TERT-Promoter Mutational Status in Glioblastoma – Is There an Association With Amino Acid Uptake on Dynamic ¹⁸F-FET PET?

Marcus Unterrainer¹,²,³ Viktoria Ruf⁴, Katharina von Rohr², Bogdana Suchorska³,⁵ Lena Maria Mittmeier², Leonie Beyer², Matthias Brendel², Vera Wenter², Wolfgang G. Kunz¹, Peter Bartenstein²,³, Jochen Hermès⁴, Maximilian Niyazi³,⁶ Jörg C. Tonn³,⁵ and Nathalie Lisa Albert²,³*

¹ Department of Radiology, University Hospital, LMU Munich, Munich, Germany, ² Department of Nuclear Medicine, University Hospital, LMU Munich, Munich, Germany, ³ German Cancer Consortium (DKTK), Partner Site Munich and German Cancer Research Center (DKFZ), Heidelberg, Germany, ⁴ Department of Neuropathology and Prion Research, LMU Munich, Munich, Germany, ⁵ Department of Neurosurgery, University Hospital, LMU Munich, Munich, Germany, ⁶ Department of Radiation Oncology, University Hospital, LMU Munich, Munich, Germany

Objective: The mutation of the ‘telomerase reverse transcriptase gene promoter’ (TERTp) has been identified as an important factor for individual prognostication and tumorigenesis and will be implemented in upcoming glioma classifications. Uptake characteristics on dynamic ¹⁸F-FET PET have been shown to serve as additional imaging biomarker for prognosis. However, data on the correlation of TERTp-mutational status and amino acid uptake on dynamic ¹⁸F-FET PET are missing. Therefore, we aimed to analyze whether static and dynamic ¹⁸F-FET PET parameters are associated with the TERTp-mutational status in de-novo IDH-wildtype glioblastoma and whether a TERTp-mutation can be predicted by dynamic ¹⁸F-FET PET.

Methods: Patients with de-novo IDH-wildtype glioblastoma, WHO grade IV, available TERTp-mutational status and dynamic ¹⁸F-FET PET scan prior to any therapy were included. Here, established clinical parameters maximal and mean tumor-to-background-ratios (TBRmax/TBRmean), the biological-tumor-volume (BTV) and minimal-time-to-peak (TTPmin) on dynamic PET were analyzed and correlated with the TERTp-mutational status.

Results: One hundred IDH-wildtype glioblastoma patients were evaluated; 85/100 of the analyzed tumors showed a TERTp-mutation (C228T or C250T), 15/100 were classified as TERTp-wildtype. None of the static PET parameters was associated with the TERTp-mutational status (median TBRmax 3.41 vs. 3.32 (p=0.362), TBRmean 2.09 vs. 2.02 (p=0.349) and BTV 26.1 vs. 22.4 ml (p=0.377)). Also, the dynamic PET parameter TTPmin did not differ in both groups (12.5 vs. 12.5 min, p=0.190). Within the TERTp-mutant subgroups (i.e., C228T (n=23) & C250T (n=62)), the median TBRmax (3.33 vs. 3.69, p=0.095), TBRmean (2.08 vs. 2.09, p=0.352), BTV (25.4 vs. 30.0 ml, p=0.130) and TTPmin (12.5 vs. 12.5 min, p=0.190) were comparable, too.
INTRODUCTION

According to the updated 2016 WHO classification of brain tumors, the molecular genetic profile plays a major role for the glioma characterization and highly affects the further clinical management and treatment strategies (1, 2). Beyond the current molecular genetic stratification using the isocitrate dehydrogenase (IDH)-mutational status and 1p/19q-codeletion, additional molecular genetic markers are increasingly identified and gradually gain access into clinical routine. In particular, mutations of the telomerase reverse transcriptase gene promoter (TERTp) were identified as important factor within the tumorigenesis and individual prognostication (3, 4), with inferior outcome in combination with an IDH-wildtype status (5, 6), which will be implemented in upcoming glioma classifications.

As recommended by the Response assessment in Neurooncology (RANO) working group in their clinical guidelines (7–9), molecular imaging using positron-emission-tomography (PET) with radiolabeled amino acids such as O-(2,18F-fluoroethyl)-L-tyrosine (18F-FET) is increasingly used for the comprehensive evaluation and characterization of brain neoplasms beyond morphological standard imaging with MRI, e.g. for treatment planning (10–13), but also for noninvasive tumor characterization at initial diagnosis (14–20). Recent studies indicated that the IDH-mutational status is highly associated with 18F-FET PET uptake in brain tumors, especially with the ‘minimal time to peak’ (TTP min) on dynamic 18F-FET PET, and has thus a high diagnostic power for the identification of IDH-wildtype gliomas (21). With regard to TERTp, no study has hitherto evaluated the association of amino acid uptake on PET and the TERTp-mutational status. Hence, we aimed to assess whether the uptake characteristics on static and dynamic 18F-FET PET are likewise associated with the TERTp-mutation status in a homogeneous group of de-novo, IDH-wildtype glioblastoma and whether PET can predict the TERTp-mutation status.

METHODS AND MATERIALS

Patients

Patients with histologically confirmed, newly diagnosed IDH-wildtype glioblastoma WHO grade IV with available molecular genetic analyses of the TERT-promoter mutation status as well as a dynamic 18F-FET PET scan prior to stereotactic biopsy or surgical resection were identified. All patients have given written informed consent prior to the PET examination as part of the clinical routine. Ethical approval of the retrospective study protocol was given by the institutional review board of the LMU.

Histological Confirmation, Tumor Grading and Molecular Genetic Analysis

Stereotactic biopsy procedures and microsurgical resections were performed at the Department of Neurosurgery, LMU Munich, Germany. As part of the clinical routine, histopathological and molecular genetic evaluations were performed at the Institute of Neuropathology, LMU Munich, Germany, and were initially classified according to the 2007 WHO classification of brain tumors (22) and were re-classified according to the updated 2016 WHO classification (1). The IDH-mutation status and TERT-promoter methylation were analyzed in clinical routine according to standard protocols (23–25). For further specification regarding the histopathological workup see also (26, 27).

18F-FET PET Image Acquisition and Data Analysis

18F-FET PET scans were performed at the Department of Nuclear Medicine, LMU. Data of the dynamic 18F-FET PET scans were acquired using an ECAT Exact HR+ scanner (Siemens). After a 15-min transmission scan with a 68Ge rotating rod source, approximately 180 MBq of 18F-FET were injected. Dynamic emission recording was accomplished after tracer injection up to 40 min post injection in 3-D mode consisting of 16 frames (7 x 10 s; 3 x 30 s; 1 x 2 min; 3 x 5 min; 2 x 10 min). Two-dimensional filtered back-projection, reconstruction algorithms using a 5 mm Hann Filter were used for image reconstruction and corrected for photon attenuation and model-based scatter. The mean background activity (BG) was assessed using 6 large crescent-shaped regions of interests (ROI) in the frontal lobe of the healthy contralateral hemisphere fused to a volume of interest (VOI), in which the mean BG was derived (28). The biological tumor volume (BTV) was estimated by a semiautomatic threshold-based delineation of a volume of interest (VOI) using a standardized uptake value (SUV) threshold of 1.6 x BG, as previously described as optimal threshold (29). The maximal SUV (SUV max) and mean SUV (SUV mean) as derived within the BTV were then divided by the BG resulting in mean and maximal tumor-to-background ratio (TBR mean/TBR max). Data on dynamic PET was evaluated using the software PET Display Dynamic implemented in the Hermes workstation: in early summation images (10-30 min p.i.), a 90%
isocontour region of interest was created to extract the time-activity-curves (TACs) on a slice-by-slice manner. Then, the time to peak (TTP) was assessed on each slice of the tumor and the shortest TTP in at least 2 consecutive slices was defined as minimal TTP (TTP$_{\text{min}}$), see also (30, 31).

### Statistics

SPSS for Windows (version 23.0; SPSS, Chicago, IL) was used for statistical analyses. Descriptive statistics are displayed as median (range). Normal distribution was assessed using the Shapiro-Wilk test. The unpaired Wilcoxon test was used for independent and not-normally distributed continuous parameters. Receiver operating curves (ROC) analyses were used to assess the diagnostic power of continuous parameters, the ‘Area under the curve’ (AUC) served as quantitative measure for the diagnostic power. Statistical significance was defined as a two-tailed p-value <0.05.

### RESULTS

#### Patients

One-hundred patients with de-novo, IDH-wildtype glioblastoma, WHO grade IV were included (62/100 (62.0%) male, 38/100 (38.0%) female). The median age was 62.0 (range, 30.1-82.7) years. Tissue samples for histological and molecular genetic analyses were obtained by stereotactic biopsy in 74/100 (74.0%) and by surgical resection in 26/100 (26.0%) cases. Overall, 15/100 (15%) did not comprise a TERTp-mutation and were classified as TERTp-wildtype. Of the remaining 85/100 (85%) patients with TERTp-mutation, 62/85 (72.9%) showed a C228T-mutation and 23/85 (27.1%) showed a C250T-mutation.

#### Overall 18F-FET-Uptake Characteristics

All included gliomas were 18F-FET-positive providing a median TBR$_{\text{max}}$ of 3.37 (2.06-7.07), a median TBR$_{\text{mean}}$ of 2.06 (range, 1.70-2.92) and a median BTV of 25.8 (range, 3.8-133.3) ml. In the dynamic analysis, median TTP$_{\text{min}}$ was 12.5 (range, 3.0-35.0) minutes with a small proportion of late TTP$_{\text{min}}$ ≥ 25 minutes in 13/100 (13.0%) cases only.

#### 18F-FET-Uptake Characteristics Comparing TERTp-Mutant and TERTp-Wildtype Glioblastomas and Predictability of TERTp Mutational Status

Comparing glioblastomas with TERTp-mutation (n=85) and those without (n=15) revealed no statistically significant difference in terms of median TBR$_{\text{max}}$ (3.41 (range, 2.06-7.07) vs. 3.32 (range, 2.32-4.67), p=0.362), TBR$_{\text{mean}}$ (2.09 (range, 1.70-2.92) vs. 2.02 (range, 1.79-2.56), p=0.349) and BTV (26.1 (range, 3.8-133.3) ml vs. 22.4 (range, 3.9-75.7) ml, p=0.377). Not only the evaluated static PET parameters, but also the dynamic parameter TTP$_{\text{min}}$ did not differ between those two groups (12.5 (range, 3.0-35.0) min vs. 12.5 (range, 7.5-25.0) min, p=0.411). By consequence, the ROC-analysis to assess the diagnostic power of 18F-FET PET for the prediction of the TERTp mutational status did not reveal reliable thresholds for the differentiation between TERTp-mutant and TERTp-wildtype glioblastomas. Analyzing the static parameters TBR$_{\text{max}}$, TBR$_{\text{mean}}$ and BTV, the AUC ranged between 0.572 and 0.576 only. Also, for the dynamic parameter TTP$_{\text{min}}$, the AUC reached only 0.562 at a best cut-off at 7.5 min. Further specifications can be found in Table 1 and Table 2. Patient examples can be found on Figure 1.

#### 18F-FET-Uptake Characteristics Comparing TERT-Mutation Subtypes (C228T vs. C250T)

Comparing the two subtypes of TERT-promoter mutation C228T (n=62) & C250T (n=23), there was also no statistically significant difference in terms of median TBR$_{\text{max}}$ (3.33 (range, 2.06-5.51) vs. 3.69 (range, 2.37-7.07), p=0.095), TBR$_{\text{mean}}$ (2.08 (range, 1.70-2.56) vs. 2.09 (range, 1.79-2.92), p=0.352) or BTV (25.4 (range, 3.8-133.3) ml vs. 30.0 (range, 5.7-102.1) ml, p=0.130). On dynamic PET analyses, the median TTP$_{\text{min}}$ was also statistically comparable between those two mutation subtypes (12.5 (range, 7.5-35.0) min vs. 12.5 (range, 3.5-35.0) min, p=0.190). For further specifications, please see Table 3.

### DISCUSSION

This is the first study evaluating the association of amino acid uptake by means of 18F-FET PET and the TERTp-mutational status in glioma patients. As the TERTp-mutational status has shown additional prognostic value in IDH-wildtype gliomas/glioblastomas (5, 6, 32, 33), a non-invasive tool for the prediction of a TERTp-mutation would be helpful for the

| Parameter | Best cut-off value | Area under the curve | Level of significance |
|-----------|-------------------|----------------------|----------------------|
| TBR$_{\text{max}}$ | 3.60 | 0.574 | p=0.323 |
| TBR$_{\text{mean}}$ | 2.21 | 0.576 | p=0.297 |
| BTV | 24.1 ml | 0.572 | p=0.395 |
| TTP$_{\text{min}}$ | 7.5 min | 0.562 | p=0.391 |

### Table 1 | Influence of TERT-mutation on 18F-FET-uptake characteristics [median (range)].

| Parameter          | Overall (n=100) | TERT-mutation (n=85) | TERT-wildtype (n=15) | Level of significance |
|--------------------|-----------------|----------------------|----------------------|----------------------|
| TBR$_{\text{max}}$ | 3.37 (2.06-7.07)| 3.41 (2.06-7.07)     | 3.32 (2.32-4.67)     | p=0.362              |
| TBR$_{\text{mean}}$| 2.06 (1.70-2.92)| 2.09 (1.70-2.92)     | 2.02 (1.79-2.56)     | p=0.349              |
| BTV                | 25.8 (3.8-133.3)ml| 26.1 (3.8-133.3)ml   | 22.4 (3.9-75.7)ml    | p=0.377              |
| TTP$_{\text{min}}$ | 12.5 (3.0-36.0) min | 12.5 (3.0-36.0) min | 12.5 (7.5-25.0) min | p=0.411              |
clinical management of glioma patients. In our large cohort with homogeneous histological and molecular genetic profile (i.e. WHO grade IV glioblastoma, IDH-wildtype only), we observed a high proportion of patients with TERTp mutation of 85%, which is in line with the proportion of patients with TERTp mutation in the current literature (4). Moreover, within the group of TERTp mutant glioblastomas, the C228T-mutation was present more frequently (72.9%) than the C250T-mutation (27.1%), which is also in line with the distribution within IDH-wildtype glioblastomas as described in the current literature (6, 34).

Comparing the TERTp-mutational status with the static PET parameters in terms of uptake intensity (TBRmax and TBRmean) and tumor extent (BTV), we observed a high overlap between TERTp-mutant and TERTp-wildtype tumors so that no cutoff could be found to differentiate between those groups. Moreover, TTPmin on dynamic PET was also indifferent between TERTp-mutant and TERTp-wildtype glioblastomas. Taken together, both groups presented with comparable imaging features as IDH-wildtype glioblastomas as described in the current literature (6, 34).

When analyzing the TERTp-mutation subtypes (i.e. C228T & C250T), expectedly, no difference in terms of uptake-intensity (TBRmax & TBRmean) and tumor extent (i.e. BTV) on PET could be observed; also, TTPmin on dynamic PET was indifferent between C228T & C250T mutations. This finding, however, is not surprising, as these two mutations of hot spot promoter regions (C228T and C250T) are basically responsible for the same molecular mechanism (32, 33).

In general, one could speculate that the pathophysiological changes that are accompanied with TERTp mutations and their influence on cell regulation might also affect the cellular metabolism in terms of amino acid metabolism. Moreover, one could argue that both static and dynamic 18F-FET PET parameters were described to be of prognostic value in the further disease course of glioma patients; as the same is true for TERTp mutations, a certain intercorrelation does not seem unlikely. On a molecular level, the TERT as a catalytic subunit of the telomerase enzyme complex is critically involved in telomere maintenance and lengthening. Abnormal upregulation and activity of TERT as a consequence of TERTp-mutations are considered one of the mechanisms of cellular immortality in cancer cells during division, particularly in gliomas (35–37). With regard to PET imaging, the activity and/or expression of the large neutral amino acid transporters, such as the 18F-FET transporter, is known to be increased in gliomas due to the high metabolic activity of these tumors. As a result, increased uptake of 18F-FET in gliomas is associated with a poor prognosis (38, 39).

### FIGURE 1

(A) a patient with TERTp-mutant glioblastoma (TBRmax 4.1; TBRmean 2.3; BTV 15.4 ml, TTPmin 5 min) shows comparable, only slightly diverging imaging features as (B), a patient with TERTp-wildtype glioblastoma (TBRmax 2.9; TBRmean 1.9; BTV 12.3 ml, TTPmin 10 min).

### TABLE 3

| TERT-mutation overall (n = 85) | C228T (n = 62) | C250T (n = 23) | Level of significance |
|-------------------------------|---------------|---------------|----------------------|
| TBRmax                        | 3.41 (2.06-7.07) | 3.33 (2.06-5.51) | 3.69 (2.37-7.07) | p=0.095 |
| TBRmean                       | 2.09 (1.70-2.92) | 2.08 (1.70-2.56) | 2.09 (1.79-2.92) | p=0.352 |
| BTV                           | 26.1 (8-133.3) ml | 25.4 (8-133.3) ml | 30.0 (6.7-102.1) ml | p=0.130 |
| TTPmin                        | 12.5 (3.0-35.0) min | 12.5 (7.5-35.0) min | 12.5 (3.0-35.0) min | p=0.190 |
acid transporter (LAT) at the tumor cells and at the brain capillary endothelial cells (38) is considered a key factor responsible for the intracellular uptake of amino acids in gliomas (39). The very exact mechanisms and the histopathological or even molecular genetic correlate resulting in diverging uptake dynamics of $^{18}$F-FET are not fully clarified yet and may be influenced by further factors such as vascularization. Our study findings suggest that the presence or, vice versa, the absence of TERTp-mutation in glioblastoma and the accompanying features on a cellular basis, although prognostically relevant, do neither result in an altered level of amino acid metabolism nor in changes of uptake dynamics on $^{18}$F-FET PET.

Notably, there is an occurrence of TERTp-mutations in different tumor types as well, also in molecular subgroups with superior prognosis compared to IDH-wildtype gliomas, e.g. in IDH-mutant gliomas. Interestingly, among IDH-mutant gliomas, IDH-mutant gliomas with TERTp-mutation comprise a superior clinical outcome compared to IDH-mutant glioma without TERTp-mutation, also with emphasis on the particular histological features (5). Therefore, the presence of TERTp-mutations in brain tumors per se is not necessarily linked to a more aggressive course in general. Particularly, in the group of oligodendrogial tumors (i.e. gliomas with both IDH-mutation and 1p/19q-codeletion), basically every tumor presents with TERTp-mutation. This molecular genetic subgroup is associated with favorable outcome compared to e.g. IDH-wildtype gliomas (32, 40), despite a basically general presence of TERTp mutations. These phenomena also warrant further investigation of PET-based imaging characteristics in the subgroup of IDH-mutant gliomas. First preliminary data using radiomic features on MRI could show certain moderate diagnostic power for the detection of TERTp mutations particularly in low-grade/IDH-mutant gliomas (41, 42).

Moreover, a vast body of literature exists dealing with radiomics, deep learning and machine learning with special emphasis on ($^{18}$F-FET) PET and hybrid imaging in neuro-oncology (43–49), not just for the differentiation of treatment-related changes from real progression (44, 50, 51), but also for the prediction of prognostically relevant mutations such as the IDH-mutation (52). Hence, it needs to be evaluated, if further PET-based analyses with the extraction of radiomic features may add value to the conventional image analysis in order to noninvasively identify the TERTp-mutational status. Interestingly, the predictability of key mutations using standard and advanced PET quantification also seems to vary depending on the used radiotracers (53–57).

Particularly, as dynamic $^{18}$F-FET PET was previously reported to show a high prognostic value in gliomas in addition to the clinically most important molecular genetic biomarkers according to the 2016 WHO classification [IDH-mutation and 1p/19q-codeletion status (14, 26)], it would be interesting to evaluate whether the additional prognostic value of PET remains even after further subgroup stratification according to the TERTp mutation status. In order to test this hypothesis, further studies with a larger number of patients (particularly in the relatively small TERTp-wildtype subgroup) are needed to perform multivariate analyses.

Limitations arise from the retrospective study design. Moreover, as mentioned above, the absolute number of tumors without TERTp mutations is relatively low (i.e. $n=15$ vs. $n=85$), however, this proportion is in line with the previously reported distribution of TERTp mutations in glioblastoma. In the current manuscript, only filtered-back projection (FBP) reconstructions were used due to the applied scanner; quantification of PET parameters could potentially be diverging using other reconstruction algorithms such as ordered subset expectation maximization (OSEM).

**CONCLUSION**

The prognostically relevant TERTp-mutational status in IDH-wildtype glioblastoma is not associated with uptake characteristics on dynamic $^{18}$F-FET PET. As both, dynamic $^{18}$F-FET PET parameters as well as the TERTp-mutation status are well-known prognostic biomarkers, but show no association in our analysis, it seems highly interesting to evaluate in larger studies if both factors are independent predictors of patients’ survival and can thereby further stratify patients into risk groups.

**DATA AVAILABILITY STATEMENT**

The raw data supporting the conclusions of this article will be made available by the authors upon reasonable request.

**ETHICS STATEMENT**

The studies involving human participants were reviewed and approved by Ethics committee, LMU Munich. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

**AUTHOR CONTRIBUTIONS**

Conceptualization, MU and NA. Methodology, MU, VR, KR, BS, LM, LB, MB, VW, and WK. Formal analysis, MU, VR, KR, and NA. Resources, all authors. Writing—original draft preparation, MU and NA. Writing—review and editing, all authors. Visualization, MU and NA. Supervision, NA, MN, JH, JT, and PB. Project administration, MU and NA. All authors contributed to the article and approved the submitted version.

**ACKNOWLEDGMENTS**

Parts of this paper originate from the doctoral thesis of KR. This work was supported by the Collaborative Research Centre SFB-824 of the Deutsche Forschungsgemeinschaft (DFG) and by the Else Kröner-Fresenius-Stiftung.
REFERENCES

1. Louis DN, Perry A, Reifenberger G, Von Deimling A, Figarella-Branger D, Cavenee WK, et al. The 2016 World Health Organization classification of tumors of the central nervous system: a summary. *Acta Neuropathol* (2016) 131:803–20. doi: 10.1007/s00401-016-1545-1

2. Weller M, van den Bent M, Tonn JC, Stupp R, Prusser M, Cohen-Jonathan-Moyal E, et al. European Association for Neuro-Oncology (EANO) Guideline on the Diagnosis and Treatment of Adult Astrocytic and Oligodendrogial Gliomas. *Lancet Oncol* (2017) 18:e315–29. doi: 10.1016/S1470-2045(17)30194-8

3. Nonoguchi N, Ohita T, Oh J-E, Kim Y-H, Kleihues P, Oghahi H. TERT Promoter Mutations in Primary and Secondary Glioblastomas. *Acta Neuropathol* (2013) 126:931–7. doi: 10.1007/s00401-013-1163-0

4. Simon M, Hosen I, Gousias K, Rachakonda S, Heidenreich B, Gessi M, et al. TERT Promoter Mutations: a Novel Independent Prognostic Factor in Primary Glioblastomas. *Neuro-oncology* (2014) 17:45–52. doi: 10.1093/neuonc/nou158

5. Eckel-Passow JE, Lachance DH, Molinaro AM, Walsh KM, Decker PA, Simon M, et al. Combined Analysis of TERT, EGFR, and IDH Status Defines Distinct Prognostic Glioblastoma Classes. *Neurology* (2014) 83:1200–6. doi: 10.1212/WNL.0000000000001814

6. Albert NL, Weller M, Suchorska B, Galldiks N, Soffietti R, Kim MM, et al. Response Assessment in Neuro-Oncology Working Group and European Association for Neuro-Oncology Recommendations for the Clinical use of PET Imaging in Gliomas. *Neuro-oncology* 2016;now058. doi: 10.1093/neuonc/now058

7. Galldiks N, Langen K-J, Albert NL, Chamberlain M, Soffietti R, Kim MM, et al. PET Imaging in Patients With Brain Metastasis—Report of the RANO PET Group. *Neuro-oncology* (2019). doi: 10.1093/neuonc/noz003

8. Law I, Albert NL, Arbizu J, Boellaard R, Drzezga A, Galldiks N, et al. PET Imaging in Patients With Brain Metastasis—Report of the RANO PET Group. *Neuro-oncology* (2019). doi: 10.1093/neuonc/noz003

9. Bachrach TJ, Arbabzadeh-Bodaghchi S, Galldiks N, Soffietti R, Kim MM, et al. Identification of Time-to-Peak on Dynamic 18F-FET-PET as a Prognostic Marker Specifically in IDH1/2 Mutant Diffuse Astrocytoma. *Neuro-Oncology* (2017). doi: 10.1093/neuonc/nox153

10. Soffietti R, Galldiks N, Giese A, Biczok A, Unterrainer M, Weller M, Drexler M, et al. Suspected Recurrence of Brain Metastases After Focused High Dose Radiotherapy: can [18 F] FET-PET Overcome Diagnostic Uncertainties? *Radiat Oncol* (2016) 11:139. doi: 10.1186/s13040-016-0713-8

11. Unterrainer M, Schweisthal F, Suchorska B, Wenter V, Schmid-Tannwald C, Fendler WP, et al. Serial 18F-FET PET Imaging of Primarily 18F-FET–Negative Glioma: Does it Make Sense? *J Nucl Med* (2016) 57:1177–82. doi: 10.2967/jnumed.115.171033

12. Verger A, Stoffels G, Bauer EK, Lohmann P, Blau T, Fink GR, et al. Static and Dynamic 18F-FET PET for the Characterization of Gliomas Defined by IDH and 1p/19q Status. *Eur J Nucl Med Mol Imaging* (2018) 45:443–51. doi: 10.1007/s00259-017-3846-6

13. Vettermann F, Suchorska B, Unterrainer M, Nelwan D, Forbirg R, Rulf V, et al. Non-Invasive Prediction of IDH-Wildtype Genotype in Gliomas Using Dynamic 18 F-FET PET. *Eur J Nucl Med Mol Imaging* (2019) 46:2581–9. doi: 10.1007/s00259-019-04452-0

14. Louis DN, Oghahi H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, et al. The 2007 WHO Classification of Tumours of the Central Nervous System. *Acta Neuropathol* (2007) 114:97–109. doi: 10.1007/s00401-007-0243-4

15. Thon N, Eigenbrod S, Kreth S, Lutz J, Tonn JC, Kretzschmar H, et al. IDH1 Mutations in Grade II Astrocytomas are Associated With Unfavorable Progression-Free Survival and Prolonged Postreurrence Survival. *Cancer* (2012) 118:452–60. doi: 10.1002/cncr.26298

16. Thon N, Eigenbrod S, Grasbon-Frodl EM, Ruitner M, Mehrkens JH, Kreth S, et al. Novel Molecular Stereotactic Biopsy Procedures Reveal Intratumoral Homogeneity of Loss of Heterozygosity of 1p/19q and TP53 Mutations in World Health Organization Grade II Gliomas. *J Neurosurg Exp Neurol* (2009) 68:1219–28. doi: 10.1093/nejbms/j318

17. Biczkó A, Kraus T, Suchorska B, Terpolilli NA, Thorstensdottir J, Giese A, et al. TERT Promoter Mutation is Associated With Worse Prognosis in WHO Grade II and III Meningiomas. *J Neuro-oncol* (2018) 139:671–8. doi: 10.1007/s11060-018-2912-7

18. Suchorska B, Giese A, Biczkó A, Unterrainer M, Weller M, Drexler M, et al. Prognostic Significance of Dynamic 18F-FET PET in Newly Diagnosed Astrocytic Low-Grade Glioma Identifies High Risk Patients. *J Nucl Med* (2014) 55:198–203. doi: 10.2967/jnumed.113.123333

19. Unterrainer M, Vettermann F, Brendel M, Holzgreve A, Lifschitz M, Zähringer M, et al. Towards Standardization of 18 F-FET PET Imaging: do we Need a Consistent Method of Background Activity Assessment? *EJNMMI Res* (2017) 7:48. doi: 10.1186/s41550-017-0295-y

20. Paulie D, Fleeth F, Hamacher K, Riemenschneider MJ, Reifenberger G, Muller HW, et al. O-(2-[18F]fluoroethyl)-L-Tyrosine PET Combined with MRI Improves the Diagnostic Assessment of Cerebral Gliomas. *Brain* (2005) 128:678–87. doi: 10.1093/brain/asw399

21. Jansen NL, Suchorska B, Wenter V, Eigenbrod S, Schmid-Tannwald C, Zwergal A, et al. Dynamic 18F-FET PET in Newly Diagnosed Astrocytic High-Grade Glioma: Does it Make Sense? *J Nucl Med* (2014) 55:198–203. doi: 10.2967/jnumed.113.123333

22. Jansen NL, Graute V, Armbruster L, Suchorska B, Lutz J, Eigenbrod S, et al. MRI-Suspected Low-Grade Glioma: Is There a Need to Perform Dynamic FET PET? *Eur J Nucl Med Mol Imaging* (2012) 39:1021–9. doi: 10.1007/s00259-012-2109-9

23. Labussière M, Di Stefano A, Gleize V, Boisselier B, Giry M, Mangesius S, et al. TERT Promoter Mutations in Gliomas, Genetic Associations and Clinico-Pathological Correlations. *Br J Cancer* (2014) 111:2024–32. doi: 10.1038/ bjc.2014.538

24. Patel B, Taiwo R, Kim AH, Dunn GP, TERT, A Promoter of CNS Malignancies. *Neuro-Oncol Adv* (2020) 2:vdaa025. doi: 10.1093/noajnd/vdaa025

25. Lee Y, Koh J, Kim S-I, Won JK, Park C-K, Choi SH, et al. The Frequency and Prognostic Effect of TERT Promoter Mutation in Diffuse Gliomas. *Acta Neuropathol Commun* (2017) 5:62. doi: 10.1007/s40478-017-0465-1

26. Horn S, Figl A, Rachakonda PS, Fischer C, Sucker A, Gast A, et al. TERT Promoter Mutations in Familial and Sporadic Melanoma. *Science* (2013) 339:959–61. doi: 10.1126/science.1230062
36. Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA. Highly Recurrent TERT Promoter Mutations in Human Melanoma. Science (2013) 339:557–9. doi: 10.1126/science.1239258.
37. Vinagre J, Almeda A, Pupulo H, Batista R, Lyra J, Pinto V, et al. Frequency of TERT Promoter Mutations in Human Cancers. Nat Commun (2013) 4:1–6. doi: 10.1038/ncomms3185.
38. Boado RJ, Li JY, Nagaya M, Zhang C, Partridge WM. Selective Expression of the Large Neutral Amino Acid Transporter at the Blood–Brain Barrier. Proc Natl Acad Sci U S A (1999) 96:12079–84. doi: 10.1073/pnas.96.21.12079.
39. Habermeier A, Graf J, Sandhöfer BF, Boissel F, Closs E. System l Amino Acid Transporter LAT1 Accumulates O-(2-fluoroethyl)-l-Tyrosine (FET). Amino Acids (2015) 47:335–44. doi: 10.1007/s00726-014-1863-3.
40. Wesseling P, van den Bent M, Perry A. Oligodendroglioma: Pathology, Molecular Mechanisms and Markers. Acta Neuropathol (2015) 129:809–27. doi: 10.1007/s00401-015-1424-1.
41. Arita H, Kinoshita M, Kawaguchi A, Takahashi M, Narita Y, Terakawa Y, et al. Lesion Location Implemented Magnetic Resonance Imaging Radiomics for Predicting IDH and TERT Promoter Mutations in grade II/III Gliomas. Sci Rep (2018) 8:1–10. doi: 10.1038/s41598-018-30273-4.
42. Ivanidze J, Ium M, Pisapia D, Magge R, Ramakrishna R, Kovanlikaya I, et al. MRI Features Associated with TERT Promoter Mutation Status in Glioblastoma. J Neuroimaging (2019) 29:357–63. doi: 10.1111/jon.12596.
43. Gutsche R, Scheins J, Kocher M, Bousabarah K, Fink GR, Shah NJ, et al. Evaluation of FET PET Radiomics Feature Repeatability in Glioma Patients. Cancers (2021) 13:647. doi: 10.3390/cancers13040647.
44. Kebir S, Schmidt T, Weber M, Lazaridis L, Gallida N, Langen K-J, et al. A Preliminary Study on Machine Learning-Based Evaluation of Static and Dynamic FET-PET for the Detection of Pseudoprogression in Patients with IDH-Wildtype Glioblastoma. Cancers (2020) 12:3080. doi: 10.3390/cancers12113080.
45. Li XT, Huang RF. Standardization of Imaging Methods for Machine Learning in Neuro-Oncology. Neuro-oncol Adv (2020) 2:av94–55. doi: 10.1093/noa/jlaa054.
46. Lohmann P, Gallidks N, Kocher M, Heinzl A, Fiss CP, Stegmayr C, et al. Radiomics in Neuro-Oncology: Workbench, Software, and Applications. Methods (2021) 188:112–21. doi: 10.1016/j.ymeth.2020.06.003.
47. Lohmann P, Kocher M, Ruge MI, Visser-Vandewalle V, Shah NJ, Fink GR, et al. PET/MRI Radiomics in Patients With Brain Metastases. Front Neurol (2020) 11. doi: 10.3389/fneur.2020.00001.
48. Lohmann P, Meißner A-K, Kocher M, Bauer EK, Werner J-M, Fink GR, et al. Feature-Based PET/MRI Radiomics in Patients With Brain Tumors. Neuro-Oncol Adv (2021) 2:av15–21. doi: 10.1093/noa/vdaa118.
49. Wüstler B, Menze B. Deep Learning for Medical Image Analysis: a Brief Introduction. Neuro-Oncol Adv (2020) 2:av35–41. doi: 10.1093/noa/vdaa092.