by titers of immunoglobulin G (IgG) against each peptide.[6] Other inclusion criteria included age between 18 and 75 years; Eastern Cooperative Oncology Group performance status 0 to 2 or 3 (only in cases with neurologic symptoms caused

Table 2. Patients' Baseline Characteristics

Characteristic	Level 1 Group (1 mg/peptide) (n = 6)		Level 2 Group (3 mg/peptide) (n = 6)		*P*
	No.	%	No.	%	
Age, years					1.000 N/S
Median	61		62		
Range	40-74		28-69		
Sex					1.000 N/S
Male	5		4		
Female	1		2		
Initiation of vaccination from recurrence or progression, days					1.000 N/S
Median	32		34		
Range	15-69		13-72		
RECIST tumor size, mm					
Median	21		42		0.078 N/S
Range	11-38		20-89		
ECOG performance status					
0	1	16.7	1	16.7	0.654 N/S
1	4	66.7	3	50.0	
2	1	16.7	1	16.7	
3	0	0.0	1	16.7	
4	0	0.0	0	0.0	
KPS					
≤ 80	2	33.3	4	66.7	0.351 N/S
90	3	50.0	1	16.7	
100	1	16.7	1	16.7	
Steroid use (systemic)					
Yes	1	16.7	4	66.7	0.242 N/S
Systemic disease					
Diabetes	1	16.7	0	0.0	1.000 N/S
Hypertension	1	16.7	3	50.0	0.545 N/S
Ischemic heart disease	0	0.0	0	0.0	

Abbreviations: N/S, not significant; RECIST, Response Evaluation Criteria in Solid Tumors; ECOG, Eastern Cooperative Oncology Group; KPS, Karnofsky performance score.

by tumors); positive for HLA-A24; life expectancy ≥ 12 weeks; no chemotherapy or immunotherapy in the previous 4 weeks; presence of some residual diseases detectable by magnetic resonance imaging; and normal hematologic, hepatic, and renal functions. Exclusion criteria included serious pulmonary, cardiac, or other systemic disease; acute infection; history of autoimmune disease; or any other cancer from which the patient had been disease-free for < 5 years.

All study protocols were approved by the institutional review board at each participating hospital. After full explanation of the protocol, written informed consent was obtained from all patients before enrollment.

Peptides

We isolated and identified more than 100 genes encoding cancer antigens.[7-16] These antigens were derived from human nonmutated proteins that participate in the proliferation of cells and were frequently expressed at high levels in cancer cells but were either not expressed or expressed at low levels in normal cells. ITK-1, consisting of 14 peptides (Table 1), was selected on the basis of the results of both basic and translational clinical research by the Immunology Group at Kurume University School of Medicine.[6-21] Concrete criteria for peptides were as follows: (1) peptides that caused no serious adverse drug reactions in previous translational clinical studies, (2) peptides capable of

Table 3. Immune Responses and Clinical Responses

Patient No.	Dose Level	Prior Therapy	Vaccinated Peptides	CTL Response*			IgG Response†			No. of Vaccinations	Best Response		OS (days)
				Prevaccination	4th	6th	Prevaccination	4th	6th		WHO	RECIST	
1	1	RT/TMZ	Lck-488	0	0	0	21	30	23	12	NC	SD	379
			MRP3-1293	0	0	0	104	125	544				
			PTH-rP-102	0	0	0	118	132	146				
			SART3-109	0	0	0	225	248	270				
2	1	RT/ACNU	Lck-486	0	0	0	75	71	79	6	PD	PD	357
			Lck-488	0	0	0	21	20	22				
			MRP3-1293	0	0	0	22	24	23				
			SART3-109	0	0	0	71	62	71				
3	1	(1) RT/ACNU/VCR	Lck-486	0	0	0	692	251	218	10	NC	SD	159
		(2) Repeat surgery/	MRP3-1293	0	70	136	63	45	44				
		TMZ	SART2-93	0	99/206/494	0	14	9	9				
			SART3-109	0	143/1,257	93/121/172	806	575	1107				
4	1	RT/TMZ	Lck-486	164	938	52	77	72	130	24	PR	SD	509
			Lck-488	0	0	176/2,156/3,274/4,232	22	19	23				
			SART2-161	0	195	258/758/2,348	39	38	37				
			SART3-109	0	94/137	70/173/419	28	23	29				
5	1	(1) RT/ACNU/IFN-β	Lck-486	231	0	0	460	470	480	6	PD	PD	197
		(2) Repeat surgery	Lck-488	0	0	3,113	106	114	187				
			PTH-rP-102	0	146	0	627	546	752				
			SART2-93	0	0	1,520	1028	1134	1865				
6	1	(1) RT/ACNU/IFN-β	Lck-486	0	369	0	335	317	400	6	NC	PD	243
		(2) Repeat surgery/	Lck-488	0	0	1,462/1,782/1,946	37	46	73				
		TMZ	MRP3-1293	626/1948	0	0	53	69	66				
			SART3-109	0	0	537/643	36	53	62				
7	2	(1) RT/ACNU/VCR	Lck-486	0	0	0	163	155	122	6	PD	SD	248
		(2) TMZ	MRP3-1293	0	582	342/619	25	21	20				
			PTH-rP-102	85	278	5,060	19	15	9				
			SART3-109	0	72	0	37	36	35				
8	2	RT/TMZ	Lck-486	606	158	1,266/1,738/2,130	89	92	108	12	NC	SD	362
			MRP3-1293	119	240	0	7	7	9				
			SART2-93	758	145	926/1,896	7	7	6.7				
			SART2-161	241	2,139/6,094	1,110/2,756	52	65	61				
9	2	RT/TMZ	Lck-486	0	0	0	463	494	476	79	PR	PR	844
			MRP3-503	0	0	0	43	33	31				
			MRP3-1293	0	0	0	46	40	36				
			SART2-161	0	0	0	42	41	34				
10	2	RT/TMZ	Lck-486	0	0	156/371/375/8,852	212	213	291	6	PD	PD	67
			Lck-488	0	606	393/1,392	35	40	56				
			SART2-93	159	354/1,112/1,526	276	50	57	72				
			SART3-109	0	112/999	0	73	90	687				
11	2	(1) RT/TMZ	Lck-486	0	0	120/212	203	159	159	6	PD	SD	287
		(2) Repeat surgery	MRP3-503	378	0	50	126	149	128				
			SART2-93	0	0	0	87	91	84				
			SART2-161	0	0	0	131	155	124				
12	2	RT/TMZ	Lck-486	0	0	78	128	150	139	15	NC	SD	384
			MRP3-503	0	716	671/4,004/10,806	124	183	250				
			SART2-93	0	0	0	58	84	108				
			SART2-161	0	0	150	121	174	216				

Abbreviations: CTL, cytotoxic T lymphocyte; IgG, immunoglobulin G; RECIST, Response Evaluation Criteria in Solid Tumors; OS, overall survival; RT, radiation therapy; TMZ, temozolomide; NC, no change; SD, stable disease; ACNU, nimustine hydrochloride; PD, progressive disease; VCR, vincristine; PR, partial response; IFN-β, interferon-β.

*Values indicate the interferon-γ production of peripheral blood mononuclear cells reactive to the corresponding peptide. (pg/mL).

†Values indicate fluorescence intensity unit of IgG antibodies reactive to the corresponding peptide.

inducing peptide-specific cellular immunity and specific humoral immunity at high frequencies in HLA-A24–positive patients with glioblastoma or other types of cancer, and (3) peptides administered to clinically responsive patients in previous translational clinical studies.

These 14 peptides were manufactured under compliance with good manufacturing practice by American Peptide Company (San Diego, CA) or PolyPeptide Laboratories (San Diego, CA). Peptides were supplied by Green Peptide (Fukuoka, Japan) in the form of freeze-dried powder (4 mg of each peptide per vial) as the investigational product ITK-1. For injection, 1, 3, or 5 mg of peptide (levels 1, 2, or 3, respectively) dissolved in water was mixed and emulsified with incomplete Freund's adjuvant (Montanide ISA51; Seppic, Paris, France) in volumes of 0.5, 1.5, and 2.5 mL, respectively. Table 1 provides information on the peptides used, including sequences, parent proteins, and references[7-16] All peptides have been reported to induce tumor-specific cytotoxic T lymphocyte (CTL) activity in an HLA class I–restricted manner in peripheral blood mononuclear cells (PBMCs) and peptide-specific IgG. According to our previous studies,[6-16] GBM cells express all of the parent proteins that encode the peptides for use in this study, except prostate acid phosphatase, prostate-specific antigen, and prostate-specific membrane antigen, which were primarily prepared from patients with prostate cancer. Indeed, none of these three proteins were injected into GBM patients in this study.

Study Design and Treatment

We enrolled 12 patients at eight hospitals between January 2006 and January 2008 in this multicenter, open-label, dose-escalation, phase I clinical study. For the assessment of patient eligibility, plasma was evaluated for titers of IgG specific to each of the 14 ITK-1 peptides before vaccination, and the peptides (up to four peptides) showing the highest IgG titers were selected for vaccination in each patient as described in previous reports.[17-21] Patients were scheduled to receive peptides weekly for a total of six times by subcutaneous injection into the upper back region at the three different dose settings (1, 3, and 5 mg/peptide). Dose escalation was allowed after evaluation of safety by the Data and Safety Monitoring Committee (DSMC). Each cycle of vaccination consisted of six consecutive injections, with the second and later cycles of vaccinations conducted after obtaining written consent from patients, if no serious adverse events or disease progression was encountered. After the last injection of every cycle, plasma was re-evaluated for IgG titers against each peptide, and the peptides (up to four peptides) with the highest IgG titers were selected again for the next cycle of vaccination. Patients who showed radiographic evidence of tumor progression or met other withdrawal criteria were excluded from the study.

Cellular and Humoral Responses to Peptides

Peptide-specific CTL precursors in PBMCs and peptide-specific IgG titers in plasma were measured according to previously reported methods[22,23] as cellular and humoral immune responses. The details are provided in the Appendix (online only).

Patient Monitoring and Toxicity Criteria

We used Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 to grade toxicity. Patients were monitored by general examinations and blood testing. Magnetic resonance images were obtained after every cycle. Radiologic responses to treatment or relapses were evaluated by each investigator at the treating institution according to WHO and Response Evaluation Criteria in Solid Tumors (RECIST) criteria. Pathologic diagnosis was initially determined and classified according to WHO criteria at the treating institution, and a subsequent central review of pathology was performed.

Assessment and Statistical Considerations

The primary end points were safety and tolerability, and secondary end points were maximum-acceptable dose (MAD), maximum-tolerated dose (MTD), and immune responses. MAD was defined as the lowest dose at which at least two thirds of patients experienced grade ≥ 2 dermal inflammation after six injections. MTD was defined as the lowest dose at which more than one third of patients experienced a grade ≥ 3 systemic adverse drug reaction. Logistic regression models were used to predict the probability of MAD and MTD.

We used the Kaplan-Meier method to estimate the distribution of progression-free survival (PFS) and OS rates. PFS was calculated from the study entry date to the date of radiographic disease progression by using either WHO or RECIST criteria. OS was calculated from the study entry date to the date of last follow-up or the date of death from any cause. The cutoff date for this analysis was August 6, 2009. Intention-to-treat statistical analysis was performed by using SAS version 9.1.3 software (SAS Institute, Cary, NC).

RESULTS

Demographics

A multicenter, open-label, dose-escalation design was planned for level 1 (1 mg/peptide), level 2 (3 mg/peptide), and level 3 (5 mg/peptide) in this trial. Doses were escalated independently for each stratum. The DSMC evaluated efficacy and safety according to the protocol schedule. Of note, this study was ended without enrollment of patients to level 3 (5 mg/peptide) on the basis of the recommendations of DSMC, which reviewed the results of another parallel ITK-1 phase I trial for advanced prostate cancer (report in progress), in which vaccine injections at level 3 (5 mg/peptide) were skipped or discontinued in all three patients because of injection site reactions.

Table 2 summarizes patient demographics and tumor characteristics. Prior therapies before enrollment are listed in Table 3. All patients received RT, and 11 underwent maximal surgical resection. Seven patients received standard RT therapy plus concomitant and adjuvant TMZ. The remaining five patients were treated with nimustine hydrochloride–based therapy synchronized to RT as the initial therapy, and three of these patients received TMZ at progression. Six patients were enrolled in this study at first recurrence, while the remainder were enrolled at second recurrence.

Patient Clinical Courses and Immune Responses

During the study period, enrolled patients received no treatments other than vaccinations. All patients completed the first cycle of six injections. Three of the six patients at levels 1 and 2 were subjected to further cycles of vaccination. According to the WHO criteria, among the 12 patients, the best response was reported as partial response (PR) in two patients, no change (NC) in five, and progressive disease in five. According to RECIST criteria, one, seven, and four patients displayed PR, stable disease (SD), and progressive disease,

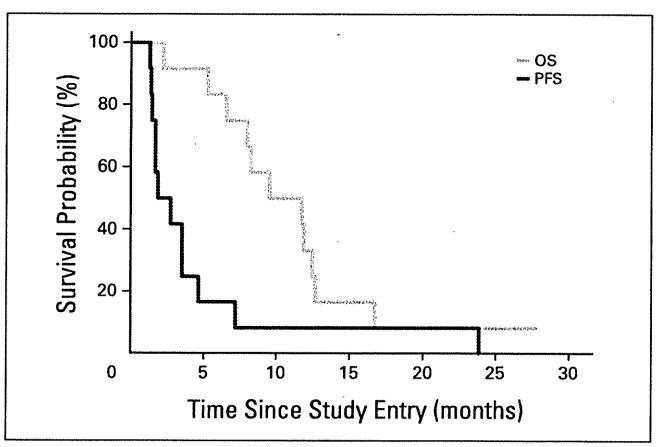

Fig 1. Progression-free survival (PFS) versus overall survival (OS) from the study entry date for glioblastoma multiforme.

respectively (Table 3). OS for each patient is given in Table 3. The two patients with PR displayed a mean PFS of 15 months. In all patients, median PFS was 2.3 months (95% CI, 1.7 to 3.5 months), and PFS rate at 6 months was 16.7% (95% CI, 2.7% to 41.3%), comparable to the historical data (8% to 21%).[5] At the time of writing, all patients have shown disease progression with 11 deaths, and most patients show disseminated disease. At 6 months as measured from the study entry date, median OS was 10.6 months (95% CI, 8.0 to 12.5 months) and OS rate was 83.3% (95% CI, 48.2% to 95.6%; Fig 1). Median OS from the date of diagnosis was 18.9 months.

Peptide-specific CTL responses were augmented for at least one vaccinated peptide after the sixth vaccination of the first cycle in four of six patients at level 1 and in five of six patients at level 2. Peptide-specific IgG responses were augmented in one of six patients in both levels 1 and 2. Immune responses showed a slight increase after the sixth vaccination (Table 3).

Representative antigen-specific immune responses in the six patients that received more than six vaccinations (patients 1, 3, 4, 8, 9, and 12) are given in Figure 2 and Data Supplement. CTL responses to two of four peptides and IgG responses to two of four peptides were augmented after the 12th vaccination in patient 1 with NC (Data Supplement). Similarly, CTL responses to two peptides and IgG responses to one peptide were augmented after the 10th vaccination in patient 3 with NC (Data Supplement). Boosted CTL responses to three peptides and IgG responses to two peptides were observed in patient 4 with PR after the 12th vaccination and continued up to the 24th vaccination (Fig 2). CTL responses to one peptide and IgG responses to two peptides were augmented after the 12th vaccination in patient 8 with NC (Data Supplement). In patient 9 with PR, who was continued up to 79th vaccination, no CTL or IgG responses were observed until the 12th vaccination, but both CTL responses to three peptides and IgG responses to four peptides became boosted after the

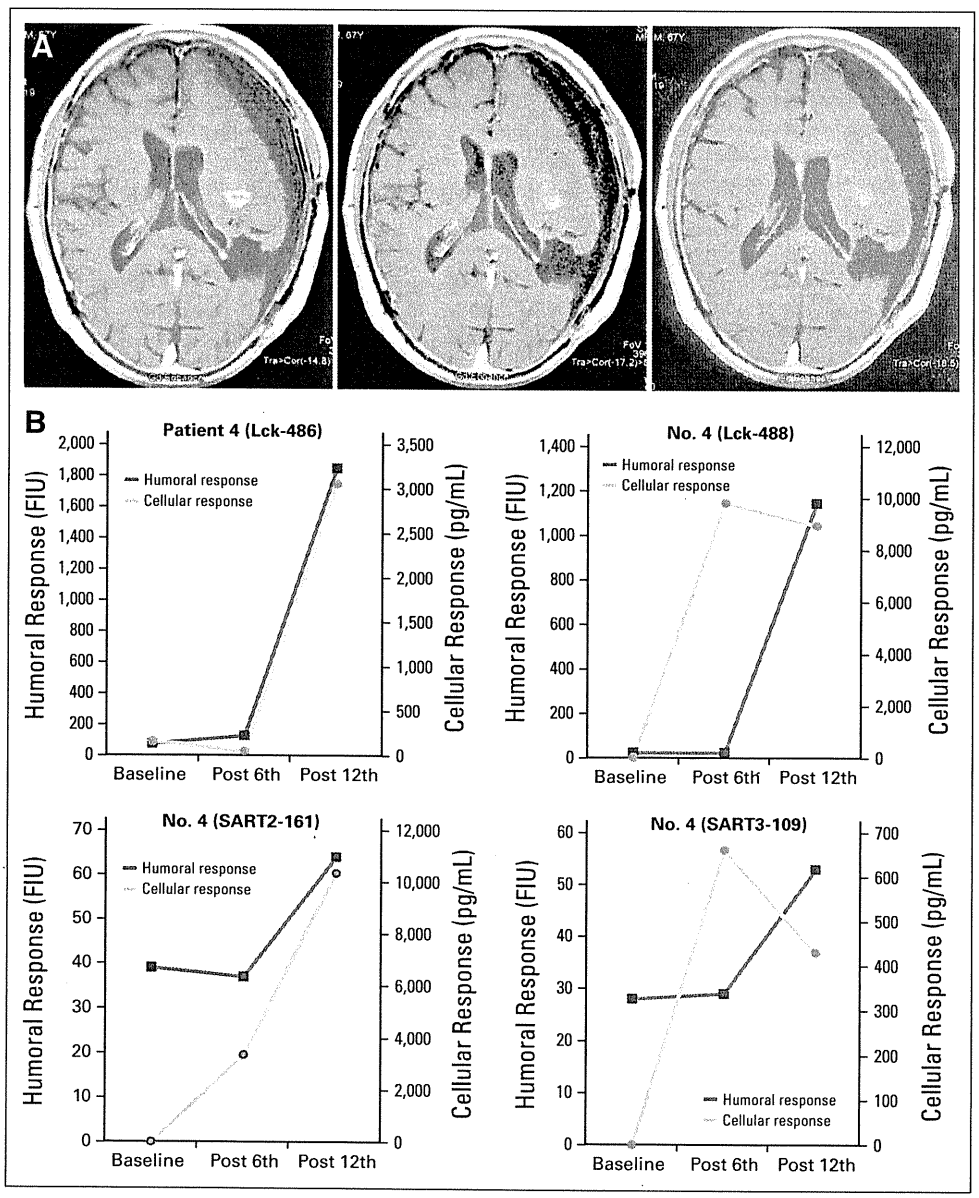

Fig 2. (A) T1-weighted magnetic resonance imaging of a representative radiologic response to ITK-1 in a patient (patient 4) with recurrent glioblastoma multiforme who achieved partial response. (B) Peptide-specific immune responses to vaccinated ITK-1 peptides. FIU, fluorescence intensity unit.

Table 4. Adverse Drug Reactions for Glioblastoma

CTCAE Category and Symptom	Grade for Level 1 Group (1 mg/peptide) (n = 6)						Grade for Level 2 Group (3 mg/peptide) (n = 6)						Total for All Grades (N = 12)	
	1		2		3		1		2		3			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Blood/bone marrow							1	16.7	1	16.7			2	16.7
Eosinophil count increased							1	16.7					1	8.3
Lymphopenia									1	16.7			1	8.3
Dermatology/skin	3	50.0	2	33.3	1	16.7	3	50.0	4	66.7			12	100.0
Eczema asteatotic									1	16.7			1	8.3
Pruritus									1	16.7			1	8.3
Injection site reaction	3	50.0	2	33.3	1	16.7	3	50.0	3	50.0			12	100.0
Lymphatics	1	16.7											1	8.3
Limb edema	1	16.7											1	8.3
Neurology							1	16.7					1	8.3
Sensory neuropathy							1	16.7					1	8.3

Abbreviation: CTCAE, Common Terminology Criteria for Adverse Events, version 3.0.

12th vaccination throughout the study (Data Supplement). In patient 12 with NC, CTL responses to three peptides were augmented after the 12th vaccination and no IgG responses were observed (Data Supplement). Collectively, both CTL and IgG responses to at least one peptide were augmented in samples after the 12th vaccination in most cases showing favorable clinical response.

Toxicities

Treatment was relatively well tolerated. Grade 1, 2, or 3 skin inflammatory reactions at injection sites occurred in all patients at both level 1 and level 2. On the basis of the logistic regression model, the MAD of ITK-1 peptides was 3.863 mg/peptide. All adverse drug reactions observed in the whole study were grade $\leq$ 3 and were considered acceptable (Table 4).

DISCUSSION

We have developed a novel strategy for personalized peptide vaccination in which the peptides most suitable for each patient are selected on the basis of cellular and/or humoral immune responses to candidate peptides before vaccination. Using this approach, we reported the phase I trial[6] of a personalized peptide vaccine for patients with high-grade glioma. Cellular and humoral immune reactions were examined against 25 HLA-A24–restricted or 23 HLA-A2–restricted peptides in PBMCs and plasma from patients before vaccination. Then the peptides showing the strongest reactions (up to four peptides) were selected as personalized vaccines. Among the 25 patients enrolled, no toxicity was reported, with a median PFS of 3 months and a PFS rate of 13% at 6 months.[6] Although the results from our previous trial seemed quite promising, the excessive complexity resulting from large numbers of peptide candidates posed a major hurdle to further clinical trials and future commercialization. To facilitate the development of personalized vaccination, we therefore selected 14 ITK-1 peptides that had been more immunogenic in patients with advanced GBM or prostate cancer in our previous translational clinical studies.[6,17-21]

This study represents, to the best of our knowledge, the first clinical report of personalized immunotherapy with ITK-1 peptide in patients who were 12 HLA-A24 positive with GBM, including 10 patients who were resistant to TMZ treatment. In this phase I trial, which was restricted to adults with recurrent or progressive GBM, no serious systemic adverse drug reactions (above grade 3) were observed, and MTD was therefore not estimated. All patients showed grade 1 to 3 skin reactions associated with the injection site of the study drug. On the basis of the results of logistic regression modeling, we calculated the MAD of ITK-1 peptides as 3.863 mg/peptide, and therefore concluded that 3 mg/peptide is a safe dosage for adults with GBM. Although the results cannot be considered conclusive because of the small number of patients in this early-phase trial, we found a clinically meaningful 6-month PFS rate of 16.7% with less toxicity and prolonged OS, consistent with the findings of a previous clinical study.[6] Considering the advanced disease status of enrolled patients, all of whom were already resistant to conventional treatments before enrollment, our results seem quite encouraging. Although most patients had SD (58.3%), this is not surprising because conventional short-term tumor responses evaluated by shrinkage of established tumor masses are not always an appropriate surrogate in cancer vaccines. Cancer vaccines often need more time to elicit beneficial immune responses that demonstrate biologic activities because of delayed vaccine effects. In fact, our patients with SD exhibited a relatively long duration of disease stabilization and survived with a median OS of 10.6 months.

The most unique aspect of this study was the personalized selection of ideal antigen peptides for individual patients on the basis of consideration of pre-existing host immunity before vaccination. In view of the heterogeneity of tumors and the complexity and diversity of immune responses, we thought that this approach would be more rational than selecting nonpersonalized universal tumor antigens. On the basis of the current paradigm that the adaptive immune system is of limited size and composition, with individual cells constantly competing against each other, inconvenient immune responses induced by nonpersonalized antigens that are either nonspecific to tumor cells or ineffective for tumor cell killing may suppress pre-existing beneficial immunity, accelerating cancer progression or early death of vaccinated patients. For example, the failure of some recent clinical trials

may be explained, at least in part, by the induction of such inconvenient immune responses induced by nonpersonalized antigens.

This study measured pre-existing antigen-specific IgG responses, but not T-cell responses, for personalized selection of antigen peptides from a panel of candidate antigens, because antigen-specific T-cell assays often showed limited sensitivity and reproducibility due to quite low frequencies of antigen-specific T cells, even after vaccinations.[22,24-26] Although antigen-specific T cells can be expanded by in vitro culture with specific antigens, cells expanded in vitro do not necessarily provide a better picture of the T-cell activity present in vivo.[22] In contrast, the multiplex bead–based LUMINEX (Luminex, Austin, TX) technology that we developed specifically for monitoring B-cell responses allows high-throughput screening of IgG responses specific to large numbers of peptide antigens with high accuracy.[23] Although T-cell assays have been more widely used than B-cell assays for immune monitoring in vaccinated patients, we have already confirmed the clinical significance of antigen-specific IgG antibody titers as surrogate biomarkers in selecting adequate peptides for personalized vaccination and monitoring vaccine-induced immune responses.[27,28] For example, our recently conducted randomized trials[29] of personalized peptide vaccinations for advanced prostate cancers in consideration of pre-existing B-cell responses to vaccine candidates in each patient resulted in clear benefits to patients. Of course, we believe that cellular immune responses might represent an important marker if appropriate assay conditions are defined and used. More sophisticated T-cell assays remain to be developed for the further evolution of cancer vaccination.

Although the mechanisms by which peptide-specific antibody responses are associated with antitumor immunity mediated by major histocompatibility complex class I restricted CTL remain to be clarified, our previous clinical results have suggested that CD45RO-positive T cells were mainly induced into tumor sites at the initial vaccination stages by personalized peptide vaccination.[30] These results suggest that this personalized regimen can activate not only $CD8^+$ memory CTLs but also $CD4^+$ memory T-helper cells, which in turn participate in the elimination of cancer cells. Further studies are currently in progress, including clarification of the biologic functions of peptide-specific antibodies.

In conclusion, personalized vaccination with ITK-1 peptide in patients who are HLA-A24 positive with GBMs could be recommended for further stages of clinical trials because of its safety and high immune-boosting effects that offer potential clinical benefits.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: Akira Yamada, Green Peptide (C) **Consultant or Advisory Role:** Tomokazu Aoki, Schering-Plough (C); Kyogo Itoh, Green Peptide (U) **Stock Ownership:** Akira Yamada, Green Peptide; Kyogo Itoh, Green Peptide **Honoraria:** None **Research Funding:** Kyogo Itoh, Green Peptide **Expert Testimony:** None **Other Remuneration:** None

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Manuscript writing: Mizuhiko Terasaki
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Appendix

Cellular and Humoral Responses to Peptides

Thirty milliliters of peripheral blood was obtained before the first vaccine injection, on the fourth injection of the first cycle, and after the last injection of every cycle for measurement of peptide-specific cytotoxic T lymphocyte (CTL) precursors in peripheral-blood mononuclear cells and peptide-specific immunoglobulin G (IgG) titers in plasma according to previously reported methods.[23,27] In brief, for the CTL assay, peripheral-blood mononuclear cells (1×10^5 cells/well in 96-well round-bottom plates; Nunc, Roskilde, Denmark) were cultured in vitro in 200 μL of culture medium containing a peptide (10 μmol/L) and interleukin-2 (20 U/mL; Serotec, Oxford, United Kingdom) in quadruplicate. Half the culture medium was removed and replaced with new medium containing the corresponding peptide (20 μmol/L) and interleukin-2 every 3 days. After culture for 14 days, cells were harvested and restimulated for 18 hours with the human leukocyte antigen (HLA) -A2402–transfected C1R cells preloaded with either the corresponding peptide or with an HLA-A24–restricted HIV-derived peptide (RYLRQQLLGI) as a negative control to test their ability to produce IFN-γ in response to specific antigens. Amounts of IFN-γ in culture supernatant were determined by enzyme-linked immunosorbent assay in quadruplicate (limit of sensitivity, 10 pg/mL), and a two-tailed t test was used for statistical analysis. A well was considered positive when the amount of IFN-γ (averages of quadruplicate determinants per well) in response to a specific peptide was significantly higher ($P < .05$ and difference $\geq$ 50 pg/mL) than that in response to the HIV control peptide. Otherwise ($P \geq .05$ and/or difference $<$ 50 pg/mL), a well was considered negative (0). Antigen-specific CTL precursors in PBMCs were considered to be increased by vaccines when the amount of IFN-γ after vaccination in response to at least one of the vaccinated peptides was at least threefold higher than the level before vaccination, or when it changed from negative to positive.

The peptide-specific IgG titers in plasma were measured by using bead-based multiplexing technology (Luminex; Luminex, Austin, TX), and results were shown as fluorescence intensity units (FIUs), as reported previously.[27] In brief, plasma (100-fold dilution) was incubated with peptide-coupled color-coded beads for 2 hours at room temperature on a plate shaker. The mixture was then washed with a vacuum manifold apparatus and incubated with 100 μL of biotinylated goat antihuman IgG (gamma chain–specific) for 1 hour at room temperature on a plate shaker. The plate was then washed, and 100 μL of streptavidin-phycoerythrin was added to wells, followed by incubation for 30 minutes at room temperature on a plate shaker. Bound beads were washed three times, followed by addition of 100 μL of Tween phosphate-buffered saline before measurement. The limit of sensitivity for this assay was 5 FIUs. IgG titers in plasma were considered to be elevated by vaccines when FIU values in postvaccination plasma were threefold higher than in prevaccination plasma.

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ORIGINAL ARTICLE

Molecular and clinical analysis of glioblastoma with an oligodendroglial component (GBMO)

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Abstract The genetic and clinical features of glioblastoma with an oligodendroglial component (GBMO), pathologically defined as anaplastic oligo-astrocytoma with necrosis, remain unclear. We investigated the correlation between genetic alterations and clinical outcomes in 19 GBMO patients we have encountered since 1997. Using single nucleotide polymorphism oligonucleotide genomic (SNP) microarrays, we analyzed gene amplification, loss of heterozygosity (LOH), and homozygous deletions in their whole genome. We also analyzed their overall survival (OS). Pathological studies revealed the presence of calcification in 11 and of a cyst in 9 of the 19 patients. Whole-genome analysis using SNP microarrays revealed LOH of chromosome 10 in 11, EGFR amplification in 8, 9p21 (INK4 locus) deletion in 12, PDGFR amplification in 2, and LOH of 1p19q in 2 patients. Median OS was 14 months (average 22.8 months). The pattern of genetic alterations was similar in GBMO and glioblastoma multiforme (GBM) patients, and the clinical outcomes were similar in GBMO and GBM patients.

Keywords Glioblastoma with oligodendroglial components · SNP microarray · Survival

Introduction

Glioblastoma multiforme (GBM) is the most malignant brain tumor in adults; it accounts for 12–15% of all intracranial tumors. Despite multimodal treatments such as surgery, radiotherapy, and chemotherapy, the survival rates of GBM patients remain extremely poor [1]. On the other hand, some of these patients experience long-term survival, possibly because the biological properties of their tumor cells are different from the aggressive type of tumor cells seen in patients with shorter survival. A small subgroup of GBM contains areas with histological features of oligodendroglial components; these cases have been reported to manifest prolonged survival [2–4]. The 2007 WHO classification defines anaplastic oligoastrocytoma with necrosis as glioblastoma with oligodendroglial components (GBMO) [5]. However, as there are currently no definite diagnostic criteria, the clinical outcome of GBMO remains controversial. In gliomas many genetic alterations have been reported; in the induction of GBM, three critical signaling pathways are thought to be important [6]. One is a signaling pathway for several growth factors necessary for proliferation, another is a G1 checkpoint, and the third is a p53 pathway. In gliomas, alterations in genes on these three pathways have been documented, and although genetic analyses of GBMO have been reported [7, 8], the specific genetic changes have not been identified. The aim of our study was to characterize GBMO genetically and to investigate clinical outcomes in patients with GBMO.

Materials and methods

We performed pathological studies to evaluate GBM patients treated at Kumamoto University hospital since 1997 retrospectively in an effort to determine the presence of oligodendroglial components in their tumors. The clinical characteristics of 19 GBMO patients are presented in Table 1. Tumor and matched blood samples were collected

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