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Phase I/IIa trial of autologous formalin-fixed tumor vaccine concomitant with fractionated radiotherapy for newly diagnosed glioblastoma

Clinical article

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Object. The objective of the present study was analysis of results of the prospective clinical trial directed toward the evaluation of therapeutic efficacy of the administration of autologous formalin-fixed tumor vaccine (AFTV) concomitant with fractionated radiotherapy in cases of newly diagnosed glioblastoma multiforme.

Methods. Twenty-four patients were enrolled into the clinical trial, while 2 cases were excluded from the final analysis of results. The treatment protocol included aggressive tumor resection, fractionated radiotherapy up to a total dose of 60 Gy, and 3 concomitant courses of AFTV administered with an interval of one week at the late stage of irradiation. Two delayed-type hypersensitivity (DTH) tests were done—one 48 hours before the initial course of vaccination (DTH-1) and one 2 weeks after the third (DTH-2). All but one of the patients received salvage therapy at the time of tumor progression. The defined primary end point was overall survival; secondary end points were progression-free survival and safety of concomitant treatment.

Results. The median duration of overall survival was 21.4 months (95% CI 13.8–31.3 months). The actuarial 2-year survival rate was 40%. The median duration of progression-free survival was 7.6 months (95% CI 4.3–13.6 months). Overall survival showed a statistically significant association with recursive partitioning analysis class ($p < 0.05$); progression-free survival showed a statistically significant association with p53 staining index ($p < 0.05$) and size of DTH-2 response ($p < 0.001$). AFTV injection concomitant with fractionated radiotherapy was well tolerated by all patients and in no case did treatment-related adverse effects exceed Grade 1 toxicity; adverse effects were limited to local erythema, induration, and swelling at the site of injection.

Conclusions. The results of this study demonstrate that AFTV treatment concomitant with fractionated radiotherapy may be effective in patients with newly diagnosed glioblastoma. Further clinical testing is warranted.
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Key Words • **brain tumor vaccine therapy** • **glioblastoma** • **autologous formalin-fixed tumor vaccine** • **oncology**

Abbreviations used in this paper: AFTV = autologous formalin-fixed tumor vaccine; CEA = carcinoembryonic antigen; DTH = delayed-type hypersensitivity; EGFR = epidermal growth factor receptor: GBM = glioblastoma multiforme; KPS = Karnofsky Performance Scale; MHC = major histocompatibility complex; MRC = Medical Research Council; OS = overall survival; PFS = progression-free survival; RPA = recursive partitioning analysis.

Despite recent advances in aggressive resection combined with radiochemotherapy, management of glioblastoma represents a significant clinical challenge. More than three-quarters of patients with this

This article contains some figures that are displayed in color online but in black and white in the print edition.

tumor die within 2 years after surgery and less than 10% survive for 5 years.[2,19,21,22] This dismal prognosis continues to stimulate the search for additional treatment options.

Recently, there has been a growing interest in therapeutic modalities based on tumor-specific immune reactions, which have a potentially high benefit-to-risk ratio. Preliminary clinical trials had revealed favorable results for immunotherapy of recurrent malignant glioma with ex vivo expanded autologous tumor-specific cytotoxic T lymphocytes.[25,26] The usefulness of this approach is, however, limited due to the necessity of propagating a large quantity of autologous tumor cells and time-consuming tedious culturing of the cytotoxic T lymphocytes; neither of these processes is always successful. An alternative approach was proposed by Ohno and colleagues, who developed use of formalin-fixed sections instead of live target tumor cells for ex vivo cytotoxic T lymphocyte induction[9,10] and later formulated the autologous formalin-fixed tumor vaccine (AFTV) for in vivo induction of killer lymphocytes.[13,14] A Phase II randomized clinical trial showed that AFTV prevents recurrence of hepatocellular carcinoma after surgery,[8] and another pilot study revealed its therapeutic effectiveness associated with absence of severe treatment-related complications in cases of recurrent and residual GBM.[6] Therefore, the present prospective clinical trial was initiated for evaluation of the therapeutic efficacy of AFTV concomitant with fractionated radiotherapy in patients with newly diagnosed GBM.

Methods

Study Design

A prospective clinical trial of AFTV concomitant with fractionated radiotherapy for management of newly diagnosed GBM was conducted by the Association of Cancer Vaccine Therapy in 2 participating hospitals, Tokyo Women's Medical University Hospital (Tokyo, Japan) and the Tsukuba University Hospital (Ibaragi, Japan). According to objectives focused on preliminary evaluation of the therapeutic efficacy and safety of treatment, the trial was designated as Phase I/IIa, and dose escalation was not planned. The study design and treatment protocol were approved by the ethics committees of both institutions and registered in the University Hospital Medical Information Network (UMIN) clinical trials registry (identification no. C000000002, Tokyo). Eligibility and exclusion criteria for patient enrollment are presented in Table 1. Written informed consent for participation in the study was obtained in each individual case. The 2-year study period started on August 10, 2005, and enrollment of 25 patients was planned. Only 24 patients, however, were actually enrolled. The trial was developed and initiated before temozolomide (Temodal, Schering-Plough) treatment (150–200 mg/m^2 daily for 5 days every 28 days) was approved by the Japanese government for malignant gliomas (September 15, 2006). When temozolomide treatment was approved, the concept of the study was reconsidered by the ethics committees of both participating institutions, and it was decided to continue the trial within the designated time period (up to August 9, 2007), with additional information on possible chemotherapy with temozolomide being provided for each patient before enrollment into the study.

TABLE 1: Eligibility and exclusion criteria*

Eligibility Criteria
age: 16–75 yrs
newly diagnosed GBM w/ histopathological confirmation of the diagnosis
manifestation of the disease w/ typical neurological symptoms
maximum possible resection of the tumor (radiologically complete removal or subtotal removal leaving the residual neoplasm w/in the vital, functionally important brain areas)
availability of at least 1.5 g of neoplastic tissue for AFTV preparation
possibility for in-house AFTV preparation & administration
completed course of postop FRT w/ a cumulative dose of 60 Gy
KPS score ≥60 before initiation of FRT
possibility of regular follow-up evaluation
Exclusion Criteria
treatment w/ glucocorticoids or antitumor chemotherapy
presence of intracranial hypertension at time of scheduled AFTV treatment
suppressed hematological function according to the Common Toxicity Criteria version 2 (National Cancer Institute) or absolute WBC count ≤2000/mm^3
decompensated function of internal organs
presence of malignant tumor other than GBM
planned or existing pregnancy
enrollment in another clinical trial w/in the 6 mos preceding the present study
ineligibility as judged by the principal investigator of the participating institution (for example, due to anticipation of problems w/ regular follow-up evaluations caused by distant address or socioeconomic issues)

* FRT = fractionated radiotherapy; WBC = white blood cell.

After confirmation of the histopathological diagnosis of GBM following resection of newly diagnosed parenchymal brain tumor, the eligible patients who provided their agreement to participate in the study were scheduled for fractionated radiotherapy and concomitant treatment with AFTV according to the standard protocol (Fig. 1). In each case the histopathological diagnosis was independently confirmed in the Japan Brain Tumor Reference Center (Y.N.) in Gunma University (Maebashi, Japan) according to the current WHO criteria, using paraffin-embedded tissue sections stained with H & E. Additional immunohistochemical analysis included evaluation of the positive cells using monoclonal antibodies for MIB-1 (Dako), p53 (Dako), and MHC Class I (Hokudo Co.). The corresponding staining indices were calculated as an average number of positive cells in the best-stained tumor areas (up to 5) with a total amount of cells not less than 1000. The MIB-1 and p53 indices were expressed as percentages, whereas MHC Class I expression was graded as 0 (absence of staining), + (up to 25% of cells stained), ++ (25%–50% of cells stained), or +++ (more than 50% of cells stained).[1,6]

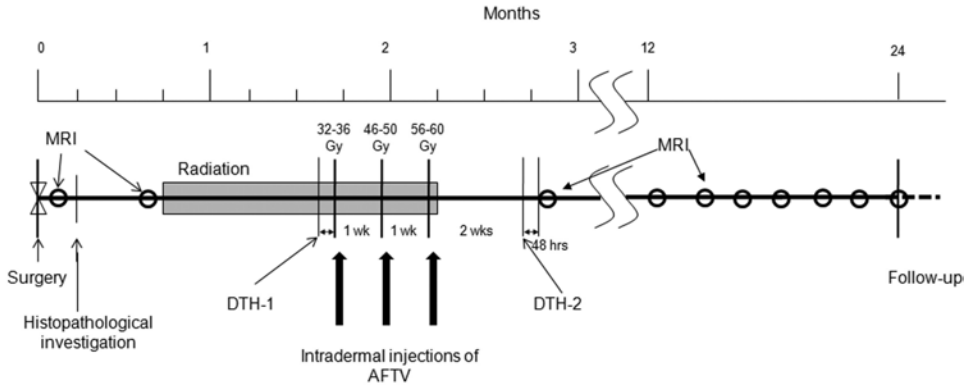

Fig. 1. Scheme of prospective clinical protocol for AFTV treatment in patients with newly diagnosed glioblastoma.

The baseline clinical investigations at the time of enrollment into the trial included physical examination with evaluation of KPS scores and determination of the MRC neurological functional grade, blood and urine tests, electrocardiogram, chest radiograph, and brain MR imaging obtained initially within 3 days after surgery and additionally just before the first fractionated radiotherapy session.

Preparation of AFTV

Autologous formalin-fixed tumor vaccine was prepared using autologous formalin-fixed GBM tissue according to an established standard operating procedure as described previously.[6] In short, the formalin-fixed histologically confirmed viable neoplastic tissue was initially thoroughly fragmented and centrifuged at 11,100 G for 5 minutes; 0.1 ml of alcohol extract prepared from 1.2 mg of freeze-dried Bacillus-Calmette Guérin (BCG) vaccine (Japan BCG Laboratory) was added to 0.22 ml of the packed tumor tissue pellet obtained after centrifugation; the pellet was washed with saline; and final concentration of the tissue fragments was adjusted to 20% (v/v, packed volume) suspended in 1 ml saline, which also contained 250 ng of tuberculin microparticles and 250 ng of soluble tuberculin (Japan BCG Laboratory).

Treatment Protocol

Fractionated radiotherapy was started within 2–3 weeks after resection of the neoplasm and included focal irradiation of the tumor cavity or residual lesion including 2 cm of perifocal margin with 2 Gy per fraction up to a total dose of 60 Gy. When the radiation dose reached 32–36 Gy the concomitant AFTV treatment was initiated. The AFTV treatment consisted of 3 courses of vaccination performed at intervals of 1 week.[6] Each course consisted of 5 local intradermal injections of 0.2 ml of AFTV per site in the upper arm.

Two delayed-type hypersensitivity (DTH) tests[6] were performed 48 hours before the first course of vaccination (DTH-1) and 2 weeks after the third course (DTH-2). For these tests, fixed autologous tissue fragments (10% v/v suspended in 0.1 ml of saline in the absence of immune adjuvant) were injected intradermally into the forearm, and response was evaluated by diameter of the local erythema and induration.

Clinical Characteristics of the Enrolled Patients

From August 10, 2005, to August 7, 2007, 24 patients with newly diagnosed GBM were enrolled into this prospective clinical trial (19 in Tokyo Women's Medical University Hospital and 5 in Tsukuba University Hospital). Two patients, however, were subsequently excluded. In Case 12, 2 separate brain tumors were present (one in the left parietotemporal lobe and one in the right temporal lobe), but only one was irradiated. The patient was excluded from final analysis because it was thought that progression of the nonirradiated neoplasm could influence survival, defined as the trial end point (see below). In Case 20, after initially agreeing to participate in the study, the patient refused scheduled AFTV injection during the course of radiotherapy. Clinical characteristics of the remaining 22 patients are presented in Table 2.

The group of 22 patients included 15 men and 7 women. Their age varied from 18 to 70 years (median 58 years). The preoperative KPS score was 90 or 100 in 14 patients, 70 or 80 in 3, and less than 70 in 5 (median 90). By the time of initiation of fractionated radiotherapy all patients had KPS scores of at least 70. With respect to RPA classification,[3] 7 patients (32%) were assigned Class III, 8 (36%) Class IV, and 7 (32%) Class V. Before surgery the maximum tumor diameter varied from 15 to 103 mm (median 50 mm). In 16 cases (73%) the resection of the neoplasm was considered total (98% or more of the contrast-enhancing lesion), and in 6 (27%) partial. In all cases the histopathological diagnosis of GBM was independently confirmed in the Japan Brain Tumor Reference Center. The MIB-1 staining index varied from 7.7% to 66.8% (median 29.1%). The p53 staining index varied from 0 to 85% (median 8.5%). The grades of MHC Class I expression were 0 in 2 cases, + in 8 cases, ++ in 7 cases, and +++ in 5 cases. There were no evident differences between the patient cohorts treated in the Tokyo Women's Medical University Hospital and the Tsukuba University Hospital, other than typically more aggressive tumor resection in the former institution due to the use of intraoperative MR imaging.

Follow-Up

Follow-up investigations were performed 2 weeks (14 ± 2 days) after completion of radiotherapy and every 2 months thereafter. Follow-up examinations included

TABLE 2: Characteristics of 22 patients*

Case No.	Age (yrs), Sex	Tumor Location	Preop Tumor Size (mm)	Preop KPS Score	RPA Class	Extent of Resection	MIB-1 Staining Index (%)	p53 Staining Index (%)	MHC Class I Expression Grade	Size of DTH-2 Response (mm)	OS (mos)	PFS (mos)
1	36, F	lt F-I	70	90	III	partial	45.5	59	++	1	42.0+	2.2
2	59, M	lt F & lt F	40	90	IV	total	26.4	37	+	10	6.1	3.3
3	34, M	bilat F	47	100	III	partial	35.9	85	+++	10	16.4	2.5
4	58, F	rt T	35	90	IV	partial	9.4	3	+++	8	14.4	2.3
5	66, M	lt P-O	50	80	IV	partial	52.6	46	+++	9	8.4	4.3
6	65, M	lt F	32	90	IV	total	13.7	0	+++	18	39.0	25.6
7	48, M	lt I	15	100	III	total	20.3	1	+	10	31.3	11.1
8	18, M	lt I	45	100	III	total	39.6	0	+++	15	41.6+	6.4
9	68, F	rt T & rt P	75	30	V	partial	34.4	0	++	8	9.5	6.6
10	64, M	rt O	60	90	V	total	26.9	3	+	11	6.4	4.5
11	58, M	rt O-T	103	50	V	total	30.4	1	+	12	26.9	14.0
13	70, M	rt F	35	70	V	total	31.0	78	+	4	12.7	3.8
14	54, F	lt T	37	50	V	total	24.9	66	+	35	22.6	13.9
15	41, M	lt F-P	50	70	IV	total	33.7	65	0	12	21.4	8.7
16	48, F	lt O	55	60	V	total	25.0	0	+	12	18.2	7.1
17	43, M	lt T	53	90	III	total	66.8	36	0	20	15.9	8.3
18	58, F	lt F	69	50	V	total	43.1	6	++	47	14.5	7.6
19	65, M	lt P	46	90	IV	total	17.5	11	++	19	13.8	5.0
21	32, M	lt F	65	100	III	partial	35.0	18	++	16	23.6+	18.5
22	61, M	lt T	52	90	IV	total	27.7	13	++	10	23.3+	13.6
23	60, F	lt T	48	100	IV	total	10.5	1	++	14	22.7+	14.4
24	26, M	rt F-T	57	100	III	total	7.7	5	+	20	20.3+	16.8

* The patients in cases 1–5 were treated at the Tsukuba University Hospital. All other patients were treated at Tokyo Women's Medical University Hospital. Multicentric gliomas were present in Cases 2 and 9. Cases 12 and 20 are not included due to violation of the study protocol (see text for details). The patient in Case 2 had 2 lesions, both in the left frontal lobe. Abbreviations: F = frontal; I = insular; O = occipital; P = parietal; T = temporal.

physical examination with evaluation of KPS score and determination of MRC neurological functional grade, blood and urine tests, and brain MR imaging. Adverse effects of treatment were graded according to the National Cancer Institute Common Toxicity Criteria version 2.0.[24]

No additional treatment was performed until tumor progression, which was defined as a 25% or greater increase in the volume of the contrast-enhanced lesion or appearance of new brain lesions.[11] At the time of neoplasm regrowth the patient was treated according to the preference of his or her doctor. In total, 21 of 22 patients underwent various types of salvage treatment (Fig. 2). In 10 cases (45%) at least 1 re-resection of the tumor was performed (with or without use of intraoperative photodynamic therapy). Chemotherapy with temozolomide (150–200 mg/m^2 daily for 5 days every 28 days) was administered in 20 patients, and 15 of them received at least 3 cycles of treatment. Among other therapies, proton-beam irradiation was performed in 1 case.

End Points and Statistical Analysis

The primary end point of the clinical trial was OS, defined as the time interval from the date of surgery to death from any cause. Secondary end points were PFS and safety of treatment. The following prespecified factors were analyzed for their association with OS and PFS: age, sex, tumor size before surgery, preoperative KPS score, RPA class, extent of tumor resection, MIB-1 staining index, p53 staining index, MHC Class I expression grade, and size of DTH-1 and DTH-2 response. Univariate analysis was performed using a log-rank test after construction of Kaplan-Meier survival curves. Continuous variables were dichotomized with regard to their median values. Factors that showed statistical significance were included in a Cox proportional hazard model for multivariate analysis. Differences were considered statistically significant if the 2-tailed p value was < 0.05.

Results

The length of follow-up varied from 6 to 42 months (median 19 months), and was 20 months for the last patient enrolled into the trial. At the time of data analysis, 6 patients (27%) were alive and 16 (73%) were dead.

Delayed-Type Hypersensitivity Test

No patient showed a positive response to the DTH-1 test, whereas response to the DTH-2 test varied in size from 1 to 47 mm (median 12 mm).

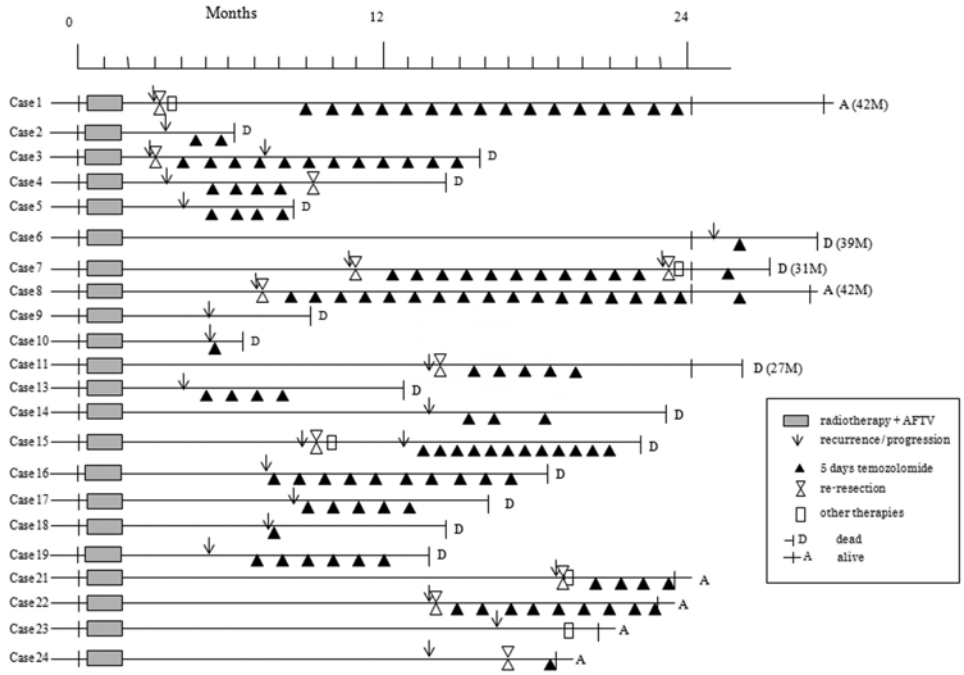

Fig. 2. Treatments applied after initial resection and outcome in each patient.

Overall Survival

The duration of OS varied from 6.1 to 42 months (median 21.4 months, 95% CI 13.8–31.3 months). The actuarial 2-year survival rate was 40% (Fig. 3).

The duration of OS showed a statistically significant association with RPA class. The median OS values in cases with RPA Class III, IV, and V, were 31.3, 21.4, and 14.5 months, respectively ($p < 0.05$).

In patients with a size DTH-2 response of 12 mm or larger, OS was longer than in those who had a response smaller than 12 mm; this difference, however, did not reach statistical significance (Fig. 4). The median OS values in these subgroups were 22.6 and 14.4 months, respectively ($p = 0.19$).

Other analyzed factors did not show statistically significant associations with OS.

Progression-Free Survival

The duration of PFS varied from 2.2 months to 25.6 months (median 7.6 months, 95% CI 4.3–13.6 months). It was significantly longer in cases in which the p53 staining index was lower than 8.5% (median PFS 7.5 months) than in cases in which the values were greater than 8.5% (median PFS 5.0 months, $p < 0.05$).

The duration of PFS in patients with a DTH-2 response of 12 mm or larger was significantly longer than in patients with a smaller DTH-2 response (Fig. 4). The median PFS in these subgroups was 13.9 months and 4.3 months, respectively ($p < 0.001$). The statistically significant difference in PFS was preserved when the cutoff level of DTH-2 response size was reduced to 10 mm (data not shown).

Other analyzed factors did not show statistically significant associations with PFS. Both the p53 staining index and size of DTH-2 response preserved their statistically significant associations with PFS in multivariate analysis.

Treatment Safety

Autologous formalin-fixed tumor vaccine treatment concomitant with fractionated radiotherapy was well tolerated by all patients. Vaccination did not result in any

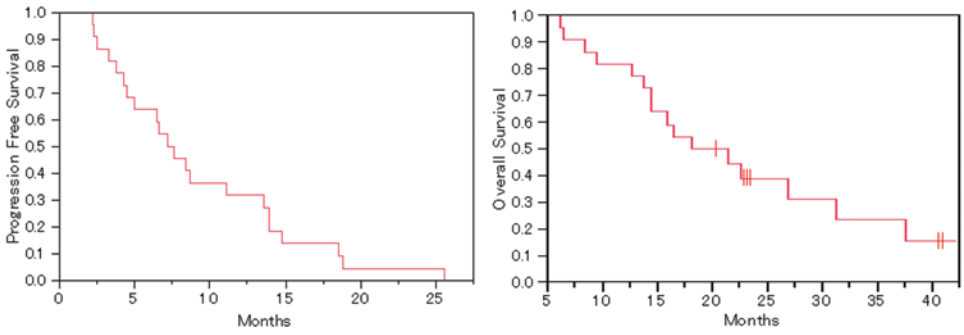

Fig. 3. Kaplan-Meier curves for PFS **(left)** and OS **(right)**. Censored observations are marked.

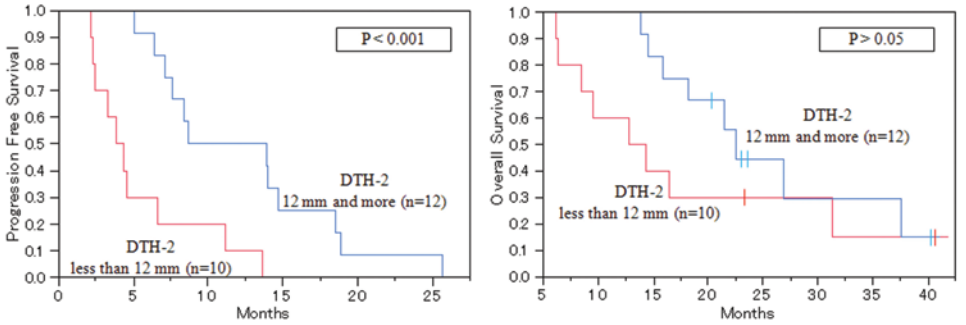

Fig. 4. Comparison of PFS **(left)** and OS **(right)** in subgroups of patients with different sizes of response to DTH testing after completion of vaccination (DTH-2). Censored observations are marked.

changes in KPS score. The treatment-related adverse effects consisted of local erythema, induration, and swelling at the injection sites and were observed in 21 of 22 patients, but in all cases these effects corresponded to Grade 1 toxicity.[24] No hematological toxicity—namely leukopenia, neutropenia, lymphopenia, thrombocytopenia, or anemia—was seen. No allergic dermatitis or anaphylaxis was observed, and there was no evidence of any autoimmune phenomena. Blood investigation before and after the vaccination did not detect significant abnormalities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, total protein, lactate dehydrogenase, albumin, Na, K, Cl, glucose, or hemoglobin levels, or hematocrit or cell counts (red blood cells, white blood cells, platelets). In one patient (Case 1) a chronic subdural hematoma was revealed on the side contralateral to the craniotomy, but it was judged to be unrelated to AFTV treatment.

Discussion

The generally dismal prognosis associated with glioblastoma stimulates the search for novel treatment strategies. Several clinical trials have investigated the effectiveness and safety of vaccines in cases of recurrent malignant gliomas with or without concomitant chemotherapy,[4,15] or in patients with newly diagnosed tumors after completion of fractionated radiotherapy (without chemotherapy).[16,18,20] Nevertheless, to the best of our knowledge, there have been no data reported on the use of vaccine concomitant with fractionated radiotherapy alone for newly diagnosed glioblastoma.

Autologous formalin-fixed tumor vaccine is a novel, stable, and clinically durable vaccine that is simple to produce. In comparison with the promising newest types of peptide vaccines, such as EGFR variant III[17] and Wilms' tumor–1 (WT-1),[7] use of AFTV does not require preselection of patients according to expression of tumor-associated antigens. Its preparation is based on the fact that peptide tumor-associated antigens derived from fixed cells or proteins are formalin resistant; therefore, formalin-fixed and/or paraffin-embedded tumor cells/tissues may be used to generate effective antitumor immune cells.[13,14] Human leukocyte antigen-A2402–restricted CEA-specific cytotoxic T lymphocytes can be induced by culturing human peripheral blood mononuclear cells (PBMCs) with formalin-fixed autologous adhesive PBMCs loaded with CEA protein–bound latex beads, or can be generated using formalin-fixed adherent cells pulsed with 9- or 10-mer CEA-derived MHC Class I–presented tumor-associated antigens.[6,13] It was demonstrated that the activity and specificity of cytotoxic T lymphocytes generated by formalin-fixed tumor cells are comparable to those induced by continuously cultured live tumor cells.[9,10]

Autologous formalin-fixed tumor vaccine showed positive immunotherapeutic effects in experimental tumor models,[13] as well as in patients with hepatocellular carcinoma[8] and glioblastoma.[6] Ishikawa et al.[6] evaluated results of such treatment in 12 patients with either recurrent or residual glioblastoma. The AFTV was given as 3 five-site intradermal inoculations performed at weekly intervals. Response to DTH testing was evaluated before and after each vaccination. Of 12 tumors, 1 showed complete response, 1 showed partial response, 2 showed minor response, 1 showed stabilization of growth, and 7 progressed. The median duration of survival after initiation of AFTV treatment was 10.7 months, but 3 of 5 responders survived 20 months or longer. A low p53 staining index and high MHC Class I expression were associated with better survival. The treatment was well tolerated by all patients with only local erythema, induration, and low-grade fever being reported.[6]

Similarly, the present study demonstrates that a treatment protocol involving 3 courses of AFTV during the late stage of fractionated radiotherapy may be effective in patients with newly diagnosed glioblastoma. The median OS in this series was 21.4 months, whereas in the similar cohort treated with radiation alone during the EORTC 26981/22981–NCIC trial, it was 12.1 months.[21] The median OS in our patients with RPA Class V (14.5 months) was comparable to those in the best prognostic category of glioblastoma (RPA Class III) in the above-mentioned cohort (14.8 months).[21] Such results probably were not caused by salvage chemotherapy with temozolomide at the time of recurrence, because 70% of patients treated with radiotherapy alone during the EORTC 26981/22981–NCIC trial also received salvage temozolomide treatment.[12,21] Therefore, while compromise due to selection bias cannot be ruled out in our study, the effect of AFTV on the outcome can be strongly suspected.

It is important, that both the OS and PFS of our patients were associated with response to the DTH test, and

patients with a greater response to DTH-2 had statistically longer PFS. Because DTH response is strongly correlated with cell-mediated acquired immune response in vivo, it can be assumed that patients with a large DTH-2 response had developed activation of the cellular immune response against the autologous tumor cells. In agreement with the previous report,[6] PFS in our series was also associated with the p53 staining index of the tumor; therefore, it can be speculated, that a p53-related mechanism is somewhat involved in AFTV-induced cytotoxic T-lymphocyte activity.

Treatment with AFTV was not accompanied by significant associated morbidity in this study. In no patient did we identify reduction of KPS score or more than Grade 1 toxicity. The latter was limited to local erythema, induration, and swelling at the site of injection. While autoimmune encephalomyelitis represents a potentially serious complication of any type of glioma cell vaccine therapy,[27] no evidence of its development was observed in any of our patients.

The main limitation of our study is the omission of concomitant temozolomide chemotherapy during the course of postoperative fractionated radiotherapy, which is currently considered standard management of newly diagnosed glioblastoma.[5,12,21,22] In 2005, Stupp et al.[22] reported that such a treatment strategy has definite prognostic advantages and extends the median PFS from 5.0 to 6.9 months, extends the median OS from 12.1 months to 14.6 months, and increases the proportion of 2- and 5-year survivors from 10.4% to 26.5% and from 1.9% to 9.8%, respectively. However, our investigation was initiated before approval of such a radiochemotherapy protocol by the Japanese government (September 15, 2006). Therefore, taking into account the results presented herein, another clinical trial on the use of AFTV therapy during irradiation of newly diagnosed glioblastoma and concomitant chemotherapy with temozolomide is definitely needed. In fact, there is a theoretical possibility that additional vaccine therapy may enhance the therapeutic effectiveness of chemotherapy. Severe lymphopenia, especially depletion of $CD4^+$ $CD25^+$ T cells, which sometimes accompanies treatment with temozolomide,[23,27] may result in augmented immune status through suppression of the $CD4^+$ $CD25^+$ regulatory T cells. Sampson et al.[17] reported results of a Phase II multicenter clinical trial on the use of an EGFR variant III–specific peptide vaccine combined with temozolomide chemotherapy in patients with newly diagnosed EGFR variant III–positive glioblastoma, and according to their data, the median PFS of treated patients was 16 months and the median OS was not reached. Further investigation of such promising results is warranted.

Conclusions

The current study demonstrates that AFTV treatment concomitant with fractionated radiotherapy may be effective in patients with newly diagnosed glioblastoma and is not accompanied by severe toxicity. In light of these results, another clinical trial on the use of AFTV therapy during radiotherapy of newly diagnosed glioblastoma and concomitant chemotherapy with temozolomide is definitely needed.

Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author contributions to the study and manuscript preparation include the following. Conception and design: Muragaki, Iseki, Takakura, Tsuboi, Matsumura, Matsutani, Sato, Ohno. Acquisition of data: Muragaki, Maruyama, Tanaka, Shinohara, Yamamoto. Analysis and interpretation of data: Muragaki, Iseki, Tsuboi, Yamamoto, Karasawa, Yamaguchi. Drafting the article: Muragaki, Shinohara. Critically revising the article: Maruyama, Iseki, Takakura, Yamamoto, Matsumura, Matsutani, Karasawa, Okada, Hori. Statistical analysis: Muragaki, Karasawa, Shimada, Yamaguchi. Administrative/technical/material support: Iseki, Shinohara, Takakura, Tsuboi, Matsumura, Matsutani, Karasawa, Ohno, Okada, Hori. Study supervision: Muragaki, Takakura, Hori. Editing of English text: Shinohara. Central review of histopathological specimens: Nakazato. Vaccine preparation: Sato, Uemae, Ohno.

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