Targeted therapy, molecular imaging and biomarkers in cancer treatment

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Chapter 4

Serial FLT-PET scanning does not discriminate between true and pseudoprogression in newly diagnosed glioblastoma patients treated with chemoradiotherapy, a prospective study

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ABSTRACT

Background: Response evaluation in glioblastoma (GBM) patients after first line radiotherapy and temozolomide (TMZ) is hampered by the occurrence of progressive, contrast-enhancing lesions on MRI not reflecting true tumor progression. ^{18}F-fluorothymidine (FLT) is a Positron Emission Tomography (PET) tracer that is taken up by proliferating cells. The goal of this study was to prospectively assess the value of FLT-PET in discriminating between true and pseudoprogression in patients with primary GBM treated with chemoradiotherapy.

Methods: FLT-PET and MRI scans were performed before start and 4 weeks after chemoradiotherapy. MRI scans were also performed after 3 cycles of adjuvant TMZ. Pseudoprogression was defined as progressive disease on MRI after chemoradiotherapy, with stabilization or reduction of enhancing lesions after 3 cycles of adjuvant TMZ. Changes in maximum standard uptake value (SUV_{max}) and tumor-to-normal brain tissue (T/N) ratios were calculated for FLT uptake and presented as the mean of the SUV_{max} in case of multiple lesions. Ki67 staining in the primary tumor and overall survival were analyzed.

Results: Thirty patients, (28 GBM, two gliosarcoma (WHO grade IV)), were included. Of 24 patients evaluable for pseudoprogression, seven showed pseudoprogression and seven true progression. No difference was found in changes of SUV_{max} and T/N ratios or changes in these parameters between these patient groups. A lower baseline FLT uptake predicted longer overall survival, but baseline FLT uptake did not correlate with Ki67.

Conclusions: FLT-PET scans do not discriminate between true progression and pseudoprogression in GBM patients. Baseline FLT uptake appears to predict overall survival (NTR3680).

INTRODUCTION

Glioblastoma (GBM) is the most common and most aggressive type of primary brain tumors, accounting for more than 50% of all gliomas with an incidence of 3.19 per 100.000 in the United States [1]. After surgery, the addition of temozolomide (TMZ) to standard 60 Gy radiotherapy has improved 2-year survival from 11 to 27% and 5-year survival from 2 to 10%. This is currently the standard of care for newly diagnosed GBM [2]. However, response evaluation of this treatment in these patients is problematic. This is due to the difficulty of distinguishing recurrent tumor (true progression) from pseudoprogression. The latter is defined as the detection of progressive gadolinium-enhanced lesions on an MRI scan immediately after the end of concurrent chemo-radiotherapy, where spontaneous improvement occurs without further treatment other than adjuvant TMZ [3,4]. This is the case in up to 64% of patients with progression on the first MRI scan after radiotherapy [5]. The difficulty of distinguishing true progression from pseudoprogression hampers clinical decision making in these patients. In case of pseudoprogression, standard treatment with adjuvant TMZ should be continued, whereas in case of true tumor progression, other treatment modalities – although scarce – or palliative care might be more appropriate.

^{18}F-fluorothymidine (FLT) is a Positron Emission Tomography (PET) tracer that is taken up by proliferating cells. It is phosphorylated in the cell by thymidine kinase 1, which is involved in DNA synthesis, and subsequently trapped. FLT uptake reflects thymidine kinase 1 activity, and can be used as a measure of cell proliferation. In several tumor types, FLT uptake corresponds with the Ki67 proliferation index and its change correlates with the response to therapy [6,7].

In glioma patients, FLT uptake has been used for tumor grading and was correlated with Ki67 [8,9]. Moreover, FLT-PET performed better in predicting survival and recurrence in glioma patients than FDG-PET and MRI [10,11]. However, no prospective studies have yet been conducted on the efficacy of FLT-PET to discriminate between pseudoprogression and true progression. An effective technique to make this discrimination is urgently needed to improve clinical decision making in these patients. Therefore, the aim of this prospective study in patients with newly diagnosed GBM was to determine whether FLT-PET scans, performed before and after chemora-diotherapy, can discriminate between true progression and pseudoprogression as measured by MRI after 3 courses of adjuvant TMZ.

PATIENTS AND METHODS

Patients and treatment

Patients with newly diagnosed GBM or gliosarcoma (WHO grade IV, hereafter referred to as GBM) who were eligible for standard treatment with radiotherapy and TMZ were prospectively included. After surgical resection or biopsy, patients were treated with radiotherapy consisting of 2 Gy irradiation 5 out of 7 days per week during 6 weeks, for a total dose of 60 Gy. Patients received concomitant TMZ orally in a dose of 75 mg/m^2 daily for 6 weeks. After a treatment break of 4 weeks, patients received up to 6 cycles of adjuvant TMZ (150–200 mg/m^2) for 5 days every 28 days. The use of corticosteroids during treatment was registered. No changes in treatment

were introduced based on the results of the FLT-PET scan. Overall survival was calculated from date of informed consent to date of death or last known date alive, censored at time of analysis (November 2013). All patients gave written informed consent to participate in the study. The protocol was approved by the local ethics committee and registered in the Dutch trial register (NTR3680).

Imaging

Patients underwent standard radiologic follow up with MRI (1.5T using T1, T2 and contrast enhanced 3D T1 Gradient echo sequences) directly after surgery (baseline), 10 weeks after start of treatment (4 weeks after completing chemoradiotherapy), 22 weeks after start of treatment (after the third cycle of adjuvant TMZ or earlier as clinically indicated) and thereafter every 3 months. MRI data for this study were assessed by an independent neuroradiologist and a radiologist in training using the Macdonald criteria for tumor response evaluation [12]. Pseudoprogression was defined as progressive disease on MRI scan at 10 weeks, with stabilization or reduction of enhancing lesions on MRI at 22 weeks. True progression was defined as progressive disease on both the MRI at 10 weeks and the MRI at 22 weeks.

FLT-PET scans were performed after surgery, but before start of radiotherapy (baseline) and 10 weeks after start of treatment (4 weeks after completing chemoradiotherapy). FLT was synthesized as described by Been et al [13]. Patients were instructed to fast for a minimum of 4 hours before tracer injection of 200 MBq FLT intravenously, injected 30 minutes before PET scanning. PET scans were made on either HR+ or mCT PET scanners (Siemens, Knoxville). The maximum Standard Uptake Value (SUV_{max}) was assessed by drawing a region of interest (ROI) around every lesion on a separate reconstruction according to the European Association of Nuclear Medicine Research Ltd [14]. In case of multiple lesions, the mean of the SUV_{max} of the different lesions was calculated. FLT-PET scans were fused with the most recent MRI to differentiate actual tumor from post-surgery effects outside the cerebrum if needed. The SUV_{mean} for normal brain tissue was assessed by drawing a ROI in the contralateral brain tissue. Tumor-to-normal ratios (T/N ratio) were determined by dividing the SUV_{max} of the tumor by the SUV_{mean} of the normal brain tissue. A PET response was defined as a 25% decrease of the SUV_{max} between the first and second FLT-PET scan.

Ki67 immunohistochemical staining

Deparaffinized GBM tissue from primary surgery was used to evaluate the proliferation fraction of tumor cells (4-µm-thick tissue slices). Antigen retrieval was performed using 10 mM Tris/1 mM EDTA (pH 9), in a microwave at 700 W. Endogenous peroxidase and biotin were blocked using routine techniques. The slides were incubated with the primary antibody, Ki67 (Clone MIB-1; Dako, Glostrup, Denmark) at room temperature for 1 hour, followed by application of the secondary antibody peroxidase-conjugated rabbit anti-mouse serum (Dako, Glostrup, Denmark), and the tertiary antibody peroxidase-conjugated goat anti-rabbit serum (Dako, Glostrup, Denmark), for 30 minutes each. The first antibody was diluted 1/100 in 1% bovine serum albumin (BSA)/phosphate buffered saline (PBS). The secondary and tertiary antibodies

were diluted 1/100 in 1% BSA/PBS with 1% AB serum. Color development was performed with 3,3′-diaminobenzidine (Sigma, Zwijndrecht, the Netherlands) for 10 minutes. The slides were scanned for hot spots of proliferative activity. In one high power field (400x magnification) the fraction of Ki67-positive nuclei/total number of nuclei was evaluated.

Statistics

Mann Whitney U tests were used to compare FLT uptake between patients with and without pseudoprogression. To discriminate between true progression and pseudoprogression, Receiver Operating Curves were used to find an optimal cutoff point for FLT uptake and changes in uptake. A Fisher's exact test was used to analyze categorical data. A Kaplan-Meier curve with a log rank test was used to analyze survival. A Pearson correlation test was used to calculate correlations between FLT uptake and proliferation index. A two-sided *P*-value of < .05 was considered significant. For the Fisher's exact test, a one-sided *P* value was given. Statistics were calculated in IBM SPSS statistics 20. Graphs were made using GraphPad Prism version 5.00 for Windows.

RESULTS

Patients

In total, 28 patients with GBM and two with gliosarcoma (WHO grade IV) were included in this study between November 2009 and November 2012. For patient characteristics, see Table 1. All but one patient completed radiotherapy. Seven patients did not complete concomitant TMZ, and of the 27 patients who started adjuvant TMZ, 16 patients did not complete the adjuvant courses. The most frequent reasons for this were progressive disease and thrombocytopenia. One patient with a secondary GBM underwent a short schedule of concomitant radiotherapy (23 x 2 Gy) and TMZ. The median overall survival for all 30 patients was 14 months (range 1-36 months). Five patients were not evaluable for pseudoprogression because of early death, salvage surgery or clinical deterioration that prevented further participation in the study. One patient was not analyzable for the pseudoprogression analysis as only a baseline MRI before tumor resection was available (Fig 1).

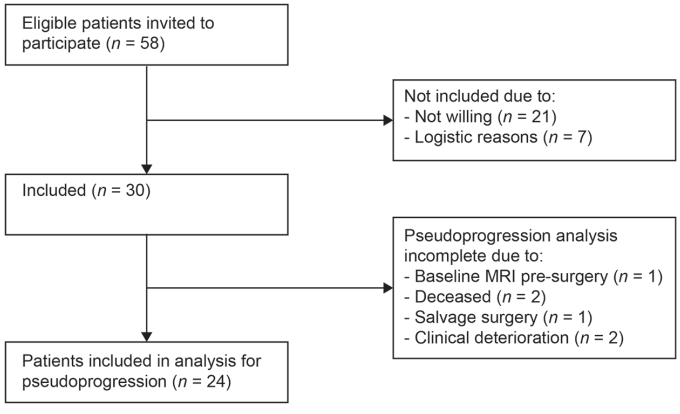

Figure 1. CONSORT diagram.

Table 1: Patient characteristics.

Characteristic	No. of patients (n = 30)	Median	Range
Age, years		58	33 - 68
Sex			
Male	17		
Female	13		
Tumor type			
Glioblastoma	26		
Secondary glioblastoma	2		
Gliosarcoma	2		
Type of intervention			
Biopsy	3		
Surgical resection	27		

Baseline FLT-PET scans were performed 5 days (median) before start of radiotherapy. Two patients had their baseline FLT-PET scan 2 and 4 days after start of radiotherapy for logistic reasons. Follow-up FLT-PET scans were made 27 days (median) after completion of radiotherapy. Three patients had their follow up FLT-PET scan 1 day after the start of adjuvant TMZ. Finally, for logistic reasons two patients had their FLT-PET scan 6 and 22 days, respectively, after the start of adjuvant TMZ.

Pseudoprogression

A total of 24 patients were evaluable for pseudoprogression analysis (Fig 1). Pseudoprogression was seen in seven patients, and true progression in seven other patients (Figures 2 and 3).Ten patients had either stable disease or a complete response on MRI after 10 weeks (Table 2).

We found no difference in or change of SUV_{max} and T/N ratio on FLT-PET scans between patients with pseudoprogression and those with true progression. With 25% reduction of SUV_{max} as a cutoff value, only two of the patients with pseudoprogressivon were identified, while three patients with true progression also showed a decrease in SUV_{max} over 25% (sensitivity 29%, specificity 43%). We also used optimal cutoff points found by others for identifying recurrent tumor of a SUV ≥ 1.34 and T/N ratio of ≥ 4.94 applied to FLT-PET scan at 10 weeks [15,16]. This approach also did not predict all cases correctly. Using a T/N ratio of ≤ 2.95 on the FLT-PET scan at 10 weeks, we identified four out of seven patients with true progression and zero patients with pseudoprogression (sensitivity 100%, specificity 57%, one sided $P = .04$). ROC curves showed no other reasonable cutoff point for any parameter to discriminate between pseudoprogression and true progression.

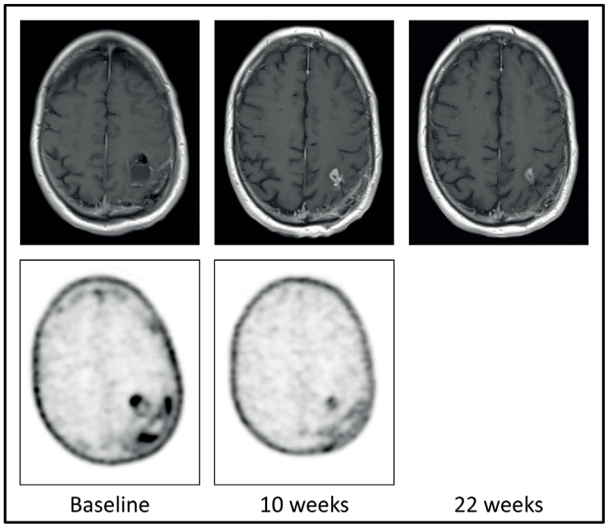

Figure 2. FLT-PET images at baseline (left) and 10 weeks (right) and MRI images at baseline (left), 10 weeks (middle) and 22 weeks (right) of a patient with pseudoprogression. SUV_{max} on baseline FLT-PET was 1.44, SUV_{max} at 10 weeks 0.74.

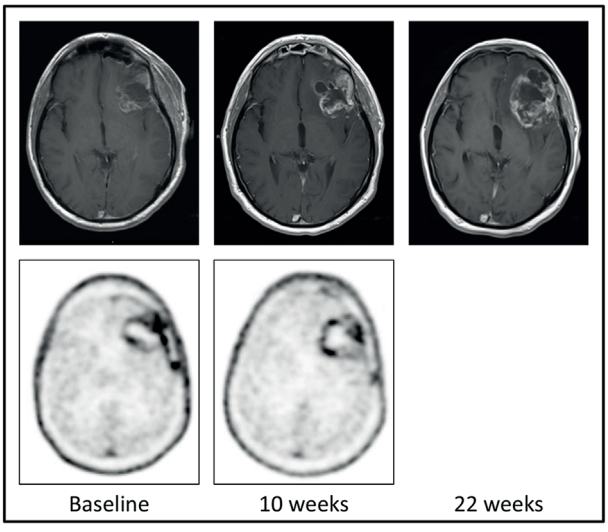

Figure 3. FLT-PET images at baseline (left) and 10 weeks (right) and MRI images at baseline (left), 10 weeks (middle) and 22 weeks (right) of a patient with true progression. SUV_{max} on baseline FLT-PET was 3.70, SUV_{max} at 10 weeks 1.80.

Table 2. Overview of results in all included patients.

Patient No.	SUV_{max} baseline	T/N baseline	SUV_{max} 10 weeks	T/N 10 weeks	Change SUV_{max} %	MRI 10 weeks	MRI 22 weeks	Ki 67 %	Overall survival
1	1.55	3.4	1.33	2.8	14.2	NE	SD	30	29
2	NU	ND	NU	ND	ND	SD	SD	40	36*
3	NU	ND	0.81	3.1	ND	PD	SD	35	32
4	1.73	5.6	1.58	6.3	8.7	PD	SD	35	17
5	1.24	3.1	1.46	3.2	-17.7	SD	PD	40	20
6	1.75	4.4	0.95	2.9	45.7	SD	PD	30	31
7	1.59	7.6	1.34	8.4	15.7	PD	NE	25	10
8	2.18	14.5	0.74	4.4	66.1	PD	PD	50	19
9	2.43	6.2	1.14	2.5	53.1	SD	SD	30	24
10	NU	ND	NU	ND	ND	CR	PD	10	14
11	NU	ND	NU	ND	ND	CR	CR	50	28
12	2.84	9.8	1.64	5.1	42.3	SD	ND	30	4
13	NU	ND	0.96	1.7	ND	PD	PD	25	9
14	1.23	3.0	1.14	3.5	7.3	PD	SD	60	27*
15	1.90	5.3	1.61	4.0	15.3	PD	SD	18	9
16	3.00	5.9	1.26	2.6	58.0	SD	PD	19	10
17	5.02	9.0	ND	ND	ND	ND	ND	50	1
18	4.17	8.7	2.67	3.3	36.0	PD	SD	ND	9
19	1.10	2.0	1.00	1.9	9.1	SD	SD	40	25*
20	1.38	3.9	1.68	2.8	-21.7	PD	PD	30	11
21	1.59	5.9	0.85	2.3	46.5	PD	PD	25	22*
22	0.65	2.0	0.68	2.7	-4.6	PD	PD	20	14
23	0.35	1.5	NU	ND	ND	CR	CR	15	19
24	1.64	6.6	ND	ND	ND	SD	ND	20	9
25	1.61	6.0	1.33	3.9	17.4	PD	SD	50	17*
26	2.85	16.8	0.93	5.5	67.4	SD	PD	60	6
27	3.70	9.5	1.80	5.3	51.4	PD	PD	50	5
28	1.44	7.6	0.74	4.1	48.6	PD	SD	50	13*
29	2.93	8.1	2.23	5.9	23.9	PD	PD	7	7
30	1.42	3.3	ND	ND	ND	SD	SD	ND	10

Abbreviations: PD = Progressive Disease, SD = Stable Disease, ND = Not Done, NE = Non Evaluable, NU = No Uptake, CR = Complete Response.

*Patients censored at date last known alive.

Overall survival

In all 30 patients, a baseline FLT-PET scan was available (Figure 1). In November 2013, 24 patients had died and six were censored at the last known alive date. The median SUV_{max} for all patients on baseline FLT-PET was 1.59. For patients with a $SUV_{max} \leq 1.59$, median overall survival was longer compared to patients with a $SUV_{max} > 1.59$ (20 vs 9 months $P = .01$) (Figure 4).

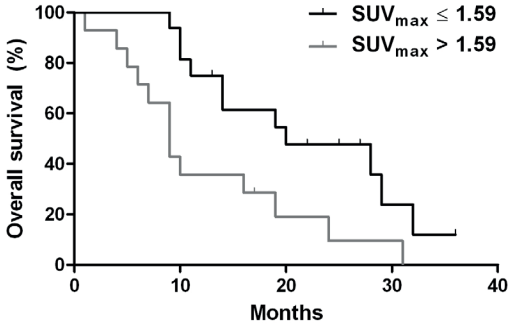

Figure 4. Kaplan-Meier curve for patients with SUV_{max} on first FLT-PET scan ≤ 1.59 and > 1.59. Overall survival is higher for patients with $SUV_{max} \leq 1.59$ ($P = .01$).

Proliferation index

In the 28 patients with available surgical specimens for Ki67 staining, the mean SUV_{max} at baseline FLT-PET did not correlate with the Ki67 index of the tumor tissue before treatment.

DISCUSSION

In this prospective trial we determined that serially measured FLT uptake in GBM patients did not discriminate between true progression and pseudoprogression. Uptake and changes in uptake was measured on FLT-PET scans before (at baseline) and after chemoradiotherapy. Low FLT uptake at baseline was predictive of longer survival, but FLT uptake did not correlate with the Ki-67 index of the primary tumor.

Despite the urgent need to distinguish between true progression and pseudoprogression in GBM patients, until now only retrospective studies have been performed on patients who had a radiological suspicion of recurrent brain tumor at different time points and showed variable results. In one such study, FLT-PET had a low specificity for identifying recurrent tumor from benign lesions in 20 patients [17]. Three other studies were able to discriminate between true progression and radionecrosis in 15, 19 and 21 glioma patients, respectively, using FLT kinetic values and the tumor-to-normal ratio [15,16,18]. A possible explanation for our findings is that FLT uptake in high-grade gliomas reflects not only trapping of FLT in proliferating tumor cells, but also disruption of the blood-brain barrier. As a result, areas with true progression as well as with pseudoprogression would show increased uptake.

To limit the burden of trial participation for the patients in this poor prognosis group, we did not perform kinetic modeling. Instead, we used SUV_{max} for the quantification of FLT uptake. SUV

is easy to obtain, mostly used in clinical practice and has been proven to be robust. However, kinetic analysis might be of interest to distinguish between true FLT uptake due to proliferation and FLT leakage that results from disruption of the blood brain barrier. There are several studies that support this, although others showed a good correlation between FLT kinetic values and SUV [19-24]. Using kinetic analysis has several disadvantages in practice. It is a time-consuming procedure, as the uptake in time needs to be assessed, and it requires collecting multiple arterial blood samples, both of which make the procedure burdensome for patients. Several studies have suggested other parameters for quantification of FLT-PET, such as proliferative volume and parametric response maps [25,26]. Due to the small numbers of patients in the studies performed so far and the different approaches used for quantification, direct comparison of the results is difficult. Consequently, we are unable to draw more definitive conclusions about the usefulness of FLT-PET in glioma. However, the use of SUV_{max} could also have limitations. For instance, the heterogeneity of the FLT uptake is not taken into account by using SUV_{max} only.

Another constraint of the present study is that the optimal time points for serial FLT-PET scanning before and during GBM treatment are difficult to choose. Because the aim of this study was to differentiate between true and pseudoprogression after chemoradiotherapy, we performed the baseline FLT-PET scan after surgery. Scanning before surgery would reveal tumor uptake, but most patients undergo a gross total resection of tumor tissue. However, scanning after surgery may have led to increased uptake of FLT due to increased blood flow and increased proliferation as part of the wound healing process.

A surprising finding was that a T/N ratio ≤ 2.95 on the FLT-PET scan at 10 weeks identified patients with true progression only, as patients with true tumor progression would be expected to have a higher proliferation rate. Because of the small numbers of patients in this analysis, this result should be interpreted with caution; future studies are needed to confirm if this is indeed a clinically relevant finding. Currently, two trials are investigating FLT as an imaging biomarker of early treatment response (NCT01880008, NCT00813566).

In earlier studies, correlations between FLT uptake in brain tumors and the Ki67 index were found [8-10]. However, in these studies FLT-PET scans were often performed before surgery, whereas in the current study post-surgery FLT-PET scans were made. This might explain the lack of correlation between FLT uptake and Ki67 index.

In the current study, pseudoprogression and true progression were determined based on the MRI results at 22 weeks. This time point was chosen due to its clinical relevance. After 3 adjuvant TMZ courses, the diagnosis of true progression results in cessation of TMZ, thus avoiding further side-effects of TMZ and enabling a timely switch to second-line therapy or inclusion in clinical trials. However, the selection of this time point may have resulted in overestimation of the number of patients with pseudoprogression. Taal et al. defined a period with stable disease of 6 months after radiotherapy for the diagnosis pseudoprogression [3]. Applying this criterion would have classified two patients with pseudoprogression as having true progression. However, this did not improve the performance of the FLT-PET scan in discriminating between true progression and pseudoprogression. On the other hand, clinical signs of pseudoprogression can take

longer than 3 months to resolve, so we might also have underestimated the number of patients with pseudoprogression [27].

Fortunately, since the start of this study, several new initiatives have been initiated. The RANO criteria for glioma response evaluation on MRI have been developed, and this reduces the number of patients found with pseudoprogression [28,29]. Also, other imaging modalities such as perfusion MRI and ^{11}C-methionine-PET have shown interesting results, although large prospective studies comparing multiple imaging modalities are still lacking [30,31].

In conclusion, our prospective study suggests that FLT-PET scanning is not useful in for discriminating between pseudoprogression and true progression in GBM patients treated with radiochemotherapy.

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