Bevacizumab Targeting Diffuse Intrinsic Pontine Glioma: Results of $^{89}$Zr-Bevacizumab PET Imaging in Brain Tumor Models

Marc H.A. Jansen$^1$, Tonny Lagerweij$^{2,3}$, A. Charlotte P. Sewing$^{1,2}$, Danielle J. Vugts$^4$, Dannis G. van Vuurden$^{1,2}$, Carla F.M. Molthoff$^4$, Viola Caretti$^{1,2,5}$, Susanna J.E. Veringa$^{1,2}$, Naomi Petersen$^2$, Angel M. Carcaboso$^6$, David P. Noske$^{2,3}$, W. Peter Vandertop$^5$, Pieter Wesseling$^{2,7,8}$, Guus A.M.S. van Dongen$^4$, Gertjan J.L. Kaspers$^1$, and Esther Hulleman$^{1,2}$

Abstract

The role of the VEGF inhibitor bevacizumab in the treatment of diffuse intrinsic pontine glioma (DIPG) is unclear. We aim to study the biodistribution and uptake of zirconium-89 ($^{89}$Zr)-labeled bevacizumab in DIPG mouse models. Human E98-FM, U251-FM glioma cells, and HSID-DIPG-007-FLUC primary DIPG cells were injected into the subcutis, pons, or striatum of nude mice. Tumor growth was monitored by bioluminescence imaging (BLI) and visualized by MRI. Seventy-two to 96 hours after $^{89}$Zr-bevacizumab injections, mice were imaged by positron emission tomography (PET), and biodistribution was analyzed ex vivo. High VEGF expression in human DIPG was confirmed in a publically available mRNA database, but no significant $^{89}$Zr-bevacizumab uptake could be detected in xenografts located in the pons and striatum at an early or late stage of the disease. E98-FM, and to a lesser extent the U251-FM and HSID-DIPG-007 subcutaneous tumors, showed high accumulation of $^{89}$Zr-bevacizumab. VEGF expression could not be demonstrated in the perinecrotic regions of subcutaneous E98-FM tumors. The poor uptake of $^{89}$Zr-bevacizumab in xenografts located in the brain suggests that VEGF targeting with bevacizumab has limited efficacy for diffuse infiltrative parts of glial brain tumors in mice. Translating these results to the clinic would imply that treatment with bevacizumab in patients with DIPG is only justified after targeting of VEGF has been demonstrated by $^{89}$Zr-bevacizumab immuno-PET. We aim to confirm this observation in a clinical PET study with patients with DIPG.

Introduction

Recent advances in molecular and cellular cancer biology have resulted in the identification of critical molecular tumor targets involved in the different phases of tumor growth and spreading.

Note: Supplementary data for this article are available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

M.H.A. Jansen, T. Lagerweij, and A.C.P. Sewing contributed equally to this work.

Corresponding Author: Esther Hulleman, VU University Medical Center-Cancer Center Amsterdam, De Boelelaan 1117, Amsterdam 1081 HV, the Netherlands. Phone: 312-0444-1779; Fax: 312-0444-2124; E-mail: e.hulleman@vumc.nl

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gliomas (HGG) reported high radiological response rates with bevacizumab treatment (7–9), but recently 2 large phase III randomized studies showed no improvement in overall survival with bevacizumab treatment in an upfront setting (10–12). Bevacizumab has been studied in a number of non-randomized trials in pediatric brain tumor patients. Efficacy in these trials has been variable, with a subset of patients showing clear radiological and/or clinical improvement (13, 14). The role of bevacizumab in the treatment of patients with DIPG is even less clear but is currently studied in several trials (refs. 15–18; NCT00890786 and NCT01182350; clinicaltrials.gov; NTR2391 TrialRegister.nl).

No validated methods are available to identify patients who may potentially benefit from bevacizumab treatment. Lack of clinical effect may be due to either poor transport of bevacizumab into the tumor microenvironment due to an intact BBB, or a lack of VEGF expression. In this study, we analyzed VEGF(R) expression in adult and childhood HGG, including DIPG tumors. Furthermore, we studied bevacizumab distribution in vivo using molecular PET imaging with 89Zirconium-labeled bevacizumab in murine DIPG models (19).

Materials and Methods

VEGF-A and VEGFR2 mRNA expression profiles

VEGF-A and VEGFR2 (KDR) mRNA expression in DIPG (n = 27) and pediatric HGG (pHGG; n = 53) were determined in silico, using publicly available datasets, and compared with a dataset of nonmalignant brain tissue (n = 44), low-grade brainstem glioma (n = 6), and adult HGG (n = 284). These datasets include tumor material from biopsy, resection, and autopsy (DIPG). Differences were analyzed by two-way ANOVA and P < 0.01 was considered significant. As a validation of these findings, VEGF-associated gene expression was studied in normal brain, low-grade brainstem glioma (LG-BSG), DIPG, and glioblastoma by creating a heatmap using K-means clustering. All expression analyses were performed using R2, a web-based microarray analysis and visualization platform (http://r2.amc.nl).

Immunohistochemistry and in situ hybridization

Formalin-fixed, paraffin-embedded slides were sectioned from xenograft tumors and brain tissue and subjected to immunohistochemical (IHC) staining. Briefly, after deparaffinization and heat-induced antigen retrieval, sections were incubated with primary mouse anti-Ki67 antibodies (clone MIB-1, DAKO) overnight at 4 °C. Thereafter, slides were washed and incubated with HRP-conjugated EnVision (DAKO) and subsequently stained by DAB with hematoxylin counterstaining.

For in situ hybridization (ISH), tumors were cut in 5-μm slices and incubated with VEGF probes against the human VEGF coding sequence using a previously described protocol (20). Samples were evaluated by microscopy with a Zeiss Axioskop microscope (HBO100W/Z) and equipped with a Canon digital camera and imaging software (Canon PowerShot A640, Canon Utilities, ZoomBrowser Ex. 5.7, Canon Inc.).

Labeling and quality control of 89Zrb bevaczumab

Bevacizumab was labeled with 89Zr using N-succinyl-desferrioxamine (N-suc-Df) as described previously (21). In short, the chelator, desferrioxamine, was succinylated to N-suc-Df. Next, the hydroxamate groups were blocked with iron and the succinyl group was activated as its TFP-ester (Fe-N-suc-Df-TPF ester). Bevacizumab (6 mg/mL) was reacted with 2 equivalents of Fe-N-suc-Df-TPF ester at pH 9 for 30 minutes at room temperature. Hereafter, iron was removed at pH 4.2 to 4.5 with an excess of EDTA for 30 minutes at 35°C, and N-suc-Df-bevacizumab was purified by size exclusion chromatography using a PD-10 column. Radiolabeling of N-suc-Df-bevacizumab was performed in HEPES buffer: to 200 μL 89Zr in 1 mol/L oxalic acid 90 μL 2M Na2CO3 was added. After 3 minutes, 300 μL 0.5 mol/L HEPES, N-suc-Df-bevacizumab, and 700 μL 0.5 mol/L HEPES were added. After 60-minute reaction time, 89Zr-N-suc-Df-bevacizumab was purified by PD10 using 5 mg/mL gentisic acid in 0.9% NaCl (pH 4.9–5.4) as the mobile phase.

Radiochemical purity and antibody integrity were determined using instant thin-layer chromatography (ITLC), high-performance liquid chromatography (HPLC), and SDS-PAGE followed by phosphor imager analysis. For analysis of immunoreactivity, an ELISA-based assay was used. ITLC analysis of 89Zr-bevacizumab was performed on TEC control chromatography strips (Biodex). As the mobile phase, citrate buffer (20 mmol/L, pH 5.0) containing 10% acetonitrile was used. HPLC analyses of bevacizumab modification and radiolabeling were performed using a Jasco HPLC system equipped with a Superdex 200 10/30 GL size exclusion column (GE healthcare Life sciences) using a mixture of 0.05 mol/L sodium phosphate, 0.15 mol/L sodium chloride (pH 6.8), and 0.01 mol/L NaN3 as the eluent at a flow rate of 0.5 mL/min. The radioactivity of the eluate was monitored using an inline NaI(Tl) radiodetector (RaytestSockett).

Cell lines and animal models

Animal experiments were performed in accordance with the European Community Council Directive 2010/63/EU for laboratory animal care and the Dutch Law on animal experimentation. The experimental protocol was validated and approved by the local committee on animal experimentation of the VU University Medical Center. Athymic nude-Foxn1nu mice (6 weeks old) were purchased from Harlan/Envigo and kept under filter top conditions and received food and water ad libitum.

The primary HSID-DIPG-007 cell line was established from DIPG tumor material obtained at Hospital Sant Joan de Deu (Barcelona, Spain) after autopsy from a 6-year-old patient and was confirmed to have a H3F3A (K27M) and ACVR1 (R206H) mutation (22). The E98 cell line was obtained from Radboud University Nijmegen Medical Center (Nijmegen, The Netherlands; ref. 23), the U251 glioma cell line from ATCC. All cell lines were transduced in our laboratory to express firefly luciferase (FLUC) and/or mCherry (24). Cell lines were mycoplasma-negative and were authenticated by STR analysis modified from De Weger and colleagues (25).

E98-FM cells were injected subcutaneously in female athymic nude mice (7–9 weeks of age) to expand the number of cells (19). When the subcutaneous tumor reached a diameter of 1 cm, the tumor was removed, and a single-cell suspension was prepared by mechanical disruption through a 100-μm nylon cell strainer. HSID-DIPG-007-FLUC was cultured in tumor stem medium (TSM; ref. 26), U251-FM in DMEM supplemented with 10% FCS and penicillin/streptomycin. Shortly before stereotactic injection, cells were washed once with PBS and concentrated to 1 × 106 cells per μL. Mice were stereotactically injected with 5 × 103 cells in a final volume of 5 μL into either the pons or striatum.
or injected subcutaneously with $3 \times 10^6$ cells in a final volume of 100 $\mu$L/flank. Coordinates used for intracranial injections were 1.0 mm X, 0.8 mm Y, 4.5 mm Z from the lambda for pontine tumors and 0.5 mm X, 2 mm Y, 2 mm Z from the bregma for the striatum tumors. Coordinates were previously validated and on the basis of "The mouse brain in stereotaxic coordinates" by Franklin and Paxinos (27). Tumor engraftment was monitored by bioluminescence measurement of the Fluc signal. For E98-FM, early-stage tumors in the pons ($n = 8$), striatum ($n = 7$), and subcutaneous xenografts ($n = 3$) were allowed to grow for 18 days and late-stage tumors ($n = 9$, striatum; $n = 3$, subcutaneous) for 35 days after xenograft injection. E98 xenograft tumors in the pons were not available 35 days postinjection because injection of E98FM cells in the pons would result in death of the mice due to tumor growth within 3 weeks. For HSJD-DIPG-007-FLUC and U251-FM, tumors (pons, striatum, subcutaneous) were evaluated, at days 78 and 22, respectively (Fig. 2A).

Two to 3 days before the endpoint of the study, mice were injected intraperitoneally with $89$Zr-labeled bevacizumab ($40 \mu$g, 5 MBq for PET analysis and ex vivo biodistribution or 185 kBq for ex vivo biodistribution only).

Figure 2. A, Schematic overview of the experiment. Cells are injected at day 0 in the pons, striatum, or subcutis (early-stage tumors) or the striatum and subcutis (late-stage E98-FM tumors). Tumor progression was monitored by bioluminescent imaging. The time point for $^{89}$Zr-bevacizumab injection was dependent on the growth speed of used cell lines. This injection was done 72 to 96 hours before PET scanning. MRI, PET, or PET/CT imaging was followed by ex vivo measurement of $^{89}$Zr-bevacizumab accumulation in tissues (biodistribution). B, Charged couple device (CCD) camera images of mice bearing E98-FM tumors. BLI images were obtained at the study endpoint, day 18 (early stage, top) and day 35 (late stage, bottom).

**PET imaging and ex vivo analysis**

The distribution of $^{89}$Zr-bevacizumab was determined 72 hours (ex vivo only) or 96 hours (PET followed by ex vivo analysis) after administration, as a minimum of 72-hour interval between injection and scanning has previously been shown to achieve optimal tumor-to-nontumor ratios (28). Scanning of E98-FM tumors was performed using a LSO/LYSO double-layer ECAT High-Resolution Research Tomograph (HRRT, CTI/Siemens): a small-animal and human brain 3-dimensional (3D) scanner with high spatial resolution (2.3–3.4 mm full width at half maximum) and high sensitivity (29). Mice were anesthetized by isoflurane inhalation anesthesia (1.5 L O$_2$/min and 2.5% isoflurane) before positioning in the HRRT. A static transmission scan (6 minutes) using a rotating 740 MBq $^{137}$Cs point source was performed. Before positioning the mice in the HRRT, a canula was placed intraperitoneally to enable later injection of $^{18}$F(-). Static images of 60-minute acquisition time were obtained. Immediately thereafter, $^{18}$F(-) was injected intraperitoneally (10 MBq/mouse) for colocalization of bone structures (static images of 60-minute acquisition). Body temperature was controlled with a heated platform (kept at 37°C).
Mice with HSJD-DIPG-007-FLUC or U251-FM tumors and where tumor growth was confirmed by increase of BLI signal were imaged using another, preclinical, PET system, (Nanoscan PET-CT, MEDISO), 72 hours after \(^{89}\text{Zr}\)-bevacizumab injection. PET images were analyzed using AMIDE software (Amide's a Medical Image Data Examiner, version 1.0.1; ref. 30).

Ex vivo analysis
Immediately after PET imaging, animals were sacrificed for ex vivo tissue distribution analysis. Blood, urine, tumor, and various tissues were excised, rinsed in PBS to remove residual blood, and weighed. Radioactivity in blood and tissues (in percentage injected dose per gram of tissue: % ID/g) was determined using an LKB 1282 gamma-counter (Compugamma, LKB Wallac). Differences in the amount of radioactivity in healthy brain regions versus subcutaneous-, pontine-, and striatal tumors were analyzed by Kruskal–Wallis test with Dunn multiple comparison posthoc testing. \(P < 0.05\) was considered statistically significant.

Results
VEGF-A-, VEGFR2-, and VEGF-associated gene expression in DIPG
A search in a publically available mRNA expression database curated by the Amsterdam Medical Center (R2.amc.nl) revealed that VEGF-A mRNA is overexpressed in DIPG compared with normal brain and compared with low-grade brainstem glioma (LG-BSG) and non-pontine adult and pediatric HGG (Fig. 1A; \(P < 0.01\), ANOVA). VEGFR2 (KDR) mRNA expression was low in both pediatric and adult glioma, compared with normal brain (Fig. 1B; \(P < 0.01\), ANOVA). A heatmap generated using K-means clustering of expression of VEGF-A–associated genes confirmed an aberrant VEGF-A pathway to be more prominent in DIPG compared with LG-BSG. Most DIPG (red) samples clustered together with pediatric GBM (green) and LG-BSG (blue) clustered with normal brain tissue (purple) (Supplementary Fig. S1).

Biodistribution of \(^{89}\text{Zr}\)-bevacizumab
After tumor growth was confirmed by an increase in BLI signal, \(^{89}\text{Zr}\)-bevacizumab was injected in these animals. Seventy-two to 96 hours after intraperitoneal injection of \(^{89}\text{Zr}\)-bevacizumab, animals were imaged by PET; a schematic overview of the experiment is given in Fig. 2, with representative BLI figures of E98-FM-engrafted mice shown in Fig. 2B. \(^{89}\text{Zr}\)-bevacizumab uptake was not visible in the E98 gliomas located in the pons (only early stage, Fig. 3A) or in the striatum at early or late stages. The rest of the brain showed no uptake of \(^{89}\text{Zr}\)-bevacizumab either, independently of the presence of a tumor. However, subcutaneous E98 tumors showed high accumulation of \(^{89}\text{Zr}\)-bevacizumab indicated by a red hotspot (Fig. 3A). Ex vivo tissue distribution measurements confirmed that no significant \(^{89}\text{Zr}\)-bevacizumab uptake was detected in the brain or brain tumor at any stage of disease, whereas there was high uptake in the subcutaneous tumor (\(P < 0.01\); Fig. 4A). Besides accumulation in the subcutaneous tumor with an average level of 50% ID/g, \(^{89}\text{Zr}\)-bevacizumab was observed in all animals (to a lesser extent) in the blood pool and in well-perfused organs, such as the liver, spleen, and lungs (Fig. 5). Experiments using the 2 other cell lines (U251FM and HSJD-DIPG-007-FLUC) showed comparable results. Using these cell lines, no uptake of \(^{89}\text{Zr}\)-bevacizumab was visualized on PET in any of the xenografts (Fig. 3B). Ex vivo biodistribution analysis showed higher \(^{89}\text{Zr}\)-bevacizumab uptake in subcutaneous HSJD-DIPG-007-FLUC tumors (\(P < 0.05\)) as compared with brain (areas) without tumor (Fig. 4B). The subcutaneous U251FM
Tumors showed no significant increase in $^{89}\text{Zr}$-bevacizumab uptake (Fig. 4C) as compared with non-tumor brain. Of note, both HSJD-DIPG-007-FLUC and U251FM cells formed only very small subcutaneous tumors during the time window in which mice baring intracranial tumors had reached human endpoints (78 vs. 22 days after tumor injection).

ISH of VEGF

To determine whether the differences in $^{89}\text{Zr}$-bevacizumab uptake in the subcutaneous and the intracranial tumors were due to an impaired distribution of $^{89}\text{Zr}$-bevacizumab into the brain or to a differential expression of its target (or both), VEGF expression was analyzed in the different xenografts. ISH confirmed expression of VEGF in the subcutaneous E98FM tumor (Fig. 6A), whereas VEGF expression was absent in E98FM brain tumors (Fig. 6B) and brain tissue without a xenograft. Of note, in the subcutaneous tumors, VEGF was preferentially expressed in perinecrotic areas, whereas necrosis was absent in all of the striatal and pontine tumors. A Ki67 (Mib-1) staining was performed to confirm presence of proliferating tumor tissue (Fig. 6C and D).

Figure 4. $^{89}\text{Zr}$-bevacizumab measured ex vivo by a gamma-counter and normalized to counts found in healthy brain tissue (brain tissue of animals without xenografted brain tumors). A, uptake is significantly higher in the subcutaneous E98-FM tumors ($^{***}, P < 0.01$), but there is no significant difference in uptake in pontine or striatal xenografts at any stage of the disease compared with normal brain. B, uptake in subcutaneous DIPG-Fluc tumors is higher than in normal brain ($^{*}, P < 0.05$). C, no significant differences between U251-FM tumors (subcutaneous or intracranial) versus normal brain.

Figure 5. $^{89}\text{Zr}$-bevacizumab uptake measured ex vivo by radioactivity of the specific organs and the tumor after dissection, expressed as percentage of the injected dose per gram tissue in E98-FM tumor-bearing animals. Note the high uptake in subcutaneous tumors, compared with the negligible uptake in the intracerebral tumors.
In HSJD-DIPG-007-FLUC and U251FM tumors, VEGF mRNA expression was not detectable in striatal and pontine gliomas. The subcutaneous tumors were too small to adequately perform VEGF ISH and therefore no conclusions could be drawn regarding VEGF expression in these subcutaneous tumors.

**MRI**

To visualize disruption of the BBB, mice with HSJD-DIPG-007-FLUC and U251-FM intracranial tumors were imaged by MRI after intravenous administration of gadolinium. In the diffusely growing HSJD-DIPG-007-FLUC tumor, gadolinium enhancement on T1-weighed images was limited (Fig. 7A, arrow), whereas gadolinium enhancement was clearly visible in the U251-FM tumor (Fig. 7C, arrow). On T2-weighed images, tumors were not clearly visible (Fig. 7B and D; arrow).

**Discussion**

The potential benefit of bevacizumab in the treatment of DIPG is unclear, as efficacy depends on expression of VEGF-A as well as appropriate drug distribution (31). We used molecular PET imaging to study the influence of location and stage of disease on biodistribution of $^{89}$Zr-bevacizumab in 3 glioma mouse models (pontine, striatal, subcutaneous) using 3 different cell lines. The E98-FM pontine and striatal and HSJD-DIPG-007 pontine xenograft models have previously been described to resemble the diffuse phenotype of human DIPG and other diffuse HGGs (19, 32, 33). U251 has been described as an intracranial murine tumor model that recapitulates most of the key features of adult glioblastoma (34). We found no significant uptake of $^{89}$Zr-bevacizumab in the intracranial tumor models at any stage of the disease, nor in the normal/nonneoplastic surrounding brain. In contrast, high accumulation of $^{89}$Zr-bevacizumab was observed in the subcutaneous E98-xenograft and moderate uptake in the subcutaneous HSJD-DIPG-007-FLUC.

We initially hypothesized that lack of $^{89}$Zr-bevacizumab uptake could be explained solely by poor distribution into the brain, as large molecules like monoclonal antibodies may not be able to pass the BBB. This hypothesis is supported by the absence of enhancement of the tumor on MRI after administration of gadolinium in animals with HSJD-DIPG-007-FLUC pontine tumors. However, MRI analysis of U251-FM tumors in the brainstem of mice showed clear gadolinium enhancement, which is indicative of “leaky” blood vessels in the tumor. Furthermore, VEGF expression of the E98FM glioma cells—analyzed by ISH—also differed between tumor locations: E98FM gliomas in both striatum and pons appeared VEGF-negative, whereas the subcutaneous E98FM tumors were partly VEGF-positive. The differences in VEGF expression of the tumors in distinct locations originating from the same cell line confirm that the orthotopic microenvironment and the resulting growth pattern significantly influence gene expression in glioma cells, a phenomenon that has been described previously (19, 20). Moreover, it has been shown that in glioblastoma, VEGF is predominantly overexpressed in hypoxic, perinecrotic cells (35, 36). Indeed, in our study, the VEGF expression in subcutaneous E98FM tumors was especially present around areas of necrosis, whereas in intracranial E98FM tumors, necrosis and VEGF expression were lacking and this also coincided with lack of bevacizumab uptake studied ex vivo and by PET. Of note, bevacizumab does not bind to murine VEGF-A (37), but as typically, the neoplastic cells are upregulating VEGF expression in tumor angiogenesis, we consider it unlikely that stromal cell–derived mouse VEGF-A plays an important role in this particular xenograft model (33).

In contrast to our preclinical findings, the in silico analysis that we performed in this study indicates that human DIPG tumors
have relatively high expression levels of VEGF mRNA. However, the majority of tumors (23 of 27) used for the microarray experiments were collected post-mortem (38, 39) and therefore these samples represent the end stage of the disease and are post-radiation therapy. In the end stage of the disease, DIPG is known to have necrotic areas with microvascular proliferations and BBB disruption, compatible with the histology of a glioblastoma, which is associated with high VEGF expression. Although the numbers are low, it is important to point out that VEGF-A expression levels in samples obtained during pretreatment were low compared with expression in post-mortem/end-stage samples (Supplementary Fig. S2). In addition, biopsy samples that were included in the analysis are frequently directed at contrast-enhancing lesions on MRI and ISH studies suggest that this relation is not so straightforward, pressing the need for studying VEGF targeting in patients treated with anti-VEGF therapy. One could however argue whether high local tumor accumulation of bevacizumab is at all needed to obtain potential therapeutic effects in DIPG. Bevacizumab is capable of decreasing VEGF levels in blood to undetectable range in less than 20 days in a large cohort of adult cancer patients (47), but only a subgroup of patients responded to anti-VEGF therapy. Also in DIPG, decreased phosphoVEGFR2 levels in peripheral blood mononuclear cells (PBMC) did not correlate with treatment response (15). In the E98 xenograft model used in this study, treatment with bevacizumab did not increase survival, nor did it influence the growth pattern in the diffusely growing parts of the tumor (36). This suggest simply decreasing VEGF in the blood pool is, at least for the tumor types studied and our xenograft model, often insufficient for adequate tumor targeting and instead, local bevacizumab accumulation seems needed (47–49). The results of immuno-PET imaging and VEGF-ISH in these DIPG models are in line with the poor clinical response rates thus far obtained with bevacizumab in children with DIPG (15). The data presented suggest that no adequate uptake of bevacizumab will occur in diffusely growing gliomas, which present with BBB disruption but without drastically increased VEGF expression. Therefore, we suggest that bevacizumab treatment is only justified if targeting of VEGF by bevacizumab has been visualized by immuno-PET scan. We aim to confirm this hypothesis in a clinical PET study with patients with DIPG.

Future directions
This study underlies the importance of using strong biological and biodistributional rationale before using any therapy in any patient. Following the results of this study, we developed a molecular drug imaging trial with 89Zr-bevacizumab in children with DIPG (study number NTR3518 www.trialregister.nl). This technique aims to further unravel the role of bevacizumab treatment in DIPG. Ideally, such molecular imaging is combined with VEGF-A and VEGFR2 expression analysis on tumor tissue originating from biopsies taken from several (contrast-enhancing and nonenhancing) parts of the tumor. Because DIPG can generally be diagnosed on the basis of its typical radiological presentation and the delicate nature of the brain involved, taking biopsies from DIPGs is still no common practice and sampling of multiple regions is even more cumbersome. In general, integrating molecular imaging with radiolabeled drugs (classic cytotoxic agents, small molecules, other monoclonal antibodies) in the treatment of childhood brain cancer provides an insight in drug targeting and might help personalize treatment and thereby to avoid unnecessary side effects of drugs that do not reach the tumor.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: M.H.A. Jansen, T. Lagerweij, A.C.P. Sewing, D.G. van Vuurden, G.A.M.S. van Dongen, G.L. Kaspers, E. Hulleman
Development of methodology: M.H.A. Jansen, T. Lagerweij, A.C.P. Sewing, V. Caretti, G.A.M.S. van Dongen

Figure 7. MRI scans of pontine tumors. The 89Zr-bevacizumab uptake in the tumors of these 2 mice is presented as %ID/g. A, limited gadolinium contrast enhancement in the tumor area (arrow) on T1 scan. B, HSJD-DIPG-007-FLUC tumor is poorly visible on T2-weighed MRI image (arrow). C, clear gadolinium contrast enhancement in the U251-FM tumor area (T1-weighed MRI scan) indicates disruption of the BBB. D, U251-FM tumor is visible on T2-weighed MRI scan.
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.); M.H.A. Jansen, T. Lagerweij, A.C.P. Sewing, D.J. Vogts, C.F.M. Molthoff, V. Caretti, N. Petersen, A.M. Carcaboso, D.P. Noske, P. Wesseling

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis); M.H.A. Jansen, T. Lagerweij, A.C.P. Sewing, D.J. Vogts, C.F.M. Molthoff, S.J.E. Veringa, P. Wesseling, G.A.M.S. van Dongen

Writing, review, and/or revision of the manuscript; T. Lagerweij, A.C.P. Sewing, D.J. Vogts, D.G. van Vuured, C.F.M. Molthoff, A.M. Carcaboso, D.P. Noske, W.P. Vandertop, P. Wesseling, G.A.M.S. van Dongen, G.J.L. Kaspers, E. Hulleman

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases); M.H.A. Jansen, T. Lagerweij

Study supervision; G.J.L. Kaspers, E. Hulleman

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