



Convection-enhanced delivery of cetuximab conjugated iron-oxide nanoparticles for treatment of spontaneous canine intracranial gliomas

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Abstract

Cetuximab conjugated iron-oxide nanoparticles (cetuximab-IONPs) have shown both in-vitro and in-vivo anti-tumor efficacy against gliomas. The purpose of this pilot study was to evaluate the safety and potential efficacy of cetuximab-IONPs for treatment of spontaneously occurring intracranial gliomas in canines after convection-enhanced delivery (CED). The use of CED allowed for direct infusion of the cetuximab-IONPs both intratumorally and peritumorally avoiding the blood brain barrier (BBB) and limiting systemic effects. A total of eight dogs participated in the study and only two developed mild post-operative complications, which resolved with medical therapy. All canines underwent a single CED treatment of the cetuximab-IONPs over 3 days and did not receive any further adjuvant treatments. Volumetric analysis showed a median reduction in tumor size of 54.9% by MRI at 1-month (4–6 weeks) follow-up. Five dogs were euthanized due to recurrence of neurological signs other than seizures, two due to recurrent seizures, and one dog died in his sleep. Median survival time after surgery was 248 days (mean 367 days).

Keywords Iron-oxide nanoparticles · Convection-enhanced delivery · Magnetic nanoparticles · Canine gliomas · Epidermal growth factor receptor (EGFR) · Cetuximab

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This study was performed at the University of Georgia, College of Veterinary Medicine in collaboration with Emory University.

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Introduction

Gliomas are the second most common type of brain tumor in canines, reported to account for up to 36% of central nervous system (CNS) tumors [1, 2]. These tumors have

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a similar prevalence and poor prognosis in humans as in canines [3, 4]. Although accurate data regarding prognosis following treatment in canines with gliomas is limited, median survival rates are reported to be < 1 year even when considered low-grade [5]. Canine spontaneous glioma tumors share many similarities including clinical, pathologic, molecular, and genetic properties with human gliomas, making dogs an attractive naturally-occurring, large animal model for validation of novel therapies [6–9].

Recently, there have been advances in understanding the molecular biology of gliomas, which has led to targeted therapy of these tumors [10, 11]. Identification of molecular signaling pathways implicated in gliomagenesis in canines can lead to development and translation of novel targeted therapies for canine gliomas [12, 13]. Potential targets that are over-expressed in spontaneous canine gliomas include proteins such as the epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor alpha (PDGFR α), insulin-like growth factor binding protein-2 (IGFBP-2), interleukin 13 receptor alpha 2 (IL-13RA2), and activating transcription factor 5 (ATF5) [14–16]. EGFR is an attractive molecular therapeutic target and a known oncogenic factor in both human and canine gliomas [9, 17]. EGFR is amplified in 40–50% of glioblastomas (GBMs) in humans and has been associated with a poor prognosis [18–21]. Additionally, EGFR was shown to be the most common glioma mutation with The Cancer Genome Atlas (TCGA) [22, 23]. In a canine glioma study, EGFR expression was found to be increased in all canine tumor types and grades, being more consistently increased than other cell surface receptors [17]. Small molecule tyrosine kinase inhibitors (TKIs) and monoclonal antibodies have been developed to target EGFR [24, 25]. Cetuximab (Erbix; ImClone LLC), a human-mouse chimeric monoclonal antibody, binds specifically to EGFR preventing dimerization and activation of the tyrosine kinase and thereby inhibiting downstream signal transduction [24, 25]. Canine and human tumor-associated antigens, ErbB-1 and -2, share over 90% amino acid homology [26]. Cetuximab is an anti-EGFR (anti-ErbB-1) antibody and was found to bind to canine mammary carcinoma cells with EGFR expression resulting in inhibition of canine tumor cell proliferation [26]. Cetuximab has been shown to reduce cell proliferation, inhibit tumor angiogenesis, and enhance radiosensitivity by promoting radiation-induced apoptosis with inhibition of radiation-induced damage repair [27]. It has shown efficacy as a single agent in advanced non-small cell lung cancer (NSCLC), ovarian cancer, head and neck cancer, and colorectal cancer [27]. Systemic administration of cetuximab has been evaluated in Phase II clinical trials in human patients with recurrent GBM and demonstrated minimal toxicity but limited efficacy and survival benefit [18, 28, 29].

Magnetic iron oxide nanoparticles (IONPs) have recently been shown to be effective for glioma targeting and can be modified by the addition of a peptide or antibody specific for tumor cells on their surface [30]. Due to their ferromagnetic properties, IONPs also provide magnetic resonance imaging (MRI) contrast enhancement and serve as a useful tool for imaging brain tumors [31]. Theranostic IONPs conjugated to an EGFR antibody have been shown to provide selective MRI contrast enhancement of human GBM tumor cells and significant antitumor efficacy with orthotopic human GBM xenografts [30]. Treatment with cetuximab conjugated IONPs (cetuximab-IONPs) resulted in a significant anti-tumor effect that was greater than with cetuximab alone due to more efficient cellular targeting and uptake, EGFR signaling alterations, EGFR internalization, and apoptosis induction in EGFR-expressing glioma stem-like cells (GSCs), as well as tumor non-stem cells [32].

Overcoming the blood brain barrier (BBB) and maximizing therapeutic efficacy directly impacts the success of a targeted therapeutic for GBM [10]. The large size of cetuximab precludes its ability to cross an intact BBB [28]. Convection-enhanced delivery (CED) of agents into the brain is designed to overcome this barrier and limit the systemic effects of pharmacologic agents by direct pressure dependent infusion into the brain [7, 33–35]. Although CED has been used in dogs with intracranial gliomas, it has only been evaluated with the animals under continuous general anesthesia using real-time MRI [36]. Our group has successfully performed intracranial CED of cetuximab-IONPs in a healthy canine model previously [37]. Robust delivery of nanoparticles into the brain was achieved by CED and effective quantitative monitoring of distribution and dispersion of nanoparticles after CED was provided by MRI. No safety, intracranial or systemic toxicity issues were found after CED of cetuximab-IONPs [37]. The aim of this pilot study was to evaluate the safety and potential efficacy of cetuximab-IONPs CED for treatment of spontaneously occurring gliomas in canines.

Materials and methods

Patient recruitment

Dogs with a pre-operative MRI documenting a suspected glioma amenable to surgery were recruited for participation in the study conducted at the University of Georgia, Veterinary Teaching Hospital (UGA VTH). Inclusion criteria were ultimately based on the following MRI characteristics consistent with spontaneously-occurring intracranial gliomas [38]: intra-axial mass, iso- to hyperintense on T2-weighted imaging, iso- to hypo-intense on pre-contrast T1-weighted imaging, with variable peri-lesional edema and contrast enhancement. All procedures were approved

by the UGA-VTH clinical research committee conforming to Institutional Animal Care and Use Committee (IACUC) regulations.

MRI and anesthesia

All dogs had a complete blood count, biochemical analysis, and urinalysis prior to anesthesia for imaging. The brain MRI scans were performed at UGA either with a 3.0 T MRI unit^{1, 2} with a single-channel quadrature knee coil or a 1.5T³ unit with a 15-channel transmit-receive quadrature knee coil. MRI scans performed at other centers were performed using the units available at the practice. Dogs received butorphanol⁴ (0.2 mg/kg IV) and diazepam⁵ (0.2 mg/kg IV) as a premedication; anticholinergic drugs (atropine⁶ or glycopyrrolate⁷) were administered based on heart rate and blood pressure. Anesthesia was induced with propofol⁸ (4 mg/kg IV or to effect) and was maintained with isoflurane⁹ or propofol CRI.

Dogs were positioned in sternal recumbency. MR images of the brain were obtained in the sagittal, transverse (slice thickness 3 mm), and dorsal planes using the following pulse sequences: T1-weighted fluid attenuated inversion recovery (FLAIR), T2-weighted, and T2W FLAIR, Gradient Echo, and additional pulse sequences based on clinician preference. Following the intravenous administration of 0.1 mmol/kg of gadopentetate dimeglumine,¹⁰ further T1W FLAIR sequences were obtained in sagittal, dorsal, and transverse planes and fast spoiled gradient echo (FSPGR) T1W images.

Surgery and CED catheter placement

A peripheral intravenous (IV) catheter was placed in all dogs prior to surgical procedures. Dogs were premedicated with IV fentanyl (5 µg/kg) and diazepam (0.25 mg/kg) separately, and anesthesia was then induced with IV propofol (4 mg/kg titrated to effect). Once intubated, dogs were maintained on oxygen, and a low percentage of isoflurane to minimize increases in intracranial pressure. During the surgical

procedure, a continuous rate of infusion (CRI) of fentanyl (5–10 µg/kg/h) was administered, in addition to a propofol CRI (6–20 mg/kg/h titrated to effect). All dogs were mechanically ventilated to maintain a low normal end tidal carbon dioxide (CO₂). Homeostasis was maintained using an IV administration of lactated Ringer's solution (5 ml/kg/h). Administration of IV buprenorphine (0.02 mg/kg) provided post-operative analgesia.

A craniotomy was performed in all cases for either open biopsy or partial tumor resection, followed by CED of the cetuximab-IONPs. The tumor extent of resection, ranged from 0 to 67.2%, with a mean extent of tumor resection of 12.5%. Intraoperative samples of the tumor were submitted for frozen histopathologic confirmation of glioma in 3/8 patients. In the remaining patients, histopathologic diagnosis was confirmed post-operatively. After partial tumor resection or open biopsy, two FDA-approved catheters (Medtronic¹¹ intrathecal catheters Indura 8709SC and Ascenda 8780) for intrathecal use, were implanted within the residual tumor. Intrathecal catheters were used for cetuximab-IONP CED based upon our prior experience with healthy canines [37]. An attempt was made to separate the catheter tips by at least 1 cm if possible. The two catheters were then tunneled through the subcutaneous tissue under the skin and connected each to an external programmable reservoir infusion pump [Medtronic¹² SynchroMed II Programmable Pump 8637] (Fig. 1). The pumps were secured on the head or body of each patient with sutures and bandaging material, after which the dogs were recovered from anesthesia. (Fig. 1).

Cetuximab-IONPs infusion

Each pump was pre-filled with cetuximab-IONPs, which were prepared in the Brain Tumor Nanotechnology Laboratory at Emory University using a technique previously described [32]. Infusions were performed after programming each external reservoir pump by utilizing a handheld device (Medtronic¹³ N'Vision Clinician Programmer 8840). Each canine patient underwent a 72 h infusion of cetuximab-IONPs at a concentration of 0.5 mg/ml and at a CED rate of 0.5 µl/min based upon our prior CED experience with healthy canines [37]. A total of 4.32 ml (2.16 ml per pump/catheter) of infusate (2.16 mg of cetuximab-IONPs) was administered by CED to each canine patient. The CED infusions were performed while the dogs were awake in the hospital. After the completion of the infusion, the catheters were removed without the need for sedation or anesthesia.

¹ GE 3.0T Signa HDx; GE Healthcare, Milwaukee, WI.

² 3.0T Siemens Skyra.

³ Siemens Symphony, TIM technology.

⁴ Butorphanol tartrate; Torbugesic®, Fort Dodge, Fort Dodge, IA.

⁵ Diazepam, Valium®; Hospira, Lakeforest, IL.

⁶ Atropine sulfate; Med Pharmex, Pomona, CA.

⁷ Glycopyrrolate; Baxter Healthcare Corp., Deerfield, IL.

⁸ Propofol; Abbott Laboratories, North Chicago, IL.

⁹ Isoflurane, MDI, Boise, ID.

¹⁰ Gadopentetate dimeglumine, Magnevist®; Bayer HealthCare Pharmaceuticals, Wayne, NJ.

¹¹ Medtronic, Inc., Minneapolis, MN.

¹² See footnote 11.

¹³ See footnote 11.



Fig. 1 CED setup. **a** Post-operative image of a canine patient's head showing the exit of two catheters used for CED. **b** Post-operative image of a patient demonstrating the attachment of two CED pumps

to the patient's body. **c, d** Two awake canine patients wearing their pumps during CED treatment

Follow-up

Follow-up MRI scans were performed in canine patients at the following time points: 24 h or immediately post-operatively (8/8), 3/8 at 7 days, 8/8 at 4–6 weeks (1 month visit), 5/8 at 3 months, 2/8 at 6 months, and at 1 year, 18 and 24 months in one dog. Postoperative SPACE (Sampling Perfection with Application optimized Contrasts using different flip angle Evolution) MRI sequences were obtained in addition to True FISP (Fast Imaging with Steady Precession) to further define catheter placement and IONP visualization. True FISP suppresses the signal from hemorrhage while maintaining the susceptibility associated with the IONPs. These produce distinct magnetic dipole areas of signal loss for direct imaging of the IONPs (Fig. 2).

Following euthanasia, brains from two dogs were fixed in 10% buffered formalin and examined microscopically for residual tumor.

Volumetric evaluation

Osirix¹⁴ imaging software was used to evaluate pre-operative and post-operative MRIs of all patients. Osirix¹⁵ was also used to determine volumetric data. Tumors were measured in the immediate post-operative scan and the 1 month post-operative scan using the following technique: on each T2-weighted FLAIR transverse image of a complete series, the tumor mass was manually outlined for each patient in Osirix¹⁶, and the program used this data to calculate the volume of the lesion in cm³.

Tumor identification, grading, and EGFR status

Formalin fixed biopsy and tumor tissue was processed routinely and embedded in paraffin. Four micron sections

¹⁴ OsiriX 3.6, Pixmeo, Bernex, Switzerland.

¹⁵ See footnote 14

¹⁶ See footnote 14

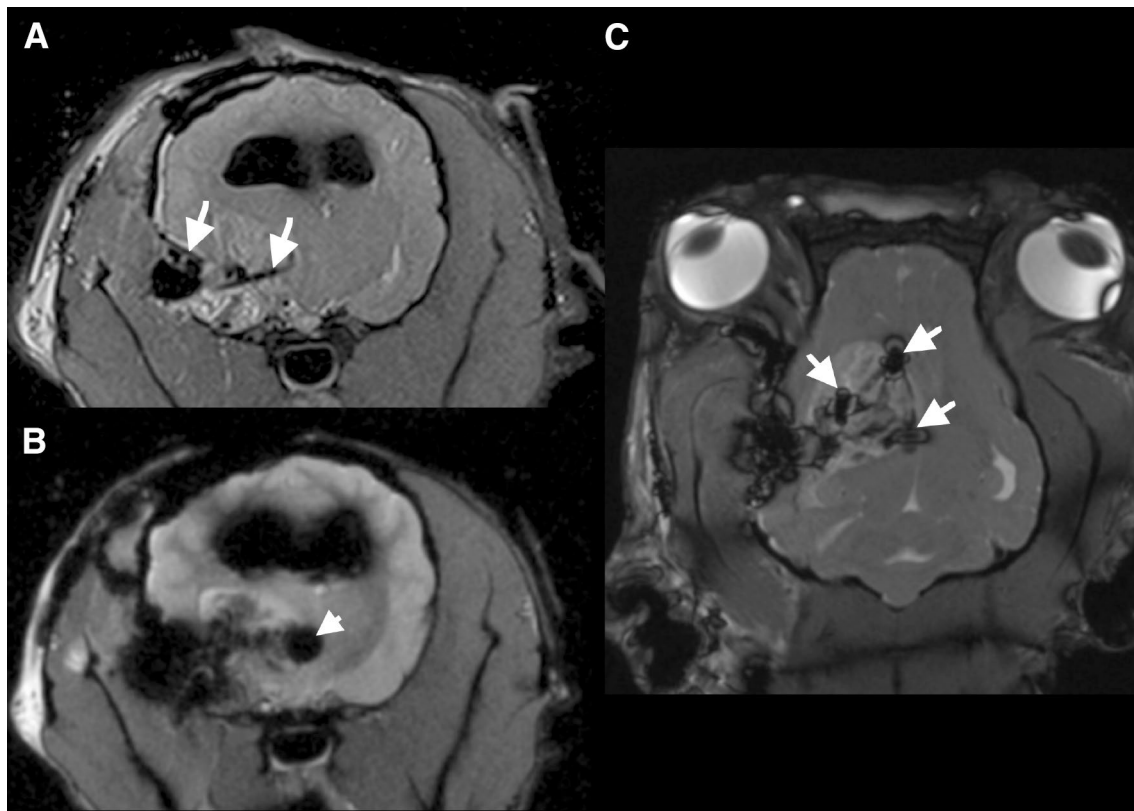


Fig. 2 Postoperative MRI of a canine glioma patient following surgical placement of two catheters and cetuximab-IONP CED. **a** Transverse 0.5 mm thick proton-density SPACE (Sampling Perfection with Application optimized Contrasts using different flip angle Evolution) MRI sequence reveals two linear hypointense catheters (white arrows) that are intra-tumoral. **b** Transverse 2.5 mm thick T2-weighted and **c** dorsal/coronal 1.2 mm thick True FISP (Fast Imaging with Steady Precession) MRI. It is more difficult to evaluate the catheter and IONPs on standard T2-weighted MRI (**b**) due to

the blooming artifact of the IONPs and post-surgical hemorrhagic products. However, IONPs can be visualized by T2-weighted imaging at the tip of the more medially extending catheter (**b**, arrow), as well as intratumorally by the other catheter. True FISP suppresses the signal from hemorrhage while maintaining the susceptibility associated with the IONPs. These produce distinct magnetic dipole areas of signal loss and permit direct visualization of the IONPs after CED (**c**, arrows)

were stained with hematoxylin and eosin (H&E), oligodendrocyte transcription factor 2 (Olig2), and glial fibrillary acidic protein (GFAP) by immunohistochemistry (IHC) to confirm tumor type. Grading of tumors was performed by a pathologist and followed the World Health Organization (WHO) classification of human brain tumors [39]. When adequate tissue was available, the tumors were also stained for EGFR by IHC and staining was subjectively scored 0–3 (0 = none; 1 = mild; 2 = moderate; 3 = marked) as compared to a positive control (canine squamous cell carcinoma that had marked immunoreactivity).

For immunohistochemistry, sections were stained with rabbit anti-Olig2¹⁷ (citrate buffer and heat retrieval, 1:400), mouse anti-GFAP¹⁸ (citrate buffer and heat retrieval,

1:4000), and mouse anti-EGFR¹⁹ (31G7 + GFR1195; citrate buffer and heat retrieval, 1:8000) followed by biotinylated secondary antibodies,²⁰ a streptavidin-HRP conjugated label²¹, and 3,3 diaminobenzidine (DAB)²² as the chromogen. Appropriate positive controls were used and in the negative control, isotype rabbit or mouse control serum²³ replaced the primary antibody.

¹⁷ Rabbit anti-olig2; GeneTex, Irvine, CA.

¹⁸ Mouse anti-GFAP, Biogenex, San Ramon, CA.

¹⁹ Mouse anti-EGFR, Lifespan Biosciences.

²⁰ Biotinylated secondary antibodies, Vector Laboratories, Burlingame, CA.

²¹ A streptavidin-HRP conjugated label, Biocare Medical, LLC, Concord, CA.

²² DAB, DAKO, Carpinteria, CA.

²³ Isotype rabbit or mouse control serum, Biocare Medical, LLC, Concord, CA.

Table 1 Clinical data

Patient #	Breed	Age (months)	Sex	Tumor type	EGFR status	% Tumor reduction at 1 month	Survival time post surgery (days)	Reason for euthanasia/death
1	Pit bull	84	MN	Oligodendroglioma	2	67.7	903	Progressive forebrain signs
2	Boston terrier	108	MN	Oligodendroglioma	N/A	100	732	Progressive forebrain signs
3	French bulldog	54	MN	Oligodendroglioma	N/A	33.4	145	Status epilepticus
4	Boxer	78	FS	Oligodendroglioma	1	15.9	209	Progressive forebrain signs
5	Mixed breed	96	FS	Oligodendroglioma	0	42.6	287	Seizures
6	Boxer	108	MN	Oligodendroglioma	1	60.3	388	Progressive forebrain signs
7	Boston terrier	108	MN	Oligodendroglioma	3	57.5	103	Progressive forebrain signs
8	Boxer	108	MN	Oligodendroglioma	N/A	0	168	Died in his sleep

Table 2 Histopathologic data

Patient	Olig2	GFAP	EGFR	Diagnosis	Grade
1	+	–	2	Oligodendroglioma	II
2	+	N/A	N/A	Oligodendroglioma	II
3	+	N/A	N/A	Oligodendroglioma	II
4	+	N/A	1	Oligodendroglioma	II
5	+	–	0	Oligodendroglioma	II
6	+	N/A	1	Oligodendroglioma	II
7	+	–	3	Oligodendroglioma	N/A
8	+	N/A	N/A	Oligodendroglioma	II

Results

The eight dogs included three Boxers, two Boston terriers, one Pit bull, one French bulldog, and one mixed breed dog (Table 1). Clinical signs at presentation included seizures (7/8), mentation changes (5/8), and circling (3/8). All tumors were rostrotentorial and oligodendrogliomas based on WHO criteria. Seven of eight were WHO grade II tumors; one had insufficient tissue for grading. There was sufficient tissue for EGFR immunohistochemistry in 5/8 tumors. One tumor did not have EGFR expression (score = 0), two tumors had mild staining (score = 1), one tumor had moderate staining (score = 2), and one tumor had marked staining (score = 3)

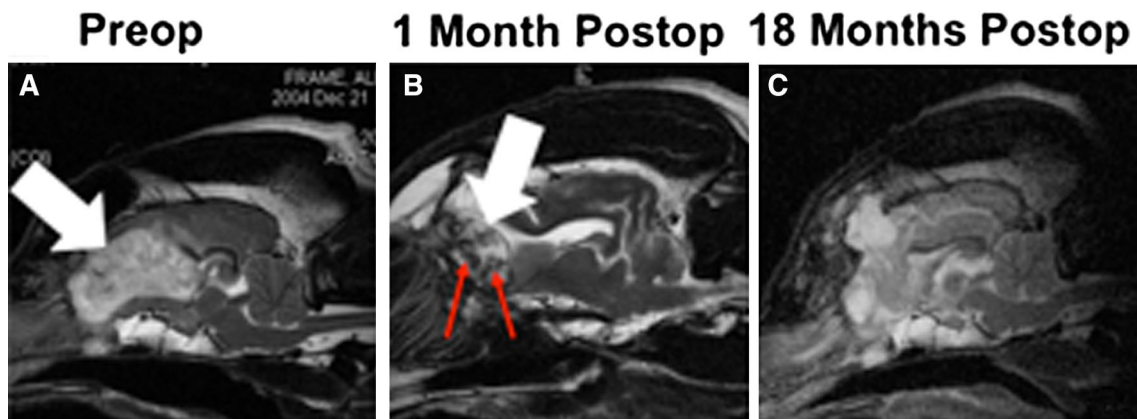
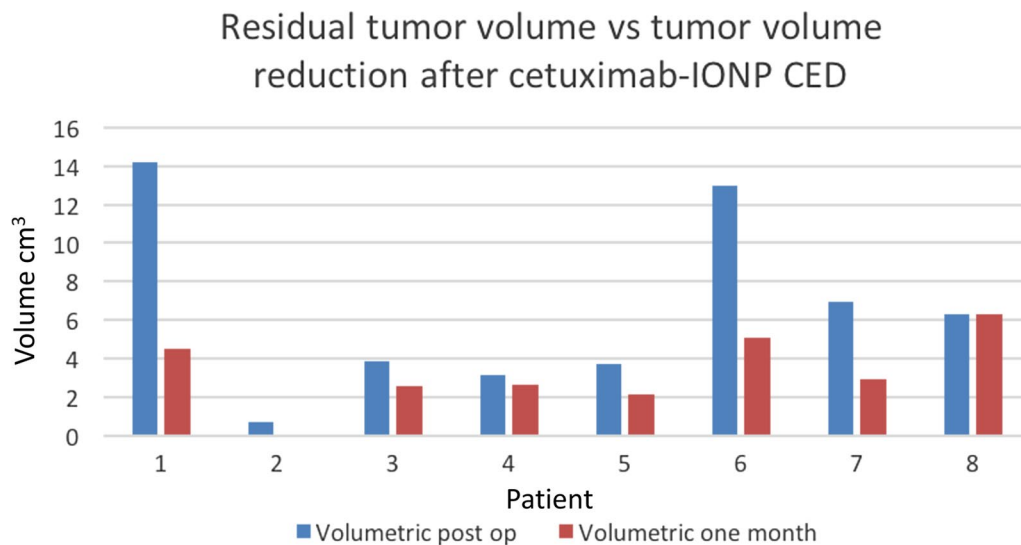


Fig. 3 Antitumor effect of cetuximab-IONP CED in a canine glioma patient. **a** Preoperative T2-weighted sagittal MRI scan revealing a large glioma tumor causing mass effect on the brain (white arrow). **b** Postoperative T2-weighted sagittal MRI at 1 month showing resid-

ual tumor (white arrow) with cetuximab-IONP artifact present within the tumor (red arrows). **c** Postoperative MRI at 18 months showing complete resolution of tumor after cetuximab-IONP CED treatment

Table 3 MRI volumetric measurements in cm³

Patient	Pre-op	Residual postop tumor volume	Tumor extent of resection (%)	1 Month post-op	Percent reduction at 1 month	EGFR status
1	14.17	14.17	0	4.5352	67.7	2
2	2.1	0.69	67.2	0	100	N/A
3	4.8	3.89	20.9	2.5891	33.4	N/A
4	3.13	3.13	0	2.6334	15.9	1
5	3.75	3.75	0	2.1882	42.6	0
6	14.6	12.94	12	5.0928	60.3	1
7	6.95	6.95	0	2.9800	57.5	3
8	6.32	6.32	0	6.2821	0	N/A

**Graph 1** Tumor volume reduction after cetuximab-IONP CED

(Table 2). Both dogs that had brains examined post mortem had tumor regrowth with tumors having similar histopathology to the original biopsy submission.

In our study, one dog had an EGFR negative tumor and three canine tumors had unknown EGFR status. The patient with an EGFR negative tumor had a 42.6% reduction in residual tumor size at 1-month postoperatively based on volumetric MRI measurements. Only one dog did not show a noticeable reduction in tumor size at the 1 month post-op MRI, but the EGFR status could not be determined for this patient due to insufficient tissue availability for histologic analysis. EGFR status was also not available for two additional patients, but these patients did have a reduction in tumor size (100% and 33.4%) (Fig. 3).

Complications

Six out of the eight dogs did not have any post-operative complications. One dog developed an elevated body temperature that resolved with antibiotics and one dog became

hypertensive, which was controlled with an antihypertensive (amlodipine).

MRI volumetric evaluation

The immediate post-operative MRI confirmed correct catheter placement of both catheters into the residual tumor in six dogs (Fig. 2). In two dogs, only one catheter resided in the residual tumor cavity. In these two dogs, one of the catheters was within the lateral ventricle and the adjacent brain parenchyma, respectively. One of these canines did not have a measurable reduction in tumor size by volumetric MRI analysis.

MRI-assisted volumetric evaluation of the neoplastic lesions was compatible with a decreased tumor size at 1 month (4–6 weeks) when compared to the post-surgical lesion size (Table 3, Graph 1) (Fig. 3). Median residual tumor volume immediately following surgery was 5.11 cm³ (mean 6.48 cm³). At 1 month, median tumor volume was 2.81 cm³ (mean 3.29 cm³), indicating a 54.9% reduction in

tumor size (mean 50.7%). One patient (patient 8) did not have a measurable reduction in tumor size.

Outcome

Five dogs were euthanized due to suspected tumor recurrence and progressive worsening of neurological signs (circling, mentation changes) and two were euthanized due to refractory seizures (one developed status epilepticus). One dog died in his sleep. Median survival time (MST) after surgery was 248 days (mean 367 days) (Table 1). None of the canines treated underwent any further adjuvant therapy after their single cetuximab-IONP CED treatment.

Discussion

Cetuximab-IONPs CED was originally evaluated by our group in a study of healthy laboratory canines and found to be a safe and effective delivery method in a larger animal model [37]. The dogs were infused with either free IONPs or cetuximab-conjugated IONPs at different CED infusion parameters (rates and volumes) and monitored with serial MRI scans. Volume of distribution was found to be linearly proportional to infusion volume and dispersion of the cetuximab-IONPs continued to occur 5 days following CED [37]. Slower infusion rates provided a more uniform distribution of IONPs and reduced infusate leak-back along the catheter track [37]. Infusion leakage along cannula tracks has also been shown in a separate study to occur at higher infusion rates (> 5 $\mu\text{l}/\text{min}$) with other therapeutics [7]. By securing the CED pumps and catheters to the patient, we were able to apply these concepts into a clinical canine model of naturally-occurring intracranial gliomas and demonstrate that infusion can be delivered in awake dogs safely and effectively over 3 days.

Cetuximab has been used to treat human GBM patients and has been shown to have an inhibitory effect on GBM cells *in vitro* and *in vivo* with pre-clinical models [40–43]. However, cetuximab has shown questionable efficacy in human GBM patients after systemic administration. Recently, cetuximab has been shown to potentially target recurrent GBM tumors by direct intra-arterial administration [44]. Multiple studies have confirmed that cetuximab can bind to the extracellular domain of human EGFR, targeting both the wild type EGFR (wtEGFR) and the EGFR variant III (EGFRvIII) deletion mutant [29, 40, 45]. Interestingly, cetuximab has also shown efficacy in EGFR negative tumors [46, 47]. We included canine patients without knowing their tumor EGFR status and found similar results. Possible reported explanations of response in EGFR negative tumors include lack of a consistent methodology and interpretation of EGFR IHC expression in tumor samples, variability in

EGFR immunoreactivity depending on fixation method, a dramatic decline in staining intensity over time, and possibly lower levels of EGFR expression in tumors (false-negative IHC) [47]. Another explanation involves variability in the EGFR especially when compared to the commercially available anti-EGFR antibody for IHC [47].

Magnetic nanoparticles, most commonly composed of ferromagnetic iron-oxide, are small theranostic particles measuring under 100 nm [48, 49], which are biocompatible and non-toxic [50]. Although uptake of magnetic nanoparticles by malignant tumor cells has been demonstrated in culture and *in vivo*, the ability to modify their surface with molecules specific for tumors allows for more specific targeting [50]. Bioconjugated cetuximab-IONPs can be used for therapeutic targeting of GBM and simultaneous MR imaging both *in vitro* and *in vivo* after intracranial CED [32]. No toxicity of normal human astrocytes was observed with cetuximab-IONPs treatment [32]. Furthermore, CED of cetuximab-IONPs has shown significant efficacy in multiple rodent glioma models with prolonged animal survival [32]. The use of cetuximab-IONPs has been found to be more efficacious than cetuximab alone by greater cellular targeting and uptake, EGFR signaling alterations, EGFR internalization, and apoptosis induction in EGFR-expressing GBM tumor cells [32]. Furthermore, cetuximab-IONP CED in a rodent glioma model has shown radiosensitivity enhancement of tumors by decreased DNA damage repair and increased reactive oxygen species formation [51]. In our current study in canines with spontaneous gliomas, volumetric evaluation showed a reduction of tumor size by an average of over 50% from the immediate post-operative MR imaging to the 1 month (4–6 weeks) time-point. We believe our single cetuximab-IONP treatment was efficacious in our canine glioma patients even in patients with EGFR negative tumors (Fig. 3).

One of the major obstacles impeding effective treatment of brain tumors is the BBB. CED avoids the BBB allowing for direct delivery of an infusate into a tumor or tumor cavity, providing high intra-tumoral drug concentrations and limiting systemic toxicity [13]. In two out of eight canine patients, only one catheter was correctly placed in the tumor bed. Although incorrect catheter placement can impact the infusate delivery, one of these patients also had the longest survival. Despite this observation, the importance of optimal CED catheter positioning has been described in human patients with high-grade gliomas [52].

Spontaneous intracranial gliomas in dogs represent an attractive model to study the delivery and efficacy of potential therapeutic agents that could be translated to human GBM patients. Although GBM tumors are the most common high-grade glioma in humans, they tend to be rare in spontaneous canine gliomas, which explains why no canine patient in this study was found to have a

GBM diagnosis [53]. Interestingly, the median survival of dogs with low-grade gliomas is reported to be about 1 year, which is similar to that of humans with GBM [54]. A decrease in the size of the residual tumor (median reduction in tumor size of 54.9%) was found in almost all of our patients by MRI volumetric analysis after a single cetuximab-IONP CED treatment. An effect was demonstrated with both EGFR-expressing and non-EGFR expressing canine glioma tumors. In the single patient that did not have an antitumor effect, only one of the catheters was properly placed within the tumor, which may have had an impact on response. Furthermore, our animals did not undergo any further adjuvant therapy and we believe the addition of fractionated external beam radiotherapy may have resulted in further enhanced treatment effect based on our preclinical studies. A future study investigating combination therapy of cetuximab-IONP CED with fractionated external beam radiotherapy after partial surgical resection in canines with spontaneous gliomas is required.

The authors understand that there are several limitations to this pilot study. It is difficult to draw conclusions about efficacy based on the low number of cases and lack of a control population. Additionally, the initial tumor size and amount of tumor removed likely affected long-term survival and varied between canine patients. Another limitation to veterinary studies is that survival is determined by euthanasia and time of euthanasia can vary between patients depending on the owner's wishes. Unfortunately, two out of the eight patients (25%) were euthanized due to recurrent seizures; however, no necropsy information or imaging was available from these patients to evaluate tumor regrowth. Finally, necropsies were only performed in two patients, so we were unable to evaluate recurrence histopathologically or toxicity associated with the infusion. Regardless, none of the canine patients showed clinical signs of either CNS or systemic toxicities after cetuximab-IONP CED treatment.

In this study, we have demonstrated for the first time that cetuximab-IONP CED is a safe and effective adjuvant therapy for spontaneous canine glioma patients at the time of their initial tumor surgery. The IONPs can be directly imaged by MRI to confirm tumor targeting (Fig. 2). Furthermore, CED infusions can be delivered in awake patients without general anesthesia over 3 days (Fig. 3), a duration which has been shown experimentally to improve the volume of distribution within the tumor. CED of cetuximab-IONPs after surgical resection in canines with spontaneous gliomas may represent a unique and translational large animal model for targeting infiltrative cancer cells away from the tumor bulk, which are responsible for tumor recurrence in human patients. A large placebo controlled blinded clinical trial is necessary to further understand the role of this treatment for canine intracranial gliomas.

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