

Molecular Drug Imaging:

⁸⁹Zr-bevacizumab PET in Children with Diffuse Intrinsic Pontine Glioma

- ⁸⁹Zr-bevacizumab PET in DIPG –

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ABSTRACT

Predictive tools to guide therapy in children with brain tumors are urgently needed. We introduced molecular imaging to facilitate this. We investigated whether bevacizumab can reach the tumor in children with diffuse intrinsic pontine glioma (DIPG) by measuring the tumor uptake of zirconium-89(⁸⁹Zr)-labeled bevacizumab by PET. In addition we evaluated the safety of the procedure in children and determined the optimal timing of imaging.

Methods

Patients received 0.1 mg/kg (0.9 MBq/kg) ⁸⁹Zr-bevacizumab, ≥ two weeks after completing radiotherapy. Whole body PET-CT scans were performed 1, 72 and 144 hours post-injection (p.i.). All patients underwent contrast (gadolinium)-enhanced MRI. Biodistribution of ⁸⁹Zr-bevacizumab was quantified as Standardized Uptake Values (SUVs).

Results

We included seven DIPG patients (4 males, age range 6-17 years) who were scanned without anesthesia. No adverse events occurred. Five out of seven primary tumors showed focal ⁸⁹Zr-bevacizumab uptake (SUVs range 1.0-6.7 at 144 hours p.i.), while there was no significant uptake in healthy brain. One patient had multiple metastases, which were all PET positive. There was inter- and intra-tumoral heterogeneity of uptake and ⁸⁹Zr-bevacizumab was predominantly (in 4 out of 5 patients) present within the MRI contrast-enhancing areas, although ⁸⁹Zr-bevacizumab uptake was variable among MRI contrast-enhancing areas. Tumor targeting was quantitatively similar at 72 and 144 hours post-injection, but tumor-to-blood SUV ratios increased over post-injection time (p=0.045). The mean effective dose per patient was 0.9±0.3 mSv/MBq.

Conclusion

⁸⁹Zr-bevacizumab PET-studies are feasible in children with DIPG. The data suggest considerable heterogeneity in drug delivery between patients and within DIPG tumors and a positive, but not 1-to-1, correlation between MRI contrast-enhancement and ⁸⁹Zr-bevacizumab uptake. The optimal moment of scanning is 144 hours p.i.. Tumor ⁸⁹Zr-bevacizumab accumulation as assessed by PET scanning might help to select patients with highest chance of benefit from bevacizumab treatment.

Keywords: Brainstem neoplasm, Positron Emission Tomography, ⁸⁹Zr-bevacizumab, pharmacokinetics.

INTRODUCTION

The need for predictors to guide therapy in the individual patient applies to the entire field of pediatric oncology but in particular to malignant brain tumors. One of the most challenging brain tumors in children is DIPG, a lethal childhood malignancy of the brainstem comprising 10% of all pediatric central nervous system tumors (1). DIPG tumors are resistant to all kinds of systemic therapies, including targeted agents, and hardly any patient survives beyond two years from diagnosis (2,3). One hypothesis of therapy failure is that drugs actually do not reach the tumor, as in most DIPGs at diagnosis MRI shows little or no gadolinium-contrast enhancement, which suggests an intact blood-brain barrier for large molecules (4). Molecular PET imaging could help to investigate this hypothesis, but despite a recent boost of molecular drug imaging in adults, thus far no immuno-PET imaging studies have been performed in children (5).

Recent mRNA profiling studies revealed overexpression of the pro-angiogenic vascular endothelial growth factor (VEGF-A) in DIPG compared to normal brain and compared to adult high grade glioma, which makes VEGF-A a potential drug target (6,7). The biologic activity of VEGF can be neutralized by bevacizumab, a recombinant humanized monoclonal antibody. Several trials are ongoing in which the potential of bevacizumab is being studied in DIPG (NCT01182350; clinicaltrials.gov, NTR2391; Trialregister.nl) and few trials have been published (8-10). The overall survival outcome in these DIPG trials was as poor as in historical controls (8,9); however, individual patients with significant responses and prolonged survival after bevacizumab treatment have been reported (10,11). Hence, the challenge is to identify those patients who will benefit from bevacizumab treatment. Zirconium-89 (^{89}Zr) labeled bevacizumab PET imaging might help assessing inter- and inpatient heterogeneity of drug biodistribution *in vivo*, hereby predicting (absence of) response in patients subsequently treated with bevacizumab. Studies in both mice and adult patients confirmed that ^{89}Zr -bevacizumab PET

imaging is feasible and able to show bevacizumab accumulation in VEGF-positive tumors (7, 12-14). In addition, in adult renal cancer tumors, ^{89}Zr -bevacizumab uptake was correlated to response to bevacizumab treatment (13). Results from our ^{89}Zr -bevacizumab preclinical research in mice suggest poor bevacizumab uptake in intracranial tumors; in three different pontine-, striatal- and subcutaneous glioma mouse models we found no significant uptake of ^{89}Zr -bevacizumab in the intracranial tumors at any stage of the disease, nor in the normal non-neoplastic surrounding brain in any of the tumor models used (7). In contrast, high accumulation of ^{89}Zr -bevacizumab was observed in the subcutaneous glioma xenograft. Poor bevacizumab distribution in the brain therefore seemed to play a major issue, however we also observed a significantly lower level of VEGF expression in the intracranial tumors (and in one intracranial tumor even absence) compared to the subcutaneous glioma xenograft.

As a first step to investigate the clinical utility of immuno-PET in this setting, we performed a pilot study to investigate whether bevacizumab can reach the tumor in children with DIPG by measuring the tumor uptake of ^{89}Zr -labeled bevacizumab. In addition, we assessed the optimal moment of PET imaging, biodistribution and radiation dosimetry of ^{89}Zr -bevacizumab, and ascertained safety and tolerability of the scanning procedures without anesthesia.

MATERIAL AND METHODS

Study population

The study was conducted at VU University Medical Center, Amsterdam. DIPG patients, aged between 4 and 18 years, were eligible. All patients were included at least 2 weeks after completing radiotherapy, the standard DIPG therapy. Patients with known hypersensitivity against humanized monoclonal antibodies were excluded, as well as those previously treated with bevacizumab or any other anti-VEGF agent. To reduce patient burden, PET-related blood

sampling and the use of anesthetics were not allowed. The latter determined the minimum age of 4 years, as children under 4 were not expected to complete a PET scan without movement artifacts without the use of anesthesia. For all subjects, both parents signed a written informed consent form. In addition, all subjects aged 12 years and older signed a written “informed assent” form. The study (registered as NTR3518 in The Netherlands National Trial Register - NTR) was approved by the Dutch Central Committee on Research involving Human Subjects and the Institutional Review Board of VU University Medical Center, and was performed according to the declaration of Helsinki.

To determine the optimal moment of scanning (72 or 144 hours post-injection), we aimed to include at least five patients with visible tumor uptake of ^{89}Zr -bevacizumab. These post-injection times were based on previous research in mice and clinical studies in adults with ^{89}Zr -bevacizumab (7,12,14).

^{89}Zr -bevacizumab labeling, infusion and PET procedures

The production and purification of ^{89}Zr and its coupling to bevacizumab was performed according to good manufacturing practice at the VU University Medical Center. The labeling process as well as quality controls have been described previously (15-17), and showed similar results as described by Bahce et al. (14). The administered dosage of ^{89}Zr -bevacizumab (0.9 MBq/kg-0.1 mg/kg) is 1% of the therapeutic bevacizumab dose in humans. Patients were imaged using a whole body Philips Gemini TF64 PET-CT scanner (18). Each PET scan was preceded by a low-dose CT using routinely applied settings. Following the CT, a 10-minute static PET-CT scan was performed covering the brain, followed by a whole body PET scan (4 minutes per bed position covering neck to upper legs and 1 min covering the legs). PET scans were performed at 1, 72 and 144 hours post-injection (p.i.). Patients were clinically observed for 3 hours p.i. to check for allergic reactions, and they were informed to contact the hospital in case of later adverse events. All patients underwent T1-weighted pre- and post-gadolinium and T2-

weighted MRI without anesthesia (Siemens Sonata 1.5 Tesla MRI, Erlangen, Germany) within 2 weeks before PET.

Image analysis

Visual analysis of the PET scans was first performed blinded to the MRI results, considering focal uptake exceeding local background as positive. Manually, volumes of interest were generated of tumors and metastases with enhanced tracer uptake, and of blood pool (1.6 mL in the aortic arch), liver, kidneys, spleen, lung and bone (vertebra). Standard Uptake Values (SUVs) were calculated as decay-corrected activity concentration (kBq/mL) per (injected dose (MBq)/body weight (kg)). PET-images of the brain were co-registered with gadolinium (Gd)-enhanced T1-weighted MRI to enable comparison of MR-post contrast-images with PET-images. T2-weighted MRI was used to determine whether ⁸⁹Zr-bevacizumab uptake was present in the whole tumor or focally. Whole body dosimetry was performed using Olinda software (19). This dosimetry model takes into account an age-dependent weight-factor.

Statistics

Statistical analyses were performed using SPSS version 19. For correlations between SUV mean ratios and post-injection time a non-parametric correlation test (Kendall's tau_b) was used.

RESULTS

Population: safety and feasibility

We included 7 patients (baseline characteristics: Table 1) with primary DIPG tumors, one of whom with metastatic disease in the myelum and subependymal space and another with tumor extension in the facial nerve. Median age was 8 years (range 6-17) and all patients were

pretreated with radiotherapy of whom 3 in combination with gemcitabine and one with temozolomide. There were no adverse events observed after injection of ^{89}Zr -bevacizumab, and all patients tolerated the scans and related procedures well. The duration of the whole body PET scans (including brain) ranged from 40 to 50 minutes. All PET and MRI images were of good quality without movement artifacts.

^{89}Zr -bevacizumab uptake in DIPG

PET scans of five patients showed focally enhanced ^{89}Zr -bevacizumab accumulation in their primary DIPG tumors [MRI transversal tumor size range 25-37mm], while two were negative at PET (Figs. 1C and 1E) [MRI transversal tumor size range 36-39mm]. Tumor PET scans were all negative at 1 hour p.i. and became positive as of 72 hours p.i. (Fig. 2A). In none of the patients ^{89}Zr -bevacizumab uptake was present in the entire tumor as depicted at T2-MRI. For example, in Fig. 1B, ^{89}Zr -bevacizumab uptake concentrates on the lower right side of the tumor, while the tumor extends into the whole pons (see T2-weighted image). There was no visually detectable ^{89}Zr -bevacizumab uptake in healthy brain tissue in any patient. Tracer tumor uptake between patients was heterogeneous, with tumor SUVs ranging from 1.0 to 5.3 and 1.0 to 6.7 at 72 and 144 hours p.i., respectively (Fig. 2B). Five DIPG tumors showed focal areas of modest to strong contrast enhancement on Gd-enhanced T1-weighted MRI (Fig. 1). Co-registration of PET-MRI images revealed focal ^{89}Zr -bevacizumab uptake in these contrast-enhancing areas in four out of five DIPG tumors. One tumor with an area of Gd-contrast enhancement (transversal enhancement size 13 mm) was PET negative (Fig. 1C). In one DIPG tumor the area of ^{89}Zr -bevacizumab uptake on PET was not in an area of Gd-contrast enhancement (Fig. 1D). SUVs varied widely between gadolinium positive tumors (Table 1).

In one DIPG patient, 11 metastases were observed which were all PET positive (Fig. 3). The two largest metastases were in the myelum at C1 level and in the subependymal space (volumes 2.0 mL and 1.2 mL, respectively), with a SUV_{mean} of 2.4 and 6.5 at 72 hours

p.i., and 2.5 and 7.2 at 144 hours p.i., respectively. In one patient extension of the tumor in the facial nerve was seen at T2-weighted MRI, without visually detectable PET signal.

Optimal moment of scanning and dosimetry

There was no significant difference between SUV_{mean} of the 5 PET positive tumors at 72 versus 144 hours p.i (Fig. 2B). Since blood pool activity declined over time, tumor/blood pool SUV ratios were positively correlated with the post-injection time interval ($p=0.045$; Fig.2C). Whole body PET evaluation revealed that ⁸⁹Zr-bevacizumab organ uptake was, in contrast to tumors, quite homogeneous among patients; uptake was highest in the liver, followed by the kidney, spleen, lung and vertebra (Fig. 4). Mean effective dose per patient was 0.9 ± 0.3 mSv/MBq (Table 2).

DISCUSSION

There is an urgent need to predict the potential of a drug ahead of therapy in children with brain tumors. In this first immuno-PET imaging study in childhood oncology patients, we have performed ⁸⁹Zr-bevacizumab PET imaging in children suffering from DIPG and showed that the procedure is feasible without the use of anesthetics from the age of six years. We observed inter- and intratumor heterogeneity of ⁸⁹Zr-bevacizumab uptake in five out of seven patients with DIPG, while normal brain showed no uptake at all. Two out of seven primary tumors showed high but focal uptake, while ⁸⁹Zr-bevacizumab was not at all detectable in two DIPG tumors. The optimal moment of scanning was 144 hours p.i., as tumor SUV/blood ratios significantly increased over time. We suggest therefore in future studies one PET-CT scan is performed at 144 hours p.i..

The ^{89}Zr -bevacizumab uptake pattern was focal in all tumors; interestingly, four out of five tumors only showed significant ^{89}Zr -bevacizumab uptake within MRI contrast-enhancing areas of the tumor. Gd-uptake in the brain is associated with BBB degradation, thus when Gd-DTPA, with an average molecular weight of 545 kDa, is able to pass the BBB, other large molecules like bevacizumab (149 kDa), might be able to pass as well, although BBB permeability is of course dependent of more than molecular size only. In addition, contrast-enhancing “leaky” tumors have been associated with higher local VEGF expression (20). We show, however, a high variability in the level of ^{89}Zr -bevacizumab uptake between Gd-enhancing areas (from intense to absent ^{89}Zr -bevacizumab uptake) which suggests large differences in local VEGF expression between DIPG. Moreover one tumor showed ^{89}Zr -bevacizumab uptake in an area without Gd-enhancement. Unfortunately, we were not able to validate the VEGF expression in tissue, as DIPG patients are not routinely biopsied. At least, the clear differences in SUVs between Gd-enhancing tumors (reflecting differences in local drug uptake), and the presence of drug uptake in a Gd-absent tumor area suggest that MRI alone is insufficient to predict tumor accumulation of large molecules like bevacizumab, and that immuno-PET is of additional value.

We realize that an important aspect is the influence of radiotherapy. In our preclinical study in non-irradiated DIPG mouse models, we observed poor uptake of ^{89}Zr -bevacizumab in the intracranial tumors (7). In our study, all patients were included at least 2 weeks after completing radiotherapy, which may have induced temporary disruption of the blood-brain barrier, as well as increased VEGF expression via the mitogen-activated protein kinase (MAPK) pathway (21). We therefore expect DIPG patients to be more eligible for bevacizumab after radiotherapy, and probably this effect on the blood-brain barrier integrity fades out with increasing time after radiotherapy. Therefore, ideally, subsequent scans before, during and after radiotherapy are performed in each patient to find out the susceptibility of the tumor for bevacizumab over time. Future studies are needed to show the correlation of ^{89}Zr -bevacizumab

tumor uptake and treatment response in DIPG. In adults with renal cell carcinoma, it has been shown that high baseline ^{89}Zr -bevacizumab uptake in the tumor prior to treatment is positively associated with time to progression in bevacizumab-treated patients (13). A difficulty in running such a study in DIPG patients is that current DIPG trials involve multi-agent therapy regimens. The finding that bevacizumab can reduce the penetration of other drugs into the tumor (22,23) underlines the need for labeled drug imaging in this multi-agent setting. On the other hand, in contrast with single agent settings, multi-agent therapy may confound the association of the imaging biomarker and outcome so that clinical validation of this immuno-PET method as a predictive biomarker of therapy response requires larger datasets. However, since immuno-PET allows for international application (long physical half-life of ^{89}Zr , relatively simple, centrally or locally performed, radiochemistry), we suggest that immuno-PET, using the data of the present pilot study, is included in future international DIPG clinical trials. Furthermore, molecular imaging data from different trials may be included in the recently established SIOPE DIPG Registry, which enables comparison of results.

Whole body molecular drug imaging can help to predict organ-related toxicity. This is particularly important in children since drugs developed to target over-expressed cancer-specific signal proteins (such as VEGF), also target tissues in which these proteins are expressed during pediatric development. We found that whole body ^{89}Zr -bevacizumab biodistribution showed relatively high organ-uptake in liver, followed by blood, kidneys, lungs, and bone. These results are comparable to the results of the two ^{89}Zr -bevacizumab clinical trials in adults and correspond to the results of toxicity trials on bevacizumab in children reporting hypertension, bleeding, aspartate aminotransferase elevation and proteinuria as the main side effects (13,14,24). We observed moderate bone uptake, however, osteonecrosis is a rarely reported symptom in bevacizumab-treated children (1%). Whether ^{89}Zr -bevacizumab uptake also correlates with long-

term organ and/or bone toxicity needs to be addressed in long-term follow up studies of children treated with bevacizumab.

The mean effective dose was slightly higher than doses of ^{89}Zr -labeled compounds published in adult studies (25), which is likely due to the age-dependent weight-factors of dosimetry models. In our study, the 29 mSv radiation burden of immuno-PET (including 3 low dose total body CT's) for an average DIPG patient (25 kg) is considerable, although in future studies the radiation dose can be reduced to 22mSv as only one PET-low dose CT of the brain will be performed. However, the possible benefits may outweigh the risks, especially in light of the poor prognosis (2-year survival of less than 10%) for patients with DIPG.

CONCLUSION

We introduced immuno-PET imaging in childhood cancer patients. The procedure is safe and feasible in children without using anesthetics and the optimal moment of scanning is 144 hours p.i.. Clear differences in ^{89}Zr -bevacizumab uptake between DIPG tumors were observed, with 2 tumors showing no uptake at all. Interestingly, 4 out of 5 tumors showed significant ^{89}Zr -bevacizumab uptake on PET within the contrast-enhancing area on MRI of the tumor. However, we show high variability in SUVs between these contrast-enhancing areas suggesting differences in local VEGF expression, and we observed one patient with a positive PET in a non-Gd enhancing area. MRI alone seems therefore insufficient to predict drug accumulation in the tumor. The addition of ^{89}Zr -bevacizumab PET imaging may therefore be of help to select potential candidates for bevacizumab treatment in DIPG, as it assesses target availability and drug accessibility of the tumor within the same procedure.

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DISCLOSURE

There is no conflict of interest to disclose. The Semmy Foundation (Stichting Semmy) and the Egbers Foundation (Egbers Stichting) who fund DIPG research in VU University Medical Center had no role in the study and/or preparation of this manuscript.

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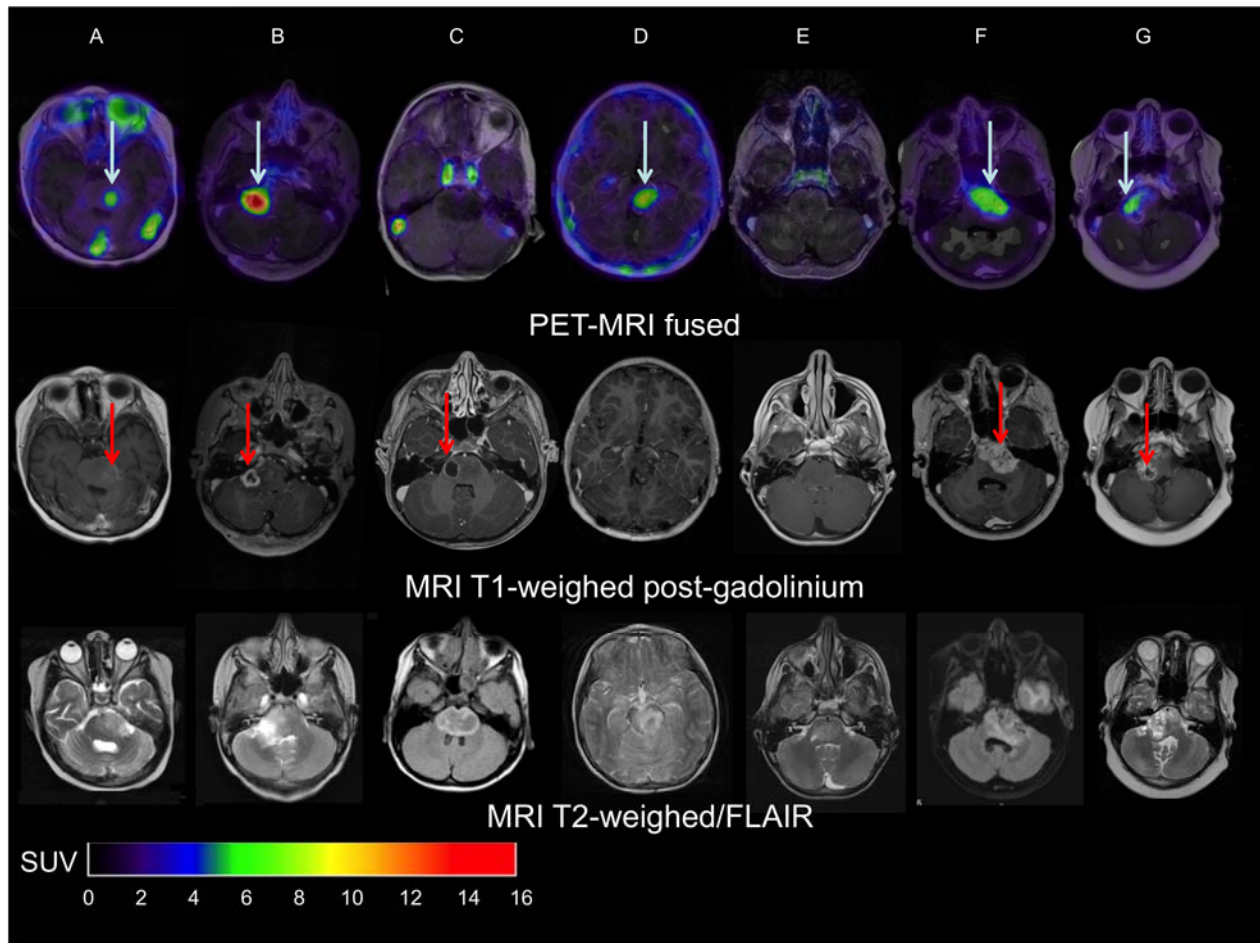


FIGURE 1. MRI AND PET-MRI FUSION IMAGES OF PATIENTS WITH DIPG. Top row: ^{89}Zr -bevacizumab PET (144 hrs p.i.) fused with T1-Gd weighted MRI per patient; middle row: T1-Gd weighted MRI; lower row: T2-weighted/FLAIR MR-images. Five tumors show variable uptake of ^{89}Zr -bevacizumab (white arrows), with both PET negative and positive areas within each tumor. Two primary tumors are completely PET negative (Fig. 1C and 1E), while the T2 weighted images show tumor infiltration in the whole pons of both patients. In the middle row, the red arrows represent the areas of contrast enhancement within the tumor. In four out of five primary tumors the PET-positive area corresponds with the contrast-enhancing area on MRI of the tumors (Fig. 1A, 1B, 1F and 1G). In Fig. 1C, the tumor shows an MRI contrast enhancing area, while there is no ^{89}Zr -bevacizumab uptake. Fig. 1D shows a PET positive tumor, while no Gd-enhancement is observed on MRI.

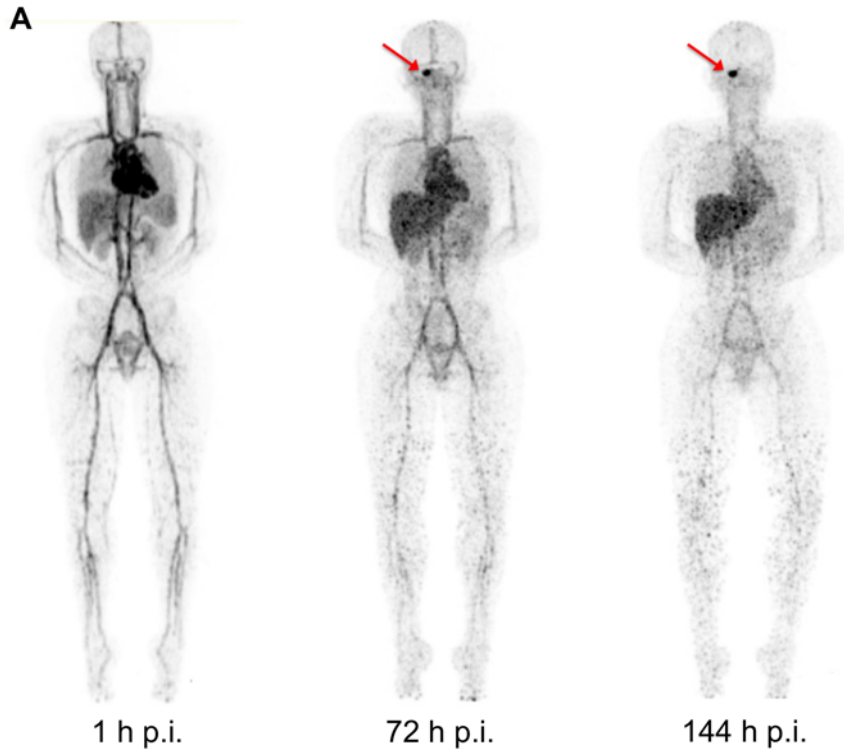
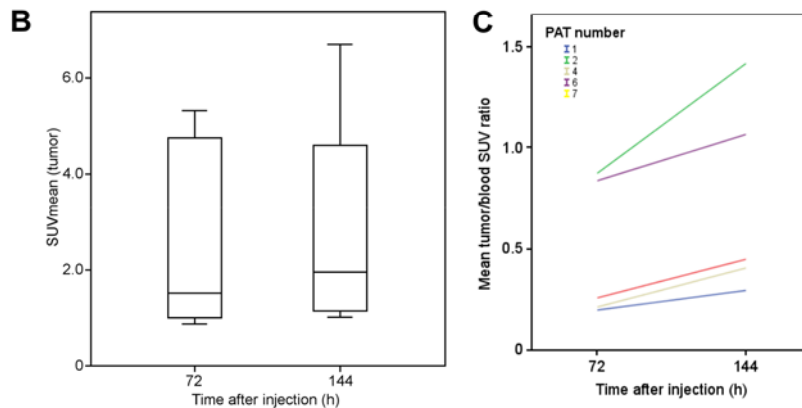


FIGURE 2. ^{89}Zr -BEVACIZUMAB TUMOR UPTAKE IN CORRELATION TO BLOOD POOL. Fig. 2A shows the whole body PET images of one DIPG patient (PAT-002) at 1, 72 and 144 hours post-injection (p.i.). At 1 hour p.i., maximum uptake is observed within the blood pool. With increasing post-injection time, the blood pool activity decreases significantly. There is no uptake of ^{89}Zr -bevacizumab in the tumor at 1 hour post-injection while there is clear



uptake both at 72 and 144 hours p.i. (red arrows). Uptake in the liver is stable and represents vascularisation (1 hour p.i.) and metabolism (72 and 144 hours p.i.) of ^{89}Zr -bevacizumab. Fig. 2B shows the boxplots of the tumor SUVs at 72 and 144 hours post-injection; SUVs were not significantly different ($p=0.6$). Fig. 2C shows SUV ratios of tumor/blood pool (measured in the aortic arch) per patient as a function of post injection time (PI). SUV ratios are positively correlated with increasing post-injection time (Kendall's tau_b correlation coefficient 0,524 $p=0.045$ (2-tailed)).

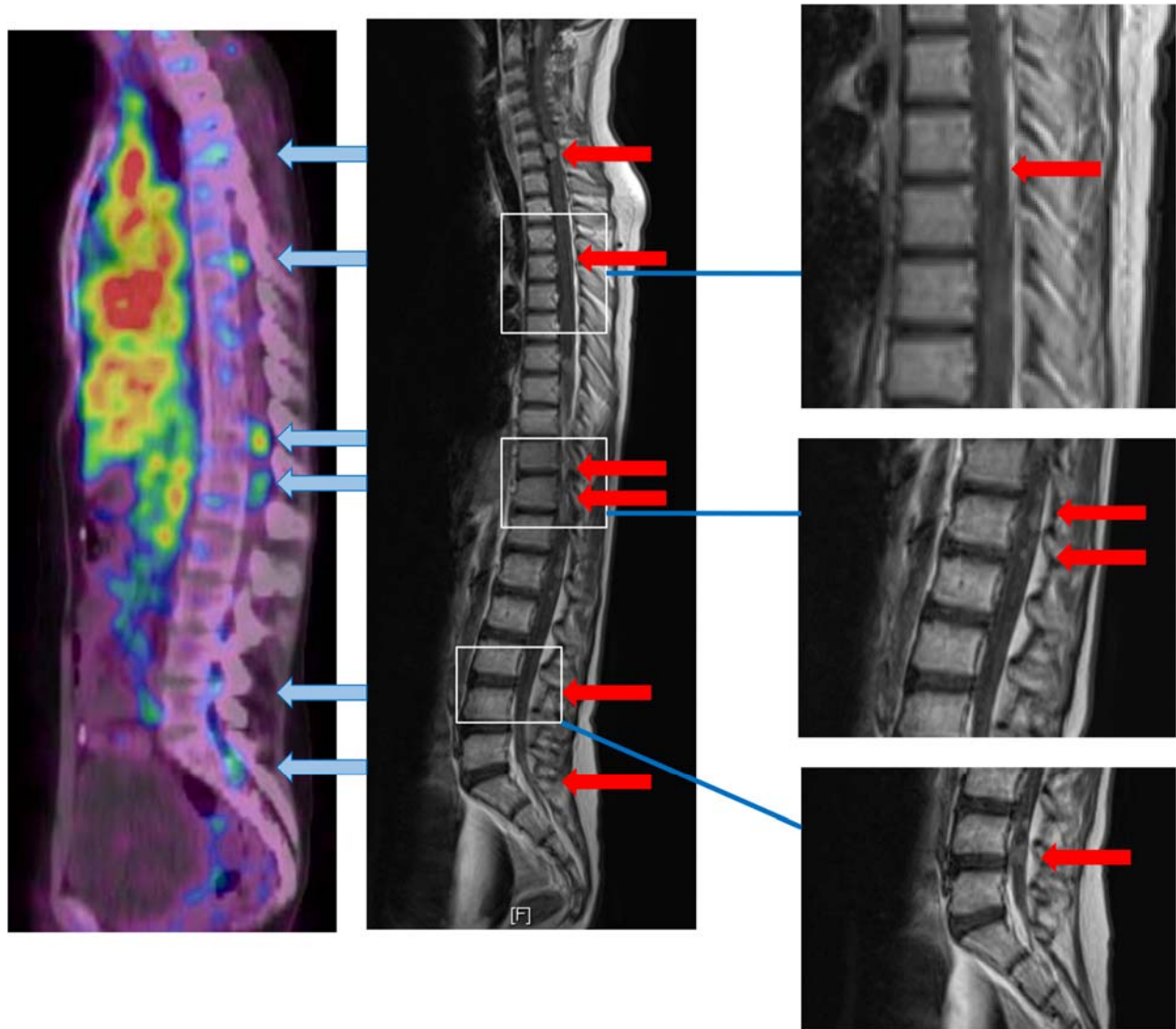


FIGURE 3. MRI AND PET-MRI FUSION IMAGES OF ONE PATIENT WITH PRIMARY DIPG AND METASTASES IN THE MYELUM. The left ^{89}Zr -bevacizumab PET-MRI image is a sagittal plane of the myelum showing six ^{89}Zr -bevacizumab PET hotspots, which were all confirmed metastases by T1-Gd weighted MRI (right image). Some of these metastases were even clearer on PET than MRI. All (11 in total) metastases were PET positive in this patient, whose primary tumor also showed high but focal ^{89}Zr -bevacizumab uptake (Fig. 1F).

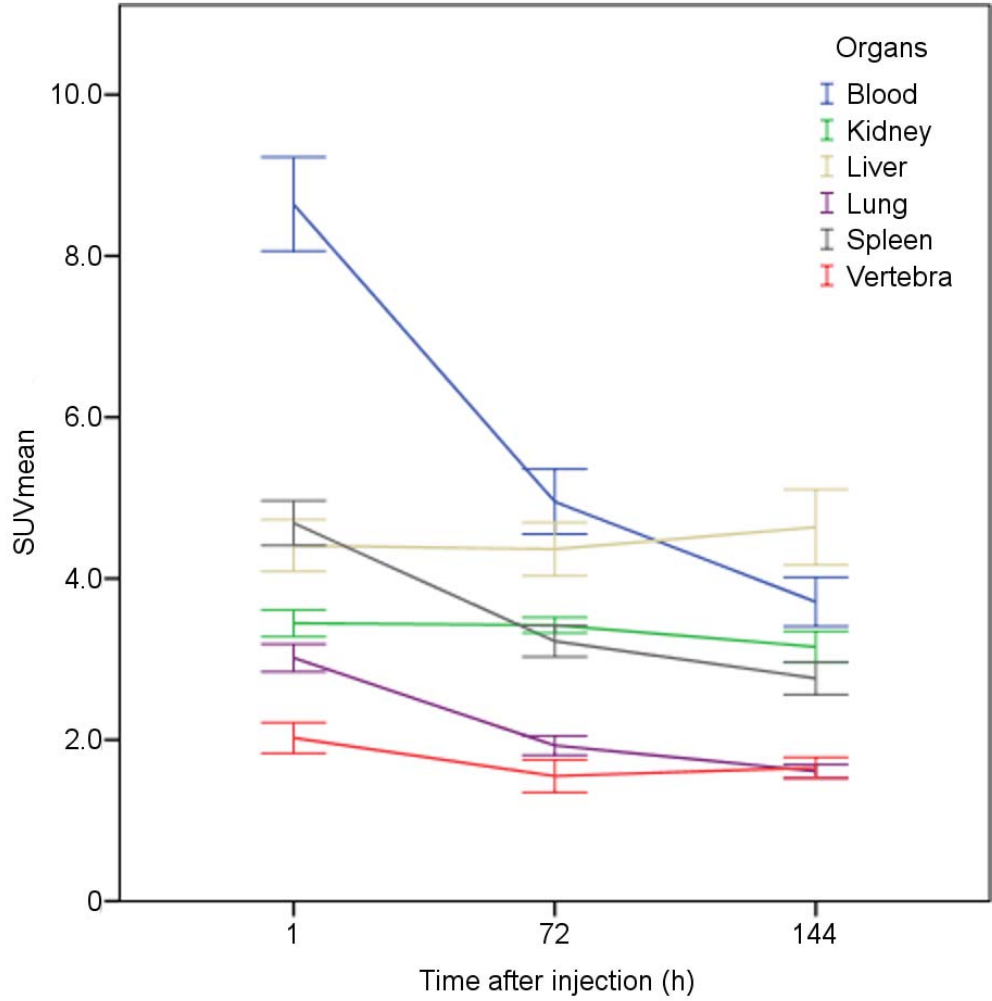


FIGURE 4. STANDARD UPTAKE VALUES OF NORMAL ORGANS. SUVs mean are shown in correlation to post-injection time (error bar = 1SD). ^{89}Zr -bevacizumab organ uptake is, in contrast to tumors, constant between patients.

Tables

Table 1. Baseline characteristics and scanning results

Patient	Sex	Age (years)	Weight (kg)	Metastases/ tumor extension	Treatment before PET study:	⁸⁹ Zr dose (Mbq)	Adverse events	Tumor size transversal diameter (mm)	Gd-Contrast enhancement on MRI	⁸⁹ Zr-Bmab tumor uptake	Tumor SUV 72 hrs p.i.	Tumor SUV 144 hrs p.i.
PAT-001	F	6	33	None	RT	30.7	None	25	Small nodular	Yes	1.0	1.0
PAT-002	F	17	55	None	RT & gemcitabine	37.1	None	37	Ring	Yes	5.3	6.7
PAT-003	M	7	28	None	RT	24.2	None	36	Ring	No	No	No
PAT-004	M	8	30	None	RT	25.6	None	33	Patchy	Yes [†]	0.9	1.2
PAT-005	M	11	37	None	RT & gemcitabine	31.2	None	39	No	No	No	No
PAT-006	F	13	44	LM and arachnoidal	RT & gemcitabine	34.7	None	32	Patchy	Yes	4.7	4.6
PAT-007	M	15	65	Tumor extension nVII	RT & temozolomide	36.7	None	37	Ring	Yes	1.5	1.9
Total	M=57%	11*	37*			31.5#		34.1#			2.7#	3.1#

F=female, M=male, LM= leptomeningeal metastasis, RT=radiotherapy, nVII= facial nerve, ⁸⁹Zr=Zirconium-89, Gd=Gadolinium, Bmab=bevacizumab, SUV=standard uptake value, hrs=hours, p.i. =post-injection , *=median, # mean, [†]PET uptake was in an area without Gd-contrast enhancement

Table 2. Absorbed doses and effective dose for individual subjects

Patient	Absorbed doses (mGy/MBq)						RoB	MIRD- age ¹	Sex	Effective dose (mSv/MBq)
	WB_ROI	Kidneys	Liver	Lung	Spleen	Bone Marrow				
PAT-001	94.3	1.8	12.9	6.5	0.8	2.1	70.3	(5yo)	F	1.37
PAT-002	77.7	1.5	12.6	9.1	1.4	4.5	48.6	(15yo)	F	0.57
PAT-003	97.9	1.2	10.5	7.9	0.6	2.8	74.8	(5yo)	M	1.43
PAT-004	98.2	1.7	11.1	7.5	0.9	2.9	74.1	(10yo)	M	0.93
PAT-005	107.0	1.3	11.0	7.7	0.8	2.9	83.3	(10yo)	M	0.99
PAT-006	115.2	1.9	13.2	8.0	1.1	3.5	87.6	(15yo)	F	0.73
PAT-007	109.3	2.0	8.5	6.2	4.1	2.7	85.7	(15yo)	M	0.73
Total (median)	98.2	1.7	11.1	7.7	0.9	2.9	74.8			0.9

Pat=patient, F=female, M=male, WB=whole body, ROI=region of interest, RoB= remainder of body, MIRD=medical internal radiation dose, F=female, M=male