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IMAGING CORRELATES OF DIFFERENTIAL EXPRESSION OF INDOLEAMINE 2,3-DIOXYGENASE IN HUMAN BRAIN TUMORS

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Abstract

Background—Tryptophan catabolism via the kynurenine pathway, mediated by indoleamine 2,3-dioxygenase (IDO), is a mechanism involved in tumor immuno-resistance. Positron emission tomography (PET) with α -[¹¹C]methyl-L-tryptophan (AMT) can quantify transport and metabolism of tryptophan in infiltrating gliomas and glio-neuronal tumors. In the present study, we investigated whether increased tryptophan metabolism in brain tumors measured by PET is related to expression of IDO in resected brain tumor specimens.

Methods—IDO expression was assessed by immunohistochemistry in tumor specimens from 15 patients (median age: 34 years) with primary brain tumors who underwent AMT PET scanning before tumor resection. Patterns of IDO expression were compared between low-grade and high-grade tumors and also to AMT transport and metabolism measured on PET.

Results—IDO immunoreactivity was seen in tumor cells in 6 of 7 low-grade tumors but only in 1 of 8 high-grade tumors ($p = 0.01$); 3 of these latter tumors showed endothelial staining only. Low-grade neoplasms showed lower transport rate ($p < 0.01$) but higher metabolic rate ($p = 0.003$) for AMT as compared to high-grade tumors. AMT metabolic rates were lower in tumor samples with no or minimal IDO expression as compared to those with widespread IDO staining ($p = 0.017$).

Conclusion—Low-grade tumors show widespread IDO expression, while IDO expression in high-grade brain tumors can be absent or largely confined to endothelial cells. AMT PET can be useful to identify brain tumors with different profiles of IDO expression, thus providing a useful imaging marker for emerging treatments targeting tumor IDO activity.

Keywords

glioma; positron emission tomography; tryptophan; indoleamine 2,3-dioxygenase; immunohistochemistry

INTRODUCTION

Catabolism of the essential amino acid tryptophan via the kynurenine pathway, mediated by the initial and rate-limiting enzyme indoleamine 2,3-dioxygenase (IDO), has been considered to be a major mechanism involved in the escape of tumors from the host immune response (Munn Mellor, 2007). IDO is constitutively expressed in various human neoplasms, including some malignant gliomas (Uyttenhove et al., 2003). IDO can also be induced by a variety of inflammatory stimuli, among which interferon-gamma (IFN- γ) is the most prominent (reviewed in King & Thomas, 2007). Strategies to target IDO in order to enhance tumor immunotherapy are under development (Löb & Konigsrainer, 2007; Jia et al., 2008). Molecular imaging methods capable of identifying tryptophan metabolism *in vivo* might be useful to predict and monitor IDO-based therapy. In a recent study, our group demonstrated increased α -[^{11}C]methyl-L-tryptophan (AMT) transport and metabolism in gliomas and glioneuronal tumors (Juhasz et al., 2006). AMT is an analog of tryptophan, and it has been shown that AMT PET is able to track the serotonergic pathway and, under certain circumstances, the kynurenine pathway (Chugani & Muzik, 2000); however, unlike other amino acid PET tracers, AMT is not incorporated into protein (Madras & Sourkes, 1965; Diksic et al., 1990). Although we previously hypothesized that increased metabolism of tryptophan via IDO may underlie increased trapping of AMT in some brain tumors (Juhasz et al., 2006), a comparison of IDO expression to AMT kinetic parameters in these tumors was not done.

In the present study we evaluated expression of IDO, using immunohistochemistry, in resected tumor tissues from patients who had undergone AMT PET scanning prior to surgery. In addition, the proliferative potential of brain tumors was evaluated using Ki-67 labeling (Quinones-Hinojosa et al., 2005). The primary goal was to study the distribution of IDO expression in various types of low- and high-grade brain tumors and compare it to AMT transport and metabolic parameters derived from PET kinetic data as a first step in the validation of AMT PET as an imaging biomarker for the guidance of IDO based tumor treatment.

METHODS

Study Population

Fifteen patients (age: 4 to 67 years; median = 34 years) with brain neoplasms were included in the study. PET data of 12 of these 15 patients were included in a previous study (Juhasz et al., 2006), although the PET scans were reanalyzed for the present study. All protocols were approved by the Human Investigation Committee at Wayne State University, and a written informed consent was obtained.

AMT PET Scanning Protocol

PET studies were performed using the CTI/Siemens EXACT/HR whole-body positron tomograph (Knoxville, TN, USA) located at the Children's Hospital of Michigan, Detroit. This scanner has a 15 cm field of view and generates 47 image planes with a slice thickness of 3.125mm. The reconstructed image in-plane resolution obtained is $7.5 \pm 0.4\text{mm}$ at full-width half-maximum (FWHM) in-plane and $7.0 \pm 0.5\text{mm}$ in the axial direction (reconstruction parameters: Hanning filter with 1.26 cycles/cm cutoff frequency) for the AMT PET. The procedure for AMT PET scanning has been described previously (Chugani et al., 1998;). In

brief, after 6 hours of fasting, a venous line was established for injection of AMT (0.1 mCi/kg) as a slow bolus over 2 minutes. Children, when needed, were also given either nembutal (5 mg/kg) or midazolam (0.2 to 0.4 mg/kg) intravenously for sedation. A second venous line was established for collection of timed blood samples (0.5 mL/sample, collected at 0, 20, 30, 40, 50, and 60 mins after AMT injection). After injection of AMT, a 20-min dynamic PET scan of the heart was performed (sequence: 12×10 s, 3×60 s, and 3×300 s) in 2D mode to obtain the left ventricular (LV) input function. Continuation of the arterial input function beyond the initial 20 minutes was achieved using the venous blood samples as described previously (Muzik et al., 1994; Suhonen-Polvi et al., 1995). At 25 minutes after tracer injection, a dynamic emission scan of the brain (7×5 min) was acquired in high-sensitivity 3D mode. Measured attenuation correction was applied to the AMT PET images of the heart, whereas computed attenuation correction was used to correct the brain images.

PET Image Analysis

To quantify unidirectional AMT transport and metabolism in tumors, a Patlak graphical analysis (Gjedde, 1981; Patlak et al., 1983) was performed using the arterial LV input function and the dynamic brain sequence (7 × 5 min frames starting 25 min after injection), as described previously (Juhasz et al., 2006). The Patlak graphical analysis is based on the following equation (Gjedde et al., 1981; Patlak et al., 1983)

$$\frac{C_T(t)}{C_p(t)} = k_3 VD \frac{\int_0^t C_p(u) du}{C_p(t)} + \varepsilon VD \quad (1)$$

with $C_T(t)$ representing tissue concentration derived from PET imaging and $C_p(t)$ representing the arterial input function. Furthermore, the rate constant k_3 characterizes the enzymatic conversion of AMT, and VD represents the volume of distribution of the tracer in the free precursor pool. Finally, the factor $\varepsilon (= k_2/(k_2+k_3))$ takes into account the time lag of the free precursor pool relative to the changing plasma tracer concentration and is dependent on the ratio between efflux of tracer from the free precursor pool back into the blood pool (k_2) and the total efflux (k_2+k_3). Upon reaching dynamic equilibrium ($dC_T/dt = 0$), the above equation (1) describes a line with slope $k_3 VD$ and intercept $\varepsilon VD (= VD')$. The quotient between slope and intercept yields then an estimate of the k_3 parameter ($k_3' = k_3/\varepsilon$).

Since all 15 tumors showed increased AMT uptake on the summed activity images (representing tracer uptake 30–60 min after injection), tumor regions of interest were directly defined on these images in all planes (except the very top and bottom planes, to avoid partial volume effects), where these increases were clearly visualized. Since some high-grade tumors showed inhomogeneous activity presumably due to a necrotic core, only tumor parts showing at least 50% of the average activity were included in the final regions of interest. Three parameters were derived from the Patlak plots: The slope parameter ($k_3 VD$) corresponds to the previously described K-complex (Chugani et al., 1998; Muzik et al., 1997) reflecting the unidirectional uptake of tracer into tissue. In the case of AMT, the value of the K-complex is thought to be proportional to the tryptophan metabolism via the serotonin and/or the kynurenine pathway when the blood–brain barrier (BBB) is intact (Chugani & Muzik, 2000). However, breakdown of the BBB in some brain tumors may influence the K-complex due to increased permeability of the tracer; therefore, an estimate of the metabolic rate constant k_3' and the volume of distribution (VD') of the tracer in the free precursor pool were also calculated. The k_3' parameter characterizes the enzymatic conversion of AMT, while the VD' parameter delineates the transport rate of AMT into the tumor tissue. We have previously shown that the VD' value is increased when the BBB is compromised (Juhasz et al., 2006).

Immuno-histochemistry (IHC) and Histopathologic Examination of Resected Tumor Tissues

Tumor specimens were fixed in 10% formalin solution, processed and embedded in paraffin, and 5 μm -thick sections were cut. Sections were deparaffinized and hydrated. Sections were stained with hematoxylin and eosin or processed for immunohistochemical staining for IDO and Ki-67. For immunostaining, endogenous peroxidase activity was quenched with 3% H_2O_2 for 20 minutes, followed by antigen retrieval in citrate buffer pH 6.0 for 20 minutes in steamer. Sections were blocked for 30 min in 5% normal horse or goat serum and incubated with primary antibodies for 2 hours at room temperature. Primary antibodies for IDO (1:50, Chemicon # MAB5412, clone 10.1) and Ki-67 (Prediluted, Cell Marque # 601 Ventana, clone K-2) were used. Following washing, sections were incubated with anti-mouse biotinylated secondary antibody IgG (H+L) (Vector Lab) for 30 min and avidin-biotin complex (Vector Lab) for 30 min. Visualization was performed with a DAB substrate (Vector Lab) chromogen and counterstained with hematoxylin. Diagnostic criteria and nomenclature for tumor grading were applied according to the World Health Organization Classification of Tumors, based on the recommendations of the Consensus Conference in Lyon, France, 1999 (Kleihues & Cavenne, 2000) and on published histological criteria (Burger & Scheithauer, 1994; Burger et al., 2002) by a board-certified neuropathologist (W.J.K.). Assessment of IDO IHC results was carried out by two investigators (C.E.A.B. and W.J.K.) independently, and discrepancies were then resolved by reviewing all specimens for which the two of them had different opinions. Positive IDO immuno-staining was defined when consistent staining was seen in at least two different fields of view throughout the sample. At least 10 fields were analyzed in all cases. Agreement between both investigators ascertained positive staining in the following elements, which could be clearly identified: tumor cells, perivascular region, endothelial cells, and neuropil.

Study Design and Statistical Analysis

Statistical analysis was performed using SPSS version 11.5 (SPSS Inc, Chicago, Ill). Comparison of AMT kinetic parameters (VD' , k_3' , and K-complex) between gliomas and DNETs, as well as between high-grade and low-grade tumors, were performed using a univariate ANOVA, using the PET parameters as dependent variables and group membership (glioma vs. DNET and low-grade vs. high-grade tumors) as predictors. In addition, tumor grade and Ki-67 scores were correlated with PET variables using the Spearman's rank correlation. Fisher's exact tests were carried out to compare the incidence of positive cases of IDO in low-grade vs. high-grade tumors. A p value < 0.05 was considered to be significant.

RESULTS

Histopathological Analysis of Surgical Specimens

A total of 15 patients with different histologic diagnoses were studied (Table 1). The histopathologic examination revealed mixed infiltrating gliomas ($n = 4$), dysembryoplastic neuroepithelial tumors (DNET) ($n = 4$), glioblastoma multiforme (GBM) ($n = 3$), pure oligodendrogliomas ($n = 2$), diffuse astrocytoma ($n = 1$), and ependymoma ($n = 1$). Tumors classified as WHO grade I or II were considered low-grade tumors, whereas anaplastic gliomas (WHO grade III) and GBM were grouped as high-grade tumors.

IDO Expression in Low-Grade vs. High-Grade Tumors

Positive IDO staining was seen in tumor cells in the majority of low-grade tumors (6 of 7 cases, 86%), including all DNETs (Table 2), while only 1 of 8 (13%) high-grade tumors showed IDO staining in tumor cells ($p = 0.01$) (Figure 1A; C). In contrast, endothelial staining was seen only in high-grade (4/8) but not in low-grade tumors (0/7; $p=0.07$) (Figure 1E). Positive IDO staining was also seen in the neuropil and perivascular tissue more frequently in low-grade

tumors than high-grade tumors (Table 2, Figure 1B–D), although this difference was not statistically significant (4/7: 57% vs. 2/8: 25%, for neuropil, and 4/7: 57% vs. 1/8: 13% for perivascular tissue, respectively; $p = 0.32$ and $p = 0.12$, respectively). IDO immunoreactivity in each tumoral component is outlined in Table 2.

PET-Derived Metabolic and Transport Rates of AMT in Different Tumor Types and Histologic Grades

AMT metabolic rates k_3' and transport rates (VD') were different in low-grade vs. high grade tumors: low-grade tumors showed higher metabolic rate k_3' for AMT as compared to high-grade tumors (0.022 ± 0.003 vs. 0.015 ± 0.004 , $p = 0.003$) (Table 1, Figure 2). The k_3' values also showed a significant inverse correlation with tumor histologic grade (Spearman's $\rho = -0.86$, $p < 0.01$). In contrast, low-grade tumors presented lower values of VD' when compared with high-grade tumors (0.42 ± 0.05 vs. 0.67 ± 0.20 , $p < 0.01$), and tumor VD' values showed a positive correlation with tumor grade (Spearman's $\rho = 0.89$, $p < 0.01$). K-complex values (which are a composite of k_3' and VD'), however, were not different between high-grade and low-grade tumors (0.01 ± 0.005 vs. 0.01 ± 0.002 , $p = 0.53$), and none of the three PET-derived variables differed between DNETs and gliomas ($p > 0.1$ in all comparisons).

Relationship between IDO Expression and AMT PET Kinetic Values

Tumor samples were divided into two groups based on the extent of IDO expression. Four cases (patient 6, #9, #13, and #14) did not show any IDO immunoreactivity in any tumoral component, while three additional cases (patient 10, #12, and #15) had IDO staining only in endothelial cells of some vessels within the tumor sample. Once the expression of IDO was limited to a very small compartment of the tumor, these three cases were included in the same group with the other four cases (no or minimal IDO expression). The other 8 cases showed widespread IDO expression including tumor cells ($n = 7$), perivascular tissue ($n = 6$) and neuropil ($n = 5$) alone or in various combinations. A comparison of the PET kinetic parameters between these two groups showed AMT metabolic rates (k_3') to be lower in the group with no or minimal IDO expression as compared to the group with widespread IDO expression (mean 0.016 ± 0.005 vs. 0.021 ± 0.004 , $p = 0.017$). Tumor VD' and K-complex values were not significantly different between the groups ($p = 0.42$ and $p = 0.52$, respectively). VD' showed a significant correlation with Ki-67 ($r = 0.74$, $p = 0.03$). Metabolic values, in turn, showed only a trend toward a significant negative correlation, i.e., higher k_3' was associated with lower values for Ki-67 ($r = -0.67$; $p = 0.09$).

DISCUSSION

Based on our previous study, which showed a relationship between tumor grade and the metabolic rates of AMT on PET in human brain tumors, we hypothesized that this increased metabolic rate may reflect increased metabolism of tryptophan via the kynurenine pathway (Juhász et al., 2006). The current study demonstrates that tumor cells in 6 of 7 low-grade, well-differentiated tumors indeed express IDO, and this immunoreactivity is associated with additional perivascular and neuropil expression in 4 cases. In contrast, most high-grade tumors either do not express IDO or the expression is confined to the endothelium. Differences in AMT metabolic rates in tumors with widespread vs. no or minimal IDO expression support the notion that high metabolic rate of AMT reflects increased metabolism of tryptophan via IDO in primary brain tumors. The only exception was seen in a grade II oligodendroglioma with no IDO staining despite relatively high AMT metabolic rate; this may be due to a sampling error or tryptophan metabolism via enzymes other than IDO. Widespread expression of IDO in most low-grade neoplasms is consistent with a tumor environment where tryptophan is metabolized via the kynurenine pathway, thus leading to local tryptophan depletion. Depletion of tryptophan in these low grade tumors may be related to the low proliferative rates, as reflected

by low Ki-67 expression. No immunoreactivity or minimal expression of IDO in some high-grade gliomas suggests that IDO inhibitors, being considered as an adjuvant immunotherapy in various malignancies, may not be effective in high grade tumors. Thus, identification of IDO-expressing tumors before such therapies are implemented would be important to optimize potential therapeutic effects. Our data further suggest that inhibition of IDO might be detrimental in low grade tumors, by removing a mechanism potentially leading to decreased cellular proliferation.

The current study has some limitations. The number of cases was relatively small and included tumors of varying histologic types, so that we could test if IDO is expressed in some common tumor types. Given this heterogeneity, we grouped the specimens based on the grade of the tumor (high vs. low), and we also compared glioneuronal tumors (DNETs) vs. infiltrating gliomas. This grouping method provided more power to detect differences across broad tumor grades and histopathological types. The results suggest that group differences found in AMT PET kinetic parameters, especially are driven by tumor grade rather than tumor type, but further refinement among various glioma types will require a larger sample size. In this study, we could reliably evaluate IDO staining in neoplastic cells and endothelial cells as well as neuropil and perivascular tissue; however, neurons and non-neoplastic glial cells can also produce IDO under certain circumstances (Guillemin et al, 2005). We did not systematically evaluate IDO staining in these other cell populations. In several cases, mostly malignant gliomas, there was no clear IDO immunoreactivity in glial cells. A few other tumors with necrosis were found to have scattered IDO-positive cells, which could represent activated microglia and/or macrophages within the tumor. In some other cases, however, the intense staining in the background, namely the neuropil, made it difficult to decide whether glial cells were also stained. It should be noted that previous studies that have demonstrated the expression of IDO by glial cells were performed in cells grown in primary cultures and in the presence of IFN- γ , well-known inflammatory mediator and one of the major regulators of IDO (see review of Kwidzinski & Bechmann, 2007). Future studies that employ colocalization methods in surgical specimens may further address the role of glial and macrophage IDO expression in human brain tumors.

Immuno-histochemistry is an excellent tool for tissue and cellular localization of protein expression. However, this method does not provide a quantitative measure of protein expression. Moreover, expression of IDO protein is not necessarily a measure of IDO enzyme activity, which relies strongly upon certain environmental conditions (Löb & Königsrainer, 2007). Real time PCR and Western blot might be employed to measure quantitatively IDO expression, as well as assays that test enzyme activity such as the level of downstream metabolites of the kynurenine pathway (Kwidzinski et al., 2003; Beutelspacher et al., 2006). We have also not distinguished between the two forms of IDO (IDO1 and IDO2), which have been recently shown to be co-expressed by some malignant tumors (Löb et al., 2008), although expression of IDO2 in malignant gliomas has not been reported thus far.

In mammals, most of the tryptophan derived from the diet is converted via the kynurenine pathway (Schwarcz & Pellicciari, 2002), which is up-regulated in several autoimmune disorders and malignant neoplasms (Schröcksnadel et al., 2006). Constitutive expression of IDO in various cancers might provoke tumoral tolerance by depleting tryptophan, which in turn is a necessary element for T-cell-mediated immune response (Uyttenhove et al., 2003). Similarly, Kai and colleagues provided evidence for a role of natural killer (NK) cells in anti-tumoral activity mediated by IDO induction (Kai et al., 2003; Kai et al., 2004). Other studies have shown that IDO might mediate immunosuppression that benefits tumor viability (Mellor & Munn, 1999; Friberg et al., 2002; Munn & Mellor, 2004). On the other hand, some studies suggest that induction of IDO may hinder tumor survival, suggesting that tryptophan depletion

mediated by IDO may inhibit tumor cell proliferation (Ozaki et al., 1988; Aune & Pogue., 1989; Taylor & Feng., 1991; Burke et al., 1995).

In the present study, we found that neoplastic cells of low-grade brain tumors expressed IDO more frequently than their high-grade counterparts, and IDO expression was also more widespread (including perivascular elements and neuropil) in some cases. High-grade gliomas, especially GBM, have a high propensity for local invasiveness (Stojic et al., 2008). Interestingly, two GBM cases reported here did not show expression of IDO in any cell type within the tumor, except in some cells within the necrotic regions of the tumor, which likely represent invading inflammatory cells (activated macrophages and/or microglia) following BBB breakdown. The third case presented positive endothelial cells, but the extension was limited to a few fields of view. Consistent with the IHC results, the tryptophan metabolic rates calculated from the dynamic AMT PET were lower in high-grade tumors compared to the ones from low-grade tumors, including four DNETs. DNET is considered a benign slow-growing tumor which may remain in the brain for many years without substantial local progression (Jensen et al., 2006). Widespread expression of IDO in these WHO grade I tumors may play a role in the low proliferative activity of these tumors via local tryptophan depletion.

The absence of IDO expression in tumor cells of all but one high-grade glioma, including three GBMs, was somewhat unexpected since a previous study reported tumor cell IDO expression in 9 out of 10 GBM cases (Uyttenhove et al., 2003). On the other hand, a previous study showed a relative preponderance of kynurenic acid, one of the downstream metabolites of the kynurenine pathway, in astrocytomas compared to less-differentiated glioblastomas (Vezzani et al., 1990). Proteomic analysis of tumor tissue may provide a more complete picture of the numerous branch points in the kynurenine pathway. A more thorough understanding of tryptophan metabolism in high-grade gliomas is necessary in order to determine whether GBMs may benefit from IDO-inhibitors.

The present study has also shown a differential expression of IDO in various cell types, and these differences might correlate with the biological behavior of these tumors. For instance, although perivascular elements, presumably including the terminal glial processes, were stained positively by IDO in both low-grade tumors (4/7) and in one high-grade tumor, endothelial IDO staining was seen only in high-grade tumors (4/8). The clinical significance of endothelial IDO content remains to be further evaluated in gliomas. It has been reported that endothelial cells can express IDO (Beutelspacher et al., 2006), and expression of IDO in tumor endothelial cells benefited survival of patients with renal cell carcinoma (Riesenberg et al., 2007). The authors suggested that IDO in endothelial cells might limit the influx of tryptophan from the blood to the tumor or generate tumor-toxic metabolites, thus restricting tumor growth and contributing to survival. IDO expression is regulated by IFN- γ (Guillemin et al., 2005), and increased IDO activity might be related in part to the therapeutic effect of IFN- γ treatment of selective cases of renal cell carcinoma. Immunotherapy with IFN- γ has been also used to treat gliomas with mixed results (Merchant et al., 1992; Fenstermaker & Ciesielski, 2004; Wolff et al., 2006). Our study suggests that malignant gliomas may not be amenable to therapies targeting IDO inhibition; indeed, in some brain tumors, not constitutively expressing IDO, induction of this enzyme may be more effective. However, further studies are required to better understand the role of IDO in biological behavior of human brain tumors, before such therapies can be designed.

In summary, we have presented preliminary results suggesting that brain tumors, particularly low-grade gliomas and glio-neuronal tumors can show widespread IDO expression associated with increased metabolism of tryptophan on PET. How increased metabolism of tryptophan via the kynurenine pathway plays a role in tumor proliferation and immune tolerance remains to be clarified. Identification of the type of cells that express IDO may help identify tumor

types that might benefit from immunotherapy—by either enhancing or inhibiting IDO or perhaps targeting other downstream metabolites of the kynurenine pathway. Furthermore, the current findings suggest that AMT PET could be considered as an imaging biomarker in future clinical trials for monitoring the effects of pharmacological agents affecting IDO activity in primary brain tumors.

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Figure 1. Immunohistochemistry comparing patterns of IDO expression in a low-grade (DNET) and high-grade tumor (glioblastoma multiforme, GBM). Positive IDO staining in tumor cells (**A**, arrows) and in perivascular tissue as well as neuropil (**B**) in the DNET (patient #4). In contrast, the GBM (patient #13) showed no staining in tumor cells or perivascular tissue (**C**, **D**). Positive IDO staining in endothelial cells in an anaplastic oligodendroglioma (patient #12) (**E**). Original magnification 40x in all images.

Figure 2.

AMT PET scans and Patlak plots of a DNET (patient #4; **A**) and a glioblastoma multiforme (GBM, patient #13; **B**). Both tumors showed high AMT activity (arrows) on summed PET images (20–60 min after tracer injection), as shown on this figure. However, on the Patlak plot, the intercept, which represents volume of distribution (VD'), is higher for GBM compared to DNET. The metabolic rate of AMT, k_3' , was calculated as the ratio between the K-complex (slope of the Patlak curve) and VD' and was much higher in the DNET than in the GBM (0.026 vs. 0.011). CP: tracer concentration in the blood, CT: tracer concentration in tumor tissue.

Table 1
Clinical data (gender, age), tumor type and grade, Ki-67 proliferation index and PET-derived AMT kinetic variables (k_3' = metabolic rate; VD' = transport rate; K-complex = unidirectional uptake rate constant).

No	Gender	Age (years)	Type of tumor	WHO Grade	Ki-67	k_3'	VD'	K-complex
1	Male	4	DNET	I	1%	.017	.45	.008
2	Female	6	DNET	I	1%	.024	.49	.012
3	Female	10	DNET	I	8%	.021	.38	.008
4	Male	12	DNET	I	1%	.026	.42	.011
5	Male	25	Mixed (oligo)*	II	6%	.023	.42	.010
6	Female	41	Oligodendroglioma	II	8%	.020	.41	.008
7	Female	41	Mixed	II	6%	.025	.34	.009
8	Male	12	Ependymoma	III	3%	.016	.61	.010
9	Male	31	Astrocytoma	III	3%	.018	.44	.008
10	Female	34	Mixed (oligo)*	III	15%	.019	.98	.019
11	Male	40	Mixed	III	20%	.019	.97	.018
12	Male	45	Oligodendroglioma	III	9%	.017	.55	.009
13	Male	35	GBM	IV	23%	.011	.71	.008
14	Male	67	GBM	IV	28%	.016	.52	.008
15	Male	67	GBM	IV	10%	.008	.55	.004

DNET: Dysembryoplastic neuroepithelial tumors

GBM: Glioblastoma multiforme

* predominance of oligodendroglioma component

Table 2

Expression of indoleamine 2,3-dioxygenase (IDO) immunoreactivity in different tumor tissue elements.

No	Tumor cells	Neuropil	Perivascular	Endothelial
1	+	+	+	—
2	+	—	—	—
3	+	+	+	—
4	+	+	+	—
5	+	—	—	—
6	—	—	—	—
7	+	+	+	—
8	+	+	+	—
9	—	—	—	—
10	—	—	—	+
11	—	+	—	+
12	—	—	—	+
13	—	—	—	—
14	—	—	—	—
15	—	—	—	+