Use of $^{11}$C-methionine PET parametric response map for monitoring WT1 immunotherapy response in recurrent malignant glioma

Clinical article

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Object. Immunotherapy targeting the Wilms tumor 1 (WT1) gene product is a promising treatment modality for patients with malignant gliomas, and there have been reports of encouraging results. It has become clear, however, that Gd-enhanced MR imaging does not reflect prognosis, thereby necessitating a more robust imaging evaluation system for monitoring response to WT1 immunotherapy. To meet this demand, the authors performed a voxel-wise parametric response map (PRM) analysis of $^{11}$C-methionine PET (MET-PET) in WT1 immunotherapy and compared the data with the overall survival after initiation of WT1 immunotherapy (OS$_{WT1}$).

Methods. Fourteen patients with recurrent malignant glioma were included in the study, and OS$_{WT1}$ was compared with: 1) volume and length change in the contrast area of the tumor on Gd-enhanced MR images; 2) change in maximum uptake of $^{11}$C-methionine; and 3) a more detailed voxel-wise PRM analysis of MET-PET pre- and post-WT1 immunotherapy.

Results. The PRM analysis was able to identify the following 3 areas within the tumor core: 1) area with no change in $^{11}$C-methionine uptake pre- and posttreatment; 2) area with increased $^{11}$C-methionine uptake posttreatment (PRM$^{+}$MET); and 3) area with decreased $^{11}$C-methionine uptake posttreatment. While the results of Gd-enhanced MR imaging volumetric and conventional MET-PET analysis did not correlate with OS$_{WT1}$ (p = 0.270 for Gd-enhanced MR imaging length, p = 0.960 for Gd-enhanced MR imaging volume, and p = 0.110 for MET-PET), the percentage of PRM$^{+}$MET area showed excellent correlation (p = 0.008) with OS$_{WT1}$.

Conclusions. This study describes the limited value of Gd-enhanced MR imaging and highlights the potential of voxel-wise PRM analysis of MET-PET for monitoring treatment response in immunotherapy for malignant gliomas. Clinical trial registration no.: UMIN000002001.


**Key Words** • glioma • $^{11}$C-methionine PET • WT1 immunotherapy • parametric response map • oncology

**Abbreviations used in this paper:** GBM = glioblastoma multiforme; MET-PET = $^{11}$C-methionine PET; OS$_{WT1}$ = overall survival after initiation of Wilms tumor 1 immunotherapy; PRM = parametric response map; RECIST = Response Evaluation Criteria in Solid Tumors; ROI = region of interest; WT1 = Wilms tumor 1.
The median overall survival time after initiating WT1 immunotherapy was 36.7 weeks. In that report, the anti-tumor effect of the treatment was assessed by determining the response of the target lesions using MR imaging 12 weeks after initiating WT1 vaccination. The tumor length, corresponding to the contrast-enhanced area on Gd-enhanced MR images, was measured and analyzed according to RECIST version 1.0,18 with results reported as complete response, partial response, stable disease, and progressive disease.

In that analysis, however, the long-term survivors were assessed as having progressive disease at 12 weeks after WT1 vaccination initiation, suggesting that evaluation by contrast-enhanced T1-weighted MR imaging is not suitable for assessing the treatment response to WT1 immunotherapy. The fact that morphological imaging often does not adequately reflect the underlying tumor biology12 imposes a considerable demand to develop alternative biological markers for therapeutic response. Recently, a voxel-wise PRM has been developed to overcome the above-mentioned issue in other treatment modalities for malignant glioma.6–8

The present report focuses on the results in 14 patients who were enrolled in the same trial but were not included in the previous report. In this study, we have attempted to apply the voxel-wise PRM method to MET-PET in the setting of WT1 immunotherapy against recurrent malignant glioma and compare its clinical value with conventional analytical methods based on MR imaging and PET.

Methods

**WT1 Immunotherapy**

Patients received intradermal injections of 3.0 mg of modified 9-mer WT1 peptide emulsified with Montanide ISA51 adjuvant. The WT1 vaccinations were given weekly for 12 consecutive weeks. Twelve weeks after the initial vaccination, the response was evaluated by means of both MR imaging and MET-PET. Our local internal review board approved this treatment and written informed consent was obtained from all patients. Details of the procedures and protocol have been reported elsewhere.9,14

**Patient Selection**

Between 2004 and 2010, 66 patients with recurrent malignant glioma were treated with WT1 immunotherapy as described above as part of an ongoing clinical trial (UMIN000002001). Nineteen of these 66 patients underwent evaluation by means of MET-PET. These patients were not included in our previous report.9 Five of these 19 patients—2 patients with intratumoral hematoma and 3 patients whose tumor volume was 2 cm³ or less as measured by MET-PET—were excluded from the current analysis. All 14 patients whose data were analyzed for this study underwent MR imaging and MET-PET before (pre-WT1) and 12 weeks after (post-WT1) WT1 vaccination. Detailed information pertaining to these 14 patients is listed in Table 1. The overall survival was measured from WT1 immunotherapy initiation, denoted as OS_{WT1}.

**Magnetic Resonance Imaging**

All MR images were obtained using a 3.0-T whole-body MR scanner (Signa, GE Medical Systems) with an acquisition time of approximately 3 minutes. After intravenous administration of Gd–diethylenetriamine penta-acetic acid (Gd-DTPA; 0.1 mmol/kg body weight), axial T1-weighted images were obtained using standard procedures. Those images were stored in 512 × 512 × 23 or 216 anisotropic voxels, with each voxel being 0.43 × 0.43 × 6.0 or 1.0 mm.

**MET-PET Scans**

All PET studies were performed using the Eminence PET system (Shimadzu Corp.). ¹¹C-methionine (111–222 MBq, 3–6 mCi), synthesized according to the method of Berger et al.,1 was injected intravenously. Tracer accumulation was recorded over 15 minutes in 99 transaxial slices from the entire brain. Total activity from 20 to 35 minutes after tracer injection was used for image reconstruction. The images were stored in 256 × 256 × 99 anisotropic voxels, with each voxel being 1 × 1 × 2.6 mm.

**Tumor Length and Volume Measurement**

Tumor length, corresponding to the contrast-enhanced area on T1-weighted MR images, was measured and analyzed according to RECIST version 1.0,18 using the ImageJ software from the National Institutes of Health (http://rsb.info.nih.gov/ij/).

Tumor volume was measured by performing a 3D threshold-based volume-of-interest analysis in all patients for contrast-enhanced lesions on Gd-enhanced MR images, using the ImageJ software. The contrast-enhanced area in each slice image was measured by manual tracking of the tumor boundaries, and the sum of the enhanced areas or high-uptake areas was multiplied by the slice interval.

**Image Fusion and Registration**

The MET-PET data were registered onto pre-WT1 contrast-enhanced T1-weighted standard anatomical images using normalized mutual information with the VINCI image analyzing software from the Max Planck Institute for Neurological Research in Cologne (http://www.nf.mpg.de/vinci/). Registration of the images was confirmed visually. The reported registration error for normalized mutual information is less than 1 mm.19 After image registration was completed, all image sets, including the standard anatomical MR images (pre-WT1) and MET-PET data (pre- and post-WT1), were converted into 256 × 256 × 256 isotropic, 1 × 1 × 1 mm images enabling further voxel-wise analysis of the images (Fig. 1).

**Data Processing and ROI Selection**

Three data sets (standard anatomical images and MET-PET data) were exported to in-house software written in MATLAB 7.6 (MathWorks) for further analysis. Regions of interest were selected as follows: for normal brain tissue, the contralateral hemisphere of the tumor was selected, including both the gray and white matter; for tumor, contrast-enhanced lesions were selected.
Parametric Response Map Calculation Algorithm

As in Fig. 1, post-WT1 \(^{11}\text{C}\)-methionine uptake was plotted as a function of pre-WT1 \(^{11}\text{C}\)-methionine uptake in both normal brain and Gd-enhancing lesions. A linear regression fitting was applied to the data obtained by the ROI placed at the normal brain (Fig. 1, blue line), which can be expressed as follows: post-WT1 MET-PET = pre-WT1 MET-PET, where “post-WT1 MET-PET” and “pre-WT1 MET-PET” are the tumor/normal tissue (T/N) ratio of pre- and post-WT1 \(^{11}\text{C}\)-methionine PET.

Next, the magnitude of deviation of each data point \(i\) from the expected linear regression fitting was calculated as follows:

\[
\text{deviation}_i = \left(\frac{(\text{post-WT1 MET-PET}) - (\text{pre-WT1 MET-PET})}{\sqrt{2}}\right)
\]

The parametric response map (PRM) of each data point was defined as follows:

\[
\text{PRM}_i = \text{deviation}_i - \mu / \rho
\]

where \(\mu\) and \(\rho\) are the mean and standard deviation of deviation, within the ROI placed at the normal brain. In other words, PRM is identical to the \(z\)-score of each data point in the lesion from the expected linear regression line calculated for normal brain.

Statistical Analysis

Statistical analyses were carried out using a Kaplan-Meyer survival analysis with the log-rank test if not specified otherwise. A p value < 0.05 was considered statistically significant, and all statistical computation was performed using Prism 5 (GraphPad Software, Inc.) or JMP 9.0 (SAS Institute, Inc.).

Results

Applying the PRM Calculation to WT1 Immunotherapy Patients

The PRM calculation, described above and in Fig. 1, was successfully performed in all 14 cases. The actual process that was performed is described below by presenting 2 representative cases, one (Case 2) in which the patient had a relatively long OS\(_{\text{WT1}}\) of 144.7 weeks and was considered a treatment responder, and another (Case 7) in which the patient had a relatively short OS\(_{\text{WT1}}\) of 20.9 weeks and was considered a treatment nonresponder.

Representative Treatment Responder. A representative case involving a treatment responder (Case 2) is illustrated in Fig. 2. First, a voxel-wise analysis was performed in normal brain tissue (Figs. 1 and 2). As shown in Fig. 2, pre- and post-WT1 \(^{11}\text{C}\)-methionine uptake showed good positive linear correlation in normal brain tissue. A linear regression line and the ± 2 SD distribution range were calculated. Subsequently, the same analysis was performed in a tumor lesion. A contrast-enhanced area was selected as the ROI for analysis. In this particular case, most voxels were distributed in the −2 SD area, suggesting that \(^{11}\text{C}\)-methionine uptake decreased after WT1 immunotherapy (Fig. 2). This area is presented as PRM\(_{\text{MET}}\) (PRM with reduced methionine uptake).

This patient survived for 144.7 weeks after initiation of WT1 immunotherapy, although the contrast-enhanced area increased after WT1 immunotherapy, categorizing this patient as having progressive disease in the Gd-enhanced MR imaging–based RECIST analysis.
Representative Treatment Nonresponder. A representative case in which the patient had only a short OSWT1 (Case 7) is illustrated in Fig. 3. The same analysis as described above was performed. In this particular case, most voxels were distributed in the +2 SD area (PRM+MET), suggesting that 11C-methionine uptake increased after WT1 immunotherapy. This patient survived for 20.9 weeks after initiation of WT1 immunotherapy.

Correlation of Treatment Response Assessment and OSWT1

Magnetic Resonance Imaging–Based Assessment. To assess the validity of evaluating the response to WT1 immunotherapy using contrast-enhanced MR imaging, the changes in length and volume of the tumor before and 12 weeks after initiating WT1 immunotherapy were calculated. As in Fig. 4A and B, both methods using Gd-enhanced MR imaging failed to show positive correlation with OSWT1 (p = 0.270 and 0.960, respectively).

Conventional MET-PET Analysis. To assess the validity of evaluating the response to WT1 immunotherapy using MET-PET, the changes in maximum 11C-methionine uptake assessed using the tumor/normal tissue ratio (T/N max) before and 12 weeks after initiating WT1 immunotherapy were calculated. Change of T/N max failed to show any statistically significant correlation with OSWT1 (p = 0.110) (Fig. 4C).

Parametric Response Map Analysis. Finally, correlation of the proposed voxel-wise PRM of MET-PET with OSWT1 was investigated. Each voxel of contrast-enhanced area on the pretreatment MR images was categorized as a no-change area, PRM+MET, or PRM−MET, according to no change, increase, or decrease, respectively, in methionine uptake 12 weeks after initiation of WT1 immunotherapy. The percentage of the 3 categories was calculated 3-dimensionally and correlated with OSWT1 (Fig. 5). While the percentage of the PRM+MET area showed moderate correlation with OSWT1 (p = 0.100) (Fig. 5 left), the percentage of the PRM−MET area showed excellent correlation with OSWT1 (p = 0.008) (Fig. 5 right). A threshold of 5% for PRM+MET yielded the best performance for discriminating WT1 immunotherapy responders from nonresponders (Fig. 5 right). When a Cox proportional hazard model was applied, adjusted by age (cutoff 50 years of age) and performance status (0 or 1 and 2), a threshold of 5% for PRM+MET still remained as the only statistically significant factor (p = 0.01).
PET monitoring of immunotherapy response

Discussion

Conventionally, MR imaging is used to evaluate response to treatment in glioma patients. The maximum length of the contrast-enhanced area is measured and the effect of treatment is analyzed according to RECIST. This method is based on previous reports showing RECIST to be useful in determining objective responses of contrast-enhancing brain tumors to therapy. Moreover, those reports showed that use of RECIST was comparable to volumetric methods. On the other hand, problems with using MR imaging–based tumor measurement as an indicator of treatment response have been reported. For example, temozolomide-based chemoradiotherapy for

Fig. 2. Case 2. A representative treatment responder with recurrent GBM (OSWT1 144.7 weeks). Images were analyzed as in Fig. 1. Voxel-wise PRM analysis revealed that most of the contrast-enhanced lesion was within the PRM−MET area. Although the OSWT1 was 144.7 weeks, conventional MR imaging evaluated the response as progressive disease. Gd-MRI = Gd-enhanced MR imaging; T/Nr = T/N max.

Fig. 3. Case 7. A representative treatment nonresponder with recurrent GBM (OSWT1 20.9 weeks). Images were analyzed as in Fig. 1. Voxel-wise PRM analysis revealed that most of the contrast-enhanced lesion was within the PRM+MET area, suggesting that the patient was not responsive to WT1 immunotherapy.
newly diagnosed GBM results in a transient increase in tumor enhancement on MR imaging in 20%–30% of patients (pseudoprogression), which is difficult to differentiate from true tumor progression. Similarly, in the present study, changes in tumor length and volume measured by contrast-enhanced MR imaging after WT1 immunotherapy did not correlate with OSWT1 (Fig. 4), suggesting that contrast-enhanced MR imaging is inappropriate for evaluating the clinical outcome of WT1 immunotherapy. Unlike chemotherapy or radiotherapy, immunotherapy causes an inflammatory reaction in the tumor, which results in infiltration of inflammatory cells, dilation of capillary vessels, and increased capillary permeability. Thus, it is possible that contrast enhancement does not reflect the tumor activity but rather represents the immune reaction in situ.

On the other hand, MET-PET provides high-resolution metabolic information about the tumor in vivo, information that is impossible to obtain using MR imaging. Previous studies have shown that the ratio of the maximum 11C-methionine uptake in tumor compared with the contralateral normal brain (T/N max) reflects prognosis. However, gliomas are heterogeneous in nature and have heterogeneous uptake of 11C-methionine. In fact, we have previously demonstrated that 11C-methionine uptake correlates with tumor cell density by comparing MET-PET images with stereotactically sampled tissue. Thus, instead of analyzing T/N max, which could result in comparisons between different locations within the tumor, a better method is to analyze the change in 11C-methionine uptake in each anatomical location to elucidate the global change in 11C-methionine uptake within the tumor. To satisfy this need, a voxel-wise PRM analysis was used in the present study and produced excellent correlation between OSWT1 and the percentage of PRM+MET (Fig. 5). This method showed far better correlation with OSWT1 than changes in T/N max by MET-PET, suggesting that the voxel-wise PRM is the most suitable method for assessing the treatment response of gliomas. Moreover, although the number of cases analyzed was small, a threshold of 5% for PRM+MET was the best indicator for discriminating WT1 immunotherapy responders from nonresponders in terms of survival time (Fig. 5 right). A similar method has already been applied for diffusion or perfusion MR imaging.

Fig. 4. Correlation of OSWT1 with changes in tumor length and volume using contrast-enhanced MR imaging and the T/N max of MET-PET. Correlations between OSWT1 and changes (from before WT1 immunotherapy to 12 weeks after immunotherapy initiation) on Gd-enhanced MR imaging–measured tumor length (A), volume (B), and T/N max of MET-PET (C) are presented. The correlations were not statistically significant (p = 0.270, 0.960, and 0.110, respectively; 14 cases).

Fig. 5. Correlation of OSWT1 with PRM−MET and PRM+MET. Correlations between OSWT1 and percentage areas of PRM−MET (left) and PRM+MET (right) are presented. The percentage of PRM−MET within the contrast-enhanced lesion before WT1 immunotherapy initiation correlated best with OSWT1 (p = 0.008; 14 cases).
PET monitoring of immunotherapy response

aging analysis in glioma treatment using temozolomide and radiation therapy and has been suggested as an early biomarker for treatment response.6–7 The main difference between voxel-wise PRM analysis and conventional imaging analysis is that voxel-wise PRM analysis allows us to identify the location and extent of areas that responded to therapy, rather than comparing the maximum values of the pre- and posttreatment evaluation modality, which could be comparing different locations.

There are, however, limitations that should be noted. Because pre- and posttreatment 11C-methionine uptake is registered and compared, this method cannot be used when the shape or size dramatically change during therapy due to cyst formation or intratumoral hemorrhage. A more advanced method that could correct for tissue deformation is required to compensate for these changes. As the images compared were obtained 12 weeks apart, it is necessary to investigate the possibility of comparing images obtained in shorter intervals. Another limitation of this study is the retrospective nature of the data analysis and the limited sample size. Although a 5% cutoff of PRM$^{MET}$ yields the best result for the survival analysis, a prospective study with a much larger sample size will be necessary to obtain the most suitable cutoff value. Moreover, other modalities, such as perfusion or diffusion MR images should also be investigated in a similar manner to elucidate whether these modalities could also be used for evaluating immunotherapy for malignant gliomas.

Conclusions

We performed a voxel-wise PRM analysis of MET-PET before and 12 weeks after WT1 immunotherapy initiation to evaluate the clinical responses to WT1 immunotherapy in recurrent malignant glioma patients. This method holds promise for evaluating the dynamics of conventional Gd-enhanced MR imaging, which can be difficult to assess using PET monitoring of immunotherapy response

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