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[^{18}F]-fluoromisonidazole (FMISO) PET/MRI hypoxic fraction distinguishes neuroinflammatory pseudoprogression from recurrent glioblastoma in patients treated with pembrolizumab

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Abstract

Response assessment after immunotherapy remains a major challenge in glioblastoma due to an expected increased incidence of pseudoprogression. Gadolinium-enhanced magnetic resonance imaging (MRI) is the standard for monitoring therapeutic response, however, is markedly limited in characterizing pseudoprogression. Given that hypoxia is an important defining feature of glioblastoma regrowth, we hypothesized that [^{18}F]-fluoromisonidazole (FMISO) positron emission tomography (PET) could provide an additional physiological measure for the diagnosis of immunotherapeutic failure. Six patients with newly diagnosed glioblastoma who had previously received maximal safe resection followed by Stupp protocol CRT concurrent with pembrolizumab immunotherapy were recruited for FMISO PET and Gd-MRI at the time of presumed progression. The hypoxic fraction was defined as the ratio of hypoxic volume to T1-weighted gadolinium-enhancing volume. Four patients diagnosed with pseudoprogression demonstrated a mean hypoxic fraction of 9.8 ± 10%. Two with recurrent tumor demonstrated a mean hypoxic fraction of 131 ± 66%. Our results, supported by histopathology, suggest that the noninvasive assessment of hypoxic fraction by FMISO PET/MRI is clinically feasible and may serve as a biologically specific metric of therapeutic failure.

Keywords

FMISO | glioblastoma | pembrolizumab | pseudoprogression | RANO

The standard of care, maximal safe resection followed by temozolomide (TMZ)-based chemoradiotherapy (CRT), for patients with newly diagnosed glioblastoma often results in neuroinflammatory changes, pseudoprogression (PSP), superimposed upon viable tumor. Pembrolizumab (anti-programmed cell death 1 [PD-1] antibody, Keytruda, Merck) has been FDA-approved for systemic tumors. Early reports in glioblastoma suggest that checkpoint blockade may improve overall survival in specific clinical settings and is expected to result in an increased incidence of PSP.[1]

Gadolinium-enhanced magnetic resonance imaging (Gd-MRI) is the standard for monitoring therapeutic response.[2–7] Gd-MRI is a measure of neurovascular leakiness. As such, it is markedly limited in characterizing therapeutic failure. The differentiation of PSP from recurrent tumor is clinically meaningful as a survival benefit associated with PSP has been reported.[8,9]

Glioblastoma therapeutic failure is distinct from PSP and is in part biologically defined by hypoxia-mediated microvascular proliferation surrounding pseudopalisading necrosis.[10] [^{18}F]-fluoromisonidazole (FMISO) positron emission tomography (PET) imaging noninvasively quantifies tissue oxygen tension.[11,12] Given that hypoxia is an important defining feature of glioblastoma regrowth, we hypothesized that FMISO PET would provide an additional physiological measure for the diagnosis of immunotherapeutic failure. Therefore, the aim of this study was to investigate the clinical utility of FMISO PET/MRI in patients treated with CRT combined with concurrent pembrolizumab for newly diagnosed glioblastoma. We report the preliminary findings of 6 patients with subsequent disease progression to demonstrate the feasibility of a novel FMISO PET/MRI-based hypoxic fraction metric to adjudicate treatment outcomes.

Case Presentation

Patient Population and Imaging Techniques

This prospective, institutional review board-approved study included the following inclusion criteria: (i) histologically confirmed diagnosis of glioblastoma (World Health Organization classification grade IV), (ii) documentation of IDH-1 mutational status, (iii) a Karnofsky performance score >50, (iv) maximal safe resection followed by Stupp protocol CRT, (v) concurrent pembrolizumab immunotherapy; 200 mg intravenous every 3 weeks (NCT03347617), and (vi) presumed disease progression based on modified RANO criteria.[6] Six patients were recruited for FMISO PET and Gd-MRI at the time of presumed progression (NCT03649880). 3.7 MBq/kg (0.1 mCi/kg; up to 370 MBq or 10 mCi; OHSU Center for Radiochemistry Research, Portland, OR, USA) of FMISO was administered intravenously 90 minutes prior to PET imaging of the brain. FMISO PET and Gd-MRI were assessed using MIM V7.0.6 (MIM Software Inc., Cleveland, OH, USA). The hypoxic volume (HV) was defined as 1.2× of mean left cerebellum uptake within the T2-hyperintense lesion. Standard uptake values were measured as the mean (SUV_{mean}) or maximum (SUV_{max}) voxel-wise values from the enhancing T2-hyperintense lesion. Standard uptake value ratios ($SUVR_{mean}$ or $SUVR_{max}$) were defined as the enhancing T2-hyperintense lesion SUV divided by the left cerebellum SUV. The hypoxic fraction was defined as the HV divided by the enhancing volume. All patients were either diagnosed as disease recurrence or PSP using surgical tissue sampling or according to modified RANO criteria using follow-up Gd-MRI.[6] In the absence of tissue sampling, >25% increase in the lesion-enhancing sum product diameter defined recurrent tumor disease status. Table 1 summarizes the FMISO PET/Gd-MRI features of the cohort.

Pseudoprogression Case Presentation

Four patients were diagnosed with PSP; two through histological tissue assessment. Minimal FMISO uptake was observed within the enhancing lesion (SUV_{mean} 1.83 ± 0.44, SUV_{max} 2.87 ± 1.14, SUVR 1.05 ± 0.09; Figure 1). The HV was 0.78 ± 0.78 mL and the hypoxic fraction was 9.8 ± 10%.

Recurrent Tumor Case Presentation

Two patients were diagnosed with recurrent tumor; one through image-guided tissue sampling. Marked FMISO uptake was observed within and about the enhancing lesion (SUV_{mean} 1.93 ± 0.3, SUV_{max} 3.61 ± 1.09, SUVR 1.31 ± 0.22; Figure 1). The HV was 7.1 ± 8.08 mL and the hypoxic fraction was 131 ± 66%.

Discussion

We investigated the clinical utility of FMISO PET/MRI in defining glioblastoma therapeutic failure in 6 patients treated with CRT combined with concurrent pembrolizumab at the time of presumed disease progression. Our preliminary findings demonstrate the feasibility of FMISO PET/MRI-based hypoxic fraction as a noninvasive metric to adjudicate treatment outcomes. The presence of minimal hypoxic fraction supports the hypothesis that PSP is a reflection of therapeutically induced neuroinflammation which can be biologically differentiated from recurrent glioblastoma. Taken together, the FMISO PET/MRI hypoxic fraction technique presented here advances neuro-oncologic research and practice by presenting a new, biologically specific metric of therapeutic failure, which may eventually improve upon the response assessment of patients with glioblastoma.

We assert that the noninvasive quantification of post-therapeutic hypoxic fraction is a feasible imaging approach for defining glioblastoma therapeutic efficacy. Hypoxia is a disease driving malignant biological feature of glioblastoma. FMISO PET HV has been explored as a response assessment metric of antiangiogenic therapy.[13–16] However, HV may fail to account for overall size of malignant biology observed with tumor recurrence. As such, its utility within the heterogeneous glioblastoma microenvironment may be misleading with

Table 1. Cohort Clinical and Imaging Demographics

Patient #	Age/ Sex	Initial Diagnosis	Pembrolizumab Duration (Wks)	SUV_{mean}	SUV_{max}	SUVR	HV (mL)	Enhancing Volume (mL)	HF (%)	Clinical Outcome at the Time of FMISO PET	Progression-Free Survival (Days)
1	39/M	IDH wildtype, MGMT unmethylated	58	1.34	1.54	0.96	0	0.7	0	PSP at day 450 based on mRANO	574 days based on biopsy at the time of subsequent progression
2	52/W	IDH mutated, MGMT methylated	148	1.74	2.31	0.99	0.21	4.0	5	PSP at day 973 based on mRANO	1095 based on mRANO with subsequent progression
3	73/M	IDH wildtype, MGMT methylated	51	2.41	3.94	1.12	1.44	14.2	10	PSP at day 393 based on biopsy	429 days as a result of perioperative death
4	59/M	IDH wildtype, MGMT unmethylated	11	1.80	3.70	1.14	1.47	6.07	24	PSP at day 92 based on biopsy	231 days based on mRANO with subsequent progression
5	73/M	IDH wildtype, MGMT methylated	41	2.14	4.38	1.46	12.80	7.2	178	Recurrent tumor at day 278 based on biopsy	292 days based on biopsy
6	43/M	IDH wildtype, MGMT methylated	40	1.72	2.84	1.15	1.38	1.62	85	Recurrent tumor at day 346 based on mRANO	399 based on mRANO

Wks, weeks; IDH, isocitrate dehydrogenase; MGMT, O-6-Methylguanine-DNA methyltransferase; $SUV_{mean/max}$, mean or maximum standard uptake value within the region of interest; SUVR, standard uptake value ratio; HV, hypoxic volume is defined by values >1.2× the normal cerebellum; enhancing volume, the volume of gadolinium-induced T1 shortening; HF, hypoxic fraction—the ratio of hypoxic volume to T1-weighted gadolinium-enhancing volume. Clinical outcome at the time of presumed progression FMISO PET examination defined by surgical tissue sampling or modified RANO (mRANO) criteria. PSP, pseudoprogression. Progression-free survival, time from surgical resection date to date of clinical outcome; recurrent tumor.

respect to the clinical significance. A small HV within a large volume of therapeutically treated tumor may have a very different clinical significance when compared with a case of small volume of therapeutically treated tumor. Therefore, we propose the use of FMISO PET/MRI-derived hypoxic fraction, as a metric of both hypoxic and lesion volume, may improve upon the clinical use of HV alone in assessing immunotherapeutic efficacy. An alternative hypothesis may be that a large hypoxic fraction prior to or at the completion of radiation therapy may result in early disease progression. We are actively addressing the mechanism of hypoxic fraction in our prospective study through longitudinal FMISO PET/MRI.

The use of biologically specific imaging metrics for assessing glioblastoma therapeutic response is likely to provide improved noninvasive diagnostic capabilities. Unfortunately, contrast-enhanced MRI response criteria lack specificity in characterizing therapeutic failure because local regional tumor regrowth cannot be prospectively distinguished from therapy-mediated neuroinflammation. Prior investigators have attempted to improve diagnostic criteria by assessing neoangiogenic features. Dynamic susceptibility weighed cerebral blood volume (CBV) is a widely used physiologic MRI metric of glioblastoma response assessment. However, this methodology fails to account for CRT-induced vasodilatory effects observed with PSP. Several recent meta-analyses provide evidence that the use of CBV has a pooled sensitivity and specificity that are both ~80%.[17–19] Radiolabeled amino acid-based PET molecular imaging has also been explored for the assessment of brain tumor response. This approach relies upon the intracellular accumulation of [^{11}C]-methionine (MET), 6-[^{18}F]-L-fluoro-L-3,4-dihydroxyphenylalanine (FDOPA), or *O*-(2-[^{18}F]-fluoroethyl)-L-tyrosine (FET) through L-type amino acid transporter (LAT). Upregulation of LAT1 has been correlated with glioma cellular proliferative index.[20] The diagnostic utility of amino acid PET imaging is still being prospectively adjudicated, but preliminary studies suggest a diagnostic accuracy of FET PET of ~85% for differentiating glioblastoma PSP from true progression.[21] While these imaging capabilities improve upon the use of T1 enhancement, there remains room for improved diagnostic utility. The clinical utilization of FMISO PET/MRI-derived hypoxic fraction may improve upon current imaging metrics by directly assessing biological features of glioblastoma therapeutic failure.

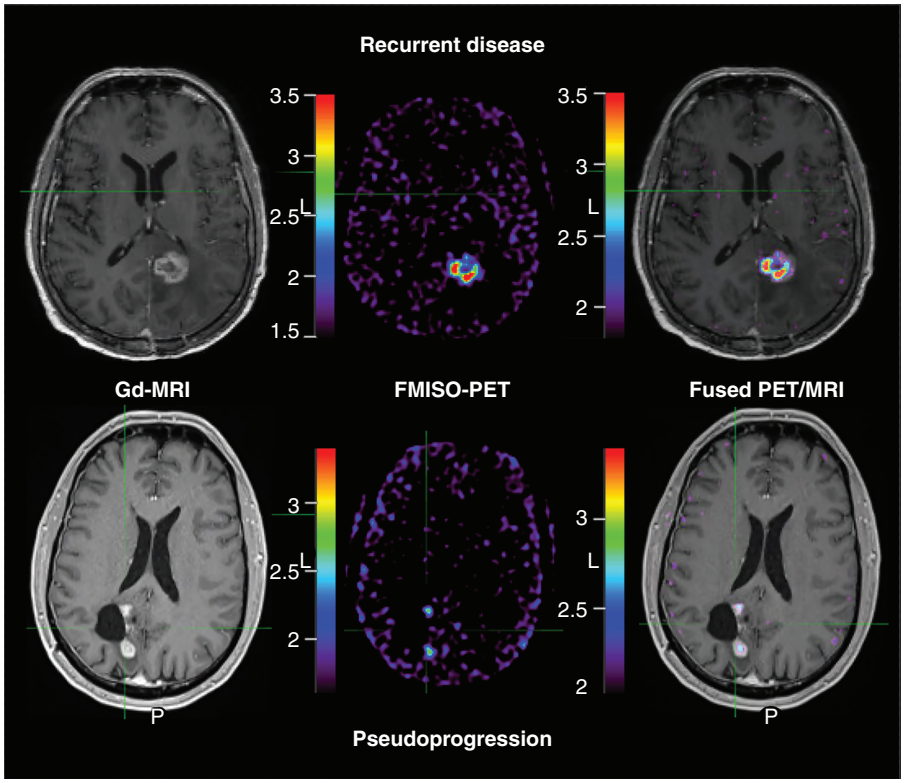

Figure 1. FMISO PET MRI at the time of glioblastoma presumed disease progression. Gadolinium-enhanced MRI (Gd-MRI; left) at the time of presumed disease progression in patients treated with Stupp protocol with concurrent pembrolizumab demonstrates similar appearing contrast-enhancing mass in patients with both recurrent tumor growth (top) and neuroinflammatory-based therapeutic changes; pseudoprogression (PSP, below). Unlike recurrent disease, patients with PSP demonstrated minimal hypoxic volume (middle column) and hypoxic fraction (right column; ratio of hypoxic volume to gadolinium-enhancing volume). In this example, the patient with recurrent disease demonstrated a hypoxic disease burden of 178%. Conversely, the example of PSP demonstrated a hypoxic fraction of 24%. Note: FMISO PET image (middle) window minimum is the mean cerebellar background and window maximum is 2× the mean cerebellar background. Fused PET/MRI image (right) window is the hypoxic volume of 1.2× the mean cerebellar background and window maximum is 2× the hypoxic volume SUV. PET intensity color scales represent the respective visualized SUV measures. Abbreviations: FMISO, [^{18}F]-fluoromisonidazole; MRI, magnetic resonance imaging; PET, positron emission tomography; SUV, standard uptake values.

The small sample size of our study precludes any interpretation beyond the demonstration of preliminary clinical feasibility. We continue to prospectively enroll patients to determine the diagnostic capability of FMISO PET/MRI to differentiate glioblastoma recurrence from PSP.

Conclusion

In a cohort of 6 patients with newly diagnosed glioblastoma treated with CRT combined with concurrent pembrolizumab, we demonstrate the clinical feasibility of FMISO PET/MRI to distinguish recurrent tumor from PSP at the time of presumed disease progression. Our results, supported by histopathology, suggest that the noninvasive assessment of hypoxic fraction may serve as a biologically specific metric of therapeutic failure.

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Conflict of interest statement. R.F.B., P.A., J.L., K.A.K., A.R., N.M., and E.A.N.: None declared.

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