Table 3: Diagnostic performance of SNB for axillary staging in AUS-negative patients

		No. of patients		
			Permanent histopathology	
Intraoperative frozen Histopathology		Total	Metastasis positive	Metastasis negative
A.	Total (n = 124)			
	Metastasis positive	24	24	0
	Metastasis negative	100	1	99
	Total	124	25	99
B.	^{18}F-FDG uptake positive (n = 12)			
	Metastasis positive	5	5	0
	Metastasis negative	7	1	6
	Total	12	6	6
C.	^{18}F-FDG uptake negative (n = 112)			
	Metastasis positive	19	19	0
	Metastasis negative	93	0	93
	Total	112	19	93

Note; SNB, sentinel node biopsy; AUS, axillary ultrasound; No., Number; SNB identification rate 99.2% (124 of 125 cases)
Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPN), and accuracy overall were 96, 100, 100, 99, and 99%, respectively in A. Sensitivity, specificity, PPV, NPV, and overall accuracy were 83, 100, 100, 86, and 92%, respectively in B.
Sensitivity, specificity, PPV, NPV, and overall accuracy were 100, 100, 100, 100, and 100%, respectively in C.

related with ^{18}F-FDG uptake at SUV cut-off point 1.8 (p = 0.15).

Categories of ^{18}F-FDG PET/CT combined with ultrasound for indications of ALND and PSC

Table 5 indicates four categories which were divided by clinical findings of ^{18}F-FDG PET/CT and AUS to the axilla. We used a visual assessment of ^{18}F-FDG uptake which proved to be a reproducible method and have acceptable sensitivity and specificity [6]. Of all 183 patients, 138 (75%) patients who had negative AUS with negative axillary ^{18}F-FDG uptake were classified as category 1. Frequency of ALNs involvement was only 21 (15%), 12 (57%) of these 21 patients had single involved ALNs. The maximum size of involved ALNs was 10 mm or smaller in 17 (81%) of 21 patients. The nuclear grade of involved ALNs was grade 1 or 2 in 16 (76%), and grade 3 in only 5 (24%).

Twenteen (7%) patients who had negative AUS but positive axillary ^{18}F-FDG uptake were classified as category 2. In category 2, ALNs involvement was detected in 6 (50%)

Table 4: Axillary nodal clinicopathological factors correlated with ^{18}F-FDG uptake

Clinicopathological factors	SUV cut-off 1.8	No. of pts	Average	SD	*p*-value
No. of involved ALNs	Low	38	3.6	5	0.15
	High	21	6.9	7	
The maximum size (mm)	Low	38	8.6	6.4	0.006
	High	21	14.6	7.9	
	SUV	No. of pts	Grade	No. ofpts	*p*-value
Nuclear grade	Low	38	Grade1/2	27	0.03
			Grade3	11	
	High	21	Grade1/2	9	
			Grade3	12	

SUV, Standardized uptake value; No, Number; pts, patients; SD, Standard derivation

Table 5: Categories of ^{18}F-FDG PET/CT combined with ultrasonography for indications of ALND/PSC

Category	1	2	3	4
AUS	-	-	+	+
^{18}F-FDG-uptake	-	+	-	+
No. of involved ALNs				
0	117 (85)	6 (50)	1 (20)	0 (0)
1	12 (9)	5 (42)	2 (40)	9 (32)
2 ≦ Involved ALNs ≦ 5	6 (4)	1 (8)	1 (20)	9 (32)
6 ≦	3 (2)	0 (0)	1 (20)	10 (36)
The maximum size of involved ALNs				
≦ 5 mm	10(48)	5 (83)	0 (0)	0 (0)
5 mm < metastasis < 10 mm	7(33)	1 (17)	2 (50)	6 (21)
10 mm ≦	4(19)	0 (0)	2 (50)	22(79)
Nuclear grade of involved ALNs				
Grade 1 and 2	16(76)	3 (50)	4 (100)	13(46)
Grade 3	5(24)	3 (50)	0 (0)	15(54)
Frequency of involved ALNs	15%	50%	80%	100%
Indications of ALND/PSC	SNB	FNAC/Bx is needed	FNAC/Bx is needed	Acceptable
Total	138 (100)	12 (100)	5 (100)	28 (100)

AUS, Axillary ultrasound; ALN, Axillary lymph node; ALND, ALN dissection; PSC, Primary systemic chemotherapy; No., Number; pts, patients; FNAC, Fine needle aspiration

of 12. A single ALN involvement was present in 5 (83%) of these 6. All 6 patients had ALNs involvement with the maximum size of less than 10 mm in category 2. Nuclear grade was 1 or 2 in 3 (50%) patients, whereas nuclear grade was 3 in 2 (50%).

Five (3%) patients who had positive AUS but negative axillary ^{18}F-FDG uptake were classified as category 3. Twenty-eight (15%) patients who had double-positive nodal status of AUS and ^{18}F-FDG uptake were classified as category 4. Four (80%) of 5 patients in category 3 had ALNs involvement and all patients in category 4 had ALNs involvement. Especially 2-or-more involved ALNs were 2 (50%) of 4 cases in category 3, and 19 (68%) of 28 cases in category 4. The maximum size of involved ALNs was 10 mm or more in 2 (50%) of 4 cases in category 3 and in 22 (79%) of 24 cases in category 4.

Nuclear grade was 1 or 2 in all 4 (100%) cases in category 3, whereas nuclear grade was 1 or 2 in 13 (46%) of 28 cases in category 4.

Diagnostic performance of SNB in ^{18}F-FDG-positive and AUS-negative patients

Table 6 shows diagnostic performance of SNB for axillary staging in ^{18}F-FDG-positive and AUS-negative patients of category 2. Six (50%) of 12 patients had involved SNs and others (50%) had no involved SNs in spite of ^{18}F-FDG uptake. No metastases were found in non-SNs in all patients that had involved SNs and received subsequent axillary dissection.

Discussion

Visual assessment of ^{18}F-FDG PET/CT for the axillary staging

In visual assessment of ^{18}F-FDG PET/CT to the axilla, we demonstrated that diagnostic accuracy of ^{18}F-FDG PET/CT was almost equivalent to that of AUS for detecting of ALN involvement in patients with primary breast cancer. Visual assessment of ^{18}F-FDG uptake to the axilla achieved higher sensitivity than AUS, and the specificity and PPV of ^{18}F-FDG PET/CT were acceptably high, 95%, and 85%, respectively.

There were 40 (22%) of 183 patients having axillary uptake of ^{18}F-FDG. Six (15%) of these patients had no metastasis of ALNs. The reason of these false positive for the ^{18}F-FDG uptake is not known, but reactive lymphadenopathy caused by breast biopsy would lead to false positive results [9,14].

AUS showed limited sensitivity equal to ^{18}F-FDG PET/CT for detecting ALN involvement, and showed almost perfect specificity and PPV. According to diagnostic perform-

Table 6: Diagnostic performance of SNB for axillary staging in ^{18}F-FDG-positive and AUS-negative patients of category 2

Patients	SUV	Involved SNs/resected SNs	Involved non-SNs/resected non-SNs	Ax dissection
1	8.1	1/1	0/10	Performed
2	2.5	1/1	0/17	Performed
3	2.4	1/2	0/12	Performed
4	1.4	1/3	0/21	performed
5	1.3	4/4	0/12	performed
6	0.7	1/4	0/6	performed
7	1.5	0/1	-	not performed
8	1	0/4	-	not performed
9	1	0/1	0/1	not performed
10	1	0/4	0/1	not performed
11	0.9	0/1	0/2	not performed
12	0.9	0/4	0/1	not performed

AUS, Axillary ultrasound;SUV, Standardized uptake value; SNs, Sentinel nodes, Ax, Axillary

ance of axillary ultrasonography, the present results showed higher outcome than others' previous studies [15-17]. In our previous study, AUS was performed using an SSD-650CL (Aloka, Tokyo, Japan), an old model of SSD-6500, and indicated sensitivity, specificity, PPV, and overall accuracy of 45, 97, 92.6, and 75%, respectively [5]. Furthermore an ultrasound specialist performed axillary investigation in the present study. From these reasons, we considered the present results were superior to those in our previous study.

Differences in criteria for judgment of axillary status or in the type of ultrasound device might have given rise to such inconsistency. Furthermore, although ultrasonography is less-invasive and relatively easy to apply, experienced skills are required to judge AUS-positive nodes. We sometimes wavered in our judgement whether ALNs were positive or not when ultrasound image of lymph nodes was less than 10 mm in diameter but homogeneously hypoechoic in centric area. From these reasons, AUS alone might be difficult to determine axillary staging.

We classified patients into 4 categories of axillary status according to ^{18}F-FDG PET/CT and ultrasonography (Table 5).

Category 1 showed the patients who have AUS-negative lymph nodes without axillary ^{18}F-FDG uptake. Fifteen percent of these 138 patients had ALNs involvement. Characteristics of ALN involvement were lesser number, smaller sizes, and lower nuclear grade of metastatic foci (Table 5). For these patients, SNB was successfully performed as shown in Table 3, and SNB is recommended to assess axillary nodal status.

Category 2 and 3 showed the patients having discrepancy between the axillary examinations of AUS and ^{18}F-FDG uptake.

Category 2 showed the patients having ^{18}F-FDG uptake but negative AUS. Half of these patients have metastatic foci in their axilla. The reason of the discrepancy was related to the fact that metastatic foci of small size (5 mm or less) and/or higher nuclear grade was detected by ^{18}F-FDG uptake but were not by AUS.

Category 3 showed the patients having positive AUS without axillary ^{18}F-FDG uptake. We found 4 (80%) of 5 patients had ALN involvement. The characteristic of these metastastic foci was lower nuclear grade. This result are in keeping with previous reports [8-10].

The conclusions could not be determined because the number of patients in categories 2 and 3 have been limited, but we could indicate lymph nodes having discrepancy in diagnosis between AUS and axillary ^{18}F-FDG uptake were found to be frequently metastasized. When the discrepancy occurred between these two modalities, therefore, we suggest further axillary investigations such as core-needle biopsy, or fine needle aspiration cytology to evaluate precisely axillary nodal status.

We confirmed the positive lymph nodes found by ^{18}F-FDG-PET matched the SNB results in all patients of category 2 that had involved SNs and received subsequent axillary dissection (shown in Table 6).

Category 4 showed the patients had double-positive ALNs of AUS and ^{18}F-FDG uptake. PPV for detecting ALN involvement was 100%. These patients were recommended to undergo ALND without SLN. In addition, it might be rational to consider that patients having AUS positive nodes and axillary ^{18}F-FDG uptake will have PSC without biopsy or fine needle aspiration cytology to the axilla.

Semiquantitative assessment of ^{18}F-FDG PET/CT for the axilla

Table 4 showed higher SUV were significantly correlated with nuclear grade 3 and maximum size of metastatic foci but not with number of involved ALNs. These results also appear to reveal biological significance of axillary ^{18}F-FDG accumulated to metastasized cancer cells.

When the cut-off of SUV exceeded 1.8, specificity and PPV of ^{18}F-FDG PET/CT were almost 100%, but sensitivity notably decreased to 36% or lower.

From the present results, appropriate determination of the cut-off of SUV appeared possible to evaluate ALN involvement by means of ^{18}F-FDG uptake. Especially by setting of cutoff of SUV, we could predict ALN involvement with excellent specificity and PPV. The cut-off of SUV for ALN involvement varies from 1.2 to 2.3 among reports previously published [9,11]. The inter-institutional standardization of the cut-off value-off SUV for ALN evaluation remains to be settled.

Thus, we found that axillary ^{18}F-FDG uptake added incremental diagnostic confidence to AUS. Richard L et al reported that ^{18}F-FDG PET may have a role in assessing patients with medially or superiorly situated breast cancers that may drain preferentially or exclusively to internal mammary or supraclavicular nodes[10]. A. Gil-Rendo et al also described that an advantage of ^{18}F-FDG PET was to be able to detect internal mammary node metastasis, which is often clinically occult and poorly visualized by conventional modality including ultrasonography [8].

We experienced a patient having ^{18}F-FDG uptake in a parasternal lymph node in spite of double-negativity in AUS and axillary ^{18}F-FDG uptake. The lymph node has been proven to be metastasized by fine-needle aspiration cytology. Another patient who have double-positivity in AUS and axillary ^{18}F-FDG uptake, also showed ^{18}F-FDG uptake in infraclavicular lymph nodes. These two patients had chosen PSC, having been ineligible for this study protocol.

Thus, we considered the whole-body ^{18}F-FDG PET/CT would be informative for imaging investigations for regional nodes involvement as well as distant metastasis [12,18].

Conclusion

In conclusion, the diagnostic accuracy of visual assessment of ^{18}F-FDG PET/CT was almost equivalent to that of AUS in sensitivity, specificity, and overall accuracy. When cut-off of SUV was set at 1.8 or more, specificity and PPV was each 100%. However, there are numerous factors that will influence SUV results and we should take into consideration the limited value of SUV in breast.

To our knowledge, this is the first study to compare between ^{18}F-FDG PET/CT and ultrasonography for detecting of ALN involvement.

Considering their limited sensitivities, the high radiation exposure by ^{18}F-FDG PET/CT and also costs of the examination, it is likely that AUS will be more cost-effective in detecting massive axillary tumor burden. However, when we cannot judge the axillary staging using AUS alone, metabolic approach of ^{18}F-FDG PET/CT for axillary staging would enable us a much more confident diagnosis.

Abbreviations

ALN: axillary lymph node; ALND: ALN dissection; AUS: axillary ultrasonography; NPV: negative predictive value; ^{18}F-FDG PET/CT: positron emission tomography/computed tomography with ^{18}F-fluorodeoxyglucose; SNB: sentinel node biopsy; SUV: standardized uptake value; PPV: positive predictive value.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SU performed the planning, acquisition of data, analysis of data, and writing of the manuscript. HT (pathologist) performed the planning, interpretation of data, and the manuscript in co-operation with SU. HA, JO, and KF (breast surgeons) performed surgery and the statistic analysis. YH, KT, JI, and YA (radiologists) performed the evaluation of tumoral SUV levels and data acquisition. NK (biochemist) performed the statistic analysis. TK (ultrasonographer) carried out axillary assessment. HM participated in its design and coordination in co-operation with SU and HT. All authors read and approved the final manuscript.

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References

1. Aarsvold JN, Alazraki NP: **Update on detection of sentinel lymph nodes in patients with breast cancer.** *Semin Nucl Med* 2005, **35**:116-128.
2. Sato K, Uematsu M, Saito T, Ishikawa H, Yamasaki T, Tamaki K, Tamai S, Kusano S, Hiraide H, Mochizuki H: **Indications and technique of sentinel lymph node biopsy in breast cancer using 99m-technetium labeled tin colloids.** *Breast Cancer* 2000, **7**:95-98.
3. van Rijk MC, Teertstra HJ, Peterse JL, Nieweg OE, Olmos RA, Hoefnagel CA, Kroon BB: **Ultrasonography and fine-needle aspiration cytology in the preoperative evaluation of melanoma patients eligible for sentinel node biopsy.** *Ann Surg Oncol* 2006, **13**:1511-1516.

4. Nori J, Vanzi E, Bazzocchi M, Bufalini FN, Distante V, Branconi F, Susini T: **Role of axillary ultrasound examination in the selection of breast cancer patients for sentinel node biopsy.** *Am J Surg* 2007, **193**:16-20.
5. Sato K, Tamaki K, Tsuda H, Kosuda S, Kusano S, Hiraide H, Mochizuki H: **Utility of axillary ultrasound examination to select breast cancer patients suited for optimal sentinel node biopsy.** *Am J Surg* 2004, **187**:679-683.
6. van der Hoeven JJ, Hoekstra OS, Comans EF, Pijpers R, Boom RP, van Geldere D, Meijer S, Lammertsma AA, Teule GJ: **Determinants of diagnostic performance of [F-18]fluorodeoxyglucose positron emission tomography for axillary staging in breast cancer.** *Ann Surg* 2002, **236**:619-624.
7. Barranger E, Grahek D, Antoine M, Montravers F, Talbot JN, Uzan S: **Evaluation of fluorodeoxyglucose positron emission tomography in the detection of axillary lymph node metastases in patients with early-stage breast cancer.** *Ann Surg Oncol* 2003, **10**:622-627.
8. Gil-Rendo A, Zornoza G, Garcia-Velloso MJ, Regueira FM, Beorlegui C, Cervera M: **Fluorodeoxyglucose positron emission tomography with sentinel lymph node biopsy for evaluation of axillary involvement in breast cancer.** *Br J Surg* 2006, **93**:707-712.
9. Chung A, Liou D, Karlan S, Waxman A, Fujimoto K, Hagiike M, Phillips EH: **Preoperative FDG-PET for axillary metastases in patients with breast cancer.** *Arch Surg* 2006, **141**:783-8; discussion 788-9.
10. Wahl RL, Siegel BA, Coleman RE, Gatsonis CG: **Prospective multicenter study of axillary nodal staging by positron emission tomography in breast cancer: a report of the staging breast cancer with PET Study Group.** *J Clin Oncol* 2004, **22**:277-285.
11. Veronesi U, De Cicco C, Galimberti V, Fernandez J, Rotmensz N, Viale G, Spano G, Luini A, Intra M, Veronesi P, Berrettini A, Paganelli G: **A comparative study on the value of FDG-PET and sentinel node biopsy to identify occult axillary metastases.** *Ann Oncol* 2007, **18**:473-478.
12. Juweid ME, Cheson BD: **Positron-emission tomography and assessment of cancer therapy.** *N Engl J Med* 2006, **354**:496-507.
13. Tatsumi M, Cohade C, Mourtzikos KA, Fishman EK, Wahl RL: **Initial experience with FDG-PET/CT in the evaluation of breast cancer.** *Eur J Nucl Med Mol Imaging* 2006, **33**:254-262.
14. Rousseau C, Bourbouloux E, Campion L, Fleury N, Bridji B, Chatal JF, Resche I, Campone M: **Brown fat in breast cancer patients: analysis of serial (18)F-FDG PET/CT scans.** *Eur J Nucl Med Mol Imaging* 2006, **33**:785-791.
15. Ohta M, Tokuda Y, Saitoh Y, Suzuki Y, Okumura A, Kubota M, Makuuchi H, Tajima T, Yasuda S, Shohtsu A: **Comparative efficacy of positron emission tomography and ultrasonography in preoperative evaluation of axillary lymph node metastases in breast cancer.** *Breast Cancer* 2000, **7**:99-103.
16. Hashimoto H, Suzuki M, Oshida M, Nagashima T, Yagata H, Shishikura T, Imanaka N, Nakajima N: **Quantitative ultrasound as a predictor of node metastases and prognosis in patients with breast cancer.** *Breast Cancer* 2000, **7**:241-246.
17. Watermann DO, Tempfer CB, Hefler LA, Parat C, Stickeler E: **Ultrasound criteria for ductal invasive breast cancer are modified by age, tumor size, and axillary lymph node status.** *Breast Cancer Res Treat* 2005, **89**:127-133.
18. Fueger BJ, Weber WA, Quon A, Crawford TL, Allen-Auerbach MS, Halpern BS, Ratib O, Phelps ME, Czernin J: **Performance of 2-deoxy-2-[F-18]fluoro-D-glucose positron emission tomography and integrated PET/CT in restaged breast cancer patients.** *Mol Imaging Biol* 2005, **7**:369-376.

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Phase II clinical trial of Wilms tumor 1 peptide vaccination for patients with recurrent glioblastoma multiforme

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Phase II clinical trial of Wilms tumor 1 peptide vaccination for patients with recurrent glioblastoma multiforme

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Object. The object of this study was to investigate the safety and clinical responses of immunotherapy targeting the *WT1* (Wilms tumor 1) gene product in patients with recurrent glioblastoma multiforme (GBM).

Methods. Twenty-one patients with WT1/HLA-A*2402–positive recurrent GBM were included in a Phase II clinical study of WT1 vaccine therapy. In all patients, the tumors were resistant to standard therapy. Patients received intradermal injections of an HLA-A*2402–restricted, modified 9-mer WT1 peptide every week for 12 weeks. Tumor size, which was obtained by measuring the contrast-enhanced area on magnetic resonance images, was determined every 4 weeks. The responses were analyzed according to Response Evaluation Criteria in Solid Tumors (RECIST) 12 weeks after the initial vaccination. Patients who achieved an effective response continued to be vaccinated until tumor progression occurred. Progression-free survival and overall survival after initial WT1 treatment were estimated.

Results. The protocol was well tolerated; only local erythema occurred at the WT1 vaccine injection site. The clinical responses were as follows: partial response in 2 patients, stable disease in 10 patients, and progressive disease in 9 patients. No patient had a complete response. The overall response rate (cases with complete or partial response) was 9.5%, and the disease control rate (cases with complete or partial response as well as those in which disease was stable) was 57.1%. The median progression-free survival (PFS) period was 20.0 weeks, and the 6-month (26-week) PFS rate was 33.3%.

Conclusions. Although a small uncontrolled nonrandomized trial, this study showed that WT1 vaccine therapy for patients with WT1/HLA-A*2402–positive recurrent GBM was safe and produced a clinical response. Based on these results, further clinical studies of WT1 vaccine therapy in patients with malignant glioma are warranted.
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Key Words • **cancer vaccine** • **glioblastoma multiforme** • **glioma** • **immunotherapy** • **Wilms tumor 1**

Currently, the standard treatment for gliomas is surgery, followed by external radiation and chemotherapy. In patients with newly diagnosed GBM, however, concurrent irradiation and temozolomide therapy, followed by adjuvant temozolomide therapy for at least 6 months, offered a modest benefit, with a median survival of 14.6 months and a 2-year survival rate of 26.5%.[22] To date, therapeutic options for patients with malignant glioma have been limited, and extensive research is ongoing.

Immune therapy against malignant gliomas includes several therapeutic approaches that involve dendritic cell–based immunotherapy and antibody-mediated immunotherapy.[31] Cancer vaccination is another novel form of therapy.[30] Recent advances in molecular biology and tumor immunology have resulted in the identification of a large number of tumor-associated antigens that could be used for cancer vaccination, since their epitopes associated with HLA Class I molecules were recognized by CTLs. One of the identified tumor-associated antigens was the product of the Wilms tumor gene, *WT1*.[17]

Abbreviations used in this paper: CTL = cytotoxic T-lymphocyte; DSMC = Data Safety Monitoring Committee; ECOG = Eastern Cooperative Oncology Group; FDG = fluorodeoxyglucose; GBM = glioblastoma multiforme; HLA = human leukocyte antigen; MR = magnetic resonance; PBMC = peripheral blood mononuclear cell; PET = positron emission tomography; PFS = progression-free survival; RECIST = Response Evaluation Criteria in Solid Tumors; SPECT = single-photon emission computed tomography; WHO = World Health Organization; WT1 = Wilms tumor 1.

The *WT1* gene was isolated as a gene responsible for Wilms tumor. It encodes a zinc finger transcription factor, which is involved in cell proliferation and differentiation, apoptosis, and organ development. Although the *WT1* gene was first categorized as a tumor suppressor gene, it was later proposed that the wild-type *WT1* gene functions as an oncogene rather than as a tumor-suppressor gene. In response to granulocyte colony–stimulation factor, growth promotion and differentiation inhibition were identified in wild-type *WT1* gene–transfected myeloid progenitor cells.[25] In many reports, the wild-type *WT1* gene was shown to be overexpressed in various types of solid tumors. The WT1 protein was found to be an attractive target antigen for immunotherapy against these malignancies.[20]

Recently, we performed a Phase I clinical trial to examine the safety of a WT1-based vaccine, as well as the clinical and immunological responses of patients with a variety of cancer types, including leukemia, lung cancer, and breast cancer.[19] The authors demonstrated that WT1 peptide vaccine emulsified with Montanide ISA51 adjuvant and administered at a dosage of 0.3, 1.0, or 3.0 mg at 2-week intervals was safe for patients other than those with myelodysplastic syndromes. Furthermore, the vaccination induced peptide-specific CTLs and was associated with clinical response. Very recently, it was confirmed that the potential toxicities of the weekly WT1 vaccination treatment schedule (3 mg per week) with the same adjuvant were also acceptable.[15]

An increasing number of central nervous system studies have reported that systemic immunotherapy is capable of inducing an antitumor response within the immunologically privileged brain.[29–31] These advances suggest the possibility of the development of a new peptide-based cancer immunotherapy. The blood–brain barrier, which was thought to be one of the hurdles hindering the development of therapeutically effective immunotherapy for gliomas, does not always function effectively in cases involving recurrent gliomas.[29]

Like many other solid tumors, gliomas have been found to express WT1 protein.[8] A definite correlation has been observed between WT1 expression and cellular proliferation activity, as indicated by WHO grade.[8] In the present study, we investigated the clinical responses to peptide-based immunotherapy targeting the *WT1* gene product in patients with recurrent GBMs. We also evaluated the correlation between the clinical response and the WT1 expression level in tumor tissues using immunohistochemical staining, as well as WT1-specific CTL frequencies (tetramer assay) in patients' PBMCs.

Clinical Materials and Methods

The WT1 Peptide

The immunization consisted of an HLA-A*2402–restricted, modified 9-mer WT1 peptide (amino acids 235–243 CYTWNQMNL), in which Y was substituted for M at amino acid position 2 (the anchor position) of the natural WT1 peptide. About 60% of Japanese have HLA-A*2402 which is the most common HLA Class I type in the Japanese population. The modified 9-mer WT1 peptide was shown to induce much stronger CTL activity against WT1-expressing tumor cells than the natural peptide.[26] The WT1 peptide (GMP grade) was purchased from Multiple Peptide Systems as the lyophilized peptide.

Patient Population

Twenty-one patients were enrolled in this study. All patients seen in our unit who had recurrent or progressive GBM were eligible to be enrolled if their disease was resistant to conventional chemotherapy and radiotherapy. Patients who had refused chemotherapy but wanted to receive WT1 vaccine therapy under the auspices of this clinical trial were also eligible. In patients who received stereotactic radiosurgery as part of their initial therapy, true recurrence or progression was distinguished from radiation necrosis by metabolic imaging or histological confirmation.

Additional inclusion criteria were: 1) age between 16 and 80 years, 2) expression of WT1 in the glioma cells determined by immunohistochemical analysis, 3) HLA-A*2402–positivity, 4) estimated survival of more than 3 months, 5) ECOG Performance Status Grade 0–2, 6) no severe organ function impairment, and 7) the written informed consent of the patient. All enrolled patients had histologically proven GBM (Grade 4) based on the WHO criteria. After initial resection of the tumor, patients underwent a course of external radiation therapy and chemotherapy. Magnetic resonance imaging was used to monitor patients for recurrence or progression of their tumor during initial therapy and during maintenance therapy. No patient was treated with chemotherapy or radiotherapy during the 4 weeks prior to WT1 immunotherapy. Registered patients had methionine-PET, FDG-PET, thallium-SPECT, and MR imaging to confirm recurrence or progression and to exclude radiation necrosis. All patients underwent electrocardiography, and blood samples were obtained to confirm that there were no abnormalities.

After informed consent was obtained, it took at least 2 weeks for the immunohistochemical analysis, HLA-typing analysis, image analysis, and other tests to be completed. Therefore, the presence of tumor recurrence or progression was again assessed > 2 weeks after registration for WT1 treatment. The DSMC independently reviewed the eligibility of each enrolled patient. Protocol compliance, safety, and on-schedule study progress were also monitored by the DSMC. The WT1 peptide–based Phase II study was approved by the ethical review boards of the Osaka University Faculty of Medicine.

Vaccine Preparation and Vaccination

Patients received intradermal injections of 3.0 mg of HLA-A*2402–restricted modified 9-mer WT1 peptide emulsified with Montanide ISA51 adjuvant. The WT1 vaccinations were scheduled to be given weekly for 12 consecutive weeks. Twelve weeks after the initial vaccination, the response was evaluated on MR imaging. If an effect was observed after the 12 vaccinations, WT1 vaccination was continued at 1-week intervals (with the patients' informed consent) until tumor progression was again noted.

Immunohistochemical Analysis

Immunohistochemical analysis was performed to confirm WT1 protein expression in malignant glioma tissue using a procedure that has been previously described.[8] Brief-

ly, formalin-fixed tissue sections were prepared from the resected tumors. Sections were microwaved in citrate buffer for antigen retrieval and incubated with anti–human WT1 mouse monoclonal antibody 6F-H2 (DAKO; diluted 1:50). The WT1 reaction was visualized with the Vectastain ABC kit (Vector Laboratories) and diaminobenzidine (WAKO). The sections were then counterstained with hematoxylin. Control positive staining was evaluated with Wilms tumor, and control negative staining was evaluated with normal brain. Expression of WT1 seen in the sections was classified on a scale from 0 to 4 based on the staining density and the pattern of the glioma cells according to the following criteria: 0, negative staining; 1, slightly increased staining in some tumor cells compared with that in normal glial cells; 2, staining at intermediate intensity in some tumor cells; 3, strong staining in some tumor cells and intermediate staining in almost all tumor cells; and 4, greatly increased staining in almost all tumor cells compared with that in normal glial cells. Three investigators scored every sample independently; scores agreed upon by at least 2 investigators were accepted.

For MIB-1 immunostaining, antibody against the Ki 67 antigen (DAKO) was diluted 1:50 and used as previously described.[11] In each case, MIB-1 immunostaining was performed on the same serial sections used for WT1 immunohistochemical evaluation. The staining index reflecting each tumor's proliferation activity was determined by calculating the percentage of positively stained tumor cell nuclei out of 1000 evaluated tumor cell nuclei. All assessments were made in areas with the greatest degree of immunostaining.

*The WT1 peptide/HLA-A*2402 Tetramer Assay of WT1-Specific CTLs*

The WT1 (a natural, HLA-A*2402–restricted, 9-mer WT1 peptide)/HLA-A*2402 tetramer was kindly provided by M. Gotoh of Sumitomo Pharmaceuticals. This tetramer stained > 90% of the TAK-1 cells, which were WT1-specific CTLs that could recognize the complex of the natural 9-mer WT1 peptide and HLA-A*2402 molecules. The PBMCs from HLA-A*2402–positive patients were double-stained with PerCP-CD8 antibody (BD Pharmingen) and phycoerythrin tetramer. The cells were analyzed by fluorescence-activated cell sorting. A double-positive fraction was considered to represent WT1-specific CD8-positive CTLs.

Evaluation of Toxicity

Blood samples were evaluated every week. Toxicities were evaluated according to the US National Cancer Institute Common Toxicity Criteria and independently reviewed by the DSMC.

Evaluation of MR Images

Magnetic resonance imaging was performed every 4 weeks. After the WT1 vaccine was administered 12 times, the antitumor effect of the treatment was assessed by determining the response of the target lesions on MR images. The tumor size, corresponding to the contrast-enhanced area on T1-weighted MR images, was measured and analyzed according to RECIST,[23] with results reported as complete response, partial response, stable disease, and progressive disease. The response rate was calculated as the percentage of the number of cases in which there was a complete or partial response divided by the total number of cases. The effective rate was calculated as the percentage of the number of cases in which there was a complete or partial response or stable disease divided by the total number of cases.

Additional Vaccinations and Calculation of the Survival Period

If an effect was observed after 12 vaccinations, further WT1 vaccination at 1-week intervals was given only with the patients' informed consent. The PFS period was calculated from the day of the first WT1 vaccination to the day of the last image prior to the detection of disease progression; this was used as the principal end point. The overall survival period after WT1 vaccination was also calculated, as was the overall survival period after tumor recurrence for WT1-vaccinated patients.

Statistical Analysis

Our main objectives were to evaluate the duration of PFS, the 6-month PFS rate, the overall response rate, the disease control rate, and toxicity based on the WHO criteria. The objective assessments of tumor response were reported using RECIST and were based on major changes in tumor size seen on Gd-enhanced MR images in comparison with the baseline images. Hematological and nonhematological toxicities were assessed using the US National Cancer Institute Common Toxicity Criteria, and the safety and tolerability of the treatment were estimated. Statistical evaluation was performed using Stat View version 4.5 (Abacus Concepts, Inc.). Probability values < 0.05 were considered statistically significant. The Kaplan–Meier method was used to analyze overall survival and PFS. The log-rank test was used to assess the strength of the association between survival time and single variables corresponding to factors that were considered prognostic for survival.

The required sample size for this Phase II trial was estimated to be 20 at 5% Type I and 20% Type II errors, under the assumption of 10 and 30% 6-month PFS rates for the null and alternative hypotheses, respectively. Allowing for the possibility that we might not be able to obtain complete data in all cases, the sample size was set at 21.

Results

Patient Characteristics

During the trial period, 37 patients were assessed for inclusion in the trial. All 37 had WT1-positive GBM, as determined by immunohistochemical analysis. Because we use HLA-A*2402–restricted WT1 peptide, 16 patients with HLA-A*2402–negative type were excluded, and 21 patients (7 women and 14 men) with HLA-A*2402–positive type were enrolled in the study (Table 1). In all the cases involving HLA-A*2402–negative excluded patients, the survival time from recurrence or progression to death was investigated. The median survival time after tumor recurrence in the HLA-A*2402–negative patients was 21 weeks, which was almost the same as that of the historical

TABLE 1

*Characteristics of and clinical results in all enrolled patients**

Case No.	Age (yrs), Sex	RT Dosage (Gy)	Chemo	Add'l Tx	Steroid Tx	KPS Score	Response	PFS (wks)	OS (wks)	WT1 Score
1	63, M	60	CE × 3	IFN	yes	50	SD	28.1	36.1	4
2	33, M	60	—	—	yes	60	PR	23.4	32.4	4
3	45, M	60	CE × 3	IFN	yes	70	PD	5.1	32.6	1
4	29, F	60	CE × 3, ACNU × 2	IFN	—	90	SD	16.0	30.1	2
5	69, M	60	—	IFN	—	80	PD	8.0	36.7	3
6	69, M	60	CE × 3	IFN	—	80	SD	24.4	106.1	3
7	42, M	50	—	—	—	60	SD	32.0	87.1	4
8	46, F	56	—	SRS	yes	60	SD	>96.0	>96.0	3
9	63, M	60	ACNU × 3	SRS	yes	80	PD	0	>87.3	4
10	67, M	60	ACNU × 3	IFN	—	90	PD	4.0	15.0	3
11	40, F	60	ACNU × 3	—	—	80	SD	51.3	69.4	3
12	76, M	60	ACNU × 3	IFN	yes	70	SD	21.1	>79.4	1
13	54, M	50	CE × 3	IFN	yes	50	PD	4.0	18.4	2
14	55, M	60	CE × 3	IFN	—	90	PD	2.0	28.4	2
15	58, F	60	CE × 3	IFN	—	90	SD	42.4	61.7	3
16	20, F	60	ACNU × 2	—	—	90	PR	20.0	29.3	4
17	42, M	60	—	—	—	90	PD	4.3	35.6	3
18	41, M	60	CE × 3, ACNU × 2	SRS	yes	100	SD	>43.6	>43.6	3
19	54, M	60	ACNU × 3	IFN	yes	90	PD	8.0	>41.6	2
20	58, F	50	—	SRS	—	50	SD	>32.1	>32.1	4
21	55, F	60	—	—	yes	100	PD	0	>31.4	4

* ACNU = nimustine hydrochloride; Add'l = additional; CE = carboplatin and etoposide; Chemo = chemotherapy; IFN = β-interferon; KPS = Karnofsky Performance Scale; OS = overall survival; PD = progressive disease; PR = partial response; RT = radiotherapy; SD = stable disease; SRS = stereotactic radiosurgery; Tx = therapy; — = not administered.

control patients at Osaka University Hospital (20 weeks, data not shown). The mean age of the 21 enrolled HLA-A*2402–positive patients was 51.4 years (range 20–76 years). Of the 21 patients, 15 had recurrent disease and 6 had disease progression after initial therapy. All patients had radiotherapy with or without chemotherapy or interferon treatment. All enrolled patients had an ECOG performance status of 0–2 (Karnofsky Performance Scale score > 50), and 10 of them were receiving a maintenance dose (1–4 mg/day betamethasone) of glucocorticoid therapy at the time of vaccination due to local symptoms or symptoms of increased intracranial pressure caused by edema in the area around the tumor. Eight patients underwent surgery after recurrence for mass reduction and confirmed recurrence, and methionine-PET, thallium-SPECT, and FDG-PET were performed in all cases to confirm tumor recurrence.

Clinical Response to Vaccination

All treated patients had a local inflammatory response with erythema at the WT1 vaccine injection site. No Grade 3 or 4 toxicities were observed. Liver dysfunction was detected in Case 9, but improved after the patient's anticonvulsant therapy was changed. This event was considered by the DSMC and was judged to have had no relationship to the WT1 treatment.

A summary of patient responses to WT1 immunotherapy is shown in Table 1. Clinical responses included partial response in 2 patients; stable disease in 10 patients; and progressive disease in 9 patients, including 2 who dropped out of the trial due to tumor progression and poor general condition (Cases 10 and 13). Patients who had an effective response continued to receive vaccinations until tumor progression was demonstrated. All responses were assessed by the DSMC.

The overall response rate was 9.5%. The disease control rate, calculated from the number of patients with complete response, partial response, or stable disease in the initial 3 months (the clinical trial period) was 57.1%. The Kaplan–Meier survival probability curves are shown in Fig. 1. Median PFS in the 21 patients with GBM who were included in the study was 20.0 weeks, and the PFS rate at 6 months (26 weeks) was 33.3%. Median overall survival after initial vaccination was 36.7 weeks. Median overall survival after tumor recurrence in WT1-vaccinated patients was 46 weeks.

Two patients (Case 2 and Case 16) experienced partial response. In both cases, immunohistochemical analysis of the tumor specimens showed high WT1 expression levels, but neither patient survived for a long period (PFS of 23.4 weeks in Case 2 and 20.0 weeks in Case 16). Both patients had disease progression after the 12-week trial period, with leptomeningeal dissemination of the glioma cells and formation of a mass at a different site.

In contrast, in the stable disease group 4 patients (Cases 8, 11, 15, and 18) experienced gradual tumor stabilization; that is, they had a response during the late period of the 3-month WT1 vaccination course. These patients survived for a long time without progression (PFS > 96.0 weeks in Case 8, 51.3 weeks in Case 11, 42.4 weeks in Case 15, and > 43.6 weeks in Case 18).

Relationship Between PFS and WT1-Immunostaining Intensity

In all 21 patients, immunostaining was positive for WT1. The WT1 expression score was 4 in 7 cases, 3 in 8 cases, 2

in 4 cases, and 1 in 2 cases (Table 1). Figure 2 shows representative photomicrographs of Score 2 (Fig. 2A), Score 3 (Fig. 2B), and Score 4 (Fig. 2C) WT1 immunostaining, and Fig. 2D shows MIB-1-immunostaining of a section from the same lesion as Fig. 2C. Both of the patients who had a partial response to vaccination had Score 4 immunostaining. The patients were grouped according to WT1 expression scores, and PFS curves were estimated for each group and then compared. The patients with Score 3 immunostaining tended to have the longest PFS time. The patients with Score 3 or 4 had a statistically longer PFS time than the patients with Score 1 or 2 ($p = 0.0020$, Fig. 3 *right*). Among the patients with high WT1-immunostaining scores (3 and 4), the patients with Score 4 had a shorter PFS time than those with Score 3, although partial response was achieved in 2 patients with Score 4. This might reflect the fact that the patients with Score 4 had high proliferation activity of the GBM cells that was recognized by the high MIB-1 staining index, although they also had the highest amount of target WT1 protein recognized by the induced WT1-specific CTLs.

Relationship Between PFS and MIB-1 Staining Index

The MIB-1 staining index, which reflects each tumor's proliferation activity, was determined by calculating the percentage of positively stained tumor cell nuclei. No statistical difference in PFS was observed between the 2 groups (Fig. 3 *left*). The proliferation activity was found not to directly affect PFS after WT1 vaccination.

Evaluation of WT1-Specific CTL Frequencies in PBMCs

The frequencies of WT1-specific CTLs before WT1 vaccination were significantly higher in patients with GBM than in healthy controls ($p = 0.0019$, Fig. 4). These results indicate that the immune system in patients with WT1-expressing GBM cells responded to the WT1 protein derived from the tumor cells and elicited WT1-specific CTLs that were present before WT1 vaccination; this suggests that the WT1 protein in GBM cells is naturally immunogenic. The existence of the high frequencies of WT1-specific CTLs before WT1 vaccination may have contributed to the favorable clinical responses in patients with GBM. There was no correlation between the induction of a clinical response and WT1-specific CTL frequencies in the PBMCs of the patients prior to vaccination (Fig. 4). Furthermore, the CTL frequencies did not increase after vaccination, even in the patients who responded.

Discussion

The WT1 gene is physiologically expressed in some organs, such as the kidneys, bone marrow, and pleura. Experimental evidence shows that WT1-specific CTLs kill WT1-expressing tumor cells without killing normal cells.[34] Consistent with these data, in the present study, patients with a clinical response had adverse effects of the WT1 vaccination that were limited to local erythema at the injection sites of the WT1 vaccine.

The primary end points of this study were PFS and the PFS rate at 6 months. The objective response rate and the disease control rate with WT1 vaccination, as well as its safety and tolerability, were also estimated.

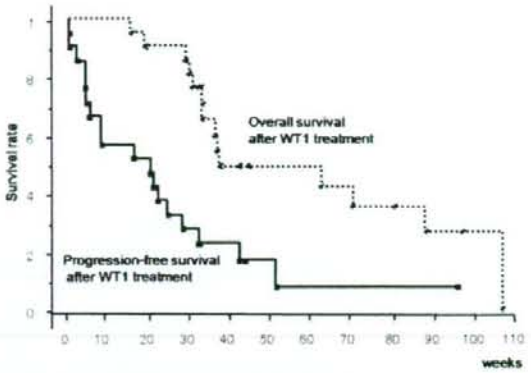

Fig. 1. Kaplan–Meier curves for PFS *(solid line)* and overall survival *(dotted line)* after initial WT1 vaccination for patients with recurrent GBM.

A review of the literature suggested that an agent demonstrating a 6-month progression-free survival rate $\geq$ 10% would be considered active.[9] A retrospective analysis of 8 Phase II chemotherapy trials conducted from 1986 to 1995 and involving a total of 225 patients with GBM was performed at the M. D. Anderson Cancer Center; a median PFS of 9 weeks and a 6-month PFS rate of 15% were reported.[28] Temozolomide, the most recent drug to be introduced for the treatment of GBM, has been shown to produce results that were not very different from those achieved with carmustine (BCNU). A study that included a series of 112 patients with GBM demonstrated a response rate of 6% with a 6-month PFS rate of 21%;[32] another study, which included a series of 138 patients with GBM, demonstrated a response rate of 8% and a 6-month PFS rate of 18%.[3] The use of BCNU chemotherapy in recurrent GBM was also recently studied; the median time to progression was 13.3 weeks, and the 6-month PFS rate was 17.5%.[4] Following these reports, 6-month PFS rates for the null and alternative hypotheses were assumed to be 10 and 30%, respectively, in this trial, and the sample size was set at 21.

In our study, the median duration of PFS was 20.0 weeks, and the PFS rate at 6 months was 33.3%. The response rate was 9.5%, whereas the disease control rate was 57.1%. The 6-month PFS rate was 33.3% in our patients with GBM—which was higher than the 10% that was set as indicating an active level—and, moreover, was higher than the 30% that was set as the alternative hypothesis before the study was started. Thus, this result suggested that WT1 vaccination was active. The median PFS and median overall survival after WT1 vaccination were 20.0 weeks and 36.7 weeks, respectively; these results are comparable to those reported in the literature for various combination regimens of chemotherapy and/or radiotherapy.

All the treated patients had an inflammatory response with erythema at the WT1 vaccine injection site, but no systemic toxicities were observed. Taken together, these findings allow one to conclude that WT1 vaccination had an anti-GBM effect, it was safe, and the patients tolerated it well.

Although the response rate in our study (9.5%) was not

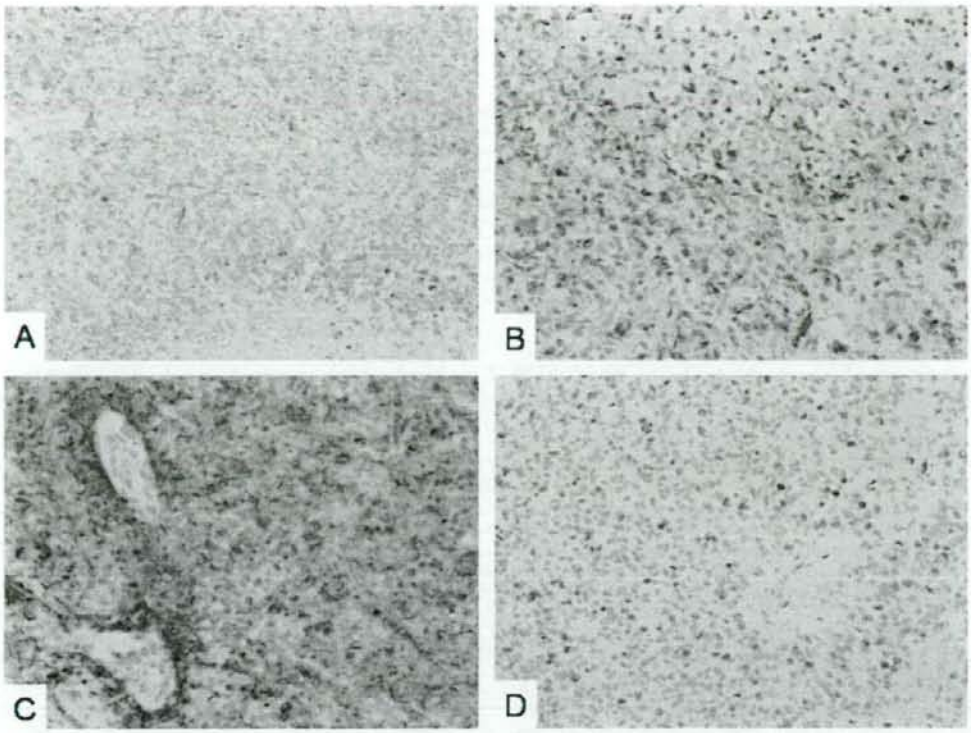

FIG. 2. Photomicrographs showing WT1 (A, B, and C) and MIB-1 (D) immunostaining. Representative WT1 immunostaining with scores of 2, 3, and 4 are shown in A, B, and C, respectively; MIB-1 immunostaining (D) in a specimen from the same case as panel C. Specimens were stained with antibody against WT1 protein (A, B, and C) or antibody against Ki 67 antigen (MIB-1) (D). Original magnification × 200.

very high compared with findings reported in chemotherapy studies, the disease control rate of 57.1% was favorable. The ability of WT1 vaccination to stabilize tumor growth might explain a good PFS of the patients treated with the vaccine. It should be emphasized that WT1 immunotherapy is less toxic than all of the chemotherapy treatments reported. Taken together, the patients in our study had a median PFS, 6-month PFS rate, and disease control rate that were comparable to those achieved using other chemotherapy regimens but with much less toxicity. These findings indicate that WT1 vaccination may be useful for the treatment of GBM.

In our study, WT1-specific CTL frequencies were higher in the PBMCs of patients with GBM than in those of healthy controls; this same phenomenon has been seen in other solid cancers.[19] The results, including good PFS and 6-month PFS rate and high stable disease rate, might be at least partly due to the high frequency of WT1-specific CTLs in the PBMCs of the patients prior to vaccination. Even in the responders, however, the CTL frequencies did not increase after vaccination. In our recent report,[19] we found a correlation between the clinical response and an increase in WT1-specific CTL frequencies in the PBMCs of cancer patients after vaccination. The correlation was clear in patients with leukemia, but it was not that clear in those with solid tumors (lung and breast cancer; unpublished data). Several cancer immunotherapy trials[2,13,14,21] have shown a poor correlation between clinical response and an increase in antigen-specific CTL frequencies. Germeau et al.[7] reported that high frequencies of the antigen-specific CTL were observed before vaccination and did not correlate the clinical response in solid cancers. They suggest that a spontaneous antitumor T-cell response that has become ineffective can be awakened by vaccination and contribute to tumor rejection. After the vaccination, CTLs in the responders might change qualitatively, but not quantitatively. The successfully activated CTLs could have more migratory ability, which would lead to the accumulation of CTLs in the brain.[12] These issues should be addressed by an intense analysis of the CTLs in WT1 vaccine-treated patients with GBM.

Immunohistochemical analysis showed that the patients with a high expression of WT1 protein in tumor specimens tended to respond well to WT1 vaccination. This finding suggests that the presence of high target antigen levels in the tumor cells plays an important role in the clinical responses. Taken together, both a high frequency of WT1-specific CTLs and a high WT1 protein expression level in tumor tissues may be needed for good clinical response to WT1 vaccination.

Under normal conditions, no lymphocytes are present in the brain parenchyma. However, tumor-infiltrating lym-

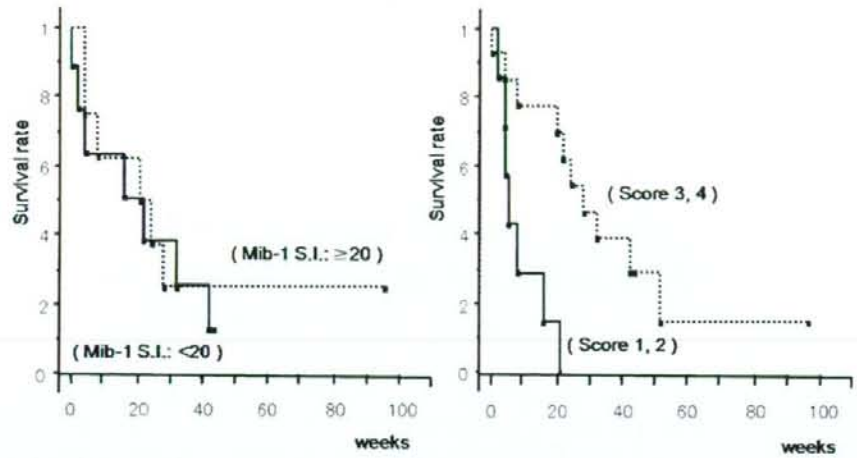

FIG. 3. *Left:* Kaplan–Meier curves for PFS after initial WT1 vaccination for patients with recurrent GBM classified by MIB-1 staining index (S.I.) determined by means of immunohistochemical analysis. The *solid line* indicates cases with an MIB-1 staining index of $< 20\%$, and the *dotted line* indicates cases with an MIB-1 staining index of $\geq 20\%$. No statistical difference in PFS was observed between the 2 groups. *Right:* Kaplan–Meier curves for PFS after initial WT1 vaccination of patients with recurrent GBM classified by WT1 expression level. The *solid line* indicates cases with low WT1 expression on tumor cells (Score 1 or 2), and the *dotted line* indicates cases with high WT1 expression on tumor cells (Score 3 or 4). Cases with scores of 3 or 4 were associated with better PFS than cases with scores of 1 or 2 ($p = 0.002$).

phocytes are found in and around the tumors in 35–80% of patients with malignant glioma;[5] this may indicate that tumor-specific CTLs would be available to attack the tumor. It has also been reported that immunosuppressive mechanisms, such as the existence of regulatory T cells,[6] hamper CTL function. Thus, the combination of a cancer vaccine with other modalities to inhibit immunosuppressive mechanisms may be useful for improving the efficacy of the vaccine.

It is probable that some cancer patients treated with cancer vaccines can survive long-term without remarkable tumor regression. On the other hand, their tumors could be stabilized or could regress following a temporary increase in size after vaccination since, in general, immunotherapy does not act as quickly as chemotherapy. In fact, some patients in the stable disease group in this study survived for a long time without the treatments achieving partial response. In Case 8, a decrease in tumor size, although it did not reach the partial response level, was observed 7 months after the initial WT1 vaccination. Furthermore, in some of the patients whose clinical response was classified as progressive disease (Cases 3 and 9), tumor stabilization was induced by WT1 vaccination at a later time during the trial. Therefore, one has to consider whether RECIST, which is the gold standard for evaluating the response of solid tumors to cancer chemotherapy, is suitable for evaluating the clinical response to cancer vaccine treatment.[18]

The mechanisms of tumor escape from immune recognition/destruction are thought to be multifactorial. They include: downregulation of major histocompatibility complex Class I molecules, loss of tumor antigens, defective death receptor signaling, lack of costimulation, and the production of immunosuppressive cytokines and suppressive cells.[1]

Given the many different potential mechanisms, combination therapy strategies that use several treatment modalities could include sequential chemotherapy, radiotherapy, and immunotherapy protocols; these will need to be considered.[27]

Conclusions

In HLA-A*2402–positive patients with GBM, immu-

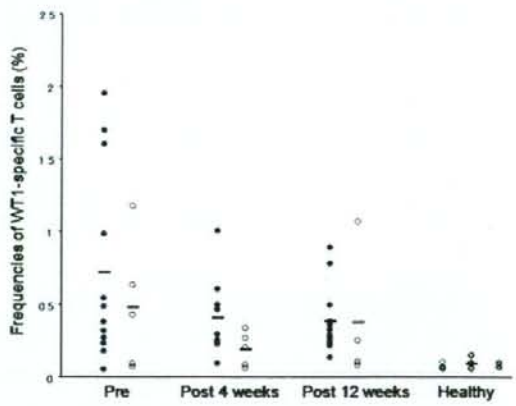

FIG. 4. Graph showing the frequencies of WT1-specific CTLs before WT1 vaccination, 4 and 12 weeks after WT1 vaccination, and in healthy controls. Patients with controlled disease (partial response or stable disease, *closed circles*) as well as those with uncontrolled disease (progressive disease, *open circles*) had a higher frequency of WT1-specific CTLs during the entire evaluation period than healthy controls *(diamonds)*. The *horizontal bars* indicate mean frequencies.

notherapy with HLA-A*2402–restricted, modified 9-mer WT1 peptide vaccination had disease-stabilizing, as well as disease progression–inhibiting, effects that were equal or superior to those of chemotherapy, with systemic toxicity that was much less than that of chemotherapy and thus allowed the vaccinations to be given for a long time. The WT1 protein is considered to be one of the most promising tumor antigens, since injection of a single WT1 peptide type can induce a clinical response. This is another advantage of the vaccine—one does not need to choose a suitable combination of peptides in the laboratory before vaccination. Compared with dendritic cell therapy, WT1 vaccination is simple. The use of a more suitable adjuvant, such as *Mycobacterium bovis* bacillus Calmette–Guérin cell wall skeleton (BCG-CWS),[16] or combination therapy involving vaccination[10] and other modalities may further enhance the clinical usefulness of this treatment for patients with GBM.

Disclaimer

The authors have no conflicts of interest related to this paper.

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References

1. Ahmad M, Rees RC, Ali SA: Escape from immunotherapy: possible mechanisms that influence tumor regression/progression. **Cancer Immunol Immunother 53:**844–854, 2004
2. Andersen MH, Keikavoussi P, Brocker EB, Schuler-Thurner B, Jonassen M, Sondergaard I, et al: Induction of systemic CTL responses in melanoma patients by dendritic cell vaccination: cessation of CTL responses is associated with disease progression. **Int J Cancer 94:**820–824, 2001
3. Brada M, Hoang-Xuan K, Rampling R, Dietrich PY, Dirix LY, Macdonald D, et al: Multicenter phase II trial of temozolomide in patients with glioblastoma multiforme at first relapse. **Ann Oncol 12:**259–266, 2001
4. Brandes AA, Tosoni A, Amistà P, Nicolardi L, Grosso D, Berti F, et al: How effective is BCNU in recurrent glioblastoma in the modern era? A phase II trial. **Neurology 63:**1281–1284, 2004
5. de Micco C: Immunology of central nervous system tumors. **J Neuroimmunol 25:**93–108, 1989
6. Fecci PE, Mitchell DA, Whitesides JF, Xie W, Friedman AH, Archer GE, et al: Increased regulatory T-cell fraction amidst a diminished CD4 compartment explains cellular immune defects in patients with malignant glioma. **Cancer Res 66:**3294–3302, 2006
7. Germeau C, Ma W, Schiavetti F, Lurquin C, Henry E, Vigneron N, et al: High frequency of antitumor T cells in the blood of melanoma patients before and after vaccination with tumor antigens. **J Exp Med 201:**241–248, 2004
8. Hashiba T, Izumoto S, Kagawa N, Suzuki T, Hashimoto N, Maruno M, et al: Expression of WT1 protein and correlation with cellular proliferation in glial tumors. **Neurol Med Chir (Tokyo) 47:** 165–170, 2007
9. Hosli P, Sappino AP, de Tribolet N, Dietrich PY: Malignant glioma: Should chemotherapy be overthrown by experimental treatments? **Ann Oncol 9:**589–600, 1998
10. Itoh K, Yamada A: Personalized peptide vaccines: a new therapeutic modality for cancer. **Cancer Sci 97:**970–976, 2006
11. Izumoto S, Suzuki T, Kinoshita M, Hashiba T, Kagawa N, Wada K, et al: Immunohistochemical detection of female sex hormone receptors in craniopharyngiomas: correlation with clinical and histologic features. **Surg Neurol 63:**520–525, 2005
12. Jomgeow T, Oji Y, Tsuji N, Ikeda Y, Ito K, Tsuda A, et al: Wilms' tumor gene WT1 17AA(-)/KTS(-) isoform induces morphological changes and promotes cell migration and invasion in vitro. **Cancer Sci 97:**259–270, 2006
13. Kammula US, Lee KH, Riker AI, Wang E, Ohnmacht GA, Rosenberg SA, et al: Functional analysis of antigen-specific T lymphocytes by serial measurement of gene expression in peripheral blood mononuclear cells and tumor specimens. **J Immunol 163:** 6867–6875, 1999
14. Mackensen A, Meidenbauer N, Vogl S, Laumer M, Berger J, Andreesen R: Phase I study of adoptive T-cell therapy using antigen-specific CD8+ T cells for the treatment of patients with metastatic melanoma. **J Clin Oncol 24:**5060–5069, 2006
15. Morita S, Oka Y, Tsuboi A, Kawakami M, Maruno M, Izumoto S, et al: A phase I/II trial of a WT1 (Wilms' tumor gene) peptide vaccine in patients with solid malignancy: safety assessment based on the phase I data. **Jpn J Clin Oncol 36:**231–236, 2006
16. Nakajima H, Kawasaki K, Oka Y, Tsuboi A, Kawakami M, Ikegame K, et al: WT1 peptide vaccination combined with BCG-CWS is more efficient for tumor eradication than WT1 peptide vaccination alone. **Cancer Immunol Immunother 53:**617–624, 2004
17. Oka Y, Tsuboi A, Elisseeva OA, Udaka K, Sugiyama H: WT1 as a novel target antigen for cancer immunotherapy. **Curr Cancer Drug Targets 2:**45–54, 2002
18. Oka Y, Tsuboi A, Kawakami M, Elisseeva OA, Nakajima H, Udaka K, et al: Development of WT1 peptide cancer vaccine against hematopoietic malignancies and solid cancers. **Curr Med Chem 13:**2345–2352, 2006
19. Oka Y, Tsuboi A, Taguchi T, Osaki T, Kyo T, Nakajima H, et al: Induction of *WT1* (Wilms' tumor gene)-specific cytotoxic T lymphocytes by WT1 peptide vaccine and the resultant cancer regression. **Proc Natl Acad Sci U S A 101:**13885–13890, 2004
20. Oka Y, Udaka K, Tsuboi A, Elisseeva OA, Ogawa H, Aozasa K, et al: Cancer immunotherapy targeting Wilms' tumor gene WT1 product. **J Immunol 164:**1873–1880, 2000
21. Romero P, Cerottini JC, Speiser DE: Monitoring tumor antigen specific T-cell responses in cancer patients and phase I clinical trials of peptide-based vaccination. **Cancer Immunol Immunother 53:**249–255, 2004
22. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, et al: European Organisation for Research and Treatment of Cancer Brain Tumor and Radiotherapy Groups; National Cancer Institute of Canada Clinical Trials Group. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. **N Engl J Med 352:**987–997, 2005
23. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al: New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. **J Natl Cancer Inst 92:**205–216, 2006
24. Tsuboi A, Oka Y, Ogawa H, Elisseeva OA, Li H, Kawasaki K, et al: Cytotoxic T-lymphocyte responses elicited to Wilms' tumor gene WT1 product by DNA vaccination. **J Clin Immunol 20:** 195–202, 2000
25. Tsuboi A, Oka Y, Ogawa H, Elisseeva OA, Tamaki H, Oji Y, et al: Constitutive expression of the Wilms' tumor gene WT1 inhibits the differentiation of myeloid progenitor cells but promotes their proliferation in response to granulocyte-colony stimulation factor (G-CSF). **Leuk Res 23:**499–505, 1999
26. Tsuboi A, Oka Y, Udaka K, Murakami M, Masuda T, Nakano A, et al: Enhanced induction of human WT1-specific cytotoxic T lymphocytes with a 9-mer WT1 peptide modified at HLA-A*2402-binding residues. **Cancer Immunol Immunother 51:** 614–620, 2002

27. Wheeler CJ, Das A, Liu G, Yu JS, Black KL: Clinical responsiveness of glioblastoma multiforme to chemotherapy after vaccination. **Clin Cancer Res 10:**5316–5326, 2004
28. Wong ET, Hess KR, Gleason MJ, Jaeckle KA, Kyritsis AP, Prados MD, et al: Outcomes and prognostic factors in recurrent glioma patients enrolled onto phase II clinical trials. **J Clin Oncol 17:**2572–2578, 1999
29. Yajima N, Yamanaka R, Mine T, Tsuchiya N, Homma J, Sano M, et al: Immunologic evaluation of personalized peptide vaccination for patients with advanced malignant glioma. **Clin Cancer Res 11:**5900–5911, 2005
30. Yamanaka R, Homma J, Yajima N, Tsuchiya N, Sano M, Kobayashi T, et al: Clinical evaluation of dendritic cell vaccination for patients with recurrent glioma: results of a clinical phase I/II trial. **Clin Cancer Res 11:**4160–4167, 2005
31. Yu JS, Liu G, Ying H, Yong WH, Black KL, Wheeler CJ: Vaccination with tumor lysate-pulsed dendritic cells elicits antigen-specific, cytotoxic T-cells in patients with malignant glioma. **Cancer Res 64:**4973–4979, 2004
32. Yung WKA, Albright RE, Olson J, Fredericks R, Fink K, Prados MD, et al: A phase II study of temozolomide vs. procarbazine in patients with glioblastoma multiforme at first relapse. **Br J Cancer 83:**588–593, 2000

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ORIGINAL ARTICLE

Capecitabine and paclitaxel combination chemotherapy for inoperable or recurrent breast cancer: a phase I dose-finding study by the Kinki Breast Cancer Study Group

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Abstract
Background The combination of capecitabine and paclitaxel (XP) has demonstrated synergistic antitumor activity in preclinical models. Three-weekly XP regimens have demonstrated excellent efficacy in phase II and III trials in metastatic breast cancer. We conducted a dose-finding study to identify the recommended 4-weekly XP regimen in patients with inoperable or recurrent breast cancer for phase II evaluation.
Methods Eligible patients had inoperable or recurrent breast cancer previously treated with chemotherapy (but not capecitabine or paclitaxel) in the (neo)adjuvant or metastatic setting. Each 4-week treatment cycle consisted of escalating doses of capecitabine (628 or 829 mg/m^2 twice daily [b.i.d.] on days 1–21) and paclitaxel (80 or 90 mg/m^2 on days 1, 8, and 15). Dose-limiting toxicities (DLT) were evaluated during the first two cycles.
Results Nine patients were treated. At dose level 1 (capecitabine 628 mg/m^2 b.i.d. plus paclitaxel 80 mg/m^2), one patient experienced a DLT (grade 3 non-hematologic toxicity). There were no further DLTs at dose level 1 or 2. Although the MTD was not reached, dose level 2 (capecitabine 829 mg/m^2 b.i.d., days 1–21, plus paclitaxel 80 mg/m^2, days 1, 8, and 15, every 28 days) is recommended for phase II evaluation, taking into consideration the single-agent doses used in Japan and the doses identified in Western studies of 3-weekly XP. The overall response rate was 44%; all patients treated at dose level 2 achieved a partial response.
Conclusions This 4-weekly XP regimen was well tolerated, active in patients with pretreated advanced breast cancer, and could be given as outpatient treatment. These results are consistent with findings of phase II and III trials evaluating 3-weekly regimens, and indicate that further investigation of a 4-weekly XP regimen is warranted.

Keywords Metastatic breast cancer · Capecitabine · Weekly · Paclitaxel · Phase I

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Introduction

Anthracycline-containing regimens, such as doxorubicin and cyclophosphamide (AC), cyclophosphamide, doxorubicin, and 5-fluorouracil (CAF), and 5-fluorouracil, epirubicin, and cyclophosphamide (FEC), are highly active and widely used as primary systemic therapy (neoadjuvant or adjuvant), reducing the risks of recurrence and death compared with non-anthracycline-containing regimens [1]. Nevertheless, most patients develop metastatic disease. Further anthracycline therapy is frequently not possible because of the increased risk of cardiotoxicity, and the benefit of re-exposure to anthracyclines is unclear. Consequently, the search for new, more effective treatments for metastatic breast cancer (MBC) is ongoing.

The taxanes, including paclitaxel, are among the most active agents for the treatment of MBC [2–6] and are increasingly being used in the adjuvant setting for high-risk patients. In a Japanese study, paclitaxel produced a 33% response rate in anthracycline-pretreated patients with advanced or metastatic disease [7]. Results of the CALGB 9840 trial indicate that weekly paclitaxel is more effective than a 3-weekly schedule, resulting in a significantly higher response rate (40 vs. 28%, respectively; OR = 1.61, $P = 0.017$) and longer time to progression (adjusted hazard ratio = 1.45, $P = 0.0008$; median = 9 vs. 5 months, respectively), although the trend towards improved overall survival with the weekly regimen did not reach statistical significance [8]. Early results from the Anglo-Celtic IV trial, presented at ASCO 2007, support these findings [9]. The administration schedule of paclitaxel also affects the safety profile, with less myelosuppression but more neurotoxicity seen with the weekly regimen [8, 10]. In Japan, weekly taxane monotherapy is currently introduced as the standard therapy after anthracycline failure. We have previously reported the antitumor activity of weekly paclitaxel in docetaxel-resistant MBC [11].

The oral fluoropyrimidine capecitabine, which was created in the Chugai Pharmaceutical Kamakura Research Laboratories (at that time the Nippon Roche KK, Research Center), generates 5-fluorouracil preferentially at the tumor site through exploitation of the significantly higher concentration of thymidine phosphorylase (TP) in tumor versus normal tissue [12]. Capecitabine monotherapy has demonstrated consistently high activity and excellent tolerability in anthracycline- and/or taxane-pretreated MBC [13–17] and in the first-line setting [18–21]. The high single-agent activity and good tolerability of capecitabine make it an attractive combination partner. Furthermore, in preclinical models, upregulation of TP by agents such as docetaxel, paclitaxel, and cyclophosphamide results in synergistic antitumor activity when co-administered with capecitabine [22, 23]. In the clinical setting, the combination of capecitabine and docetaxel in anthracycline-pretreated MBC significantly improves response rate, time to progression, and overall survival compared with docetaxel alone [24]. In addition, results of a randomized, phase II trial presented at ASCO 2006 indicated that in the first-line setting, the combination of capecitabine and docetaxel significantly improved response rate, time to progression, and overall survival compared with sequential docetaxel followed at progression by capecitabine [25]. Several phase II and III trials have demonstrated that the combination of capecitabine and paclitaxel (XP, with paclitaxel administered using a weekly or a 3-weekly schedule) is also highly active [26–30]. In a recently reported randomized, phase III trial, XP demonstrated similar efficacy to epirubicin plus paclitaxel, with median overall survival of 25.6 months and median progression-free survival of 12.0 months at interim analysis [30].

Previously reported clinical trials have evaluated XP administered using 3-weekly cycles, with capecitabine given according to the standard schedule used in Europe and the USA (capecitabine twice daily (b.i.d.) for 14 days followed by a 7-day rest period). In Japan, however, a 4-weekly regimen with capecitabine given on days 1–21 followed by a 7-day rest period has been investigated [31–33]. This regimen was selected for further development based on cutaneous effects observed in an early dose-finding study of a continuous regimen in Japanese patients [34]. Treatment with capecitabine monotherapy is covered by National Health Insurance only if administered using this 4-weekly schedule at a dose of 829 mg/m^2 b.i.d. As a result, a 4-weekly schedule is commonly adopted in clinical practice in Japan. Similarly, paclitaxel treatment for MBC typically consists of weekly administration of 80 mg/m^2 on days 1, 8, and 15 every 28 days. Therefore a 28-day XP combination regimen may be a valid alternative to the 21-day regimen developed in most Western countries.

Patients and methods

Study design

The primary objective of this multicenter, phase I, dose-finding study conducted by the Kinki Breast Cancer Study Group (KBCSG) was to identify the maximum tolerated dose of a 28-day regimen of capecitabine in combination with weekly paclitaxel in patients with advanced or recurrent breast cancer, and determine the recommended dose for a phase II study. Secondary objectives included estimation of the proportion of patients achieving a response or stable disease 6 months after treatment initiation and assessment of the safety profile. A standard 3 + 3 dose–escalation design was used.

The protocol was approved by the institutional review board at each center. The study was conducted in accordance with Good Clinical Practice Guidelines (Sixth International Conference on Harmonisation and the Declaration of Helsinki). All patients provided written informed consent.

Eligibility criteria

Female patients with histologically or cytologically confirmed breast cancer and evidence of measurable metastatic disease using Response Evaluation Criteria in Solid Tumors (RECIST) were eligible for this study. Main inclusion criteria were: age 20–75 years, ECOG performance status 0 or 1, normal renal, hepatic, and hematologic function confirmed by a prestudy examination (white blood cell count $\geq$ 4,000/mm^3 and $\leq$12,000/mm^3, neutrophil count $\geq$ 2,000/mm^3, platelet count $\geq$ 100,000/mm^3, hemoglobin $\geq$ 9.0 g/dl, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) $\leq$ 2.5 $\times$ upper limit of clinic normal (ULN), total bilirubin < 1.25 $\times$ ULN, serum creatinine < 1.5 $\times$ ULN, creatinine clearance > 50 ml/min), and no prior radiotherapy to the target lesion. At least 2 weeks must have elapsed since completing previous endocrine therapy and 4 weeks must have elapsed since completion of previous chemotherapy, radiotherapy, surgery, or treatment with an investigational new drug. Patients were ineligible if they had previously received a taxane or capecitabine.

Treatment

Capecitabine was administered orally at a twice-daily dose of 628 mg/m^2 (dose level 1) or 829 mg/m^2 (dose levels 2 and 3) taken within 30 min after morning and evening meals, for 21 consecutive days. Paclitaxel was administered intravenously as a 60-minute infusion at a dose of 80 mg/m^2 (levels 1 and 2) or 90 mg/m^2 (level 3) on days 1, 8, and 15. These initial doses were selected based on the anticipated increase in both efficacy and toxicity compared with single-agent administration, as is usual in combination studies. Table 1 shows the dose–escalation scheme. Premedication prior to paclitaxel administration was mandatory and consisted of i.v. dexamethasone 20 mg, oral diphenhydramine hydrochloride 50 mg, and i.v. ranitidine 50 mg. If no hypersensitivity reactions occurred after the first dose of paclitaxel, the dexamethasone dose could be reduced to 8 mg from cycle 2 onwards at the discretion of the treating physician. If the first two cycles of XP combination therapy were well tolerated, patients could continue to receive combination therapy until disease progression or unacceptable toxicity.

Table 1 Dose-escalation scheme

Dose level	Paclitaxel days 1, 8.15 (mg/m^2)	Capecitabine days 1–21 (mg/m^2) b.i.d
−1	70	628
1	80	628
2	80	829
3	90	829

b.i.d. twice daily

Dose–escalation scheme

The first cohort of three patients was treated at dose level 1. Dose-escalation decisions were based on the occurrence of dose-limiting toxicities (DLT) during the first two cycles. DLTs were defined as any of the following during the first two cycles of chemotherapy: (1) grade 4 leukopenia (<1,000/mm^3) or neutropenia (<500/mm^3) lasting for >4 days; (2) toxicity resulting in omission of two or more paclitaxel doses; (3) fever (>38.0°C) associated with grade 3 or 4 neutropenia (<1,000/mm^3) or clinical infection; (4) grade 4 thrombocytopenia (<25,000/mm^3); (5) grade 3 hand–foot syndrome [12]; (6) other grade 4 non-hematologic toxicity (excluding hair loss, nausea/vomiting, and anorexia); (7) toxicity resulting in a delay of >7 days before administration of the second or third cycle. Any events other than those described above could be classified as a DLT at the discretion of the data and safety monitoring committee.

If none of the three patients in a cohort experienced a DLT, three patients were to be treated at the next dose level. If one of the three patients experienced a DLT, three additional patients were to be treated at the same dose level. If no further DLTs occurred, three patients were to be treated at the next dose level. If a DLT occurred in two or more of a cohort of six patients, or two or more of a cohort of three patients, that dose level was to be defined as the maximum tolerated dose and the previous dose level was to be defined as the recommended dose.

Study assessments

Adverse events were graded using the National Cancer Institute Common Toxicity Criteria (CTCAE v3.0). Details of all adverse events and laboratory abnormalities and their relationship to study treatment were to be recorded on the Case Report Form. Tumor lesions were measured at baseline and after every second cycle using RECIST.

Results

Patient characteristics

Between November 2003 and June 2005, nine women were enrolled, all of whom had previously received chemotherapy. Six received dose level 1 and three received dose level 2. Baseline characteristics are summarized in Table 2. XP was given as first-line chemotherapy for MBC in three patients, second-line therapy in five patients and third-line therapy in one patient.

Tolerability

Table 3 shows all adverse events occurring in more than one patient during the first two cycles of therapy. Among the first cohort of three patients treated at dose level 1 (capecitabine 628 mg/m^2 b.i.d., paclitaxel 80 mg/m^2), one experienced grade 3 non-hematologic toxicity (peripheral neuropathy, malaise, muscle pain, difficulty in walking due to pelvic pain), meeting the criteria for DLT. Therefore, three additional patients were recruited to dose level 1. There were no further DLTs at this dose level. Three patients were enrolled to dose level 2, none of whom experienced any DLT. Hand-foot syndrome was observed at grade 1 intensity in two patients and grade 2 intensity in four patients. There were no grade 3 cases in any of the patients treated in this study and there was no evidence that concomitant use of paclitaxel exacerbated hand-foot syndrome.

When all three patients had completed combination treatment at level 2, tolerability was evaluated by the data and safety monitoring committee. The panel decided that as both capecitabine and paclitaxel had been administered at their full, Japanese regimen, single-agent doses (capecitabine 829 mg/m^2 b.i.d., paclitaxel 80 mg/m^2), and the dose intensity of level 2 exceeded the doses adopted in clinical trials of this combination in the USA, escalation to dose level 3 should not proceed. Consequently, the recommended regimen for phase II evaluation is capecitabine 829 mg/m^2 b.i.d. on days 1–21 in combination with paclitaxel 80 mg/m^2 on days 1, 8, and 15, both repeated every 28 days.

Table 2 Baseline characteristics ($n = 9$)

Median age, years (range)	55 (47–66)
ECOG performance status	
0	4
1	5
Menopausal status	
Premenopausal	1
Postmenopausal	8
Sites of metastases	
Liver	6
Lung	3
Pleura	1
Bone	5
Skin	1
Lymph node	4
Prior adjuvant chemotherapy	
None	4
CMF	2
Anthracycline	2
Oral 5-fluorouracil	1
Prior chemotherapy for MBC	
None	3
Anthracycline	5
Docetaxel	1

CMF cyclophosphamide methotrexate 5-fluorouracil, *MBC* metastatic breast cancer

Table 3 Adverse events occurring in more than one patient during the first two cycles of therapy

	Level 1 ($n = 6$)		Level 2 ($n = 3$)	
Capecitabine (mg/m^2 b.i.d., days 1–21)	628		829	
Paclitaxel (mg/m^2, days 1, 8,15)	80		80	
Grade	2	3	2	3
Hematologic				
Leukopenia	3	0	3	0
Neutropenia	0	1	3	0
Non-hematologic				
Anorexia	0	0	1	0
Nausea	0	0	1	0
Vomiting	1	0	2	0
Diarrhea	0	0	2	0
Constipation	1	0	1	0
Neurotoxicity (sensory)	1	1	0	0
Fatigue	1	1	0	0
Muscle pain	0	1	0	0
Arthralgia	1	0	0	0
Hand–foot syndrome	3	0	1	0
Alopecia	2	0	0	0
Dysgeusia	0	0	2	0
Hypertension	0	0	1	0
Desquamation	0	0	2	0
Laboratory				
AST	1	0	0	0
ALT	0	0	1	0

ALT alanine aminotransferase, *AST* aspartate aminotransferase

Antitumor efficacy

Partial responses were confirmed in all three patients receiving dose level 2 and one of the two patients receiving dose level 1 as first-line therapy, giving an overall response rate of 44% (4/9 patients). The other patient receiving dose level 1 as first-line therapy achieved stable disease. Among the remaining four patients receiving dose level 1 as second- ($n = 3$) or third-line ($n = 1$) therapy, three-showed disease progression and one was not evaluable.

Discussion

The 4-weekly XP schedule investigated in this study is well tolerated and active. We recommend a regimen of capecitabine 829 mg/m^2 b.i.d. on days 1–21 plus paclitaxel 80 mg/m^2 on days 1, 8, and 15, both repeated every 28 days, for phase II evaluation. Our results provide an early indication that our 4-weekly XP regimen is a valid alternative to the 3-weekly regimen used in other parts of the world.

In patients with less aggressive, asymptomatic, small-volume breast cancer following anthracycline- and/or taxane-containing (neo)adjuvant therapy, oral capecitabine monotherapy is considered an acceptable first-line therapy, enabling patients to maintain good quality of life. At disease progression, more intensive chemotherapy, including intravenous taxane therapy, may be considered.

In patients with more aggressive disease who require rapid reduction in tumor burden, the regimen offering the highest response rate should be considered. This strategy is important because if first-line therapy is ineffective, resulting in increased tumor volume, further treatment may be compromised because of extensive tumor growth, deterioration in general health, or worsening symptoms. This concept is supported by results of the randomized, phase III trial reported by O'Shaughnessy et al. [24], in which 35% of patients initially randomized to docetaxel received no further chemotherapy at progression, thus denying them the opportunity to benefit from other agents [35]. Further support comes from a randomized clinical trial comparing capecitabine and docetaxel combination treatment versus sequential administration of these two agents [25]. In this trial, response rate, progression-free survival, and overall survival were significantly superior in patients receiving combination versus sequential treatment. Similarly, the authors of the Mexican Oncology Group Study concluded that "when rapid response is the primary goal, patients should receive combination therapy (capecitabine plus taxane). If long-term outcomes and quality of life are more important, capecitabine followed by a taxane is an equally appropriate choice" [21].

The value of capecitabine/taxane combination regimens has been seen in numerous clinical trials [24–30, 36–38]. Phase I and II studies conducted in Europe and North and South America have explored different doses and schedules of XP. In several of these studies [28, 36, 38], a capecitabine dose of 1,000 mg/m^2 b.i.d., days 1–14 every 21 days, has been adopted. This dose was also used in combination with 3-weekly paclitaxel in the randomized phase III trial versus epirubicin/paclitaxel as first-line therapy for MBC [30]. The capecitabine dose recommended based on results of the present study provides a dose intensity of 8,699 mg/m^2/week, very similar to the 9,333 mg/m^2/week intensity in the studies using 1,000 mg/m^2 b.i.d. in a 3-weekly regimen. Interestingly, in the US Oncology phase II study reported by Blum et al. [29], the US phase II study reported by Gradishar et al. [27], and the randomized trial by the Mexican Oncology Study Group [21], the dose intensity was only 7,700 mg/m^2/week. This difference can probably be explained by regional differences in the tolerability of fluoropyrimidines that have recently become apparent. In an analysis of more than 3,000 patients treated with 5-fluorouracil- or capecitabine-based regimens for colorectal cancer, fluoropyrimidine therapy was least well tolerated in US patients and best tolerated in Asian patients [39]. Tolerability in European patients was intermediate. Thus, subtle differences in dose intensity may be appropriate when these differences are taken into account. Recently, a 2-weekly regimen with capecitabine given on days 1–7 every 14 days has been investigated in the USA [40], based on preclinical findings and mathematical modeling [41]. Although the average weekly dose delivered using this 7/7 schedule is no higher than with the conventional schedule, it is attracting some interest in the USA. Hand–foot syndrome and diarrhea remain the DLTs. The paclitaxel dose intensity in our study was identical to that used in the study by Susnjar et al. [36].

Both the randomized phase III trial of capecitabine plus 3-weekly paclitaxel [30] and the US phase II study investigating capecitabine plus paclitaxel on days 1 and 8 [29] demonstrated remarkable efficacy. Response rates were 52 and 55%, respectively, and median overall survival was 25.6 and 17 months, respectively. The slightly lower response rate in the present small study was to be expected given that most patients received XP in the second- or third-line setting in our study, contrasting with the first-line setting in the larger studies. Moreover, this Japanese study included only nine patients and was not designed to assess efficacy. Nevertheless, antitumor activity was evident.

To further assess the efficacy and tolerability of a 4-weekly XP schedule in Japanese patients, a multicenter, phase II trial (KBCSG 0609) has been initiated using the regimen identified in the present study.