Diagnostic impact of additional O-(2-[18F]fluoroethyl)-L-tyrosine (^{18}F-FET) PET following immunotherapy with dendritic cell vaccination in glioblastoma patients

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Abstract

Objective

Vaccination therapy using tumour antigen-loaded, autologous dendritic cells (DC) is a promising therapeutic approach alongside standard treatment for glioblastoma (GBM). However, reliable diagnostic criteria regarding therapy monitoring are not established. Here, we analysed the impact of additional ^{18}F-fluoroethyl-tyrosine positron emission tomography (^{18}F-FET PET) imaging following DC vaccination therapy.

Methods

We analysed data of GBM patients who received DC vaccination therapy. Following MRI diagnosis of tumour recurrence, additional ^{18}F-FET PET imaging was performed. Vaccination was performed five times by intradermal injections, either weekly between concomitant radio/-chemotherapy and intermittent chemotherapy or after tumour recurrence, before re-radiation therapy. MRI and ^{18}F-FET PET results were compared and correlated with clinical data.

Results

Between 2003 and 2016, 5 patients were identified who received DC vaccination and ^{18}F-FET PET imaging (1 female/4 males; mean age: 44 ± 14 y). 3/6 patients showed congruent results of tumour progression. In three patients ^{18}F-FET PET indicated treatment related changes, which was in contrast to MRI findings that indicated tumour progression. In these patients ^{18}F-FET PET results could be confirmed by either neuropathological diagnosis or according to the RANO criteria

Conclusions

Despite the small patients number our results indicate an additional impact of ^{18}F-FET PET for monitoring outcome following vaccination therapy.

Keywords: glioblastoma multiforme; dendritic cell vaccination; ^{18}F-FET PET; pseudoprogression

Introduction

Glioblastoma multiforme (GBM) is the most frequent malignant brain tumour, accounting for 81% of all gliomas (Wen and Santosh, 2008, Ostrom et al. 2014). Current multimodal standard first line therapy, combining maximal safe resection, radio- and concurrent chemotherapy with temozolomide (TMZ) followed by further 6 intermittent cycles of TMZ, increased median overall survival (OS) to 14.6 months (Stupp et al., 2005). Recent trials demonstrate a further OS increase to 20.9 months and 37.9 months by combining standard therapy with tumour- treating fields (Stupp et al., 2017) or additional CCNU chemotherapy in O6-methylguanine–DNA methyltransferase (MGMT) methylated patients (Herrlinger et al., 2017).

Dendritic cell (DC) vaccination is a promising active immunotherapeutic approach, aiming at inducing GBM-specific immune responses, which specifically kill tumour cells. Patients are vaccinated with tumour antigen-loaded DC, inducing DC migration to local lymph nodes, presenting tumour antigen-derived peptides on human leukocyte antigen molecules and finally initiating anti-tumoural T-cell response (Palucka and Banchereau, 2013). Preclinical studies in animal models provided a proof of principle (Mac Keon et al., 2015) and several smaller or non-controlled studies have documented feasibility as well as safety and suggested a clinical benefit (Rutkowski et al., 2004, Cho et al., 2012, Cao et al., 2014).

In GBM, magnetic resonance imaging (MRI) scans are assessed according to the criteria defined by the Response Assessment in Neuro-Oncology (RANO) working group to monitor therapy responses and tumour recurrence (Wen et al., 2010). However, immunotherapy might require a different approach, since it may result in inflammatory reactions, which mimic tumour progression and may even include the appearance of ‘new lesions’ on MRI scans. Moreover, immunotherapy may result in delayed responses, i.e.

patients may still benefit from therapy although showing signs of progressive disease initially (Okada et al., 2015). Therefore, distinguishing progression from pseudoprogression unequivocally can require repeated MRI scans over a prolonged (≥ 3 months) observation period as summarized by Okada et al. (2015).

Metabolic imaging using amino acid positron emission tomography (PET) provides information on tumour metabolism. ^{18}F-Fluoroethyl-tyrosine (FET)-PET imaging in combination with MRI has been shown to allow more accurate diagnosis of tumour progression or recurrence after standard therapy compared to conventional MRI alone (Galldiks et al., 2015).

Therefore, we were interested in the additional impact of ^{18}F-FET PET imaging on therapy monitoring after DC vaccination of GBM patients, which has not been addressed previously. Here, we retrospectively analysed GBM patients, treated with DC vaccination therapy at our neurooncological department who underwent additional ^{18}F-FET PET imaging for the differentiation between tumour progression and therapy related changes.

Patients and Methods

Study design

The retrospective ^{18}F-FET PET imaging study was approved by the Ethics Committee of the Medical Faculty of the University of Düsseldorf (internal study number: 2438). The medical records of all GBM patients receiving DC vaccination therapy between December 2003 and December 2016 at the Department of Neurosurgery of the University Clinic Düsseldorf were reviewed. Inclusion criteria were 1) surgical resection of histopathological confirmed GBM, 2) DC vaccination therapy, 3) suspicious tumour recurrence on MRI scans according to the RANO criteria and 4) ^{18}F-FET PET imaging for further differentiation within 6 weeks following MR imaging.

Surgery

For all resections, 5-aminolaevulinic acid-based fluorescence (5-ALA)-guided surgery was used. Awake surgery with intraoperative neurophysiological monitoring was performed for patients with eloquently located GBM in an asleep-awake-asleep protocol as described before (Beez et al., 2013).

Dendritic cell (DC) vaccination therapy

DC vaccination therapy was performed on a compassionate-use basis with written informed consent of the patients. Mature, monocyte-derived DC, loaded with autologous tumour lysate as a source of tumour antigens were manufactured as described previously with production permission of the local authorities (Bezirksregierung Düsseldorf) (Sorg et al., 2003, Rapp et al., 2006).

DC vaccination was performed five times, mainly in weekly intervals, by intradermal injections in the upper arm between concomitant radio/-chemotherapy and intermittent chemotherapy, or in relapse situations, before re-radiation therapy. For each vaccination, patients received a dose of $11.3 \pm 7.3 \times 10^6$ ($0.6 - 20 \times 10^6$) tumour-antigen loaded, mature DC.

Magnetic resonance imaging (MRI)

Pre-, postoperative and follow-up MRI were performed at the Institute of Radiology, University Clinic Düsseldorf. Standard imaging was performed by contrast-enhanced 1.5 Tesla MRI (Avanto, Siemens, Erlangen, Germany) before and after administration of a gadolinium-based contrast agent (0.5 mmol/kg, Guerbet, Sulzbach, Germany),

including T1- and T2-weighted and fluid attenuated inversion recovery sequences. MRI-based diagnosis of tumour recurrence was based on RANO criteria (Wen et al., 2010).

^{18}F-FET PET imaging and data analysis

The amino acid O-(2-^{18}F-fluoroethyl)-L-tyrosine (^{18}F-FET) was produced via nucleophilic ^{18}F-fluorination as described previously with a specific radio-activity of more than 200 GBq/mmol (Hamacher and Coenen, 2002). Acquisition of PET scans was performed after intravenous injection of 200 MBq of ^{18}F-FET. For all images, an ECAT EXACT HR1 scanner (Siemens Medical Systems) in 3-dimensional mode (32 rings; axial field of view, 15.5 cm) was used, and transmission was measured with three ^{68}Ge/^{68}Ga rotating line sources for attenuation correction. Following Fourier rebinning and correction for attenuation, scattered coincidences, random coincidences and decay, 63 image planes were reconstructed in an iterative process (ordered-subsets expectation maximization, 6 iterations, 16 subsets) using the ECAT 7.2 software. Summed PET data from 20 to 40 min after injection was used for further evaluation. ^{18}F-FET PET and contrast-enhanced MRI scans were co-registered with MPI tool software (version 6.48; ATV). Region of interest (ROI) analyses were performed at the trans axial slice, which showed the highest ^{18}F-FET accumulation in the tumour and at a slice showing the contralateral hemisphere in an area of normal-appearing grey and white matter to gain ^{18}F-FET uptake in the unaffected brain tissue. A tumour-to-brain ratio (TBR) of at least 1.6, evaluated in earlier studies (Pauleit et al., 2005), was used to determine the ^{18}F-FET uptake in the tumour by an auto contouring, 2-dimensional process. By dividing the mean and maximum standardized uptake volume (SUV) of the tumour ROI by the mean SUV of normal brain in the ^{18}F-FET PET scan, mean and maximum TBR (TBR_{mean}, TBR_{max}) were calculated. Furthermore, time-activity curves (TAC) of mean SUV of ^{18}F-

FET uptake in the tumour and in the brain were generated by application of a spherical Volume-of-Interest with a volume of 2 ml centred on maximal tumour uptake and of a reference ROI in the unaffected brain tissue (as described above) to the entire dynamic data set.

Data collection and follow-up

Epidemiological data, data regarding tumour location and histology as well as pre-, postoperative and follow-up images were collected from the files and electronic records. Patients were followed from the time of surgery until death or referral to a palliative care ward 3 monthly, including a contrast-enhanced MRI.

Statistical analysis

Descriptive statistics including minimum, maximum, mean and standard deviation were calculated for all continuous variables. Progression-free survival (PFS) was measured from the day of surgery before vaccination therapy until the date of tumour progression or death and overall survival (OS) until death or last follow-up according to the Kaplan-Meier method with 2-sided log rank statistics for comparison.

Results

Patients

Five GBM patients (mean age: 44 ± 14y) could be included in the study who were treated between 2003 and 2016 with DC vaccination therapy and were assessed by ^{18}F-FET-PET in addition to MRI scans. Clinical data are summarized in table 1. At the time point of vaccination therapy, two patients were firstly diagnosed with GBM, in two patients the first and in one patient the third tumour recurrence was diagnosed.

As first line therapy, all patients received postoperative radiotherapy, concomitant TMZ chemotherapy and 6 cycles of intermittent TMZ chemotherapy according to the protocol established by the EORTC (European Organization for Research and Treatment of Cancer)/NCIC (National Cancer Institute of Canada) trial (Stupp et al., 2005).

After tumour recurrence patients were treated individually different depending on clinical status, tumour extent and localisation. Second and third line therapy schemes are specified in table 1.

Further molecular tumour diagnostics revealed isocitrate dehydrogenase (IDH) mutations in two tumour samples; O6-methylguanine-DNA-methyltransferase (MGMT) promotor methylation was present in two tumour samples (table 1).

Vaccination therapy was well tolerated in all patients and no side effects relating to the immunotherapeutic treatment have been reported.

MRI/ ^{18}F-FET-PET imaging

MRI and ^{18}F-FET-PET findings are summarized in table 2. After suspicious tumour recurrence via MR diagnostic within six weeks additional ^{18}F-FET-PET imaging was performed for further evaluation. In three patients, MRI scans indicated tumour recurrence based on the RANO criteria, which was confirmed by ^{18}F-FET-PET (figure 1). In the framework of further photodynamic therapy in one patient a biopsy was performed, and histopathological findings verified tumour recurrence. In the other two patients, further follow-up MRI confirmed tumour recurrence based on the RANO criteria.

In two patients, MRI and ^{18}F-FET-PET imaging findings were discordant. In these patients MRI imaging demonstrated tumour recurrence according to the RANO criteria whereas ^{18}F-FET-PET imaging revealed decreased tracer-uptake, suspicious for therapy

induced changes. (figure 2 and 3). Because of the discordant imaging in one patient an open biopsy was performed, and histopathological findings demonstrated post radiogenic reactive and necrotic tissue. Follow up MR imaging two months later demonstrated decreased contrast enhancement as well as decreased hyperintensity on T2- weighted imaging confirming therapy induced changes in the second patient (figure 4).

Discussion

In the last years survival of glioblastoma patients could be clearly increased by the combination of surgery, radio- chemotherapy and new therapeutic approaches such as tumour treating fields (Stupp et al., 2017). However, therapy evaluation is getting more and more complicated by the combination of different therapies (de Wit et al. 2004). Early recognition of tumour progress plays a key role in defining further therapeutic strategies as long as the patient is not severely affected by neurological deficits. Thus, a critical issue in treatment of GBM patients is to distinguish progressive disease from therapy related changes as early as possible. The phenomenon of pseudoprogression, observed in approximately 10–20% of newly diagnosed glioblastoma patients following concomitant radio-/ chemotherapy, and on the other hand, pseudoresponses following anti-angiogenic therapy underline the problems of MRI interpretation (de Wit et al., 2004, Brandsma et al. 2008). In 2010, the RANO criteria were introduced to address the complexity of imaging assessment following radio- and chemotherapy (Wen et al., 2010).

^{18}F-FET PET imaging has not been considered as standard therapy monitoring in GBM patients yet, but it has already found its way into clinical application for patients with uncertain progressive disease, due to its potential contribution to better distinguish progressive disease from pseudoprogression: Compared to the diagnostic accuracy of conventional MRI to diagnose tumour progression or recurrence (85%), a higher accuracy

(93%) was achieved by the addition of ^{18}F-FET PET (Galldiks et al., 2015). However, the impact of ^{18}F-FET PET imaging on therapy monitoring after immunotherapy has not extensively been studied, although preliminary promising data in mouse models exist (Antonios et al., 2017).

DC vaccination besides blockade of checkpoint regulators or chimeric antigen-receptor (CAR) T-cells is a promising immunotherapeutic approach in GBM (Rutkowski et al., 2004, Cao et al., 2014, Brown et al., 2016, Migliorini et al.,2018). Recent studies and review articles (De Vleeschouwer et al., 2006, De Vleeschouwer et al., 2008, Polyzoidis et al., 2015, Chen et al. 2016, Finocchiaro and Pellegatta, 2016, Reardon and Mitchell, 2017) discuss the possible impact of DC based vaccination therapy for malignant glioma patients and present promising data of potential efficacy in combination with other adjuvant treatment regimens without striking side-effects. As an active immunotherapy, it may result in local inflammatory reactions at sites of residual tumour, irrespective of whether the tumour can be detected on MRI scans or not. Such reactions, which are signs of therapy response, mimic tumour progression on MRI scans, including the appearance of new suspected lesion (Okada et al., 2009, Okada et al., 2011). Therefore, evaluation of MRI scans following immunotherapy is challenging and the RANO group recently underlined the need of a different monitoring strategy and additional monitoring intervals (Okada et al., 2015). For patients, who demonstrate imaging findings that meet RANO criteria for progressive disease within six months of initiating immunotherapy, including the development of new lesions and without significant worsened neurological deficits, a follow-up imaging in three months is recommended before defining a treatment failure.

Here, progressive disease was diagnosed by MRI and ^{18}F-FET PET scan in three out of five GBM patients vaccinated with DC. In two patients, however, MRI and ^{18}F-FET PET scans showed discordant results. Whereas MRI showed new contrast enhancement,

suspicious for progressive disease, ^{18}F-FET PET did not show increased tracer uptake, thus indicated pseudoprogression. Pseudoprogression was confirmed histopathologically in one of these patients. There was no evidence of GBM, but of necrosis and leukocyte infiltrates, including macrophages and effector T-cells. Pseudoprogression was confirmed by subsequent MRI scans after two and four months according to the RANO criteria in the other patient (figure 4). These results emphasize the importance of additional MRI scans for patients undergoing immunotherapy as suggested by the RANO working group, to prevent premature diagnosis of progression (Okada et al., 2015). On the other hand, our results also indicate a benefit of additional ^{18}F-FET PET imaging following DC vaccination therapy.

Here, we present preliminary data retrospectively observed in a single centre. We acknowledge several limitations of the present study: (1) our results are limited by the retrospective study design, (2) the small number of patients, (3) different time-point of vaccination therapy and (4) non-comparable different additional therapy-regimes. Because of the small and incongruent patient cohort it is not possible to (1) perform statistical analysis regarding sensitivity or specificity of MRI or ^{18}F-FET PET diagnostics or to evaluate the different observed phenomenon on MR and PET imaging. For further elucidation of our observations a randomized, prospective trial is needed. This year our study group will initiate a phase II trial regarding DC vaccination in primary glioblastoma patients. Based on our observations we are planning to preform additional FET PET imaging for further differentiation of tumour progression and therapy induced changes.

Conclusions

In this preliminary observational study ^{18}F-FET PET appears to be more accurate than contrast-enhanced MRI in distinguishing tumour recurrence from reactive changes following DC vaccination therapy of GBM patients. Although the retrospective design, the small number of patients and the variable therapeutic schemes after tumour recurrence limit this analysis, it indicates a potential role for ^{18}F-FET PET for monitoring upcoming GBM immunotherapy studies, in defining disease progression and preventing premature termination of treatment, due to false declaration of treatment failure.

Disclosure of interest

The authors report no conflict of interest.

Table 1:

patient	sex	age	MGMT status	IDH-1 mutation	tumor localization	DC vaccination time-point	2nd line therapy	3rd line therapy	PFS	OS
1	f	37	methylated	positive	bifrontal	ID	TMZ, bevacizumab	none	11	26.9
2	m	49	methylated	positive	frontal left	1. recurrence	resection + carmustin, re-radiation	none	7	10.2
3	m	58	unmethylated	wildtype	frontal right	ID	PDT	none	3	11.8
4	m	44	unmethylated	wildtype	temporal left	1. recurrence	re-radiation, TMZ	none	1	9.5
5	m	20	unmethylated	wildtype	parietal right	3. recurrence	resection+ carmustine, TMZ	resection, bevacizumab+ irinotecan	4	11.5

Table 1: Clinical patients' data, therapy schemes and molecular tumour data. All patients were diagnosed with a glioblastoma, as first line therapy all patients received concomitant radio/TMZ- chemotherapy followed by 6 cycles of intermittent TMZ chemotherapy. PFS - progression-free survival (months); OS - overall survival (months); ID: initial diagnosis; f - female; m - male; TMZ - temozolomide; PDT - photo dynamic therapy; MGMT - O6-methylguanine-DNA-methyltransferase; IDH - Isocitrate dehydrogenase

Table 2:

patient	MRI suspected PD	^{18}F-FET PET TBR_{mean}	^{18}F-FET PET TBR_{max}	^{18}F-FET PET TAC	^{18}F-FET PET suspected PD	surgery	neuropathological result	follow-up MRI suspected PD
1	yes	2.3	2.7	increasing	no	biopsy	necrosis	no
2	yes	1.8	2.5	increasing	no	no	-	no
3	yes	2.0	2.6	decreasing	yes	biopsy	GBM	-
4	yes	3.7	5.4	decreasing	yes	no	-	yes
5	yes	3.6	5.0	decreasing	yes	no	-	yes

Table 2: Summary of MRI and ^{18}F-FET PET findings of all patients. PD-progressive disease

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Figures

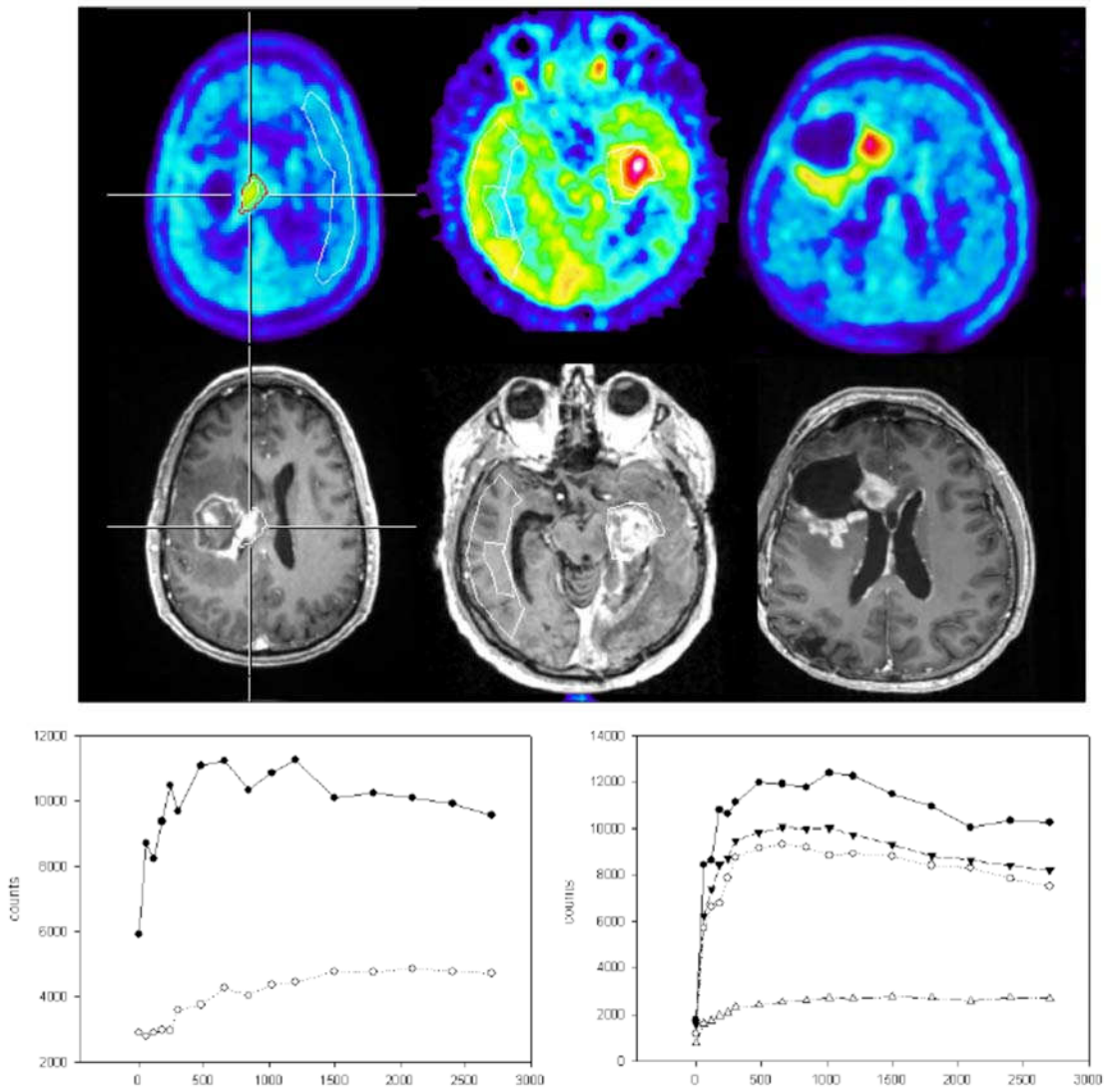

Figure 1:

^{18}F-FET PET and MR (T1 with gadolinium) imaging of patient 3,4 and 5. Dynamic data of patient 3 and 5. MRI demonstrated increased contrast enhancement accompanied by increased ^{18}F-FET uptake (TBRmax 2.6, 5.4, 5.0) as well as rapid, steep increasing time–activity curves symptomatic for active high-grade tumour progression

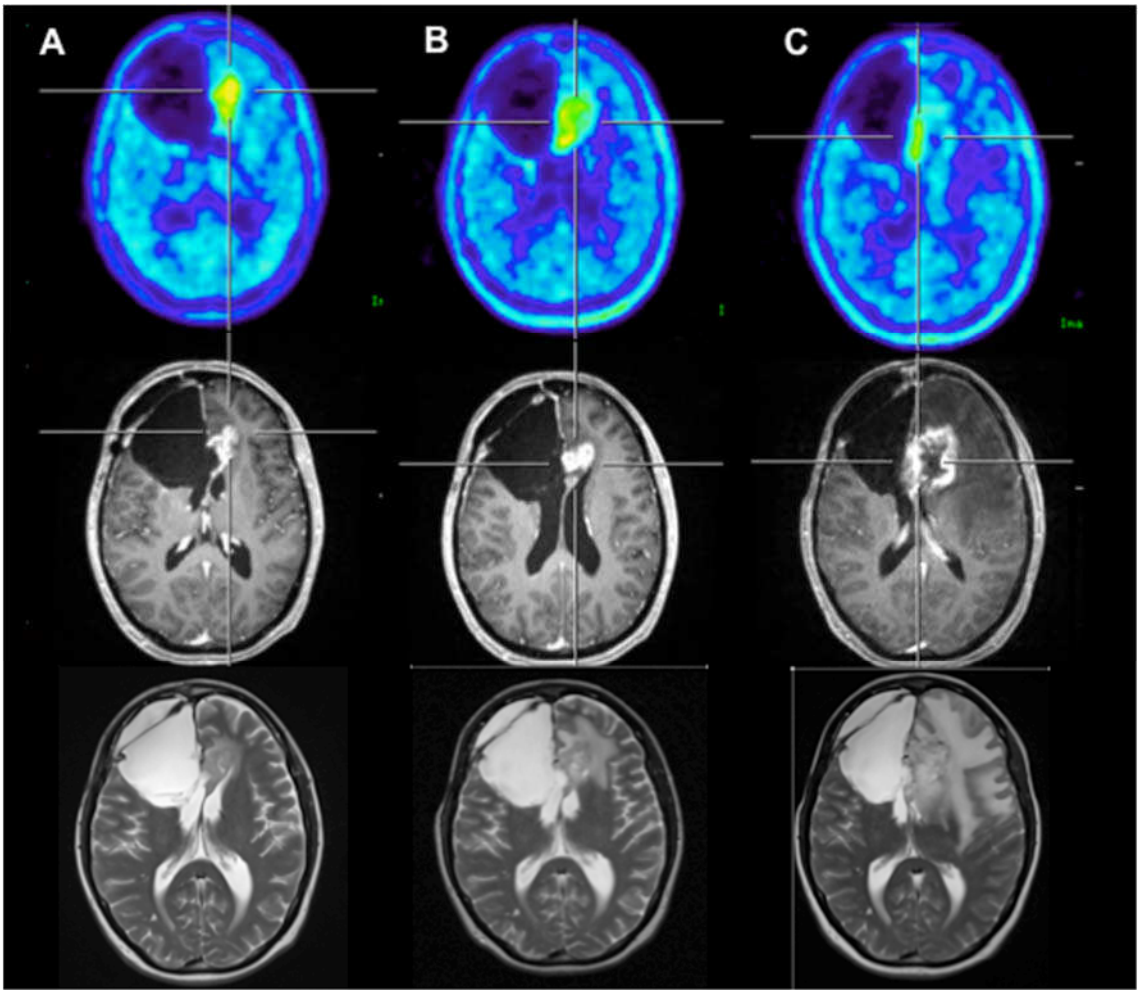

Figure 2:

^{18}F-FET PET and MR imaging of patient 1 (A) 6 weeks (B) 10 weeks and (C) 14 weeks after completion of concomitant radio /chemotherapy. MR imaging (T1 contrast enhanced, T2- weighted) demonstrated an increasing oedema and contrast enhancement mimicking tumour progression (RANO criteria). ^{18}F-FET PET imaging reveals a decrease ^{18}F-FET uptake (TBRmax 3.7, 3.3, 2.7) as result of therapy response.

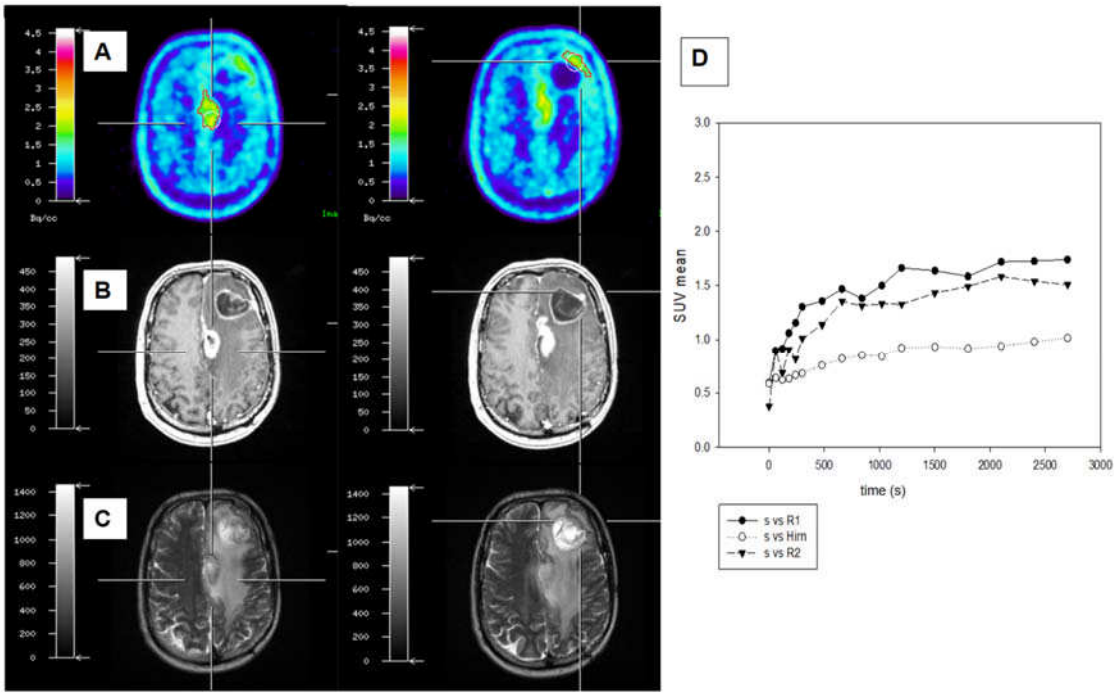

Figure 3:

^{18}F-FET PET (A) and MR imaging (B: contrast enhanced T1; C: T2 weighted) of patient 2. MR imaging indicates tumour progression according to the RANO criteria by perifocal oedema and progressive contrast enhancement. In contrast PET imaging reveals only minimal increased FET uptake (TBRmax 2.5). D: ^{18}F-FET PET kinetics of both lesions demonstrating only a slight uptake followed by a flattened curve. Kinetic findings in combination with the uptake measurement indicate therapy induced changes.

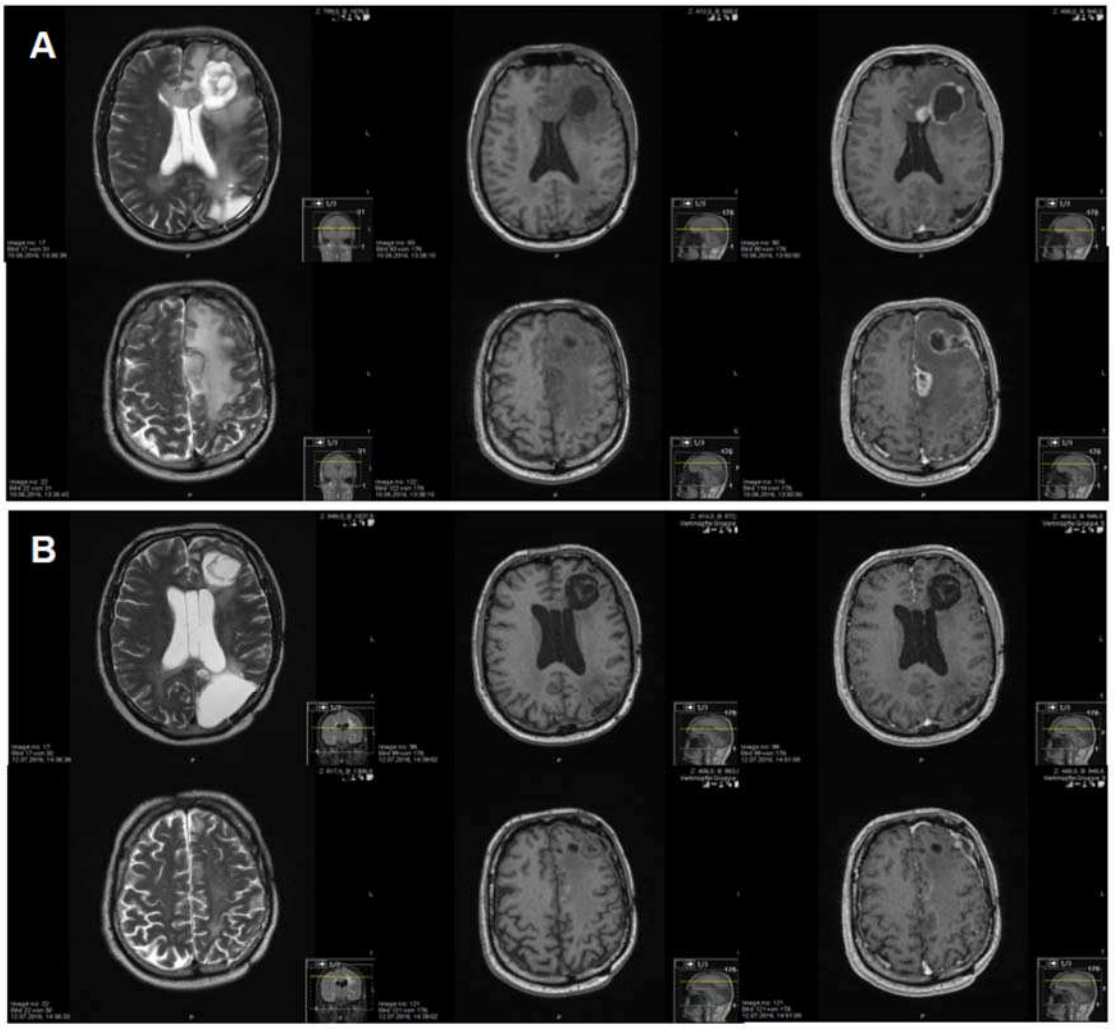

Figure 4:

MR imaging of patient 2. A: MR imaging (T1 with and without contrast enhancement, T2 weighted imaging) demonstrating suspicious tumour recurrence at both lesions. B: Follow up MR imaging 8 weeks later with decreased perifocal oedema and decreased contrast enhancement, confirming now therapy induced changes.