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PET With ^{11}C-Methyl-L-Methionine as a Predictor of Consequential Outcomes at the Time of Discontinuing Temozolomide-Adjuvant Chemotherapy in Patients With Residual *IDH*-Mutant Lower-Grade Glioma

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Purpose: The aim of this study was to clarify whether PET with ^{11}C-methyl-L-methionine (^{11}C-met PET) can predict consequential outcomes at the time of discontinuing temozolomide (TMZ)-adjuvant chemotherapy in patients with residual isocitrate dehydrogenase gene (*IDH*)–mutant lower-grade glioma.

Patients and Methods: Among 30 patients showing residual lesions of *IDH*-mutant lower-grade glioma, we compared the tumor-to-normal brain tissue ratio of standardized uptake values ($SUV_{T/N}$) from ^{11}C-met PET at the time of discontinuing TMZ-adjuvant chemotherapy with putative predictive factors including age, Karnofsky Performance Scale, number of courses of adjuvant therapy, residual tumor size, and promotor methylation status of O^6-methylguanine-DNA methyl-transferase gene (*MGMT*). For each factor, progression-free survival (PFS) was compared between groups divided by cutoff values, determined to predict tumor relapse using receiver operating characteristic curves for each factor. Univariate and multivariate analyses were conducted using log-rank testing and Cox regression analysis, respectively. In addition, PFS was compared between patients grouped by combined findings from multiple predictors identified from univariate and multivariate analyses.

Results: Univariate and multivariate analyses identified $SUV_{T/N}$ from ^{11}C-met PET and *MGMT* methylation status as independent predictors of outcomes after TMZ discontinuation. When comparing 3 groups assigned by the combination of *MGMT* and $SUV_{T/N}$ findings, PFS differed significantly among groups.

Conclusions: The present study suggested that ^{11}C-met PET at the time of discontinuing TMZ-adjuvant chemotherapy allows prediction of outcomes at least comparable to *MGMT* methylation status in patients with residual *IDH*-mutant lower-grade glioma. Further, ^{11}C-met PET allows more precise prediction of outcomes by assessment in combination with *MGMT* findings.

Key Words: *IDH*-mutant lower-grade glioma, ^{11}C-methionine PET, temozolomide adjuvant chemotherapy, *MGMT* methylation status

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Diffuse astrocytic tumor is the most intractable primary brain tumor encountered in adults and is classified morphologically into diffuse astrocytoma (DA), anaplastic astrocytoma (AA), and glioblastoma. In 2016, the World Health Organization urged the addition of molecular profiles of tumors such as isocitrate dehydrogenase gene (*IDH*) status, as molecular markers of prognostic relevance, to conventional morphological diagnoses.[1] Without chromosome 1p/19q codeletions as a molecular hallmark of oligodendroglial tumor, no significant differences in prognosis were identified between *IDH*-mutant DA and *IDH*-mutant AA.[2,3] As a result, *IDH*-mutant DA and *IDH*-mutant AA can be combined under the term "lower-grade glioma," accounting for the majority of DA and AA cases in adults.[1,4] Although no standard therapy for *IDH*-mutant lower-grade glioma has yet been established, temozolomide (TMZ), an oral active DNA alkylating agent, is considered one of the key antitumor drugs for patients with *IDH*-mutant lower-grade glioma.[5,6] Patients with residual *IDH*-mutant lower-grade glioma are commonly treated with TMZ-adjuvant chemotherapy on an outpatient basis after surgical intervention with or without radiotherapy. TMZ-adjuvant chemotherapy is administered for 5 consecutive days every 4 weeks for 6 cycles, 12 cycles, or even more, because the ideal number of TMZ cycles remains unclear. However, TMZ necessarily has to be discontinued due to reasons such as compliance with therapeutic protocols, patient requests, and adverse effects from TMZ, even if residual tumor remains in the brain. Under such circumstances, physicians inevitably have concerns regarding consequential tumor relapse following the discontinuation of TMZ. Precise assessment of the biological activity of residual tumor at the time of discontinuing TMZ-adjuvant therapy may provide clues to predicting consequent outcomes.

PET with radiotracer to assess metabolism is one method for precisely and reliably evaluating the biological activity of glioma in real time. Methionine (met) is an α-amino acid used in the biosynthesis of proteins in humans, and ^{11}C-methyl-L-met is a met analog tracer that is classically established and widely available for quantitative assessment of amino acid metabolism in diffuse astrocytic tumors.[7,8] To the best of our knowledge, however, few reports regarding the contribution of PET to TMZ-adjuvant therapy have been documented.[9,10] The present study focused on the assessment of amino acid metabolism within tumor using ^{11}C-met PET at the time of discontinuing TMZ-adjuvant chemotherapy in patients with residual tumor confined to *IDH*-mutant lower-grade glioma without chromosome 1p/19q codeletions. We expected that real-time assessment of amino acid metabolism using ^{11}C-met PET at the time of discontinuing TMZ-adjuvant chemotherapy would allow more precise prediction of consequential outcomes than other prognostic factors. The ability of ^{11}C-met PET to predict outcomes was evaluated by comparison with other putative prognostic factors, comprising age,

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Karnofsky Performance Scale (KPS), size of residual tumor, and number of courses of TMZ-adjuvant therapy, along with the methylation status of the O^6-methylguanine DNA methyltransferase gene (*MGMT*) promotor, as a predictor of response to alkylating and methylating chemotherapies such as TMZ in glioma.[1,5]

PATIENTS AND METHODS

Entry Criteria

The present study was performed in accordance with the precepts established by the Declaration of Helsinki. All study protocols were approved by the ethics committee at our institute (approval no. H22-96). Subjects met the following entry criteria: presence of residual tumor diagnosed as *IDH*-mutant lower-grade glioma as either DA or AA without codeletion of chromosome 1p/19q on tumor specimens from the initial surgery between January 2013 and April 2018; receipt of TMZ-adjuvant chemotherapy (150 or 200 mg/m^2 per day for 5 days every 4 weeks) in the outpatient department of our institute; residual tumor ≥1 cm in diameter located in the cerebral white matter or insular cortex had sustained stable disease according to the criterion of the response assessment of neuro-oncology (RANO)[11] during TMZ-adjuvant chemotherapy; discontinuation of TMZ-adjuvant therapy due to any reasons other than tumor relapse; performance of ^{11}C-met PET at the time of discontinuation of TMZ-adjuvant therapy; age ≥20 years but <75 years; KPS ≥ 70%; no history of treatment for brain diseases other than lower-grade glioma; and voluntary provision of written informed consent to participate in this study.

Molecular Diagnosis

Histological diagnosis and *IDH* status were based on tumor specimens obtained from the initial surgical intervention. On 3-μm-thick sections from paraffin-embedded tumor tissues, *IDH* protein was stained immunohistochemically using a mutation-specific monoclonal mouse anti–*IDH*-1 R132H antibody (1:80, clone, H09; Dianova, Hamburg, Germany). When most tumor cells showed the presence of *IDH*-1 antigen, we identified the tumor as *IDH*-mutant type. Histopathological procedures were performed by 2 investigators (N.Y., T.S.) who were blinded to other data in this study. Noncodeletion of chromosome 1p/19q was determined by fluorescence in situ hybridization at an outsourcing laboratory (SRL, Tokyo, Japan).

We evaluated *MGMT* promotor methylation status by methylation-specific polymerase chain reaction using paraffin-embedded tumor specimens obtained from the initial surgery. DNA was extracted from paraffin-embedded tumor specimens using Dexpat solution (Takara Bio, Kusatsu, Japan). Extracted DNA was then further refined by the alcohol precipitation method using the Ethachinmate (Nippon Gene, Tokyo, Japan). Bisulfite modification of genomic DNA was performed using the Epitect Bisulfite Kit (Qiagen, Hulsterweg, the Netherlands) according to recommendations of the manufacturer. Polymerase chain reaction amplification was carried out using a polymerase chain reaction kit (Zymo Taq PreMix; Zymo Research, Irvine, CA), using the methods previously described by Sonoda et al.[12] Polymerase chain reaction products were loaded onto 4% agarose gels, stained with ethidium bromide solution (Genesee Scientific, San Diego, CA), and methylated or unmethylated status of each patient was determined by detection of DNA fragments under ultraviolet illumination. All procedures for MSP were conducted by an investigator (T.I.) who was blinded to all clinical data.

Entry Patients

A total of 30 patients comprising 16 patients with *IDH*-mutant DA (age range, 25–61 years; median age, 40 years; 9 men, 7 women) and 14 patients with *IDH*-mutant AA (age range, 20–67 years; median age, 42 years; 11 men, 3 women) met the entry criteria and were enrolled to this study. All 14 patients with *IDH*-mutant AA underwent aggressive tumor resection. For *IDH*-mutant DA, 6 patients underwent needle biopsy, and the remaining 10 patients underwent aggressive tumor resection. After surgery, all patients with *IDH*-mutant AA received radiotherapy with TMZ-concomitant chemotherapy followed by TMZ-adjuvant therapy. In *IDH*-mutant DA, only 5 patients received radiotherapy before TMZ-adjuvant therapy. The remaining 11 patients decided against radiotherapy and switched to TMZ-adjuvant therapy after surgery. After initial treatments, all patients received TMZ-adjuvant therapy in the outpatient department of our institute. The number of cycles of the adjuvant therapy differed between individuals, but all patients were determined to have discontinued adjuvant therapy due to reasons other than tumor relapse. Prescription of TMZ was stopped in patients who completed the scheduled courses of TMZ under any therapeutic protocol and/or showed severe adverse effects from TMZ. For patients who requested discontinuation of TMZ, we prepared an option of restarting TMZ-adjuvant therapy according to the result from ^{11}C-met PET as described in the next section.

PET at Time of Discontinuing TMZ-Adjuvant Therapy

All patients underwent ^{11}C-met PET within 28 days after the date of the final administration of TMZ. Tracer synthesis and scan conditions have been provided in previous reports.[13,14] At 20 minutes after IV injection of ^{11}C-met at a dose of 317–346 MBq (mean, 6.5 MBq/kg body weight), PET was performed using a PET/computed tomography system (SET3000 GCT/M; Shimadzu, Kyoto, Japan). SUV_{mean} was automatically determined for all regions of interest as areas of 6 mm in diameter centered on the point of highest ^{11}C-met accumulation within the residual tumor, and in 3 regions of apparently normal cerebral white matter in the contralateral hemisphere, as previously reported.[13] The mean value for those 3 normal regions was calculated and used as the SUV_{mean} of apparently normal tissue. The tumor-to-normal brain tissue ratio of standardized uptake values ($SUV_{T/N}$) was calculated for each scan. All procedures for PET were performed by 2 investigators (T.S., K.T.) who were blinded to all clinical data. When the residual tumor showed $SUV_{T/N}$ ≥ 1.6, as a provisional cutoff suggesting high amino acid metabolism according to previous reports regarding differentiation between active glioma and other diseases,[10,15,16] patients were able to choose to either discontinue or restart TMZ after discussions between the patient and physicians.

Assessments and Analyses

We kept track of patient conditions using physical tests and conventional MRI including fluid-attenuated inversion recovery (FLAIR) and gadolinium-enhanced T1-weighted imaging in all patients. Baseline MRI was performed within 2 weeks of PET, with different dates for MRI and ^{11}C-met PET. Consecutive MRI scans were performed every 3 months for all patients, using a 3.0 T MRI system (Discovery MR750; GE Healthcare Japan, Tokyo, Japan). For each patient, findings from follow-up MRI were compared with those from the baseline MRI according to the criterion of the RANO.[11] MRI and patient conditions were evaluated during follow-up (median, 434 days; range, 84–1304 days) after baseline MRI. The period between the date of baseline MRI and the date of clinical or radiological progression or death was defined as the progression-free survival (PFS). Because subjects in this study comprised patients with *IDH*-mutant DA and *IDH*-mutant AA, we compared PFS between patients with *IDH*-mutant DA and patients with *IDH*-mutant AA using log-rank testing, to verify no difference in PFS between groups.

We aimed to clarify whether $SUV_{T/N}$ from ^{11}C-met PET is better as an outcome predictor after discontinuation of TMZ-adjuvant therapy than other putative prognostic factors of age, KPS, size of residual tumor on baseline MRI, number of cycles of TMZ-adjuvant

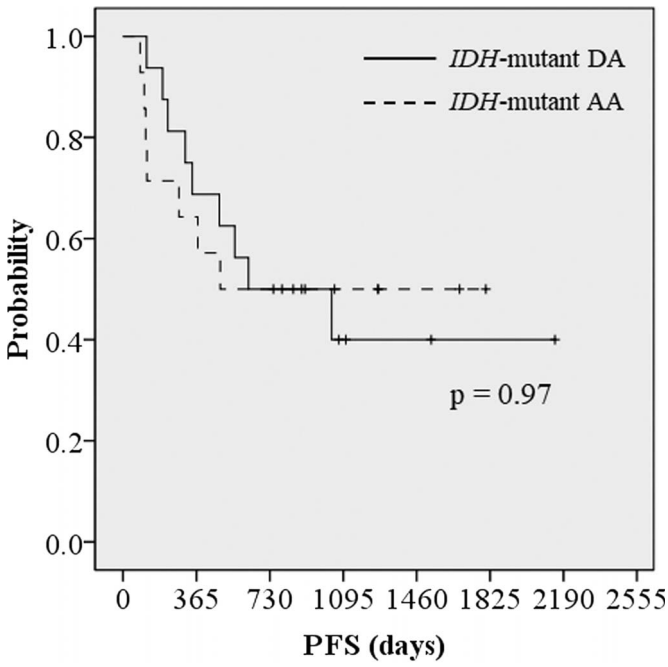

FIGURE 1. Kaplan-Meier curves in a comparison of PFS between groups of patient with *IDH*-mutant DA and *IDH*-mutant AA.

therapy, and *MGMT* promotor methylation status. For each factor except *MGMT* promotor methylation status, we determined the cutoff value maximizing both sensitivity and specificity for predicting tumor progression, using receiver operating characteristic analysis. Size of residual tumor was measured using 2-dimensional measurement as the maximum diameter multiplied by the maximum perpendicular diameter of residual tumor according to procedures defined in RANO.[11] Progression-free survival was compared between groups of patients showing equal or more than and less than the cutoff value for each factor using log-rank testing in univariate analyses. With regard to *MGMT* promotor methylation, PFS was compared between patients with methylated *MGMT* and those with unmethylated *MGMT*. Differences in PFS between groups were delineated using the Kaplan-Meier curves. We then performed a multivariate analysis using Cox proportional hazards regression modeling, applying prognostic factors conjectured to represent predictive factors based on values of $P < 0.1$ in univariate analyses. In the case that multiple predictive factors were identified from both univariate and multivariate analyses, PFS was compared between groups of patients using combined findings from the multiple identified predictors. All statistical analyses were conducted by one investigator (S.F.) using PASW Statistics version 18 software (SPSS Japan, Tokyo, Japan). Values of $P < 0.05$ were considered significant in all statistical analyses.

RESULTS

Reasons for discontinuing TMZ-adjuvant therapy were, according to the individual therapeutic protocol in 22 patients, request from the patient in 7 patients and adverse effects from TMZ in 1 patient. Twenty-two patients who needed to discontinue TMZ in accordance with their therapeutic protocol had received TMZ-adjuvant therapy, including 5 patients with 6 cycles, 12 patients with 12 cycles, and 5 patients with 24 cycles (median, 12 cycles). In the 7 patients who discontinued TMZ on their own request, the median number of courses of TMZ-adjuvant therapy was 30 cycles (range, 14–58 cycles), which was more than the number of cycles in patients who discontinued TMZ in accordance with therapeutic protocols. One patient who experienced grade 4 blood toxicity due to TMZ discontinued TMZ at 11 cycles. Since all 7 patients who requested discontinuation of TMZ showed $SUV_{T/N} < 1.6$ as the provisional cutoff, none restarted TMZ during the study period. After discontinuing TMZ, 16 patients (9 of 16 patients with DA and 7 of 14 patients with AA) experienced tumor relapse, and the remaining 14 patients survived without disease progression. The median PFS was 624 days in all patients, 624 days in DA, and 484 days in AA. Progression-free survival did not differ significantly between *IDH*-mutant DA and *IDH*-mutant AA ($P = 0.97$, Fig. 1).

On ^{11}C-met PET at the time of discontinuing TMZ-adjuvant chemotherapy, mean $SUV_{T/N}$ was 1.41 ± 0.43 in all patients, 1.42 ± 0.48 in patients with DA, and 1.41 ± 0.39 in patients with AA. Mean $SUV_{T/N}$ for 16 patients with tumor relapse was 1.51 ± 0.53 (range, 0.67–2.9), whereas that for 14 patients surviving without relapse was 1.25 ± 0.22 (range, 0.78–1.59). $SUV_{T/N}$ tended to be lower in patients without relapse than in patients with relapse, but no significant difference was identified (Mann-Whitney *U* test, $P = 0.1$). We determined the best cutoff $SUV_{T/N}$ for predicting subsequent disease progression using receiver operating characteristic analyses. Among the putative prognostic factors, optimal cutoffs were 42 years for age (sensitivity, 37.5%; specificity, 50.0%), 95% for KPS (sensitivity, 50.0%; specificity, 35.7%), 8.9 mm^2 for tumor size (sensitivity, 52.9%; specificity, 61.5%), and 13 cycles for TMZ-adjuvant therapy (sensitivity, 31.3%; specificity, 50.0%). The resulting cutoff $SUV_{T/N}$ was 1.27, offering 68.8% sensitivity and 64.3% specificity, as the highest values among the factors established in this study (Fig. 2). With regard to *MGMT*, methylation status was unable to be determined in 1 patient with *IDH*-mutant

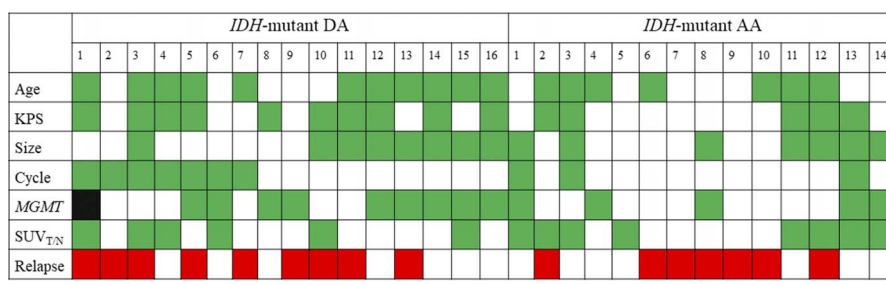

FIGURE 2. Assignment to groups with favorable outcomes according to receiver operating characteristic analysis for each factor in each patient. Numerals indicate each patient. Green squares indicate that the patient was assigned to a group with favorable outcome according to receiver operating characteristic analysis: age, <42 years; KPS, 100%; tumor size, <8.9 mm^2; adjuvant therapy, ≥13 cycles; methylated *MGMT*, and $SUV_{T/N}$ <1.27. Blank squares indicate that the patient was assigned to a group with unfavorable outcome in each factor. The black space in Case 1 for *IDH*-mutant DA indicates that *MGMT* methylation status was unavailable. Red squares indicate patients who developed tumor relapse.

TABLE 1. Univariate and Multivariate Analyses of Prognostic Factors for Tumor Progression

	Log-Rank Test	Cox Proportional Hazards Test	
Variable	***P***	**HR (95% CI)**	***P***
Age (≥ 42 vs <42 y)	0.61		
KPS (100% vs <100%)	0.21		
Size (≥8.9 vs <8.9 mm^2)	0.39		
Cycles (≥13 vs <13)	0.098	4.14 (1.11–15.5)	0.035*
MGMT (methylated vs unmethylated)	0.048*	9.70 (2.27–41.4)	0.002*
$SUV_{T/N}$ (≥1.27 vs <1.27)	0.022*	8.44 (2.19–32.5)	0.002*

*Statistical significance.
HR, hazards ratio; CI, confidence interval.

DA, due to the insufficient amount of DNA extracted from tumor specimens (case 1 in Fig. 2). In the remaining 29 patients, *MGMT* promotor methylation status was judged as methylated in 14 patients (9 patients with DA, 5 patients with AA) and unmethylated in 15 patients (6 patients with DA, 9 patients with AA).

Progression-free survival was then compared between groups divided by the cutoffs for each prognostic factor of age, KPS, residual tumor size, number of cycles of TMZ, and $SUV_{T/N}$, and between patients with methylated *MGMT* and unmethylated *MGMT*. Log-rank testing showed that $SUV_{T/N}$ and *MGMT* promotor methylation status were significantly associated with disease progression (Table 1). Kaplan-Meier curves also demonstrated significant differences in PFS for $SUV_{T/N}$ and *MGMT*, not but for the other factors (Figs. 3A–F). Considering the results from univariate analyses, we performed multivariate analysis on 3 selected factors: the number of cycles of adjuvant therapy; $SUV_{T/N}$; and *MGMT* promotor methylation status. As a result, both unmethylated *MGMT* and $SUV_{T/N} \geq 1.27$ were significantly associated with poor outcomes after discontinuing TMZ-adjuvant therapy (Table 1). The number of cycles of adjuvant therapy was identified as a relevant factor for outcome only in multivariate analysis and showed no significant relevance to PFS in univariate analysis. Assignments to groups for favorable outcomes in *MGMT* promotor methylation status and $SUV_{T/N}$ did not necessarily coincide (Fig. 2). Even in patients showing methylated *MGMT*, outcome was often reflected by the findings from $SUV_{T/N}$ (Fig. 4). All patients could be categorized into 3 groups: both $SUV_{T/N} < 1.27$ and methylated *MGMT* (5 patients); either $SUV_{T/N} < 1.27$ or methylated *MGMT* (17 patients); and neither $SUV_{T/N} < 1.27$ nor methylated *MGMT* (7 patients). Comparison of PFS between these 3 groups revealed that all 5 patients showing both $SUV_{T/N} < 1.27$ and methylated *MGMT* survived without tumor relapse, whereas tumor relapse was seen in all 7 patients showing neither $SUV_{T/N} < 1.27$ nor methylated *MGMT*. The group with findings of either $SUV_{T/N} < 1.27$ or methylated *MGMT* showed outcomes that were intermediate between the other

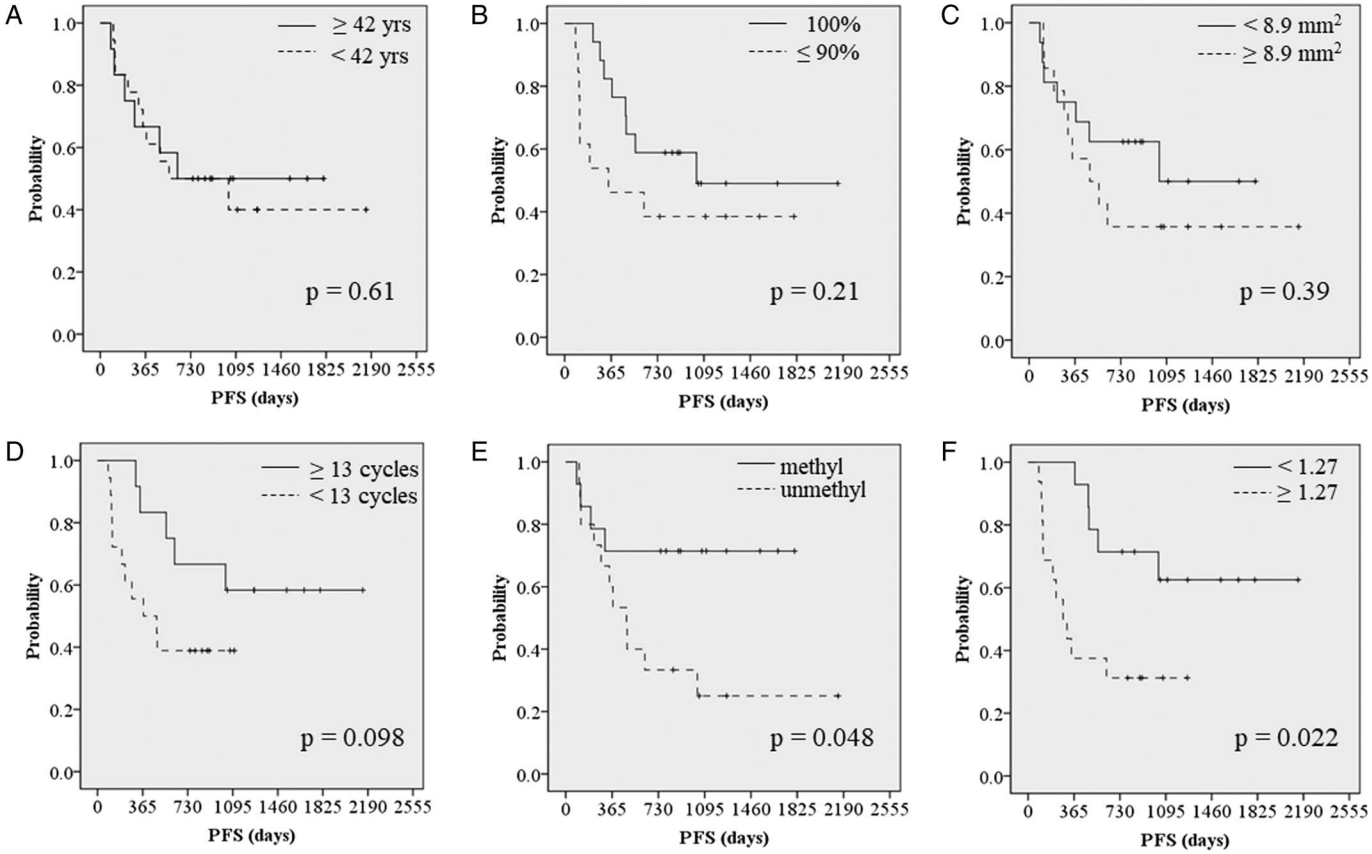

FIGURE 3. Kaplan-Meier curves for comparisons of PFS between groups of patients divided by cutoff values for each factor of age (**A**), KPS (**B**), size of residual tumor (**C**), number of cycles of TMZ-adjuvant therapy (**D**), *MGMT* promotor methylation status (**E**), and $SUV_{T/N}$ from ^{11}C-met PET (**F**).

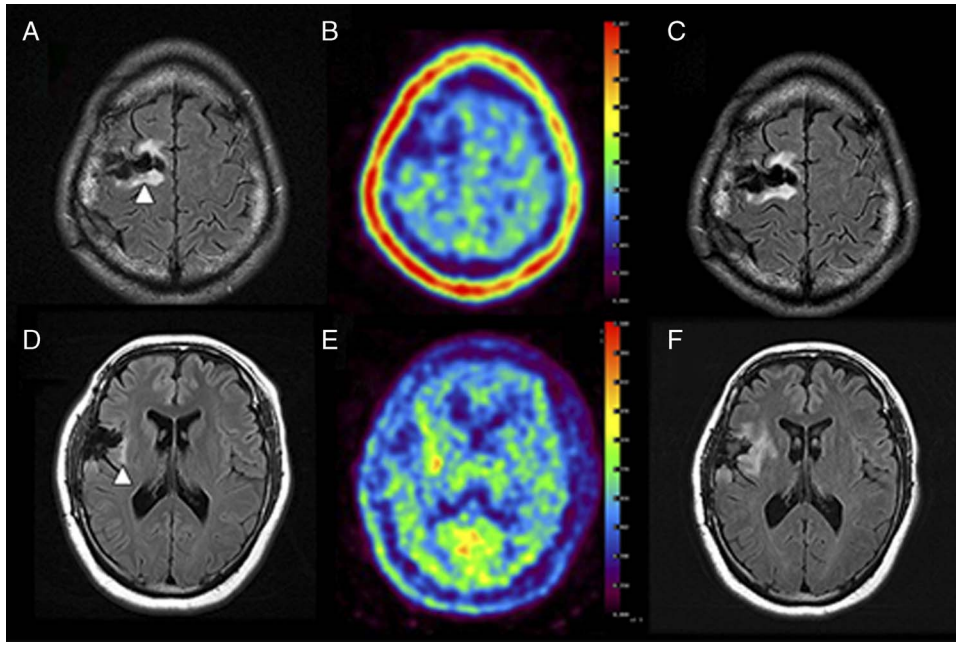

FIGURE 4. Two illustrative cases: a patient with methylated *MGMT* and $SUV_{T/N}$ of 1.08 (**A**–**C**) and a patient with methylated *MGMT* and $SUV_{T/N}$ of 1.51 (**D**–**F**). FLAIR as baseline MRI (**A**, **D**), ^{11}C-met PET (**B**, **E**) at the time of discontinuing TMZ-adjuvant therapy, and FLAIR imaging at the end of follow-up (**C**, **F**). The former patient remained tumor relapse-free as of 2 years after discontinuation of TMZ (**C**), whereas the latter patient experienced tumor relapse 10 months after discontinuing TMZ (**F**), even with methylated *MGMT*. Arrowheads indicate residual tumor.

groups. Kaplan-Meier curves demonstrated significant differences in PFS between the 3 groups ($P < 0.01$, Fig. 5).

DISCUSSION

This study confined subjects to those with lower-grade glioma comprising *IDH*-mutant DA or *IDH*-mutant AA. Recent reports have found no significant differences in prognosis between *IDH*-mutant DA and *IDH*-mutant AA.[2,3] The finding that median PFS did not differ significantly between *IDH*-mutant DA and *IDH*-mutant AA may corroborate the validity of mixing DA and AA cases in the present study.

All 30 patients in this study received adjuvant chemotherapy with TMZ, because some clinical trials have proven that TMZ-adjuvant therapy is essential for *IDH*-mutant, chromosome 1p/19q noncodeletion lower-grade glioma.[5,6] However, most patients have to discontinue TMZ-adjuvant therapy due to various reasons, as with patients in this study. Independent predictors of clinical outcomes at the time of discontinuing TMZ-adjuvant therapy are therefore desired. We identified only a few previous reports regarding the utility of PET for TMZ-adjuvant therapy. Galldiks et al[9,10] reported that PET with ^{11}C-met or ^{18}F-fluorothymidine enabled prediction of therapeutic response to TMZ-adjuvant therapy in various glioma subtypes, including glioblastoma. In those reports, change in tracer uptake on serial scans reflected therapeutic response during TMZ-adjuvant therapy, thereby predicting outcomes. In contrast, the present study focused on the utility of ^{11}C-met PET at the time of discontinuing TMZ-adjuvant therapy specifically in patients with residual *IDH*-mutant lower-grade glioma. We consider that the timing of ending adjuvant therapy is important for both patients and physicians, because such patients will not receive any further therapy until tumor relapse is identified, and this would be chronologically nearest to tumor recurrence. In the present study, $SUV_{T/N}$ from ^{11}C-met PET at the time of discontinuing TMZ-adjuvant therapy has been clearly confirmed as a predictor of consequential outcomes after TMZ-adjuvant therapy. We consider PET using amino acid tracers such as ^{11}C-met (thus enabling direct assessment of amino acid metabolism within the tumor in real time) as the best modality at the time of discontinuing adjuvant therapy for predicting outcomes after adjuvant therapy.

The present study also showed that *MGMT* promotor methylation status in tumor specimens obtained from the initial surgery provides a factor independently associated with tumor relapse. *MGMT* promotor methylation status has been universally recognized as an independent prognostic biomarker for response to TMZ in glioblastoma,[12,17] and also in *IDH*-mutant AA[5] and *IDH*-mutant DA with high-risk factors,[18] because promotor methylation of *MGMT* brings about epigenetic silencing and loss of *MGMT* protein expression leading to the protection of cells against TMZ. From the perspective of predicting outcomes after TMZ-adjuvant therapy, however, we assumed that assessment of tumor metabolism using ^{11}C-met PET at the time of discontinuing TMZ-adjuvant therapy would be more practical than determining *MGMT* promotor methylation status using tumor specimens obtained from the initial surgery. Unexpectedly, the results of the present study suggest that ^{11}C-met PET allowed the prediction of outcomes at least comparable to *MGMT* promotor methylation status in patients with residual *IDH*-mutant lower-grade glioma. However, assessment using either *MGMT* or ^{11}C-met PET may be insufficient to predict outcomes after discontinuing TMZ-adjuvant therapy. Findings for methylated *MGMT* and $SUV_{T/N} \leq 1.27$ did not necessarily coincide in our patients (Fig. 2). That is, results for *MGMT* status from tumor specimens at the initial surgery and ^{11}C-met PET at the time of discontinuing adjuvant therapy are unrelated. In fact, tumor progression was seen even in some patients with methylated *MGMT* (Figs. 2–4). We therefore assessed combined findings from $SUV_{T/N}$ from ^{11}C-met PET and *MGMT* methylation status. Assessment of combined findings from $SUV_{T/N}$ from ^{11}C-met PET and *MGMT* methylation status was found to provide

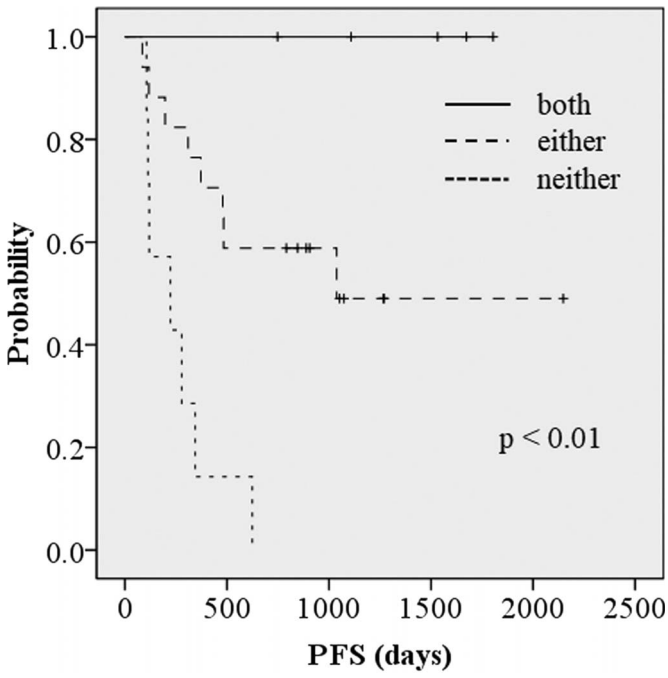

FIGURE 5. Kaplan-Meier curves for comparisons of the 3 groups of patients, assigned by the combined findings from methylated *MGMT* and $SUV_{T/N} < 1.27$. Solid line, patients with both methylated *MGMT* and $SUV_{T/N} < 1.27$; dashed line, patients with either methylated *MGMT* or $SUV_{T/N} < 1.27$; dotted line, patients with neither methylated *MGMT* nor $SUV_{T/N} < 1.27$.

more precise predictions of outcomes after discontinuing TMZ-adjuvant therapy than that from each examination alone (Fig. 5). This suggests that ^{11}C-met PET would offer a supplement to *MGMT* methylation status in predicting outcomes.

Some limitations must be considered when interpreting the results of this study. First, the cohort size was small, because relatively few patients met all inclusion criteria for the study. This issue would presumably exert a substantial effect on the cutoff values for predicting outcomes in each factor. Further studies including larger populations are desired. Second, not all patients received the same treatment before TMZ-adjuvant therapy. Patients with *IDH*-mutant DA were arbitrarily assigned to receive or not receive radiotherapy before TMZ-adjuvant therapy, because the efficacy of radiotherapy for *IDH*-mutant DA remains unclear.[19] Some patients with *IDH*-mutant DA who did not receive radiotherapy before adjuvant therapy might thus have shown shorter PFS, potentially contributing to the lack of difference in PFS between DA and AA. On the other hand, the median PFS of 484 days (1.3 years) for *IDH*-mutant AA in this study seemed shorter than that in 2 large-scale clinical trials of 2–3 years[6] and 3.5 years.[5] This discrepancy could be attributable to the inclusion criterion in this study that PFS excluded a period exceeding 1 year for radiotherapy followed by TMZ-adjuvant therapy, and all patients showed residual tumor. Third, the present study included some degree of bias in determining the number of cycles for adjuvant therapy. The number of cycles of TMZ-adjuvant therapy varied among patients due to the protocol of the specific clinical trial, severe blood toxicity, the arbitrary discretion of the attending physicians, and/or the decisions of the patient, because the optimal number of cycles for *IDH*-mutant lower-grade glioma has yet to be established. Although multivariate analysis identified the number of cycles of TMZ-adjuvant therapy as a relevant factor for outcomes after adjuvant therapy, confirming whether TMZ-adjuvant therapy for long periods leads to favorable outcomes is difficult. Further studies enrolling patients who received the same therapy may be anticipated.

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