

REVIEW ARTICLE

^{11}C -L-Methionine Positron Emission Tomography in the Clinical Management of Cerebral Gliomas

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Abstract

Positron emission tomography (PET) using L-[methyl- ^{11}C]-methionine (MET) is the most popular amino acid imaging modality in oncology, although its use is restricted to PET centers with an in-house cyclotron facility. This review focuses on the role of MET–PET in imaging of cerebral gliomas. The biological background of tumor imaging with methionine is discussed with particular emphasis on cellular amino acid transport, amino acid utilization in brain, normal metabolism of methionine, and its alterations in cancer. The role of MET–PET in clinical management of cerebral gliomas in initial diagnosis, differentiation of tumor recurrence from radiation injury, grading, prognostication, tumor-extent delineation, biopsy planning, surgical resection and radiotherapy planning, and assessment of response to therapy is also reviewed in detail.

Key words: L-[Methyl- ^{11}C]-methionine (MET), Positron emission tomography (PET), Gliomas

Introduction

Cerebral gliomas are the most frequent primary brain tumors. These tumors are also the most treatment-resistant and difficult to image because of the diffuse infiltrative nature of glioma cells. Prognosis of cerebral gliomas has continued to remain dismal for several decades [1], notwithstanding significant advances in multimodality diagnostic (magnetic resonance imaging, nuclear medicine, molecular genetics) and therapeutic procedures (image-guided surgery, targeted radiation, chemo-, and immunotherapy). Inter- and intratumoral heterogeneity and diffuse infiltration of glioma cells into normal brain pose a formidable challenge to the therapists as well as imaging scientists engaged in tumor detection and precise delineation of its boundaries. The exact determination of the tumor volume in its entirety and

assessment of the tumor sensitivity to the proposed/ongoing therapy are necessary for successful treatment planning. Therefore, development of a highly specific and sensitive non-invasive imaging modality that can enhance the ability to closely correlate diagnosis with pathology, distinguish inflammation from tumors, differentiate tumor grades, accurately delineate tumor volume, monitor treatment responses, and identify residual tumor/recurrence remains a desirable goal to improve the current clinical management of brain tumors. Availability of a suitable imaging modality or a multimodality combination to obtain the information of interest non-invasively is also vital for the basic research and development of novel and effective experimental therapeutics required to improve prognosis [2–6].

The conventionally employed contrast-enhanced computed X-ray tomography (X-ray CT) and magnetic resonance imaging (MRI) provide excellent anatomical information on the localization of brain lesions but are inadequate in distinguishing between non-specific pathologies and tumor grades, exact delineation of tumor volumes, treatment-induced changes, and tumor recurrence [7, 8]. To overcome

these deficiencies, the structural information is supplemented by functional imaging, providing information on the biological processes characterizing tissue biology. The radioactive tracer technologies like single-photon computed emission tomography (SPET/SPECT) and positron emission tomography (PET), along with magnetic resonance-based methods (functional MRI, fMRI; magnetic resonance spectroscopy, MRS), can be used to obtain information on the molecular events (specific gene expressions), physiological processes (such as blood flow), and metabolic pathways (for example, glucose usage, protein synthesis, and DNA synthesis) characterizing tumor biology.

Living cells require a continuous supply of essential nutrients. Transmembrane transport and biosynthesis generate and maintain an internal pool of these molecules that are used for production of metabolic energy, synthesis of biomolecules, maintenance of cellular functions, and proliferation. The transport of the nutrient molecules across the cellular membranes is, therefore, tightly coupled with their intracellular utilization and cellular physiology for efficient and economic utilization; the demand for nutrients regulates their supply. Therefore, strategies to image tissues/organs by mapping the uptake/usage of nutrients such as glucose, amino acids, and nucleotides have been useful in non-invasive studies of normal functions and detection of pathologies.

Malignant tumors are known to have higher rates of glucose utilization and glycolysis [9]. Based on this knowledge, non-invasive PET methods to image glucose uptake using 2-deoxy-2-[¹⁸F] fluoro-D-glucose (FDG), a positron emitting analogue of glucose, have been developed. FDG is transported inside the cell by specific glucose transporters and is phosphorylated by hexokinase but is not metabolized further to any significant extent. Thus, FDG-6-phosphate accumulates in the cell, permitting tissue distribution of FDG to be imaged by PET. Applications of FDG-PET for tumor imaging have proved to be highly useful in predicting pathology as well as prognosis in primary brain tumors [10, 11]. A recent study of 336 patients with primary brain tumors demonstrated that tumor grading according to FDG tumor uptake was more accurate than histopathology in prediction of survival [12]. Histopathological diagnosis from stereotactic biopsy specimen from a small region in a highly heterogeneous tumor is prone to sampling errors; in contrast, FDG-PET scans are obtained from the whole brain and tumor. Despite the promising advantages and its increasing applications in oncology, FDG-PET is far from an ideal imaging modality for cerebral gliomas. Extensive studies carried out on glioma patients have revealed several limitations in the applications of FDG-PET:

- (a) Sensitivity of detection by FDG-PET for low-grade gliomas is low, as tumor to normal brain contrast is insufficient because of relatively small differences in the rates of glucose utilization between normal brain and low-grade tumors [13].
- (b) Additionally, increase in FDG uptake is non-specific, as it has also been observed in inflammatory lesions [14,

15]. Even within tumors, about 24% of the FDG concentration in a tumor mass is actually in macrophages and other inflammatory cells [16]. Because of these reasons, FDG-PET demonstrates low sensitivity and specificity in distinguishing recurrent brain tumors from radionecrosis [17, 18].

- (c) Furthermore, FDG-PET has difficulty in precisely delineating tumor boundaries because of high uptake of FDG in the gray matter and difficulties in imaging the tumor infiltrative areas in the normal brain.

Combinations with the current MRI and MRS techniques also have not been entirely successful in overcoming these limitations. Therefore, search for alternative techniques to overcome the deficiencies of FDG-PET for tumor imaging has gained importance in recent years, and attempts to explore combinations of newer modalities for metabolic mapping are ongoing. Increased amino acid transport, in addition to increased glucose usage, has been shown to be associated with early events in carcinogenesis [19]. Besides being substrates for the synthesis of a variety of nitrogen-containing compounds such as proteins and nucleotides, amino acids also act as regulators of fluxes through major metabolic pathways. The carbon skeletons of amino acids are often utilized as oxidative fuel source for adenosine triphosphate (ATP) generation in addition to glucose and fatty acids. Because of their essential role in cell metabolism, survival, and proliferation, cancer cells and tumors may display higher demand and utilization of amino acids.

The natural amino acids are transported inside the cells by specific carrier-mediated transport systems and get incorporated into proteins and intermediary metabolites to different extents. Many synthetic derivatives, such as methyl-aminoisobutyric acid (MeAIB), and various halogenated derivatives of phenylalanine and tyrosine have also been shown to enter the cells using similar transport systems but are not metabolized. Therefore, many natural amino acids (such as methionine, glycine, tyrosine, phenylalanine, and leucine) and their synthetic analogues have been labeled with radioactive isotopes and are being investigated as tumor imaging agents for PET/SPECT [20]. Because the uptake of amino acids (AA) in normal brain is low, the contrast between tumor and normal brain is generally better with amino acid scanning as compared to FDG-PET. It would be interesting and useful to identify the most suitable AA tracer for imaging of gliomas; however, of the several amino acid tracers investigated for tumor imaging, only a few have been evaluated beyond the initial feasibility studies in human patients. Currently, PET using L-[methyl-¹¹C]-methionine (MET) is the most popular AA-imaging modality for tumors, although its use is restricted to PET centers with an in-house cyclotron facility. Clinical studies have indicated MET-PET to be more efficient than FDG-PET in delineating the tumor extent, especially in low-grade gliomas and detecting tumor recurrences [21–24].

To enable a more widespread use of AA imaging, promising results shown by MET-PET have stimulated the

development and evaluation of amino acids labeled with longer life radioisotopes such as fluorine-18 (half-life=110 min). A few clinical studies demonstrated the suitability of ^{18}F -fluorophenylalanine [25] and L-2- ^{18}F -fluorotyrosine [26] for tumor imaging, but the synthesis and labeling procedures proved to be tedious for routine applications. More recently, a non-metabolizable analogue of tyrosine, *O*-(2- ^{18}F -fluoroethyl)-L-tyrosine (L- ^{18}F -FET) and ^{18}F -labeled 1-amino-3-fluoro-cyclobutane carboxylic acid (^{18}F -FACBC), another non-metabolized amino acid have been prepared in high yield by rapid methods that can be easily automated [27, 28]. Initial clinical studies indicate results that compare favorably with MET-PET [29, 30, 31]. Similar results have also been reported by SPECT imaging using ^{123}I -iodo- α -methyl tyrosine (^{123}I -IMT), a less expensive technique, although somewhat inferior in resolution compared to PET [32–34].

MET-PET has been successfully used in oncology. This review summarizes molecular mechanisms of methionine uptake into tumor cells and the available clinical studies on MET-PET in cerebral gliomas with the goal of extending and enhancing the role of MET-PET imaging in the clinical management of cerebral gliomas.

Scientific Background of Tumor Imaging with MET-PET

Because of the operation of evolutionary economics, it may be assumed that demand and supply of nutrients are tightly coupled; therefore, under conditions of sufficient bioavailability, the uptake and biodistribution of nutrients in tissues and organs are related to organ function that determines the demand of the nutrients. This is the basic assumption underlying the metabolic imaging. The transport of nutrients inside the cell being the first step in the metabolic network, it constitutes the major control element of the metabolic flux. The relevant available information on the mechanisms of transport of neutral amino acids including methionine and its metabolic pathways in the normal brain and gliomas is summarized below.

Cellular Amino Acid Transport

Amino acids constitute an important group of nutrients required for a variety of metabolic pathways providing building blocks for the synthesis of proteins and precursors in the formation of several other biomolecules like nucleotides, fats, ketone bodies, glucose, signaling molecules, and neurotransmitters. Amino acids can be used as fuels in the generation of metabolic energy and also play important roles in the biochemical processes of transmethylation, transamination, and transsulfuration. To carry out the diverse functions according to the needs of various tissues and organs, it is important to maintain the plasma levels of the amino acids relatively constant. Inter-organ fluxes of amino acids, transport across cellular membranes, and modulation of enzyme activities by competition kinetics are the key elements in this

homeostasis; however, because of the multiple interactions and networking between various pathways, the regulation of amino acid metabolism is very complex.

From the blood, amino acids enter the cells mainly through multiple carrier-mediated amino-acid-specific (neutral, acidic, and basic) transport systems, passive diffusion processes contributing only marginally. The amino acid transport systems are protein complexes that recognize, bind, and transport the amino acids across cellular membranes. Because the isolation and determination of the molecular structures of the AA transporters have been undertaken only in recent years, they have been identified and characterized [35] depending upon their functional properties (such as substrate specificities, kinetics, ion dependence, etc.). Generally categorized as Na⁺-dependent and Na⁺-independent, many AA transporters have broad and overlapping substrate specificities, permitting an individual amino acid to be transported in parallel by more than one transport system. The Na⁺-dependent systems utilize the potential energy present in the transmembrane Na⁺ electrochemical gradient, maintained largely by the Na⁺/K⁺-ATPase, to drive the uptake of amino acids against their concentration gradient. The neutral amino acids are mainly transported through Na⁺-independent system L (leucine preferring) and Na⁺-dependent systems A (alanine preferring), ASC (alanine, serine, cysteine), and N (preferring amino acids with N in the side chains). Charged (cationic or anionic) amino acid transport is mediated largely through systems B⁰, +, y⁺, y⁻ + L X_{AG}⁻ and X_C⁻. Each cell type usually expresses a unique complement of amino acid transport systems. Current knowledge on the structure and functions characterizing different AA transporters isolated from various organs and tissues have been reviewed [35–39].

Methionine, a sulfur-containing essential amino acid, is transported mainly through systems L, A, and ASC. System L has a broad substrate specificity and transports large neutral amino acids (LNAA) with linear or branched side chains with high affinity including several essential amino acids and also amino acid-related compounds such as L-DOPA, triiodothyronine, thyroxine, and certain drugs like melphan. Two membrane-spanning proteins, LAT1 (high substrate affinity) and LAT2 belonging to system L, have been isolated; both require an additional membrane-spanning protein identified as heavy chain of 4F2 surface antigen (4F2hc/CD98) for their functional expression [40–42]. The 4F2 antigen (CD98) associated with a variety of cellular activities, such as cell proliferation, cell transformation, and cell adhesion, forms a heterodimeric complex with LAT1/LAT2 via a disulfide bond. LAT1, strongly expressed in malignant glioma cells [43], is also present in the membranes of brain capillary endothelial cells and mediates permeation of amino acids through the blood-brain barrier (BBB) [44]. The second system L isoform, LAT2, is more ubiquitously expressed than LAT1 and transports not only LNAA but also small neutral amino acids [45].

Sodium-dependent transport system A is highly pH sensitive and preferably transports short non-branched side-

chain NAA, including alanine, glycine, serine, glutamine, and methionine, into and out of cells [46]. A major feature of system A observed in numerous cell types is its positive correlation with the rate of cell proliferation and its capability to undergo adaptive regulation in response to changes in cellular environment, presence of growth factors, hormones, and cellular amino acid availability [46].

Amino Acid Utilization in the Brain

Amino acids serve special roles in the brain, contributing to cerebral protein synthesis, intermediary metabolism, and inter-neuronal synaptic transmission. At least four amino acids [glutamic acid (Glu), aspartic acid (Asp), glycine (Gly), and γ -aminobutyric acid (GABA)] function as neurotransmitters within the central nervous system, some others [tryptophan (Trp), tyrosine (Tyr), and histidine (His)] serve as precursors to neurotransmitters including serotonin, catecholamine, and histamine. Amino acids are also required for the formation of neuroactive peptides including substance P, the enkephalins, vasopressin, neurotensin, and somatostatin. Taking into account the recycling of the amino acid pool because of protein degradation, regional differences in the rates of protein synthesis have been estimated in rats using L-[1-¹⁴C]-leucine; the protein synthesis in the gray matter is reported to be about twofolds higher than in white matter [47]. More recently, the results have been confirmed using PET in anaesthetized monkeys [48].

The essential amino acids (Arg, Val, His, Ile, Leu, Lys, Met, Phe, Trp, and Thr) cannot be synthesized by mammalian cells and, therefore, must be obtained from diet through intestinal absorption and release into the blood supply. Availability and contents of amino acids in the brain depends primarily on the characteristics of the BBB, which separates the blood supply from the interstitial fluid, and the type of amino acids; exchange and utilization of nutrients between glia and neurons cooperate in maintaining the extracellular concentrations. Cerebrovascular endothelial cells express specific carriers that mediate the entry and efflux of amino acids across the luminal (blood facing) and abluminal (brain facing) membranes of the BBB.

Transport of essential neutral amino acids from blood to brain is greater than that of nonessential amino acids. Presence of neutral amino acid carriers, systems L1, A, and ASC in BBB has been demonstrated both *in vivo* and *in vitro* [49]. The kinetic properties of the various transport systems in BBB endothelial systems have been summarized recently [50]. LNAA, such as asparagine, cysteine, glutamine, histidine, isoleucine, leucine, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine, and valine, are transported mainly via a high-affinity sodium-independent carrier system belonging to the L (leucine preferring) system [35]. The two carriers, L1 in the BBB and L in other cell types, differ in one important aspect—the affinity of the L1 transporter for NAA in rat brain (Km values around 10 μ M) in the abluminal membranes exceeds that reported for

the L system in most other tissues by 100- to 1,000-fold [51–53]. The Km of the various NAA in human capillaries *in vitro* has been shown to vary several folds; for example, Km for phenylalanine in human capillaries is 0.22 μ M, whereas the estimated value for methionine is 5.1 μ M [50]. The uptake of individual amino acids is considerably inhibited by the increase in plasma concentration of other amino acids because of competition kinetics [54, 55].

Transport of NAA at the BBB is bidirectional with rate coefficients for brain AA efflux exceeding those for influx by more than five- to tenfold [56]. This difference maintains the manifold lower concentrations of amino acids in brain extracellular fluid (ECF) as compared to plasma. Interestingly, the distribution of the AA transporters in the luminal and abluminal membranes is asymmetric. Sodium-dependent carriers, which transfer amino acids from the ECF of brain to the endothelial cells and, thence, to the circulation, are not present in the luminal side, although at least four different Na⁺-dependent NAA transporters have been identified in the abluminal membrane. System A transports Pro, Ala, His, Asn, Ser, and Gln; system ASC transports Ser, Gly, Met, Val, Leu, Ile, Cys, and Thr; system N transports Gln, His, Ser, and Asn; Na-LNAA transports Leu, Ile, Val, Trp, Tyr, Phe, Met, Ala, His, Thr, and Gly. Together, these four systems present on the abluminal membrane can actively transfer every naturally occurring NAA from the ECF to the endothelial cells and, thence, to the circulation, thus, providing a mechanism to maintain manifold lower NAA concentrations in the ECF of the brain than in the plasma.

Protein synthesis and degradation rates undergo numerous changes in development and aging, and they can be influenced by pathological and environmental factors. The developmental changes in protein metabolism occur in all brain areas and brain cells, with metabolic rates in young being two to three times that in adult [57]. Aging is associated with significant decreases in rates of protein synthesis in the brain as a whole, as well as in several specific brain regions [58]. Aging at the cell level involves functional changes of membranes, in turn associated with alterations in the transport of nutrients [59]. Age-associated changes in amino acid transport from blood to frontal cortex were reported in humans [55].

Methionine Metabolism

Methionine, an essential sulfur amino acid, is necessary for growth and development. The major metabolic functions of methionine are:

- (a) In protein synthesis
- (b) Conversion to S-adenosylmethionine (AdoMet), which is required in multiple metabolic pathways: (1) as the predominant biological methyl group donor, (2) a precursor in polyamine synthesis, and (3) as an intermediate in the transsulfuration (TS) pathway leading to cystathionine, cysteine, and further derivatives of cysteine such as glutathione

AdoMet is the methyl donor in numerous, biologically significant, transmethylation (TM) reactions. *S*-Adenosylhomocysteine (AdoHcy), a product of these reactions, is hydrolyzed to yield homocysteine (HCY), the immediate precursor of methionine. HCY, which does not occur in the normal diet, can be remethylated (RM) to methionine by accepting a methyl group from 5-methyltetrahydrofolate or betaine. Thus, in the normal cells, methionine can be reformed from HCY by two pathways in methionine recycling, one catalyzed by a betaine-homocysteine methyltransferase (BHMT) and the other by a methyltetrahydrofolate-vitamin-B12-dependent methionine synthetase (MS). Thus, HCY could replace methionine in the presence of vitamin B12 and folic acid or if betaine or its precursor choline were in the diet.

AdoMet is also utilized as the propylamine donor in the synthesis of spermidine, spermine, and other higher polyamines. Polyamines have multiple functions: as growth factors [60], in stabilization of membranes and subcellular particles [61], and in stabilization of DNA [62]. Polyamine synthesis closely parallels the RNA and protein synthesis in cell multiplication. Rapidly growing tissues, both normal and neoplastic, contain high concentrations of polyamines [63–65]. Puterine levels and rates of its metabolism in human brain tumors are higher than in normal brain and could be correlated with the degree of malignancy [66, 67]. The activity of ornithine decarboxylase, the rate-limiting enzyme in polyamine biosynthesis, in tumor samples from glioma patients show higher values than in peritumoral non-neoplastic tissue and are correlated with tumor grades [68, 69].

Methionine also acts as a precursor of cysteine and its derivatives such as glutathione, which plays an important role in maintaining the cellular redox potential. Through transsulfuration reactions, HCY is converted to cystathionine and, subsequently, to cysteine and glutathione.

Alterations of Methionine Transport and Metabolism in Cancer

Enhanced demand for methionine in cancer cells caused by increased fluxes in the pathways of protein synthesis, transmethylation, and transsulfuration is reflected in the higher uptake. Metabolic defects in cancer cells often manifest in the inability to grow in media where Met has been replaced by its precursor HCY. The molecular mechanisms underlying this methionine dependence remain yet to be completely elucidated; however, methionine-dependent cell lines have much higher basal transmethylation rates than methionine-independent cell lines [70, 71].

Increase in amino acid uptake is one of the earliest events associated with *in vitro* transformation [19]. Facilitated transport of amino acids is known to be enhanced across glioma capillaries, and tumors can induce upregulation of amino acid transporter expression in their supporting vasculature [72]. The role of enhanced expression of transporter systems of neutral amino acids in cancer cells has been comprehensively reviewed [39]. Methionine is mainly transported by

systems L, A, and ASC. Important differences in the L-system transporters in glioma cells and normal astrocytes have been recently reported [43]. LAT1, which preferably transports LNAA has been shown to be strongly expressed in malignant tumors [41, 42, 73] including glioma [43]. In contrast, LAT2 with its associated subunit 4F2hc is expressed in normal astrocytes, shows broader substrate selectivity than LAT1, and transports not only large neutral amino acids but also small neutral amino acids [43, 45, 74, 75].

Overall rates of transmethylation are frequently increased in human tumor cells [76]. Methionine dependence *in vitro* culture has been shown in a number of human cell lines of different cellular origin [77–79] including gliomas. Methionine dependence may reflect an overall imbalance in transmethylation that results in the hypermethylation of some substances and hypomethylation of others within cancer cells.

Transfer RNA (tRNA) is the nucleic acid that has the highest percentage of its bases and nucleoside methylated. Altered methylation of tRNA may affect its coding properties [80, 81], the ability of the tRNA to bind ribosomes [82], and may inhibit the aminoacylation of tRNA [83]. Unfractionated tRNA from human gliomas showed higher amount of methylated bases and nucleosides compared to normal brain [84]. In a study of eight different human ovarian carcinoma, it was found that more rapid metastasizing, poorly differentiated carcinomas had higher tRNA methylase activity than did slower metastasizing, well differentiated, and intermediately differentiated carcinomas [85].

Methionine Uptake, Biodistribution, and Tumor Imaging in Model Systems

Cell Cultures In Vitro Investigations in a human glioblastoma cell line have demonstrated that the uptake of ³H-Met and a non-metabolized tyrosine analogue IMT is higher in proliferating cells than in resting plateau-phase cells and is mediated largely by L-system of transport [86]. It has been also shown that transport through system L and 4F2 antigen expression correlated with the proliferation rate [87]. More recent studies in glioma cell lines have indicated that AA transporters are upregulated under starvation conditions [88].

Incorporation of ³H-Met in human brain-14 astrocytoma-III cell line into protein, RNA, DNA, and lipids was reported by Narayanan et al. [89]; kinetic studies showed that maximum incorporation into protein was at 2 h (55%) followed by DNA (11%), RNA (5.8%), and lipids (2.6%) at 4 h of incubation in growth medium containing 1 μ Ci of ³H-Met. These observations indicate that in glioma cells, considerable amount of methionine could be utilized for non-protein synthesis; estimation of the rate of protein synthesis from MET uptake must take this into account.

Comparison of the effects of hypoxia on the uptakes of tritiated thymidine, methionine, and FDG in human tumor cell lines showed that, whereas methionine uptake did not change under hypoxic conditions, an increase in the

glucose usage and a decrease in thymidine uptake were observed [90].

Animal Models Ishiwata et al. [91] compared the tissue uptake and protein incorporation of ^3H -Met, ^{14}C -Leu, and ^{18}F -Tyr in the brain and in tumors of mice bearing FM3A mammary carcinoma. The tissue distribution profile after 60 min was observed to be similar for the three amino acid tracers with pancreas showing the highest uptake followed by the liver, tumor, and brain. ^3H -Met showed higher uptake and was also incorporated in lipids and RNA besides proteins, whereas most of ^{14}C -Leu was found in the protein fraction without any significant incorporation in other macromolecules. Comparison of the metabolism of methionine using carboxyl carbon-labeled L-(^{14}C)-Met with L-(methyl- ^{11}C)-Met in rats bearing Walker 256 carcinosarcoma, showed significant differences in the amounts and patterns of methionine incorporation in the protein and non-protein metabolites in plasma, brain, and tumor as measured by the two methionines [92]. These observations stress the importance of minor metabolic pathways of methionine (in addition to protein synthesis) and must be considered in kinetic modeling necessary for quantitative analysis of methionine uptake by PET.

Effects of radiotherapy on the cellular uptake of L-[methyl- ^{11}C] methionine in a rat tumor model demonstrated a rapid decrease in the Met uptake by tumor cells. The radiation dose-dependent decrease in ^{11}C -Met was observed earlier than reduction in tumor volume and, subsequently, before the recurrent growth of the tumor [14, 93]. Microautoradiographic studies on the distribution of ^{14}C -Met in various cellular elements of mouse malignant tumor tissues demonstrated that the tracer uptake by the tumor was mostly by viable cancer cells, whereas it was low in macrophages and other cellular components [16]. In contrast, 2-DG uptake in irradiated tumor remained higher than in normal muscle because of high glucose usage by macrophages around the rim of necrotic regions [94]. Thus, in residual tumors after radiotherapy, uptake of ^{11}C -Met appears to be related to the viable cancer cells and would be more suitable than FDG for differentiating between necrosis and viable tumor in monitoring early tumor radiotherapy responses.

Regional differences in the incorporation of L-[^{14}C] Met in proteins have been demonstrated in rat brain, the gray matter showing higher values than the white-matter-enriched regions, whereas no significant variations were observed in concentrations of free methionine and its labeled metabolites [95]. However, in these studies, a rather low value (~0.5) of brain/plasma-specific activity ratio for free methionine was measured, suggesting the presence of an endogenous source of free methionine (likely from protein breakdown) in the brain regions. These results are in agreement with the studies using other amino acids such as leucine [96] and imply that the contribution of protein breakdown to the cellular amino acid pools needs to be considered while estimating protein synthesis rates (PSR) from PET studies.

Methionine Uptake in Benign Pathological Conditions

Increased methionine uptake in the perivascular mononuclear infiltrate and gliotic reaction in the collagen capsule surrounding hematomas and BBB breakdown have been postulated as potential mechanisms for a positive MET-PET scan in these patients [97]. Similarly, increased blood flow and BBB breakdown have been discussed as possible causes of MET accumulation in some cases of brain abscess [98]. As mentioned earlier, ^{14}C -Met has been shown to have a low accumulation in macrophages as compared to ^{18}F -FDG [16], so the relative contribution of MET accumulation in inflammatory cells to overall MET accumulation in benign inflammatory conditions is not clear.

Clinical Studies

Soon after publication of rapid high-yield procedures for labeling of methionine with ^{11}C [99–101], studies to explore its use in PET imaging were initiated. During the last two decades, interest in the AA imaging in oncology has grown after promising results shown by MET-PET in better detection and delineation of viable tumor as compared to FDG-PET, especially in low-grade gliomas. A number of second-generation amino acid tracers labeled with radioisotopes with longer half-life are under active development. The impact of applications of MET-PET in improving the clinical management of glioma patients needs to be evaluated in depth. Currently available information is summarized in this context. The clinical role of MET-PET is reviewed in this paper under the following headings: (1) Diagnostic/Detection Accuracy, (2) Differentiation of Tumor Recurrence Versus Radiation Injury, (3) Grading, (4) Prognosis, (5) Assessment of Tumor Extent, (6) Biopsy Planning, (7) Surgical Planning, (8) Radiotherapy Planning, and (9) Assessment of response after therapy.

Diagnostic Accuracy

The differential diagnosis of intracranial mass lesions includes primary and metastatic brain tumors, hemorrhage, infarction, infections like abscess, viral encephalitis, proliferative multifocal leukoencephalopathy, and inflammatory pathologies like multiple sclerosis and post-infectious encephalomyelitis. Although characteristic features exist, accurate differentiation may be difficult on MRI/CT alone. Several studies that have tried to determine the diagnostic accuracy of ^{11}C -methionine PET (MET-PET) for brain tumors are summarized in Table 1.

The overall sensitivity of MET-PET for gliomas, including both high- and low-grade gliomas, has been estimated to be around 76–95% in various studies [102–105]. The variation in estimated overall sensitivity in different series is likely to be because of their relative proportion of low-grade and high-grade gliomas. In low-grade gliomas, the range of

Table 1. A table of studies evaluating the role of MET–PET in detection of cerebral gliomas

Author	Year	Number of patients	Patients	Results
Moskin et al. [23]	1989	10	Supratentorial gliomas	Positive MET in 9/10 glioma cases
Kameyama et al. [114]	1990	14	Gliomas	Positive MET in all cases irrespective of grade
Ogawa et al. [159]	1995	8	Hematoma-4 neoplastic hematomas; 4 non-neoplastic hematomas	More intense accumulation in neoplastic than non-neoplastic hematomas; greater extent of uptake than MRI in neoplastic hematomas
Herholz et al. [102]	1998	196	121 untreated and 61 treated patients with suspected gliomas (2/3 of confirmed gliomas scans in LGG; 1/3 in HGG); 14 non-gliomatous brain tumors	Overall accuracy=79%; sensitivity=76%; specificity=87% (T/N cut off value=1.47)
Sasaki et al. [116]	1998	23	Astrocytomas-16 high-grade gliomas, 7 low-grade gliomas, comparison with FDG, Tl	SUV=1.49±0.44 in grade II, SUV=3.29±1.44 in grade III SUV=3.20±0.92 in grade IV; more sensitive than FDG, Tl
Weber et al. [29]	2000	16	Suspected primary or recurrent glioma/metastatic tumor; 13 tumors, 3 post treatment changes; comparison with 18F-FET	Comparable uptake contrast ratios for MET and 18F-FET
Massager et al. [109]	2000	30	Brainstem lesions (14 had MET, 11 had tumor, 3 had non-neoplastic etiology)	Sensitivity of MET=82%; aided in biopsy planning
Chung et al. [104]	2002	45	Hypo- or isometabolic lesions on FDG–PET (35 neoplasms, 10 non-neoplastic lesions)	Sensitivity=89% (92% in gliomas); specificity=100%
Braun et al. [103]	2002	32	Intracranial tumors (34 lesions: 11 low-grade gliomas, 19 high-grade gliomas, 4 non-neoplastic)	Nine out of 11 LGG detected by MET, overall sensitivity=87%, specificity=75%, PPV=96%, NPV=43%
Becherer et al. [160]	2003	20	12 HGG, 6 LGG, 1 metastatic lesion, 1 demyelination; comparison with 18F-FDOPA	Mean T/N ratio=2.05±0.91 for MET, comparable ratio for FDOPA, false positive uptake in demyelination
Kracht et al. [105]	2004	30	22 Primary brain tumors, 8 recurrent brain tumors, 100 biopsy specimens with tumor, 18 specimens with non-tumorous brain tissue	Sensitivity=87%; Specificity=89% (values based on biopsy specimens) at a T/N cut-off of 1.3

MET ¹¹C-Methionine, PPV positive predictive value, NPV negative predictive value, T/N ratio of uptake in tumor to contralateral normal brain, HGG high-grade gliomas, LGG low-grade gliomas, N number of patients, Tl thallium, FDG fluorodeoxyglucose, FET fluoroethyltyrosine

reported sensitivities lies between 65–85% [102, 105, 106]. Because of the high contrast, MET–PET allows the identification of low-grade gliomas, although its uptake may be lower in low-grade gliomas than in high-grade gliomas. Even when no uptake is visible on FDG–PET, increased methionine uptake is seen in several types of brain tumors, including gliomas (Fig. 1). In a study by Chung et al., 89% of 35 brain tumor patients with hypo- or isometabolic lesions on FDG–PET scan showed a high uptake on MET–PET scan [104]. Low-grade gliomas that are difficult to identify on anatomic imaging modalities like CECT and MRI can also be seen in MET–PET. For example, in a study by Ribom et al., 30 in 32 (94%) of low-grade gliomas had an increased methionine uptake, while only 12 in 32 (38%) showed contrast enhancement [107, 108].

Specificity of MET–PET for distinguishing non-tumoral brain lesions is also determined to be high. Herholz et al. determined a specificity of 87% using a tumor/normal tissue ratio of 1.47 as the diagnostic cut-off in a sample of 28 non-tumoral cases [102]. A specificity of 100% was determined in hypo- or isometabolic FDG–PET [104]. None of the ten non-tumorous lesions hypometabolic on FDG showed a high uptake on MET–PET scan in this study. The reported

causes of false positives on a MET–PET brain scan include demyelination, necrosis, subacute, or chronic ischemia [102, 103, 105], leukoencephalitis [109], brain abscess, acute infarct, and hematoma [97, 98, 110]. Other intracranial tumors demonstrating a high methionine uptake include pituitary adenomas, benign, and malignant meningiomas, hemangioblastomas, ependymomas, juvenile desmoplastic gliomas, lymphomas, and metastases. An overall accuracy of 79% was reported for MET–PET in a large study of 196 patients by Herholz et al. [102]. Almost two thirds of the scans in confirmed glioma cases in this series were done in patients with low-grade gliomas.

Whereas sensitivity and specificity serve the purpose of establishing a test's accuracy from the epidemiological point of view, the clinician is more interested in the positive and negative predictive values of the test [111], that is, the probability of having the disease with a positive test result and that of not having the disease in case of a negative test result, respectively. These values are in turn determined by the prevalence of the condition of interest in the tested population (pre-test probability) apart from the test's sensitivity and specificity. Because of the high pre-test probability for brain tumors in patients undergoing a MET–PET scan for an intracranial mass, the positive predictive value (PPV) of

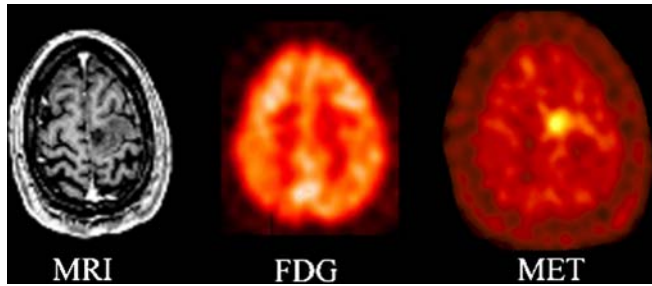


Fig. 1. Grade II glioma. The contrast-enhanced T1-weighted MR, co-registered images of F-18 FDG and C-11 methionine in a proven case of grade II glioma. No contrast enhancement in MR and no FDG uptake but mild to moderate Met uptake in the same area.

MET-PET is high, but the negative predictive value (NPV) is expected to be low, which has been confirmed in a recent analysis [103]. In other words, the probability of having a brain tumor is high if the MET-PET scan is positive, but its presence cannot be ruled out if MET-PET is negative in the setting of a suspicious mass lesion on CT/MRI referred for the MET-PET scan before histopathological diagnosis.

Moreover, reproducibility of PET tracer measurements is a key concern in cross-sectional comparisons between groups of patients and longitudinal comparisons between successive studies of the same patients. Calculation of tumor-to-normal brain tissue ratios is a frequently used method for this purpose. To standardize the results, Kaschten et al. [112] have recommended the use of tumor-to-mean cortical uptake ratio for MET-PET where the mean cortical uptake in the contralateral cortex is calculated as a mean of seven cortical regions—medial and lateral frontal, medial and lateral temporal, parietal, and medial and lateral occipital cortex—and is, thus, subject to a low-error margin. Tumor-to-contralateral gray matter and tumor-to-contralateral white matter ratios are also routinely used.

In summary, MET-PET has a high sensitivity and specificity in detecting brain gliomas. However, because of the occurrence of both false positives as well as false negatives on MET-PET scan, histopathological examination may be needed in individual cases. MET-PET scan is particularly useful in detecting low-grade gliomas when other imaging modalities are inconclusive.

Grading

Conventionally, FDG uptake has been shown to have a strong association with the histopathological grade of cerebral gliomas. Padma et al. demonstrated that 86% (143/166) of the patients with a low FDG uptake (defined as an uptake less than or equal to contralateral white matter on visual analysis) had a low-grade glioma, whereas 94% (154/165) with a high FDG uptake had a high-grade glioma [12]. MET-PET on the other hand has not revealed such clear prediction value for grading through visual analysis [113]. On semi-quantitative

analysis, although a significant difference in the MET uptake indices of low-grade and high-grade tumors has been demonstrated [114], there is a significant degree of overlap among tumors of several grades. In a study of 194 patients, Nariai et al. [115] demonstrated that while the T/N ratio (ratio of SUV in tumor to contralateral normal brain) was significantly different in the low-grade and high-grade gliomas, there was no significant difference between grades I and II, and grades III and IV gliomas. Sasaki et al. have reported a significant difference in the mean SUV of 1.49 ± 0.44 (mean \pm SD) for grade II gliomas and 3.20 ± 0.92 for grade IV gliomas [116]. In a recent analysis, Ceysens et al. reported no significant overall difference in MET uptake index between tumor grades. However, their results might have been affected by a significant delay between histopathological examination (HPE) and acquisition of MET-PET scan, owing to the tendency of gliomas to undergo malignant transformation [117]. In an interesting study on 59 patients, Borbely et al. reported that tracer distribution was more heterogenous within the tumors for FDG as compared to MET and the heterogeneity for both tracers was more in higher grade tumors. They found that peak tumor tissue FDG uptake to white matter FDG uptake ratio had the maximum value for differentiating high-grade from low-grade gliomas. The same index for MET was not found to be significantly different for low- and high-grade gliomas. Mean tumor tissue MET uptake to white matter ratio, however, did show a significant difference between the two groups. Mean tumor tissue uptake to mirror image uptake ratio did not show significant association with tumor grade for either of the tracers [118]. A significant correlation between the extent of methionine uptake and microvessel density was reported by Kracht et al. [119]. They suggested that methionine uptake may be useful for selection and follow-up of patients for anti-angiogenic therapy.

Herholz et al. [102] did not find any significant difference between the methionine uptake in treated and untreated gliomas. The methionine uptake in recurrent grade II astrocytomas, however, was found to be significantly higher than in untreated grade II astrocytomas, owing probably to malignant transformation. Moreover, it was also found that there was no significant effect of corticosteroid treatment on methionine uptake in low grade gliomas. However, in high-grade gliomas, a 25% reduction in methionine uptake after corticosteroids was found reflecting a higher contribution of BBB breakdown to methionine uptake in this subset of gliomas [102].

In summary, statistically significant differences in semi-quantitative MET uptake have been demonstrated between high- and low-grade gliomas, although visual analysis may not aid in accurate grading unlike in FDG scanning.

Prognosis

Several investigators have found a higher methionine uptake in oligodendrogliomas than astrocytomas of similar grade [103,

105, 120] in spite of their apparently better prognosis. Grade, age, performance status, and associated indices like Ki-67 proliferation index are some of the conventional prognostic factors in patients with brain gliomas. FDG uptake has also been demonstrated in several studies to be a predictor of grade and prognosis in cerebral gliomas [12, 13].

In a study of 85 patients followed up for a median duration of 13 months, De Witte et al. searched for prognostic value of methionine uptake within each histological class. They used both qualitative visual scale and semiquantitative scales. “Hot spots” on visual interpretation with intense methionine concentration were associated with a lower survival. Grades II and III gliomas with a tumor to contralateral counts ratio >2.2 and >2.8 , respectively, were associated with a reduced survival. No such association was observed within the grade IV glioma group because of the overall poor prognosis in this subset [120]. Grade II oligodendrogliomas with a low methionine uptake were shown to have a longer time to progression of disease in another study [108]. In a multivariate analysis, MET uptake was found to be an independent significant prognostic factor in cerebral gliomas as compared to FDG [121] (Fig. 2). Also, a significant correlation was found between MET uptake and Ki-67 proliferation index but not with FDG in this study. A higher T/N ratio of methionine uptake was found to be associated with shorter survival in another study of 73 patients followed for over a year [115]. In summary, a lower semiquantitative MET uptake has been associated with a better prognosis in patients with similar tumor grade and vice versa.

Tumor Extent Determination

Several studies have demonstrated that conventional MR imaging underestimates the tumor extent in cerebral gliomas [122–124]. The borders of the abnormal MRI lesion in the autopsy specimens of brains affected by gliomas can have viable tumor cells on histopathological examination [122, 124]. MET-PET has been investigated to compare its tumor delineation with other modalities’ by several investigators.

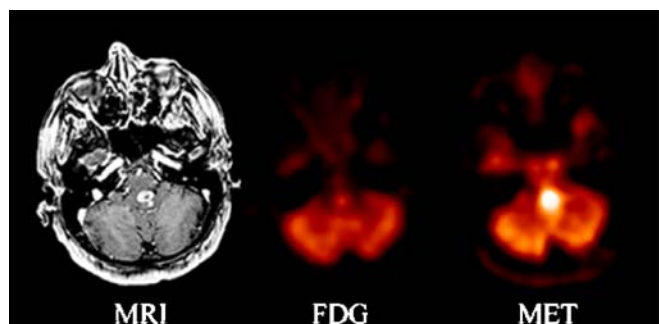


Fig. 2. Grade III glioma. Patient with anaplastic astrocytoma with negligible FDG uptake but high methionine uptake. There is variable glucose uptake in grade III astrocytomas, some even show very high glucose uptake.

Several methods have been used in different studies for tumor delineation with MET-PET. Qualitative visual method [125], threshold value of tumor uptake at 120% of mean weighted activity in cerebellum [126], T/N ratio of 1.3–1.5 [105, 127], or SUV of 1.8–2.2 [127], and automatic software-based segmentation algorithm [128] are some of the examples of such techniques. Improved resolution and slice thickness of up to 3.6 and 3.125 mm, respectively, with the modern PET scanners, have resulted in better delineation of tumor margin [105] using MET-PET.

The extent of tumor delineated by MET-PET was found to be larger than contrast-enhanced MRI or CT scan in 67% and same in 33% of 46 brain tumor patients [129]. Similar results that showed the MET uptake area to be larger than or at least as large as the contrast-enhanced area on MRI or CT were reported in another study [122]. MET-PET has been found to delineate a larger area of increased uptake than FDG-PET [106, 130]. Increased methionine uptake is also seen in tumor-infiltrated areas of the brain where the degree of uptake maybe more than in the solid tumor areas (1.7 versus 1.4 times as compared to normal) [105]. In a recent quantitative PET and MR image fusion study, MET uptake area was found to extend up to more than 30 mm beyond the contrast-enhanced area on the T1-weighted MRI in glioblastoma multiforme patients [125]. In the same study, T2-weighted images revealed an area of abnormal signal that was larger than the MET uptake area, but the MET uptake area extended partly beyond the high T2 area in nine out of ten patients. It was inferred that the T2-high area beyond the MET area may represent peritumoral edema [125]. In that study, recurrence was noted in three out of five patients in the region of increased MET uptake after resection of the gadolinium-enhanced tumor region [125]. This supports the presence of tumor beyond the area of BBB breakdown reflected by contrast enhancement and the need for incorporation of MET-PET in treatment planning. Automatic software fusion of MET-PET and CT/MRI has been validated for radiotherapy planning and enabled a better delineation of residual gliomas than CT/MRI alone [131]. Other intracranial tumors, particularly skull-base meningiomas, were also better delineated by MET-PET [131]. With the increasing availability of PET/CT scanners [132], more studies using “hardware fusion” of MET-PET and CT for treatment planning are expected in the future to improve the outcome in these patients.

Surgical Resection The role of MET-PET in planning a surgical resection is closely linked to its advantages in relation to delineating tumor extent for a complete resection and localizing metabolically active areas within a tumor in case of a subtotal resection. It has been used in conjunction with ^{15}O -water for identifying the functional areas of the brain and planning an appropriate surgical approach [127, 133] and extent of resection (Table 2). In a recent large series of 103 surgical resections in 91 patients where no clear delineation

Table 2. A table of studies evaluating the role of MET–PET in biopsy and surgical resection planning

Author	Year	Number of patients	Patients	Results
Biopsy planning				
Moskin et al. [161]	1987	36	Supratentorial gliomas, comparison with CECT, multiple stereotactic biopsies	MET-PET more accurate than CT in 22 cases for tumor extent. False-positive and false-negative MET–PET findings in 5 and 4 cases, respectively
Goldman et al. [141]	1997	14	High-grade gliomas, comparison with FDG for biopsy planning, 93 biopsy samples	High FDG and MET uptake in anaplastic areas within tumor; significantly low MET uptake in necrosis within anaplastic areas
Pirotte et al. [143]	1997	25	Non-resectable lesions (23 tumors, 2 non-tumorous lesions), comparison with FDG	MET guided biopsy in 11 FDG-inconclusive tumor lesions (where FDG uptake was equal to or less than cortical uptake); MET-guided biopsy particularly useful in cortical lesions
Massager et al. [109]	2000	30	Brainstem lesions (18 underwent MET–PET), comparison with FDG	Combined FDG and MET-guided biopsy planning reduced the number of required biopsy trajectories; precise HPE results possible in all cases
Pirotte et al. [142]	2003	9	Children with infiltrating, ill-defined brain lesions	MET–PET led to reduction in number of trajectories and protecting functional areas
Pirotte et al. [140]	2004	32	Biopsy planning in glioma patients (61 trajectories, 22 HGG, 10 LGG)	MET guided biopsy in 39 out of 61 FDG negative or equivocal areas. All gliomas had MET uptake. MET-defined tumor extent greater than FDG defined extent
Surgical resection planning				
Nariai et al. [127]	1997	16	Glioma patients, combined with H2 15O activation study	Total or near-total resection possible in 8 patients, decompression of functionally active part in 4 patients
Pirotte et al. [135]	2005	22	Children with ill-defined LGG on MRI close to functional areas	MET improved tumor delineation in 20 out of 22 cases. Total resection possible in 17 cases with tumor negative surgical margins
Pirotte et al. [134]	2006	91	82 procedures with MET (59 in LGG, 23 in HGG); comparison with FDG	FDG contributed to HGG only. MET altered resection planning in 88% of LGG and 78% of HGG. MET–PET can replace FDG–PET for resection planning

MET ¹¹C-Methionine, CECT contrast-enhanced computed tomography, HGG high-grade gliomas, LGG low-grade gliomas, N number of patients, FDG fluorodeoxyglucose

of tumor was possible on MRI [T1, T2, and fluid-attenuated inversion recovery (FLAIR) sequences], Pirotte et al. [134] have reported that of the 82 cases that had a MET–PET scan, MET–PET altered resection planning in 88% of low-grade gliomas (52/59) and 78% of high-grade gliomas (18/23). Alteration included either an extended or focused resection in 39/52 and 13/52 (75 and 25%) low-grade gliomas, respectively, and 10/18 and 8/18 (55 and 45%) high-grade gliomas, respectively. The authors presented a classification of patterns of MRI and PET findings. According to the authors, six broad patterns can be observed: MET–PET-defined contour may be fully incorporated in MRI-defined contour, there may be an overlap between the two contours, MRI-defined contour may be fully incorporated in MET–PET contour, MET–PET and MRI contours coincide fully, and only PET or only MRI-defined contours could be used for tumor delineation [134]. They found that MRI-defined contour was fully incorporated in MET–PET-defined contour in 41% and vice versa in 33% cases. There was an overlap between the two contours in 18% cases. In 3/82 (5%) cases (all low-grade gliomas), there was no increase in MET–PET uptake and, hence, MET–PET was non-contributory. In the same study, FDG–PET contributed to resection planning in high-grade gliomas only. The authors have reported an altered imaging strategy where MET–PET is the only tracer employed for glioma PET imaging, replacing their older approach where FDG was used when contrast enhancement was positive and MET when

contrast enhancement was negative on anatomical imaging. Similarly, in a study of 22 children with ill-defined low-grade brain tumors located close to functional areas, MET–PET improved tumor delineation in 20/22 cases, and a total resection was possible in 17 cases, with a negative tumor margin [135]. More studies incorporating short- and long-term patient outcome measures, integration of functional activation images using fMRI, PET and white matter spread imaging using diffusion tensor imaging [136] in surgical planning, histopathological confirmation of completeness of MET–PET-based resection [134], and widespread use of mutual information multi-modality image coregistration software [137] for MRI and PET image fusion are expected in the future.

Radiotherapy and Radiosurgery Planning The feasibility of MET–PET incorporation in external radiotherapy, brachytherapy, and stereotactic radiosurgery planning and follow-up has been demonstrated [129, 128, 138]. Both frame-based and frameless methods of PET acquisition have been described [128, 131]. Like the experience in biopsy and surgical resection planning, various patterns of relative contour delineation by MET–PET and MRI were seen. A similar classification as above was described [128, 139]. Grosu et al. found that of the 39 patients undergoing RT planning for malignant gliomas after surgical resection, 74% had a MET-

defined tumor volume larger than contrast-enhanced T1-weighted MRI-defined volume. The region of increased MET uptake extended up to 45 mm beyond Gd enhancement in their study. In 50% of the 18 patients who underwent both T2-weighted MRI and MET-PET in the same study, increased MET uptake extended beyond the abnormal T2 signal area. In all 18 patients, some area of abnormal T2 signal was noted outside increased MET uptake area. The mean MET-defined tumor volume was determined as 19 versus 11 cc on Gd-enhanced T1-weighted MR images and 23 versus 42 cc on T2-weighted MR images [139]. In a study of 57 patients [40 patients with primary central nervous system (CNS) tumors, 7 with CNS metastases, 10 with pituitary adenomas] undergoing stereotactic radiosurgery using Leksell gamma knife, 72 target volumes were defined of which PET findings were found useful in 43 cases (69% of 62 PET positive volumes). These included both MET-PET (particularly, in low-grade gliomas) and FDG-PET (particularly in high-grade gliomas because MET-PET was not frequently performed in them) and led to a smaller target volume in 25 (58%) patients and a larger target volume delineation in 18 patients (42%) [128]. However, as the authors of this study discussed, the final target delineation for such a procedure is determined by several factors including anatomical tumor extent, metabolic extent as delineated by PET, surrounding functional areas, clinical status of the patient, previous treatment, and tumor histology [128]. Although histological verification of targets delineated for stereotactic radiosurgery and radiotherapy is obviously not possible, coupled with the high sensitivity (87%) and specificity (89%) of MET-PET reported in 100 biopsy specimens of solid/infiltrating tumor tissue and 18 specimens of non-tumorous brain tissue by Kracht et al. [105] MET-PET is likely to play an increasingly important role in the planning of radiation treatment of cerebral gliomas, as it adequately delineates tumor volume, although more studies investigating the role of the same are warranted.

Biopsy Planning

Brain tumors are histologically heterogenous. There are areas of different grades of malignancy and necrosis within a solid tumor mass. Dependence on contrast enhancement on CT/MRI alone for identifying the biopsy target can lead to non-diagnostic tissue samples and inaccurate grading of the tumor. FDG-PET has been conventionally used in a complementary role to the anatomic imaging modalities for identifying the hypermetabolic foci within brain tumors to guide biopsy. However, there are two disadvantages of FDG as the sole metabolic marker in brain tumors as described earlier. It has a low uptake in low-grade gliomas. Secondly, a hypermetabolic tumor focus in or near the cortex may be indistinguishable from the already high uptake in the gray matter. MET has been found to complement and may even be capable of replacing FDG for this purpose, as it has many-fold higher uptake in low-grade gliomas and provides high contrast in

intra- and pericortical tumors because of its lower uptake in normal gray matter (Table 2). Although the correlation between the degree of methionine uptake and tumor grade is not as robust as that of FDG, the focus of maximum uptake of MET was found to correspond to the point of maximum FDG uptake in brain tumors when there is high uptake of both tracers [140] (Fig. 3). An increased uptake of MET and FDG was found in areas of anaplastic changes within brain tumors [141]. Necrotic areas, on the other hand, showed reduced methionine uptake in spite of an increased FDG uptake, probably because of the well-known FDG uptake in inflammatory cells [141], supporting the use of MET-PET for identifying these areas. Pirotte et al. demonstrated that incorporation of MET-PET in biopsy planning reduced the number of required attempts and protected functional areas in children with brain tumors [142]. In a study of non-resectable brain lesions, it was found that MET-PET showed increased radiotracer concentration in all 23 brain tumors. FDG-PET-based trajectories were used in 12 of these patients. In all the other 11 patients where FDG-PET showed a reduced or equivocal increased uptake, MET-PET-based biopsy trajectories were used successfully [143]. Similar advantages of MET-PET were reported in a study on patients with brain-stem lesions [109]. In another study comparing FDG-PET with MET-PET in 32 glioma patients, only 22 out of 61 trajectories were planned based on FDG because of equivocal or reduced FDG uptake in other cases. All these other diagnostic trajectories were planned based on MET [144]. The non-diagnostic biopsy samples in this study were obtained from MET-negative areas based on MRI/CT alone. The investigators reported a higher contribution of MET-PET in biopsy planning in cortical tumors than in subcortical (basal-ganglia and brain stem) tumors as compared to FDG-PET. A same day protocol with injection of MET followed by scanning after 20–40 min and injection of FDG after 80 min followed by scan after 40–60 min has been proposed [144, 145]. With a reported pixel dimension of 2.591×2.591

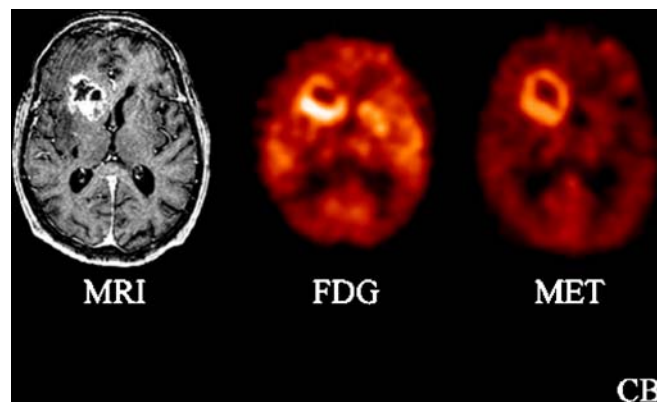


Fig. 3. GBM. The contrast-enhanced T1-weighted MR, co-registered images of F-18 FDG and C-11 methionine in a proven case of glioblastoma multiforme. Contrast-enhanced areas show good FDG and Met uptake.

mm and 6.75 mm slice thickness, the target volume of 45 μ l was reported in this study [144]. Bernays et al. have reported planning biopsy directed toward hypermetabolic focus on FDG-PET in conjunction with intraoperative MRI, demonstrating the complimentary nature of various modalities [146]. Further studies using MET-PET in this setting in conjunction with FDG-PET and intraoperative MRI are needed.

Therapy Assessment

The accuracy of MET-PET in differentiating recurrent tumor from radiation injury has been reviewed earlier in this article. Several other questions, however, need to be answered for analyzing the role of MET-PET in assessing response to treatment and follow-up after treatment. Which scan parameters need to be considered? When is the optimum time of performing the scan after treatment? How frequently should the scan be repeated? What is the prognostic value of MET uptake after treatment? How often might it help to optimize the treatment plan in case of persistent uptake or an absence of uptake? Are there any studies on controlled comparisons of patient outcome after using such an approach? Prolonged survival in grades 3 and 4 glioma patients with no methionine uptake after surgical resection as compared to those with

residual MET uptake was noted [115]. No such difference in survival was noted in low-grade glioma patients in this study. A significant reduction in methionine uptake after radiotherapy and/or chemotherapy was also reported by the authors for the high-grade gliomas. However, the authors did not report the time interval between surgery and scan [115]. Piroette et al. used an early MET-PET scan to determine the amount of residual tumor in children with gliomas after surgery. A residual uptake in 14 children led to a second look surgery in 11 of them. A conservative approach was followed in six children without residual MET uptake, and subsequently, no progression was seen on follow-up MR imaging [135]. The authors have reported performing MET-PET within 2–8 days or after 6 weeks of surgery to avoid a potential false positive result because of inflammation [134]. In a study of 30 grade II glioma patients, a similar methionine uptake was noted after surgery irrespective of adjunctive radiotherapy [145]. In another study with 12 patients with low-grade astrocytomas, six monthly MET-PET scans were performed after radiotherapy. A stable or decreasing MET uptake was seen in patients with no evidence of disease or stable disease. An increasing uptake was seen in patients with progressive disease. A transient increase in SUV in a clinically stable patient who had an equivocal uptake on visual analysis was seen, which subsequently declined on follow-up scanning.

Table 3. A table of studies evaluating the role of MET-PET in differentiating recurrent tumor from radiation injury

Author	Year	Number of patients	Patients	Results
Lilja et al. [150]	1987	4	Long-term high-grade glioma survivors (2 with recurrent tumor, 2 with apparently no recurrence)	Recurrent tumor extent larger on 11 C-methionine than CT in both patients with recurrence, false positive uptake in one clinically stable patient
Ogawa et al. [151]	1991	15	Suspected recurrence (3 out of 5 with radiation injury and 7 out of 10 with recurrent tumor had MET-PET)	HPE confirmation in 14 out of 15 cases, no false positives on MET, all cases of tumor detected by MET
Viader et al. [107]	1993	1	Suspected recurrence presenting with seizures	Normal CT and MRI in recurrent low-grade glioma patient with seizures, detected by MET-PET
Sasaki et al. [152]	1996	1	Suspected recurrence (21 years post-therapy) presenting with seizures	Increased FDG, methionine and Tl uptake in histopathologically confirmed radiation necrosis; resolved following surgery
Sonoda et al. [153]	1998	12	Suspected recurrence on MRI (5 with recurrent tumor, 7 with radiation necrosis)	True positive MET uptake in all cases of recurrent tumor. False positive MET uptake in 1 out of 7 cases with radiation injury. False positive Tl uptake in 4 out of 7 cases. All clinical diagnoses
Tsuyuguchi et al. [157]	2003	21	Suspected metastatic tumor recurrence (9 with recurrence, 12 with necrosis)	Sensitivity=78%, specificity=100% (T/N cut off value=1.42)
Tsuyuguchi et al. [154]	2004	11	Suspected glioma recurrence (6 with recurrence, 5 with radiation injury)	Sensitivity=100%, specificity=60% (visual and semiquantitative analysis)
Van Laere et al. [155]	2005	30	Suspected glioma recurrence/progression, comparison with FDG	MET more sensitive than FDG, 100% inter-observer agreement for MET, 73% for FDG; combination of FDG and MET provided maximum prognostic accuracy
Tang et al. [158]	2006	33	Suspected pituitary adenoma recurrence (24 secreting adenomas, 9 non-functional adenomas)	MET positive in 30/33 cases. Altered management plan in 9 out of 14 cases inconclusive on MRI

HPE Histopathological examination, MET ^{11}C -methionine, T/N ratio of uptake in tumor to contralateral normal brain, N number of patients, Tl thallium, FDG fluorodeoxyglucose

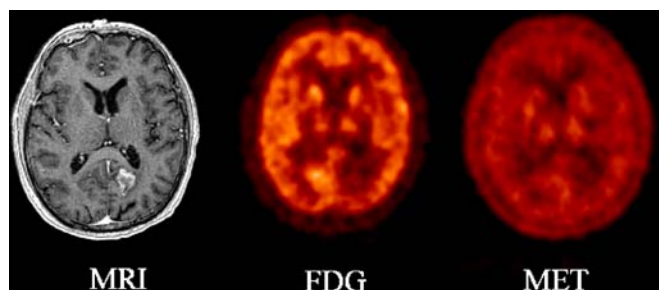


Fig. 4. Radionecrosis. Status grade III astrocytoma in left parieto-occipital region, post-surgery and post-radiotherapy recurrence of symptoms after 6 months of last treatment. MRI clearly shows the lesion in the same area; however, both FDG and methionine show no uptake consistent with the diagnosis of radiation-induced necrosis.

However, the SUV did not increase above the pre-treatment level in this case [138]. Measuring both MET-defined tumor volume and MET-uptake has been recommended for following up on glioma patients [108, 126]. A significant reduction in MET uptake and a semiquantitative index based on both MET uptake and MET-defined volume was noted in seven low-grade oligodendroglioma patients after chemotherapy with procarbazine, CCNU, and vincristine [126]. Herholz et al. [147] have estimated a reduction rate in methionine-defined active tumor volume of approximately 2.4% per day in a case of anaplastic oligoastrocytoma after chemotherapy. In another study on 32 untreated grade II glioma patients, an increase in MET uptake or MET-defined volume on follow-up scans was associated with a reduced time to progression of disease [108]. In a study on MET-PET scan performed 1 year after seed implantation of ^{125}I for brachytherapy of cerebral glioma, a significant reduction in MET uptake was evident but no significant change was noted in ten patients with FDG scan [129]. Sorensen et al.

[148] noted a prompt reduction in methionine uptake after therapy in two children with prolactinomas within days of starting therapy. In summary, the feasibility and usefulness of MET-PET for therapy assessment and follow-up in conjunction with anatomical imaging after surgery, chemotherapy, and radiotherapy have been demonstrated in several studies. The optimal time and frequency of MET-PET scanning deserves to be established in future studies.

Differentiation of Tumor Recurrence Versus Radiation Injury

Non-invasive differentiation of recurrent tumor from radiation injury in a symptomatic patient on post-therapy follow-up can be difficult. Contrast enhancement patterns on CT/MRI can occur in both the conditions caused by the breakdown of the BBB. FDG-PET has been shown to be useful for differentiating tumor recurrence from radiation injury [149]. However, there may be absence of high FDG uptake in recurrent low-grade tumors and presence of increased FDG uptake in the inflammatory tissue because of radiation. MET-PET has been investigated by several authors for its usefulness in correctly identifying recurrent tumor after surgery and radiation (Table 3).

In an early study, Lilja et al. [150] reported that MET-PET showed a larger tumor area than CT in two of their four patients with residual tumor. In one patient with no recurrence, no uptake of MET was found. Nonspecific methionine uptake was reported in one clinically stable patient assumed to have no recurrence [150]. Ogawa et al. [151] found that none of the three patients in their series who had radiation injury and MET-PET scan showed either methionine or FDG uptake. Out of 10 patients who had recurrent tumor, MET-PET was done in seven patients, all of whom showed an increased MET concentration [151]. Viader et al. [107] have reported a case of recurrent low-grade gliomas that was missed by CT/MRI but was seen only by MET-PET.

Table 4. Summary of the role of ^{11}C -methionine in clinical management of cerebral gliomas

Clinical management	Comments
Initial diagnosis	High sensitivity and specificity High positive predictive value Low negative predictive value More useful than FDG, complementary to MRI Particularly useful for low-grade gliomas
Recurrence versus radiation injury	High positive predictive value High negative predictive value More useful than FDG and MRI/CT
Grading	Less Useful than FDG on visual analysis Semiquantitative techniques can distinguish high and low grade gliomas
Prognostication	“Hot spots” associated with poorer prognosis Higher semiquantitative uptake associated with poorer prognosis
Tumor extent	Better delineation of tumor margin than FDG and/or contrast enhancement on T1 weighted MRI and T2 MRI
Surgical resection biopsy planning	Clinical feasibility and usefulness demonstrated in several studies
Radiotherapy planning	Alteration in target volume demonstrated in several studies
Therapy assessment	Decline in ^{11}C -methionine uptake after surgery, chemotherapy, radiotherapy demonstrated. Prognosis associated with degree and extent of uptake after therapy. More studies on optimization of time and frequency of scanning for regular follow-up needed

Sasaki et al. have reported increased MET, FDG, ^{201}Tl , and HMPAO uptake in a patient with radiation necrosis that was epileptogenic. The focus of increased uptake resolved after surgery [152]. A case of residual high-grade glioma detected by MET-PET before the appearance of contrast enhancement and clinical symptoms has been reported by Nariai et al. [115]. Sonoda et al. [153] have reported that all five of their patients with recurrent tumor showed increased MET uptake, whereas only one of seven patients with radiation necrosis showed an increased MET concentration (Fig. 4). ^{201}Tl showed an increased accumulation in four of these seven patients with radiation necrosis [153]. Tsuyuguchi et al. found that MET correctly identified all six recurrences and three of five radiation injury patients. The mean T/N was 1.8 in the recurrent tumor group, whereas the mean T/N was 1.3 in the radiation injury group [154]. Von Laere et al. have performed a comparison of FDG and MET for identifying recurrence and found that 28 out of 30 patients (15 high-grade gliomas and 15 low-grade gliomas on initial diagnosis) had an increased MET uptake, whereas only 17 out of 30 patients had increased FDG concentration. Only three patients in their study had histopathological confirmation [155]. The classification of all cases of death as recurrence and all cases that were alive at the end of follow-up period as radiation injury, as done in this study, may not be accurate. A patient who had recurrence but received treatment and was alive at the end of follow-up as a result of treatment might have been wrongly classified as radiation injury when he/she actually had a recurrence at the time of the PET scan. An accurate increased MET uptake in such a case would be misclassified as a false-positive. The mildly increased MET uptake in radiation injury patients observed in this and other studies can be caused by the proliferation of glial cells that is a known feature of radiation injury [17].

Moreover, there is a possibility of overlap between the T/N values in radiation injury and low grade recurrence, and thus, low-grade tumor recurrence may sometimes be indistinguishable from radiation injury. However, the underlying mechanism for methionine uptake in the two conditions is different. The uptake in low-grade gliomas is caused by the active uptake of methionine in the tumor cells, and the uptake in radiation injury is predominantly caused by passive diffusion across the breakdown of BBB. In fact, in cases with borderline methionine uptake, repeat MET-PET scanning after corticosteroid administration may serve to distinguish between the two conditions, as corticosteroids may reduce the methionine uptake because of BBB breakdown in radiation injury cases while leaving the methionine uptake due to active transport in low-grade gliomas intact. Coregistration of MET-PET with contrast-enhanced MRI revealed recurrent low-grade glioma in non-enhancing but high MET uptake area of the brain in a patient with suspected recurrence, whereas the region showing contrast enhancement but minimal methionine uptake in the same patient was found to be necrotic [156].

Tsuyuguchi et al. [157] and Tang et al. [158] have performed comparisons in recurrent brain metastasis and

pituitary adenomas and found that MET was useful in differentiating recurrent tumor from radiation injury. Overall, in spite of few and small-sized studies and absence of histopathological confirmation in many instances, there is evidence that MET-PET has a high sensitivity for detecting tumor recurrences. Further, in most cases of radiation injury, it is likely to be negative. However, in some cases, it may show mildly increased uptake. In the six studies reviewed in this paper [107, 150–154], there were 18 cases of radiation injury that had a MET-PET scan of which five showed increased MET uptake according to the observers. This estimates the specificity of MET-PET to be 72% (95% confidence interval (CI)=50–94%). All 21 of the tumor recurrence cases that had a MET-PET scan in these studies showed an increased MET uptake, estimating the sensitivity to be 100% (95% CI=96–100%). Assuming a pre-test probability of 57% of having a tumor recurrence from this analysis yields an estimated PPV of 81% (95% CI=65–97%) and a NPV of 100% (95% CI 97–100%) with its inherent limitations. In summary, MET-PET appears to have a high sensitivity, specificity, positive predictive value, and negative predictive value in differentiating recurrence from radiation injury.

Future Research Directions

Investigations conducted during the last three decades notwithstanding the role of MET-PET in the management of gliomas remain yet to be properly evaluated and defined. This is a difficult task in the absence of data from large series of patients obtained in prospective multi-center clinical trials using standardized methodology. The data currently available from largely retrospective analysis on series of relatively small number of patients, however, indicate a few emerging useful applications of amino acid imaging, particularly in (a) the detection and differential diagnosis of low-grade gliomas (oligodendrogliomas), (b) more accurate delineation of tumor extent and integration of the data in treatment planning, and (c) post-treatment detection of tumor recurrence and differentiation from delayed radiation-induced lesions (Table 4).

Based on the above trends, the future clinical research should, therefore, be designed and coordinated to obtain reliable information on large patient populations in different glioma subgroups recruited in multi-center studies to answer specific questions regarding the role of amino acid imaging (especially MET-PET and FET-PET) in improving therapeutic outcome. International organizations such as WHO or IAEA can be helpful in the standardization of methodology and coordination of the multi-center trials.

The high uptake of methionine in low-grade tumors is useful for initial diagnosis of low-grade gliomas. However, the same property makes ^{11}C -methionine a less useful agent for grading and prognostication than FDG, the difference in whose uptake between high- and low-grade tumors is more marked. Therefore, a semi-quantitative or quantitative approach to ^{11}C -methionine uptake for grading and prognostication becomes necessary.

The potential utility of MET-PET in the clinical management of gliomas could be enhanced by quantitative analysis of data through more sophisticated computer-assisted image processing to minimize subjectivity and inter-observer variability. Objective, accurate, and reproducible measurements of tumor volume are of great importance in treatment planning and assessing the response to treatment. Visual assessment of tumor size and alterations induced by therapy regimens suffer from large inter-observer variability, especially in diffusely infiltrating, residual or recurrent tumors. Therefore, development of suitable computer-assisted automatic methods for segmentation tasks in PET images with poor contrast and low signal to noise ratio is a challenging research area.

Recently, there is an ongoing debate in the literature about the need for randomized controlled trials in establishing the role of diagnostic modalities in patient management. Lord et al. [162] have recently suggested that a randomized controlled trial for a diagnostic study may be only necessary when the treatment for a given condition is not efficacious. However, accuracy studies may suffice if there is a clear improvement in patient outcome after treatment [162]. In the case of MET-PET in gliomas, a case for a randomized trial may be made in the light of the above argument and relative lack of efficacy of current treatment modalities in gliomas.

Because of the 20-min half life of ^{11}C -methionine, there are logistical hindrances in its use at centers that do not have an on-site cyclotron. Therefore, considering the apparent usefulness of ^{11}C -methionine in brain gliomas, future clinical studies should also prospectively study the accuracy of ^{18}F -labeled amino acid tracers in cerebral gliomas.

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