21

Maximizing Local Access to Therapeutic Deliveries in Glioblastoma. Part V: Clinically Relevant Model for Testing New Therapeutic Approaches

JOHN ROSSMEISL[1,2,3]

[1]Brain Tumor Center of Excellence, Wake Forest Baptist Medical Center Comprehensive Cancer Center, Winston Salem, NC, USA; [2]School of Biomedical Engineering and Sciences, Virginia Tech-Wake Forest University, Blacksburg, VA, USA; [3]Department of Small Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine; Blacksburg, VA, USA

Author for correspondence: John H. Rossmeisl, Jr., Veterinary and Comparative Neuro-oncology Laboratory, Virginia-Maryland College of Veterinary Medicine, 215 Duckpond Drive, Virginia Tech Blacksburg, VA 24061, USA. E-mail: jrossmei@vt.edu

Doi: http://dx.doi.org/10.15586/codon.glioblastoma.2017.ch21

Abstract: A significant obstacle to the development of new brain tumor therapeutics remains the lack of rodent models that faithfully reproduce the *in vivo* complexities of human glioblastoma. Dogs and humans are the only species that frequently develop spontaneous brain tumors. Remarkable clinical, phenotypic, and molecular similarities exist between human and canine malignant glioma. Our research has focused on the development of pharmacologically tractable molecular targets common to human and canine gliomas, as well as the discovery

In: *Glioblastoma*. Steven De Vleeschouwer (Editor), Codon Publications, Brisbane, Australia
ISBN: 978-0-9944381-2-6; Doi: http://dx.doi.org/10.15586/codon.glioblastoma.2017

and refinement of novel methods of drug delivery to the brain, such as convection-enhanced delivery (CED), irreversible electroporation (IRE), and focused ultrasound, that can overcome the limitations imposed by the blood–brain and blood–tumor barriers. Through the conduct of early phase clinical trials in dogs, we demonstrate the safety, feasibility, and preliminary efficacies of IL-13RA2- and EphA2-targeted bacterial cytotoxins and IRE for the treatment of spontaneous malignant glioma, illustrate the clinical utility of real-time imaging monitored CED as a robust drug delivery platform, and describe the use of the tumor-bearing dog in transcranial-focused ultrasound applications related to neuro-oncology. The dog brain cancer model offers unique opportunities to expedite the clinical translation of cancer therapeutics through the design of preclinical investigations that ask and answer drug and medical device development questions that cannot be sufficiently addressed in rodent models.

Key words: Convection-enhanced delivery; Dog; Electroporation; Focused ultrasound; Glioma

Introduction

Although significant advancements in the understanding of the biology of human cancers have been made in the past two decades, clinical translation of new drugs that improve the survival and quality of life of patients with many aggressive malignancies continues to be challenging. The unmet clinical need for beneficial cancer therapeutics is highlighted by the fact that in the United States, approximately one in four deaths is attributed to cancer annually (1). Malignant primary brain tumors, and in particular malignant gliomas (MGs), represent some of the most treatment-refractory human cancers, and are leading causes of cancer-related death in adults and children (1). The median survival of adults with glioblastoma (GBM), the most aggressive and common MG variant, treated with the current standards of care is ~16 months, and the 2-year survival rate is approximately 25% (2, 3). The MG landscape also poignantly illustrates the current obstacles to the development of novel therapeutics, as only two new drugs and two medical devices have been approved for the treatment of these tumors in the last 20 years (2, 4).

The majority of preclinical studies aimed at the development of new therapies for gliomas have been conducted in small animal rodent models. While chemically induced, xenograft and genetically engineered murine glioma models have contributed significantly to the current body of knowledge regarding the pathobiology and treatment of MG, none of these modeling systems is capable of recapitulating the complex *in vivo* environment that characterizes human MG (5). As far back as 2002, a report from the National Institute of Neurological Disorders and Stroke (NINDS) and National Cancer Institute (NCI) stated, "…currently available cellular, tissue, and animal models do not accurately represent the biology of human brain tumors…" (6). Recognizing the benefits and limitations of rodent models of human brain tumors, it would be desirable to have animal models that could fill the gaps presented by current model systems, and thus better predict the therapeutic outcome in humans.

In this context, the identification and use of novel preclinical models that allow for the study of fundamental cancer drug and device development questions would meet a critical and shared need among stakeholders in the cancer research and global health care communities. The potential of companion animals, and particularly dogs, with naturally occurring cancers to provide answers to these questions is being increasingly recognized and realized (7–10). A growing body of evidence indicates that several spontaneous canine cancers are clinically, phenotypically, and molecularly similar to their human analogs, thus providing unique avenues for preclinical discovery and testing (7, 8, 10). Translational studies of investigational agents in, for example, tumor-bearing dogs can provide a variety of pharmacokinetic, mechanistic, toxicity, and anti-tumor activity data in an immunocompetent host, and thus offer numerous opportunities to more accurately guide the drug development process (8–10). It has been suggested that inclusion of preclinical canine studies in the drug development pathway could result in billions of dollars of research savings, principally by improving the design of Phase II human clinical trials and thus potential avoidance of the historically high late-stage failure and attrition rates of new cancer agents (9, 11). Dogs with spontaneous brain tumors have been assimilated into several comparative neuro-oncology research programs in an effort to accelerate the development and translation of cancer drugs to the clinic, and to mutually improve the lives of dogs and humans with brain tumors (5, 10, 12–15).

Clinically Relevant Model for Testing New Therapeutic Approaches in Gliomas

SPONTANEOUS CANINE GLIOMAS AS A FAITHFUL MODEL OF HUMAN DISEASE

Canines and humans are the only mammalian species in which spontaneous primary central nervous system (CNS) tumors are common. Estimated incidences of canine nervous system tumors range from 14.5 to 20/100,000 dogs (16, 17), which closely approximates epidemiological data indicating a primary CNS tumor incidence of 20.5/10,000 people (18). Postmortem surveys indicate that intracranial tumors are found in 2–4.5% of all dogs in which necropsy is performed (19–21), a frequency comparable to a study reporting brain tumors in 2% of humans undergoing autopsy (22). Gliomas account for 35% of all primary brain tumors in dogs, and collectively represent the second most frequently diagnosed primary tumor type after meningiomas (19, 20, 23). The median age of dogs diagnosed with glioma is 8.5 years, corresponding to the fifth and sixth decades of life in humans (21, 23). In both people and dogs, the risk for developing glioma increases with age (16, 18, 21, 24). Gliomas are significantly overrepresented in certain brachycephalic breeds of dogs, namely, Boston terriers, Boxers, and Bulldogs, which strongly suggests a genetic contribution to tumor development, and a glioma susceptibility locus has been identified on canine chromosome 26 (19, 21, 23, 25). The existence of a predisposition to gliomas in these select and highly related dog breeds with relatively limited genetic variation provides unique

opportunities to probe the canine genome for glioma-associated genetic aberrations that may not be as easily discernible amidst the much more diverse genetic background that exists in humans (25).

Considerable similarities exist between human and canine anatomy and physiology, and the physical size of the canine brain is amenable to the testing and optimization of diagnostics and therapeutics developed for human patients, without a need to rescale instrumentation. Dogs with brain tumors present with significant clinical signs, including seizures, alterations in consciousness, and motor and sensory dysfunction that can be objectively characterized and annotated using instruments comparable to those used in humans including the neurological examination, modified Glasgow Coma Scale, canine Karnofsky performance score, Engel seizure classification, and Modified Rankin Scale (26–28). As two-thirds of canine gliomas occur in the forebrain, seizures and behavior changes are the most commonly reported clinical signs (23, 28, 29). In addition, health-related quality of life surveys for use in the assessment of clinical disability in dogs with cancer do exist, although the current iterations have not been specifically developed for or validated in dogs with brain tumors (30). The prognosis for dogs with gliomas is also poor, with death occurring weeks to months following diagnosis in the absence of treatment.

The histopathological and diagnostic imaging features of canine gliomas (Figure 1) are also remarkable similar to their human counterparts (31–35). These shared morphologic features facilitate comparative classification and grading of tumors using World Health Organization criteria (36) and performing objective imaging–based therapeutic response assessments using the Response Assessment in Neuro-Oncology (RANO) system criteria (28, 37). However, the frequency of glioma subtypes encountered in dogs differs from that seen in humans (Table 1), with oligodendrogliomas accounting for a significantly higher proportion of all canine gliomas compared to humans (19–21, 23, 37).

Molecular and genetic profiling of brain tumors is becoming a routine procedure in human neuro-oncology (38, 39). These analyses have led to evolutions in the classification and prognostic stratification of human brain tumors, and are fundamental to the rational translational application of molecularly targeted therapies (38–40). The characterization of the molecular and genomic landscapes of canine brain tumors has been facilitated by the increasing availability of canine-specific reagents and advancements in high-throughput sequencing platforms (25). To date, studies in dogs have demonstrated that hallmark alterations in proteins involved in cellular proliferation, apoptosis, and cell-cycle regulation, such as the RTK, p53, and RB1 pathways that participate in tumorigenesis, parallel those seen in human gliomas (31, 38, 40–42). Also similar to humans, overexpression of alpha3-beta1integrin, c-Met, EGFR, EphA2, IGFBP2, IL-13RA2, MMP-2, and-9, PDGFRa, uPAR, and VEGF/VEGFR1/2 have been observed in canine gliomas (43–51). Homologous overexpression of cell surface receptors in canine and human gliomas, such as EGFR, EphA2, and IL-13RA2, have driven the preclinical investigation of molecularly targeted therapeutics in glioma-bearing dogs (48, 52).

Continuing the global genetic characterization of canine gliomas, as well as the confirmation of the molecular signatures of individual canine patient tumors are paramount to the rational design of preclinical investigations, especially in

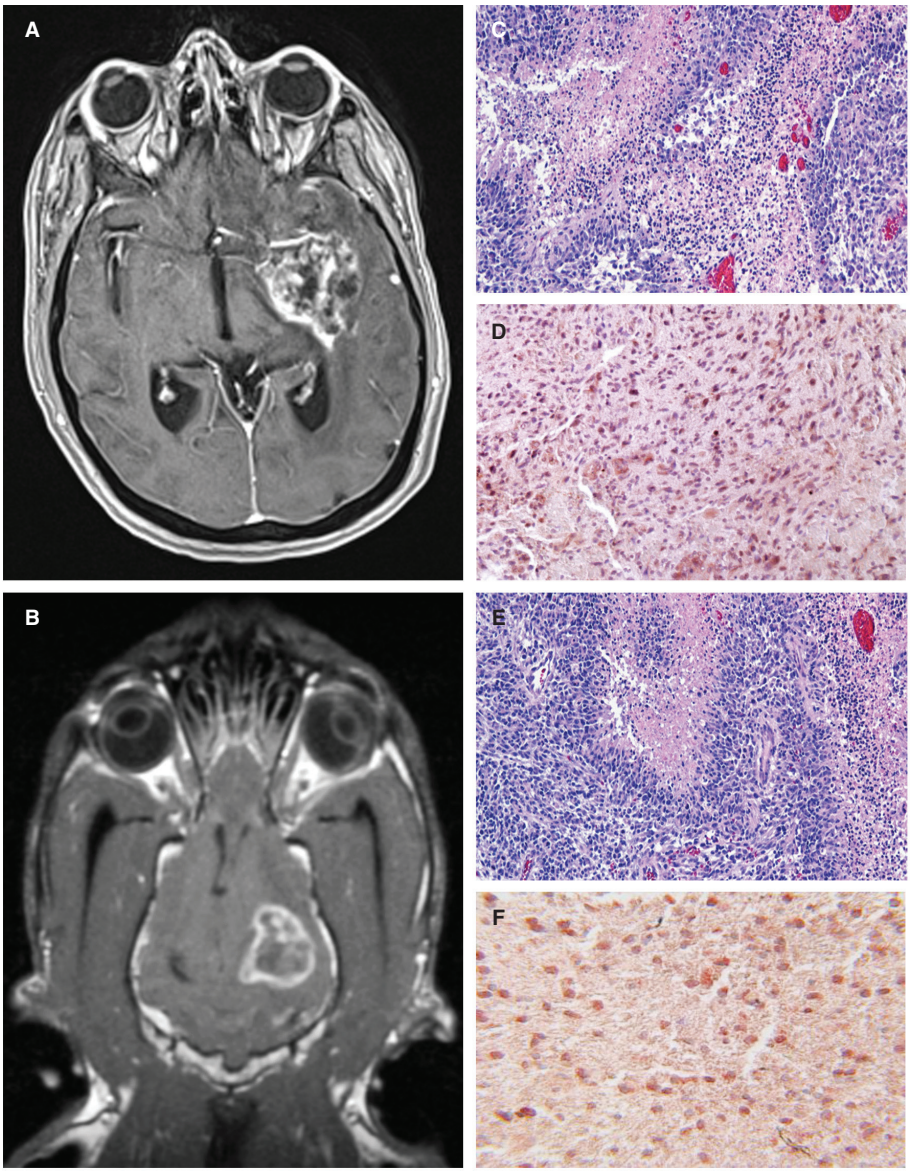

Figure 1 Comparative morphological and immunophenotypical features of human and canine glioblastoma (GBM). Post-contrast T1-weighted magnetic resonance images from a human (**A**) and dog (**B**) demonstrating ring-enhancing cerebral GBM. Classic microscopic features of hypercellularity and pseudopalisading necrosis in a human (**C**) and canine (**E**) GBM (H&E stain, bar = 150 µm). GBM from both species demonstrate intense immunoreactvity to IL-13RA2 (**D**, **F**).

TABLE 1 Comparative Frequencies of Glioma Subtypes and Grades in Dogs and Humans

Tumor type	Grade	*Grade distribution within tumor type*	
		Canine (%)‡	Human (%)
Astrocytoma (1, 18, 19, 21, 23) • 30–60% of all canine neuroepithelial tumors • 60–70% of all human neuroepithelial tumors	I (Pilocytic)	<1	5
	II (Diffuse)	~40	10–15
	III (Anaplastic)	~20	10–20
	IV (Glioblastoma)	~30	60–75
Oligodendroglioma (1, 18, 19, 21, 23) • 30–50% of all canine neuroepithelial tumors • 10–15% of all human neuroepithelial tumors	II (Oligodendroglioma)	70	70
	III (Anaplastic)	30	30

‡Grade distribution data obtained from archived specimens in Veterinary and Comparative Neuro-oncology Laboratory tissue biorepository.

the context of the rapidly growing library of targeted agents available for cancer diagnostics and treatment (53). Although the discovery of additional common denominators shared among canine and human tumors is likely with the use of more robust whole genomic sequencing and single-nucleotide polymorphism platforms, it is also probable that aberrations in key gliomagenesis pathways that are unique to the dog will also be revealed, as some fundamental species-specific differences have already been documented. For example, the favorable prognostic hallmark in human oligodendroglioma of co-deletion of chromosome 1p/19q has not been identified in canine gliomas, nor have the classical genetic mutations in TP53 or IDH1 that define human astrocytomas (41, 54, 55).

The value of dogs with spontaneous brain tumors as faithful preclinical models of human disease has been demonstrated in several additional areas of neuro-oncology. A study investigating dendritic cell vaccination of glioma-bearing dogs with tumor cell lysates containing a toll-like receptor ligand adjuvant in combination with *in situ* adenoviral interferon-gamma gene transfer demonstrated sufficient safety and promise to result in rapid translation of this immunogenetic therapy to a human clinical trial (56, 57), and promising active immunotherapeutic approaches using dogs with intracranial meningiomas have recently been published (58). Pioneering work in dogs with gliomas illustrated the feasibility and importance of real-time MR imaging monitoring of convection-enhanced delivery (CED) for confirmation of target coverage, as well as providing an opportunity to detect and remedy any local adverse effects of CED treatment, including reflux of the infusate along the catheter (59–61).

PRECLINICAL TESTING OF VARIOUS THERAPEUTIC METHODS (CONVECTION-ENHANCED DELIVERY, IRREVERSIBLE ELECTROPORATION, TRANSCRANIAL-FOCUSED ULTRASOUND) IN DOGS WITH SPONTANEOUS TUMORS OF THE BRAIN

Recognizing the translational relevance of and collaborative opportunities offered by the spontaneous canine brain tumor model, our laboratory's research focuses on the multi-scale, comparative targeting of brain tumors. Our efforts include the identification of pharmacologically tractable molecular targets common to human and canine brain tumors, as well as the development of novel macroscopic methods of CNS drug delivery that overcome the limitations imposed by the blood–brain barrier (BBB) and blood brain tumor barrier (BBTB) (13, 14). The design and conduct of clinical trials in dogs with naturally occurring brain tumors is a major mechanism by which we assess our drug and device discoveries (10, 13).

CONVECTION-ENHANCED DELIVERY

The CED technique involves the pressurized infusion of therapeutic agents directly into tumor other target tissues using specialized catheters (13, 62–64). By bypassing the BBB, CED allows for delivery of high concentrations of macromolecular drugs directly to the tumor with negligible or no systemic drug exposure, and CED is capable of achieving clinically relevant drug distribution volumes by bulk fluid flow without significantly increasing intracranial pressure when infusions are administered at low pressures over several hours or days (60, 62–64). CED can increase drug distribution volumes in the brain by at least an order of magnitude relative to simple diffusion, and it can be performed safely throughout the CNS in humans and animals (65). It has been demonstrated that liposomal CPT-11 and EGFRvIII-antibody conjugated to iron oxide nanoparticles can be safely delivered via CED to canine gliomas, and these studies have provided evidence of the efficacies of these approaches in this model (52, 60).

Historically, major technical impediments to the widespread adoption of CED for the treatment of human glioma has been an inability of the technique to distribute drugs to the entire heterogeneous tumor volume and margin (60, 61, 66), as well as inherent limitations of catheters adopted for use in CED. To overcome these obstacles, advancements in CED have included the incorporation of predictive computational imaging analyses into therapeutic planning, real-time MR imaging of infusions to facilitate and confirm target coverage, and the design and utilization of novel catheters appropriate for CED (59–61, 64).

Building upon these advancements and cognizant of the lessons learned from prior CED clinical trials, we are investigating the use of CED to deliver high-molecular weight-targeted therapeutics to canine gliomas. Given the potential efficacy of first generation of IL-13RA2 conjugated pseudomonal exotoxins in human GBM (67), and common overexpression of IL-13RA2 and EphA2 in canine and human gliomas (Figure 1), potent IL-13 and ephrin-A1-based cytotoxins containing modified *Pseudomonas* exotoxin A or *Diphtheria* toxin targeted to IL-13RA2 and EphA2 receptors were generated, respectively (47, 48, 68, 69).

We are actively conducting a clinical trial in dogs with gliomas to evaluate the tolerability and preliminary efficacy of this targeted bacterial cytotoxic cocktail administered by delivered using MRI-monitored CED.

Canine subjects enrolled in the trial have mild-to-moderate clinical signs of brain dysfunction and histopathologically confirmed gliomas demonstrating immunoreactivity to IL-13RA2 and/or EphA2. The trial is designed using a 3+3 dose-escalation scheme, with cohorts administered 0.05, 0.1, 0.2, or 0.4 μg of each cytotoxin/ml of infusate. To optimize the CED procedure, an inverse therapeutic planning method, using a spherical shape–fitting algorithm generated from patient-specific, segmented MRI/CT images, is used to simulate ideal cannula placement and target coverage prior to treatment (69, 70). CED is performed in the anesthetized dog using reflux-preventing cannulae to co-administer the cytotoxins with a gadolinium tracer (Figure 2) to allow for intraoperative MRI visualization of infusate distribution. Tolerability is defined as the absence of dose-limiting toxicities (DLT) within 28 days of infusion. DLT are considered the development of Grades 3, 4, or 5 adverse events, as defined by the Cancer Therapy Evaluation Program CTCAE standards (71). Serial clinical, laboratory, and brain MRI examinations are performed for 6 months following CED treatment, and the

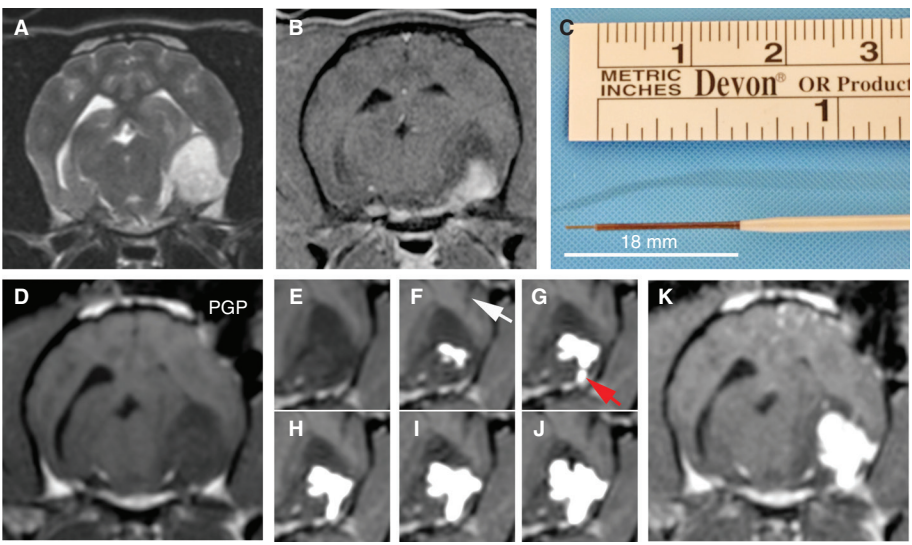

Figure 2 Intratumoral convection-enhanced delivery (CED) of molecularly targeted therapeutics into a canine astrocytoma. Pre-treatment transverse T2-weighted (**A**) and post-contrast T1-weighted (**B**) images demonstrating the tumor in the temporal-piriform lobes of the brain. (**C**) Fused silica and ceramic reflux-preventing cannula (RPC) with multistep tip design used for CED. (**D**) Intraoperative, transverse T1-weighted images obtained immediate prior to infusion, showing probe guide pedestal (PGP) implanted in the skull, through which RPC (**F**, white arrow) will be stereotactically placed into the tumor. (**E–J**) Time-lapsed 3DT1-weighted images taken over approximately 2 h of MR-monitored infusion showing progressively increasing volume of distribution of the infusate co-delivered with gadolinium (white) within the tumor. An additional RPC has been inserted (G, red arrow) to facilitate tumor coverage. (**K**) Immediate post-infusion T1-weighted image demonstrating tumor coverage and infusate containment achieved at completion of CED.

CED infusions can be repeated in the event of tumor progression or suboptimal target coverage is achieved during the initial infusion. Efficacy is determined by characterizing objective tumor responses using RANO and volumetric criteria modified for use in canine patients (72).

Using this approach, we have achieved robust and clinically relevant volumes of infusate distribution in unresected canine MGs (Figure 2). In addition, inclusion of real-time MR-monitoring-facilitated intraoperative cannulae revisions that allowed continued target coverage after observation of ventricular leakage or infusate reflux in some procedures. Clinical and partial tumor volumetric responses (≥50% volumetric tumor reductions) have been observed in 55% (5/9) of the dogs treated to date. Necropsy examinations performed in four dogs with progressive disease have revealed tumor necrosis in infused regions. In the first three dosing cohorts, significant DLT have not been observed. Results from this trial indicate that improvements in CED cannula design, therapeutic planning, and MRI monitoring allow for safe and effective intratumoral delivery of IL-13RA2- and EphA2-targeted cytotoxins. This ongoing study also provides preliminary evidence of the efficacy of these cytotoxins when used as a monotherapy in a spontaneous animal glioma model.

In our continuing effort to more precisely and specifically target gliomas with locally delivered therapies, we have clinical trials planned that will incorporate infusion our next generation multivalent cytotoxin, QUAD-CTX, that simultaneously targets the IL-13RA2, EphA2, EphA3, and EphB2 receptors into canine gliomas (see Part I, page xxx). Similar to humans, we have also demonstrated that canine gliomas overexpress EphA3. To further increase the efficiency and utility of CED, we will administer the QUAD-CTX using our innovative convection-enhanced arborizing catheter (see Part III, page xxx).

IRREVERSIBLE ELECTROPORATION

Electroporation is a technique in which electrical pulses are used to permeabilize tissue through formation of nanoscale pores in cellular membranes (73). When the applied electric field strength exceeds a critical value, irreversible electroporation (IRE) is achieved, which creates permanent defects in cellular membranes resulting in cell death (73, 74). IRE is a novel, minimally invasive, rapid, and non-thermal method of tissue ablation that has been demonstrated to be safe and effective for the treatment of solid tumors in animals and humans (75–78). It has been shown that IRE therapy has also been shown to have sparing effects on the vasculature, ductal networks, and extracellular matrix, which facilitates posttreatment healing (73, 74, 79).

We have developed a novel technology, coined high-frequency irreversible electroporation (H-FIRE) that represents a significant advancement in IRE therapy, the specifics of which have been covered in Part III of this chapter (80). Briefly, the treatment of patients with high-amplitude IRE pulses (1–3 kv, ~100 μs) requires administration of neuroparalytic agents in order to abolish muscle contractions associated with pulse delivery (79). The requirements for general anesthesia and neuroparalytics may complicate or exclude IRE treatment of some tumors in some debilitated patients. The H-FIRE generator is capable of delivering bipolar bursts of pulses with individual pulse durations two orders of

magnitude shorter than in IRE (~1 µs). This allows H-FIRE to non-thermally ablate tissue without causing muscle contractions, which negates the need for neuroparalytic use during treatment, achieves more predictable zone of treatment by mitigating tissue heterogeneities (80, 81), and may allow for selective tumor cell ablation based on altered cellular morphology (82).

In addition, we and others have demonstrated that IRE and H-FIRE pulses are capable of transiently disrupting the BBB outside the region of irreversible tissue ablation in a voltage-dependent manner (83–85). This provides an opportunity for the delivery of otherwise impermeable macromolecules to a penumbra of tissue surrounding the macroscopic tumor volume exposed to the electrical field, which could be exploited for delivery of therapeutics to microscopic tumor infiltrates extending beyond the gross tumor margins, which account for the majority of local treatment failures in MG (83, 85). We believe that these unique features of IRE and H-FIRE make them particularly attractive for use in intracranial surgery, and have been developing these platforms for the treatment of brain cancer.

We have evaluated the safety and preliminary efficacy both IRE (Figure 3) and H-FIRE (Figure 4) in dogs with spontaneous brain tumors (77, 79, 86).

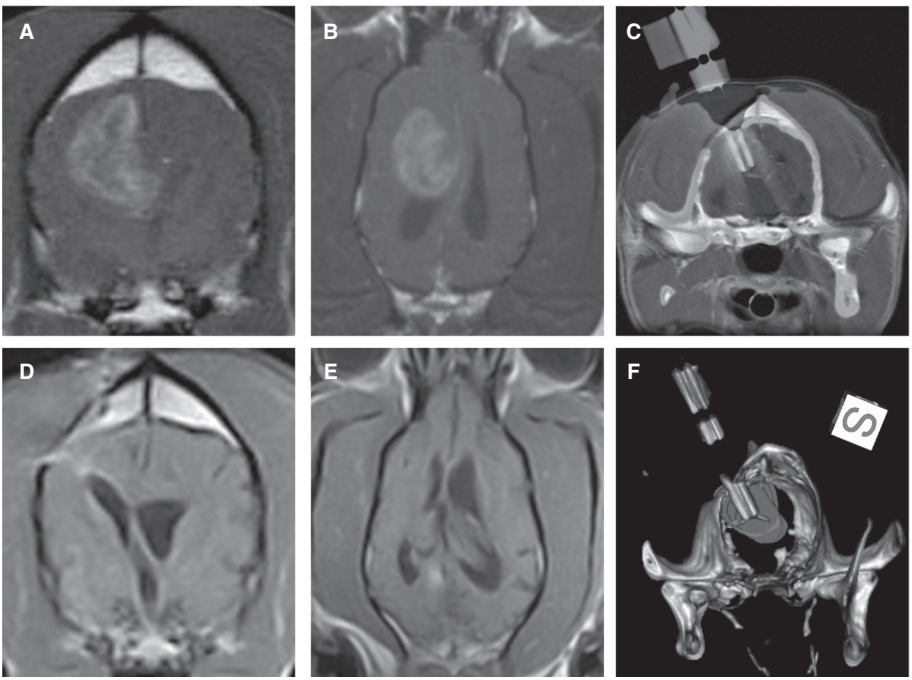

Figure 3 Stereotactic glioblastoma ablation with irreversible electroporation (IRE). Pre-treatment transverse (**A**) and dorsal planar (**B**) post-contrast T1-weighted MR demonstrating ring-enhancing glioblastoma in the frontoparietal lobe of the cerebrum. Co-registered intraoperative CT and pre-treatment MR images (**C**) and three-dimensional reconstructed CT (**F**) with IRE electrodes in situ within the tumor in preparation for ablation. Three-month post-IRE treatment transverse (**D**) and dorsal planar (**E**) post-contrast T1-weighted MR illustrating 95% reduction in tumor burden.

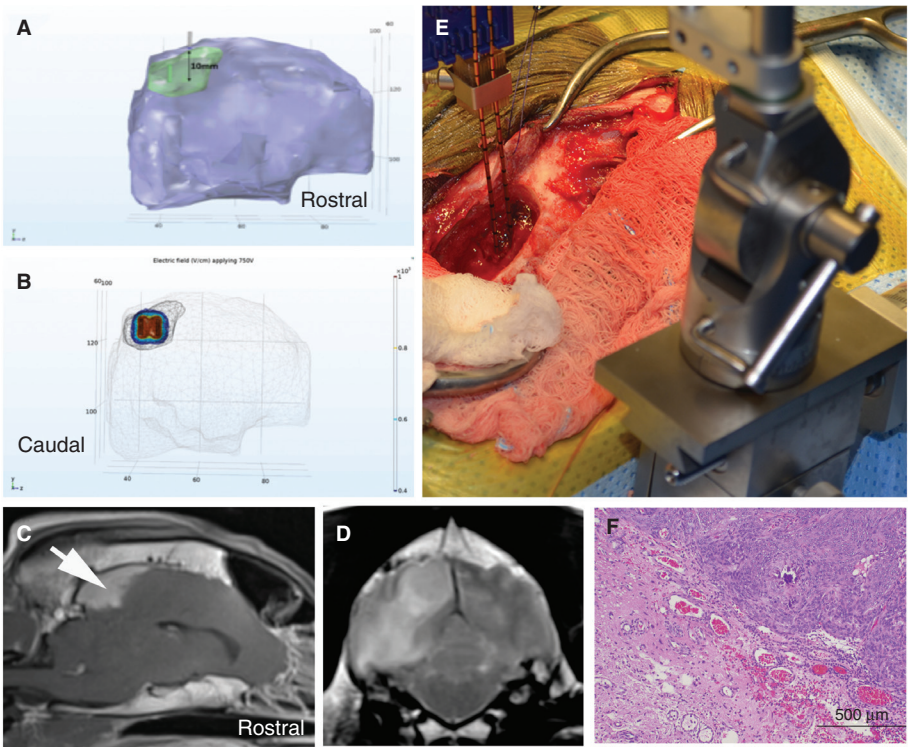

Figure 4 High-frequency irreversible electroporation (H-FIRE) treatment of a canine Type I parasagittal meningioma. Treatment planning (**A**, **B**) involves segmentation of the tumor (green) and brain (purple) from the patient's MR images (**C**, **D**), and determination of the electrode placement trajectory (**A**). The resulting electric field distributions are then simulated (**B**) using finite element analysis software (**B**). The H-FIRE electrodes are placed using intraoperative stereotaxy (**E**) according to the treatment plan, and the pulses delivered. After-HFIRE treatment, the tumor was resected and serially sectioned to correlate the predicted with actual ablation volume. Photomicrograph of the treatment margin (**F**), illustrating a sharp line of demarcation between H-FIRE ablated (lower left) and viable tumor (upper right); **H**&**E** stain.

An integral component of the preclinical evaluation of IRE and H-FIRE was the development of anatomically accurate numerical treatment planning models that maximize tumor coverage while minimizing damage to surrounding healthy tissue and also account for the increase in tissue conductivity that occurs during pulse delivery (86–88). Incorporating therapeutic plans developed from patient-specific, segmented medical images imported into finite element analysis modeling software, we have confirmed the ability of IRE and H-FIRE to safely and precisely ablate normal and neoplastic canine brain tissues with a submillimeter line of demarcation between ablated and non-treated tissues (79, 86, 89). IRE treatment of canine gliomas resulted in significant objective tumor responses in 4/5 dogs with quantifiable target lesions (Figure 3), and these radiographic responses were accompanied by improvements in Karnofsky

performance scores and posttreatment seizure control (72, 86). Similarly, using a treat and resect treatment paradigm, we have confirmed the ability of H-FIRE to safely and precisely ablate clinically relevant volumes of canine brain tumors without the induction of muscular contractions during pulse delivery (Figure 4).

To overcome previously recognized barriers to the translation IRE and H-FIRE therapies to the clinic, such as the inability to incorporate MR image-guidance into treatments and need to use multiple software programs for therapeutic planning, we have been developing a comprehensive solution that combines all of the necessary components of the workflow in a user-friendly platform that can be incorporated into contemporary neurosurgical theaters (86, 90, 91). The foundation for this platform is an open-source, online interface that uses a treatment planning approach similar to that employed in radiotherapeutic applications. The software allows for tissue-specific segmentation, determination of the tumor dimensions, and formulation of virtual electrode insertion approaches that can be used in surgery (91). These volumetric representations are then used to perform computational simulations of the electric field distribution surrounding the active electrodes during pulse delivery to determine tumor coverage (Figure 4) and cell kill probabilities (90, 92). Validation of the predicted therapeutic outcomes generated with this platform is currently underway using clinical data from IRE-treated dogs with intracranial gliomas (90).

Another fundamental step which we have undertaken to clinically implement this technology is the development MR compatible electrodes for use in IRE and H-FIRE procedures. This provides for coupling of the imaging-based computational predictive models to near real-time imaging-derived feedback with regard to the electrode location and electrical properties of the tumor through the use of magnetic resonance electrical impedance tomography, which allows for intraoperative monitoring of the electrical field distribution during electroporation-based treatments (93). Using this anatomical and biophysical imaging-guided approach, the expected outcome of the treatment can be confirmed after pulse delivery is completed, and the treatment can be revised, if necessary, to accommodate any suboptimally treated areas that are identified.

TRANSCRANIAL MR-GUIDED-FOCUSED ULTRASOUND SURGERY

The use of acoustic energy for therapeutic applications in the CNS was first described more than a half century ago in seminal studies performed in a feline model by Fry and colleagues (94–96). Ultrasound transducers are capable of focusing acoustic waves on targets located deep within tissues. By manipulating the sonication parameters, focused ultrasound is capable of thermal tissue ablation, mechanical tissue ablation (histotripsy), neuromodulation, and BBB disruption, and thus has many potential applications in the treatment of brain disease (97–105). Although early studies showed the promise of focused ultrasound for the treatment of intracranial disorders, obstacles associated with the control and monitoring of the procedure coupled with the limitations associated with application of acoustic waves through the skull have, until recently, impeded the widespread application of this technology in neuro-oncology.

The skull has been a major challenge to the clinical adoption of focused ultrasound in the brain. The attenuation of acoustic waves that occurs in bone is approximately 50 times higher than that of soft tissue, and this causes rapid heating of the skull which limits the safe energy exposures that can be delivered (101). The skull also has a significant effect on the propagation of acoustic waves, as variations in skull shape and thickness make it difficult to reliably focus the ultrasound beam. In early focused ultrasound trials, the barriers posed by the calvarium required delivery of ultrasound through a craniectomy defect, which negated the benefits of a noninvasive transcranial procedure (98, 99, 106).

Vast improvements in technology have resulted in the development of several focused ultrasound systems which incorporate MR imaging and allow for the precision targeting and control of the procedure with real-time feedback obtained from quantitative MR thermometric imaging (103, 107, 108). The precision offered by MR-guidance, coupled with the incorporation of active tissue cooling measures, the design of large geometric phased transducer arrays, application-specific tuning of the ultrasound frequency, and computational phase offset beam correction, have allowed the successful mitigation of the heating and beam focusing problems traditionally posed by the skull, and ushered in the era of noninvasive transcranial MR-guided-focused ultrasound (TcFUS).

In parallel with advancements made in humans, non-human primates, and other animal models, we have been working toward the use of TcFUS for thermal ablation of canine tumors and focused disruption of the blood–brain barrier to facilitate drug delivery to the canine brain (109). The preclinical evaluation of TcFUS in dogs has posed additional and unique challenges. The tremendous inherent variations in skull size, conformation, and thickness within and among dog breeds has required expanding and refining engineering solutions developed to reliably achieve transcranial beam focusing. Although beam focusing aberrations associated with skull variability can be corrected using large arrays of individually controllable transducing elements, the geometry of existing FUS hemispheric arrays and the size and conformation variability, as well as positioning constraints of the canine cranium within these arrays complicates treatment delivery in the dog (Figure 5). Using computed tomographic scans of the head obtained prior to treatment co-registered with diagnostic MR data sets and a customized multi-element elliptical array, we are in the process of optimizing patient and canine species-specific phase offset simulations to correct for differences in acoustic wave propagation associated with skull heterogeneity, and to allow for electronic steering of the focal position.

We have also attempted transcranial BBB opening in the normal canine brain using existing FUS systems (Figure 5). In our preliminary studies in dogs, we observed that the assessment of BBB opening using passive cavitation detection (PCD) resulted in considerable variability that was poorly associated with other measures of BBB opening, such as the opening volume (110). This is in contrast to findings indicating that PCD is an acceptable surrogate of BBB permeability in rodent models. We believe that these differences may be attributable to the gyrencephalic structure and increased white/gray matter, vascular, and ventricular heterogeneity of the canine brain compared with rodents, as other investigators have demonstrated similar PCD variability when using a non-human primate model (100). Optimization of PCD monitoring remains a focus of our current canine TcFUS work, and will be paramount to answering questions associated

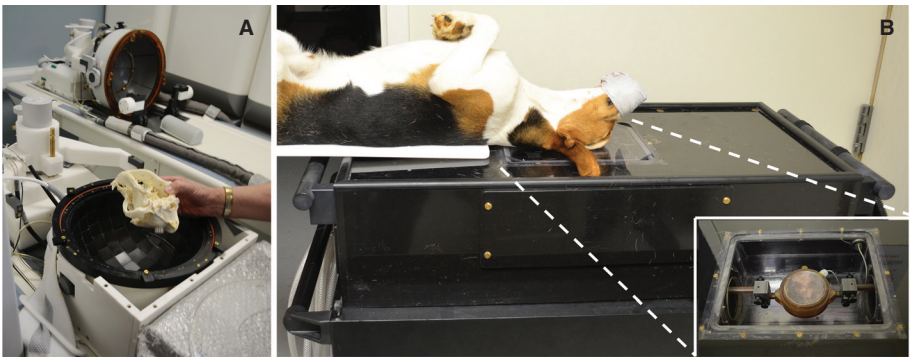

Figure 5 Evaluation of TcFUS instrumentation in the canine model. Canine skull (**A**) positioned in the Exablate platform (InSightec Ltd., Dallas, TX, USA) hemispheric transducer array. In the background, an additional Exablate hemispheric transducer and couch are visible illustrating the equipment configuration that would be used to treat a human brain. Canine positioned on the RK-100 couch (FUS Instruments, Toronto, Ontario, Canada) system in preparation for transport into the MR suite for TcFUS BBB opening. Note the size difference in the transducers between the two systems (**B**, inset).

with quantifying drug delivery and efficiency that are fundamental to assessment of TcFUS in the canine brain tumor model, and translation of these drugs and technologies to humans.

Conclusion

Dogs with spontaneous brain tumors represent an immunocompetent model that recapitulates many key clinical and pathobiological features of human tumors, and thus provide a unique avenue for the assessment of novel therapeutics. Integration of the canine brain tumor model into neuro-oncology research programs offers an opportunity to accelerate the development of effective treatments that will mutually benefit humans and dogs. The potential translational impacts of clinical trials in dogs with spontaneous brain tumors on neuro-oncologic technologies and techniques have been demonstrated in investigations focused on CED and immunotherapy. Continued critical analyses of the natural biology and molecular genetics of canine brain tumors will be paramount to defining standards of care for specific canine tumor types, the expansion and validation of canine-specific reagents and techniques necessary for quantitative and reproducible end-point evaluations, and ultimately, the optimal design of investigational clinical trials that incorporate brain tumor–bearing dogs that attempt to evaluate therapeutic outcomes.

Acknowledgment: Portions of this section were supported by grants from the Wallace H. Coulter Foundation, National Science Foundation CAREER Award, NIH/NCI R01CA139099, NIH/NCI R01CA213423, Wake Forest University Translational Sciences Institute, and the Virginia Biosciences Health Research Corporation.

The author would like to thank the following institutions and collaborators for their contributions to the generation of data and material support of experiments presented in this section:

- Wake Forest University Comprehensive Cancer Center and Brain Tumor Center of Excellence: Drs. Waldemar Debinski, Stephen Tatter, Thomas Ellis, Constance Stanton, John Olson, and Mrs. Denise Herpai;
- Virginia Tech-Wake Forest University School of Biomedical Engineering and Sciences: Drs. Rafael Davalos, John Robertson, Scott Verbridge, Paulo Garcia, Michael Sano, Chris Arena, Robert Neal, Mr. Eduardo Latouche, Mr. Rudy Andriani, and Ms. Jill Ivey;
- University of Texas, Austin, Department of Mechanical Engineering: Drs. Chris Rylander and Robert Hood, Ms. Egleide Elenes, Mr. Jason Mehta;
- University of California, Davis, School of Veterinary Medicine: Dr. Peter Dickinson;
- University of Virginia Department of Biomedical Engineering and Focused Ultrasound Center: Drs. Richard Price and Yuval Gruber;
- University of Ljubljana, Faculty of Electrical Engineering, Laboratory of Biocybernetics, Slovenia: Drs. Damijan Miklavcic, Bor Kos, and Denis Pavliha;
- Sheikh Zayed Institute for Pediatric Surgical Innovation, Children's National Health System: Dr. Pavel Yarmolenko.

The author recognizes the following investigators at the Virginia–Maryland College of Veterinary Medicine for clinical care and patient professional services provided to dogs enrolled in clinical trials described here: Drs. Theresa Pancotto, John Robertson, Jeffery Ruth, Kemba Clapp, Gregory Daniel, Samatha Emch, Kelli Kopf, Thomas Cecere, Jamie King, Tanya LeRoith, Philip Sponenberg, Natalia Henao-Guerrero, Noah Pavlisko, Joao Soares, Rachel Carpenter, Kevin Lahmers, Ms. Mindy Quigley, Ms. Barbara Wheeler, Ms. Maureen Sroufe, and Ms. Kelli Hall-Manning.

Conflict of Interest: Dr. Rossmeisl holds patents and has patents pending in the use of irreversible electroporation (IRE) for the treatment of cancer, IRE for blood–brain barrier disruption, and fiberoptic microneedle convection-enhanced delivery catheter systems.

References

1. Cancer Facts & Figures 2015. Available from: www.cancer.org (accessed March 12, 2016).
2. Omuro A, DeAngelis DM. Glioblastoma and other malignant gliomas: A clinical review. JAMA. 2013;310(17):1842–50. http://dx.doi.org/10.1001/jama.2013.280319
3. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJB, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 2005;352(10):987–96. http://dx.doi.org/10.1056/NEJMoa043330

4. Kang JH, Adamson C. Novel chemotherapeutics and other therapies for treating high-grade glioma. Expert Opin Investig Drugs. 2015;24(10):1361–79. http://dx.doi.org/10.1517/13543784.2015.1048332
5. Candolfi M, Curtin J, Nichols W, Muhammad AKM, King G, Pluhar G, et al. Intracranial glioblastoma models in preclinical neuro-oncology: Neuropathological characterization and tumor progression. J Neurooncol. 2007;85(2):133–48. http://dx.doi.org/10.1007/s11060-007-9400-9
6. Brain Tumor Progress Review Group. Strategic plan for addressing the recommendations of the Brain Tumor Progress Review Group. Washington, DC: NINDS/NCI; 2002.
7. Vail DG, MacEwen EG. Spontaneously occurring tumors of companion animals as models for human cancer. Cancer Invest. 2000;18(8):781–92. http://dx.doi.org/10.3109/07357900009012210
8. Schiffman JD, Breen M. Comparative oncology: What dogs and other species can teach us about humans with cancer. Philos Trans R Soc Lond B Biol Sci. 2015;370:1673. http://dx.doi.org/10.1098/rstb.2014.0231
9. Khanna C, London C, Vail D, Mazcko C, Hirschfield S. Guiding the optimal translation of new cancer treatments from canine to human cancer patients. Clin Cancer Res. 2009;15:5671–7. http://dx.doi.org/10.1158/1078-0432.CCR-09-0719
10. LeBlanc AK, Mazcko C, Brown DE, Koehler JW, Miller AD, Miller R, et al. Creation of an NCI comparative brain tumor consortium: Informing the translation of new knowledge from canine to human brain tumor patients. Neuro Oncol. 2016;18(9):1209–18. http://dx.doi.org/10.1093/neuonc/now051
11. Gordon I, Khanna C. Modeling opportunities in comparative oncology for drug development. ILAR J. 2010;51:214–20. http://dx.doi.org/10.1093/ilar.51.3.214
12. Hicks J, Platt S, Kent M, Haley A. Canine brain tumours: A model for the human disease? Vet Comp Oncol. 2017;15(1):252–72. http://dx.doi.org/10.1111/vco.12152
13. Rossmeisl JH. New treatment modalities for brain tumors in dogs and cats. Vet Clin North Am Small Anim Pract. 2014;44(6):1013–38. http://dx.doi.org/10.1016/j.cvsm.2014.07.003
14. Dickinson PJ. Advances in diagnostic and treatment modalities for intracranial tumors. J Vet Int Med 2014;28(4):1165–85. http://dx.doi.org/10.1111/jvim.12370
15. Bentley RT, Ahmed AU, Yanke AB, Cohen-Gadol AA, Dey M. Dogs are man's best friend: In sickness and in health. Neuro Oncol. 2017;19(3):312–22. http://dx.doi.org/10.1093/neuonc/now109
16. Dorn CR, Taylor DO, Frye FL, Hibbard HH. Survey of animal neoplasms in Alameda and Contra Costa Counties, California. I. Methodology and description of cases. J Natl Cancer Inst. 1968;40:295–305.
17. Dobson JM, Samuel S, Milstein H, Rogers K, Wood JL. Canine neoplasia in the UK: Estimates of incidence rates from a population of insured dogs. J Sm Anim Pract. 2002;43:240–6. http://dx.doi.org/10.1111/j.1748-5827.2002.tb00066.x
18. Dolecek TA, Propp JM, Stroup NE, Kruchko C. CBTRUS statistical report: Primary brain and central nervous system tumors diagnosed in the United States in 2005–2009. Neuro Oncol. 2012;14(Suppl 5):v1–v49. http://dx.doi.org/10.1093/neuonc/nos218
19. McGrath JT. Intracranial pathology of the dog. Acta Neuropathol (Berl). 1962;Suppl I:3–4. http://dx.doi.org/10.1007/978-3-642-92847-5_2
20. Priester WA, Mantel N. Occurrence of tumors in domestic animals. Data from 12 United States and Canadian colleges of veterinary medicine. J Natl Cancer Inst. 1971;47:1333–1344.
21. Song RB, Vite CH, Bradley CW, Cross JR. Postmortem evaluation of 435 cases of intracranial neoplasia in dogs and relation-ship of neoplasm with breed, age, and body weight. J Vet Intern Med. 2013;27:1143–52. http://dx.doi.org/10.1111/jvim.12136
22. Klotz M. Incidence of brain tumors in patients hospitalized for chronic mental disorders. Psychiatr Q. 1957;31:669–80. http://dx.doi.org/10.1007/BF01568758
23. Snyder JM, Shofer FS, Van Winkle TJ, Massicotte C. Canine intracranial primary neoplasia: 173 cases (1986–2003). J Vet Intern Med. 2006;20:669–75.
24. Hayes HM, Priester WA, Pendergrass TW. Occurrence of nervous-tissue tumors in cattle, horses, cats and dogs. Int J Cancer. 1975;15:39–47. http://dx.doi.org/10.1002/ijc.2910150106
25. Truve K, Dickinson P, Xiong A, York D, Jayashankar K, Peilberg G, et al. Utilizing the dog genome in the search for novel candidate genes involved in glioma development-Genome wide association mapping followed by targeted massive parallel sequencing identifies a strongly associated locus. PLoS Genet. 2016;12(5):e1006000. http://dx.doi.org/10.1371/journal.pgen.1006000

26. Platt SR, Radaelli ST, McDonnell JJ. The prognostic value of the modified Glasgow Coma Scale in head trauma in dogs. J Vet Int Med. 2001;15(6):581–584. http://dx.doi.org/10.1111/j.1939-1676.2001.tb01594.x
27. Valladao ML, Scarpelli KC, Metze K. Clinical utility of a life quality score in dogs with canine transmissible venereal tumor treated by vincristine chemotherapy. Arq Bras Med Vet Zootec. 2010;62(5):1086–1093. http://dx.doi.org/10.1590/S0102-09352010000500010
28. Rossmeisl JH, Garcia PA, Pancotto TE, Robertson JL, Henao-Guerrero N, Neal RE, et al. Safety and feasibility of the NanoKnife system for irreversible electroporation ablative treatment of canine spontaneous intracranial gliomas. J Neurosurg;2015;123(4):1008–1025. http://dx.doi.org/10.3171/2014.12.JNS141768
29. Bagley RS, Gavin PR, Moore MP, Silver GM, Harrington ML, Connors RL. Clinical signs associated with brain tumors in dogs: 97 cases (1992–1997). J Am Vet Med Assoc. 1999;215:818–819.
30. Iliopoulou MA, Kitchell BE, Yuzbasiyan-Gurkan V. Development of a survey instrument to assess health-related quality of life in small animal cancer patients treated with chemotherapy. J Am Vet Med Assoc. 2013;242(12):1679–87.
31. Lipsitz D, Higgins RJ, Kortz GD, Dickinson PJ, Bollen AW, Nayadan DK, et al. Glioblastoma multiforme: Clinical findings, magnetic resonance imaging, and pathology in five dogs. Vet Pathol. 2003;40:659–69. http://dx.doi.org/10.1354/vp.40-6-659
32. Stoica G, Kim HT, Hall DG, Coates JR. Morphology, immunohistochemistry, and genetic alterations in dog astrocytomas. Vet Pathol. 2004;41:10–19. http://dx.doi.org/10.1354/vp.41-1-10
33. Stoica G, Levine J, Wolff J, Murphy K. Canine astrocytic tumors: A comparative review. Vet Pathol. 2011;48(1):266–75. http://dx.doi.org/10.1177/0300985810389543
34. Young BD, Levine JM, Porter BF, Chen-Allen AV, Rossmeisl J, Platt SR, et al. Magnetic resonance imaging features of intracranial astrocytomas and oligodendrogliomas in dogs. Vet Radiol Ultrasound. 2011;52:132–41. http://dx.doi.org/10.1111/j.1740-8261.2010.01758.x
35. Rossmeisl JH, Clapp K, Pancotto TE, Emch S, Robertson JL, Debinski W. Canine butterfly glioblastomas: A neuroradiological review. Front Vet Sci. 2016;3:40.
36. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, et al. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathologica. 2007;114:97–109. http://dx.doi.org/10.1007/s00401-007-0243-4
37. Ostrom QT, Gittleman H, Fulop J, Liu M, Blanda R, Kromer C, et al. CBTRUS statistical report: Primary brain and central nervous system tumors diagnosed in the United States in 2008–2012. Neuro Oncol. 2015;17(S4):iv1–iv62. http://dx.doi.org/10.1093/neuonc/nov189
38. Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell. 2010;17:98–110. http://dx.doi.org/10.1016/j.ccr.2009.12.020
39. Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature. 2008;455:1061–1068. http://dx.doi.org/10.1038/nature07385
40. Boudreau CE, York D, Higgins RJ, LeCouteur RA, Dickinson PJ. Molecular signalling pathways in canine gliomas. Vet Comp Oncol. 2017;15(1):133–50. http://dx.doi.org/10.1111/vco.12147
41. York D, Higgins RJ, LeCouteur RA, Wolfe AN, Grahn R, Olby N, et al. TP53 mutations in canine brain tumors. Vet Pathol. 2012;49:796–801. http://dx.doi.org/10.1177/0300985811424734
42. Ide T, Uchida K, Kikuta F, Suzuki K, Nakayama H. Immunohistochemical characterization of canine neuroepithelial tumors. Vet Pathol. 2010;47:741–50. http://dx.doi.org/10.1177/0300985810363486
43. Higgins RJ, Dickinson PJ, Lecouteur RA, Bollen AW, Wang H, Corely LJ, et al. Spontaneous canine gliomas: Overexpression of EGFR, PDGFRalpha and IGFBP2 demonstrated by tissue microarray immunophenotyping. J Neuro Oncol. 2009;98:49–55. http://dx.doi.org/10.1007/s11060-009-0072-5
44. Dickinson PJ, Roberts BN, Higgins RJ, Leutenegger CM, Bollen AW, Kass PH, et al. Expression of receptor tyrosine kinases VEGFR-1 (FLT-1), VEGFR-2 (KDR), EGFR-1, PDGFRalpha and c-Met in canine primary brain tumours. Vet Comp Oncol. 2006;4:132–40. http://dx.doi.org/10.1111/j.1476-5829.2006.00101.x
45. Dickinson PJ, Sturges BK, Higgins RJ, Roberts BN, Leutenegger CM, Bollen AW, et al. Vascular endothelial growth factor mRNA expression and peritumoral edema in canine primary central nervous system tumors. Vet Pathol. 2008;45:131–9. http://dx.doi.org/10.1354/vp.45-2-131

46. Rossmeisl JH, Duncan RB, Huckle WR, Troy GC. Expression of vascular endothelial growth factor in tumors and plasma from dogs with primary intracranial neoplasms. Am J Vet Res. 2007;68:1239–45. http://dx.doi.org/10.2460/ajvr.68.11.1239
47. Gibo DM, Debinski W, Bonomo J, Rossmeisl J, Robertson J, Dickinson P, et al. Interleukin 13 receptor alpha-2 is widely over-expressed in human and canine primary brain tumors as detected by novel bispecies-specific monoclonal antibodies. Neuro Oncol. 2012;14:vi47.
48. Debinski W, Dickinson P, Rossmeisl JH, Robertson J, Gibo DM. New agents for targeting of IL-13RA2 expressed in primary human and canine brain tumors. PLoS One. 2013;8:e77719. http://dx.doi.org/10.1371/journal.pone.0077719
49. Sturges BK, Dickinson PJ, Aina OH, York D, LeCouteur RA, Lam KS. Identification of novel targeting peptides for canine glioma. J Vet Intern Med. 2008;22:771.
50. Mariani CJ. Mariani CJ. Matrix metalloproteinase-2 and -9 in canine central nervous system tumors. In: Proceedings of the American College of Veterinary Internal Medicine Forum, New Orleans, LA, May 30–June 2, 2012, p. 421.
51. Rossmeisl JH. Exploiting proteases and their inhibitors as targets for the treatment of canine brain tumors. In: Proceedings of the American College of Veterinary Internal Medicine Forum, Denver, CO, June 8–11, 2016, pp. 518–21.
52. Platt S, Nduom E, Kent M, Freeman C, Machaidze R, Kaluzova M, et al. Canine model of convection-enhanced delivery of cetuximab-conjugated iron-oxide nanoparticles monitored with magnetic resonance imaging. Clin Neurosurg. 2012;59:107–13. http://dx.doi.org/10.1227/NEU.0b013e31826989ef
53. Betensky RA, Louis DN, Cairncross JG. Influence of unrecognized molecular heterogeneity on randomized clinical trials. J Clin Oncol. 2002;20:2495–9. http://dx.doi.org/10.1200/JCO.2002.06.140
54. Reitman ZJ, Olby NJ, Mariani CL, Thomas R, Breen M, Bigner DD, et al. IDH1 and IDH2 hotspot mutations are not found in canine glioma. Int J Cancer. 2010;127:245–6. http://dx.doi.org/10.1002/ijc.25017
55. Thomas R, Duke SE, Wang HJ, Breen TE, Higgins RJ, Linder KE, et al. "Putting our heads together": Insights into genomic conservation between human and canine intracranial tumors. J Neurooncol. 2009;94:333–49. http://dx.doi.org/10.1007/s11060-009-9877-5
56. Pluhar GE, Grogan PT, Seiler C, Goulart M, Sanatcruz KS, Carlson C, et al. Anti-tumor immune-response correlates with neurological symptoms in a dog with spontaneous astrocytoma treated by gene and vaccine therapy. Vaccine. 2010;28:3371–8. http://dx.doi.org/10.1016/j.vaccine.2010.02.082
57. CBS Interactive, Inc. Man's best friend: Key to brain cancer cure? CBS News (www.cbsnews.com). 2011. Available from: http://www.cbsnews.com/video/watch/?id=7390631n&tag=cbsnewsMainColumnArea (accessed January 5, 2017).
58. Andersen BM, Pluhar GE, Seiler CE, Goulart MR, Sanatacruz KS, Schutten MM, et al. Vaccination for invasive canine meningioma induces in situ production of antibodies capable of antibody-dependent cell-mediated cytotoxicity. Cancer Res. 2013;73:2987–97. http://dx.doi.org/10.1158/0008-5472.CAN-12-3366
59. Dickinson PJ, LeCouteur RA, Higgins RJ, Bringas JR, Roberts B, Larson RF, et al. Canine model of convection-enhanced delivery of liposomes containing CPT-11 monitored with real-time magnetic resonance imaging: Laboratory investigation. J Neurosurg. 2008;108:989–98. http://dx.doi.org/10.3171/JNS/2008/108/5/0989
60. Dickinson PJ, LeCouteur RA, Higgins RJ, Bringas JR, Larson RF, Yamashita Y, et al. Canine spontaneous glioma: A translational model system for convection-enhanced delivery. Neuro Oncol. 2010;12:928–40. http://dx.doi.org/10.1093/neuonc/noq046
61. Varenika V, Dickinson PJ, Bringas J, LeCouteur R, Higgins R, Park J, et al. Detection of infusate leakage in the brain using real-time imaging of convection-enhanced delivery. J Neurosurg 2008;109:874–80. http://dx.doi.org/10.3171/JNS/2008/109/11/0874
62. Bobo RH, Laske DW, Akbasak A, Morrison PF, Dedrick RL, Oldfield EH. Convection-enhanced delivery of macromolecules in the brain. Proc Natl Acad Sci U S A. 1994;91:2076–80. http://dx.doi.org/10.1073/pnas.91.6.2076
63. Vogelbaum MA. Convection-enhanced delivery for treating brain tumors and selected neurological disorders: A review. J Neurooncol. 2007;83:97–109. http://dx.doi.org/10.1007/s11060-006-9308-9

64. Debinski W, Tatter SB. Convection-enhanced delivery for the treatment of brain tumors. Exp Rev Neurother. 2009;9:1519–27. http://dx.doi.org/10.3171/jns.1995.82.6.1021
65. Lieberman DM, Laske DW, Morrison PF, Bankiewicz KS, Oldfield EH. Convection-enhanced distribution of large molecules in gray matter during interstitial drug infusion. J Neurosurg. 1995;82:1021–9.
66. Sampson JH, Archer G, Pedain C, Wembacher-Schroder E, Westphal M, Kunwar S, et al. Poor drug distribution as a possible explanation for the results of the PRECISE trial. J Neurosurg 2010;113: 301–9. http://dx.doi.org/10.3171/2009.11.JNS091052
67. Kunwar S, Chang S, Westphal M, Vogelbaum M, Sampson J, Barnett G, et al. Phase III randomized trial of CED of IL13-PE38QQR vs Gliadel wafers for recurrent glioblastoma. Neuro Oncol. 2010;12(8):871–81. http://dx.doi.org/10.1093/neuonc/nop054
68. Gibo DM, Dickinson P, Robertson J, et al. Highly potent toxin targeting IL-13Ra2 in canine and human glioblastoma. Neuro Oncol. 2012;14:vi31–2.
69. Debinski W, Dickinson PJ, Rossmeisl JH, Roberetson JL, Gibo DM. Canine gliomas over-express IL-13Ralpha2, EphA2 and Fra-1 in common with human high-grade astrocytomas. Neuro Oncol. 2009;9:535.
70. Sampson JH, Raghaven R, Brady ML, Provenzale JM, Herndon JE, II, Croteau D, et al. Clinical utility of a patient-specific algorithm for simulating intracerebral drug infusions. J Neuroocol. 2007;9: 343–53. http://dx.doi.org/10.1215/15228517-2007-007
71. Rosenbluth KH, Martin AJ, Mittermeyer S, Eschermann J, Dickinson PJ, Bankiewicz KS. Rapid inverse planning for pressure-driven drug infusions in the brain. PLoS One. 2013;8(2):e56397. http://dx.doi.org/10.1371/journal.pone.0056397
72. Rossmeisl JH, Garcia PA, Daniel GB, Bourland JD, Debinski W, Dervisis N, et al. Invited review-Neuroimaging response assessment criteria for brain tumors in veterinary patients. Vet Radiol Ultrasound. 2014;55:115–132. http://dx.doi.org/10.1111/vru.12118
73. Davalos RV, Mir LM, Rubinsky B. Tissue ablation with irreversible electroporation. Ann Biomed Eng. 2005;33:223–31. http://dx.doi.org/10.1007/s10439-005-8981-8
74. Al-Sakere B, Andre F, Bernat C, Connault E, Opolon P, Davalos RV, et al. Tumor ablation with irreversible electroporation. PLoS One. 2007;2:e1135. http://dx.doi.org/10.1371/journal.pone.0001135
75. Rubinsky B. Irreversible electroporation in medicine. Technol Cancer Res Treat. 2007;6:255–9. http://dx.doi.org/10.1177/153303460700600401
76. Neal RE, II, Rossmeisl JH, Jr, Garcia PA, Lanz OI, Henao-Guerrero N, Davalos RV. Successful treatment of a large soft-tissue sarcoma with irreversible electroporation. J Clin Oncol. 2011;29:e372–7. http://dx.doi.org/10.1200/JCO.2010.33.0902
77. Garcia PA, Pancotto TE, Rossmeisl JH, Jr, Henao-Guerrero N, Gustafson NR, Daniel GB, et al. Non-thermal irreversible electroporation (N-TIRE) and adjuvant fractionated radiotherapeutic multimodal therapy for intracranial malignant glioma in a canine patient. Technol Cancer Res Treat. 2011; 10:73–83. http://dx.doi.org/10.7785/tcrt.2012.500181
78. Thomson KR, Cheung W, Ellis SJ, Fdereman D, Kavnoudias H, Loader-Oliver D, et al. Investigation of the safety or irreversible electroporation in humans. J Vasc Interv Radiol. 2011;22(5):611–21. http://dx.doi.org/10.1016/j.jvir.2010.12.014
79. Ellis TL, Garcia PA, Rossmeisl JH, Henao-Guerrero N, Robertson J, Davalos RV. Nonthermal irreversible electroporation for intracranial surgical applications: Laboratory investigation. J Neurosurg. 2011;114:681–8. http://dx.doi.org/10.3171/2010.5.JNS091448
80. Arena CB, Sano MB, Rossmeisl JH, Caldwell JL, Garcia PA, Rylander MN, et al., High-frequency irreversible electroporation (H-FIRE) for non-thermal ablation without muscle contraction. Biomed Eng Online. 2011;10(1):102. http://dx.doi.org/10.1186/1475-925X-10-102
81. Arena CB, Sano MB, Rylander MN, Davalos RV. Theoretical considerations of tissue electroporation with high-frequency bipolar pulses. IEEE Trans Biomed Eng. 2011;58(5):1474–82. http://dx.doi.org/10.1109/TBME.2010.2102021
82. Ivey JW, Latouche EL, Sano MB, Rossmeisl JH, Davalos RV, Verbridge SS. Targeted cellular ablation based on the morphology of malignant cells. Sci Rep. 2015;5:17157. http://dx.doi.org/10.1038/srep17157
83. Garcia PA, Rossmeisl JH, Jr, Robertson JL, Olson JD, Johnson AJ, Ellis TL, et al. 7.0-T magnetic resonance imaging characterization of acute blood-brain-barrier disruption achieved with

intracranial irreversible electroporation. PLoS One. 2012;7:e50482. http://dx.doi.org/10.1371/journal.pone.0050482
84. Hjouj MD, Last D, Guez D, Daniels D, Sharabi S, Lavee J, et al. MRI study on reversible and irreversible electroporation induced blood brain barrier disruption. PLoS One. 2012;7:e42817. http://dx.doi.org/10.1371/journal.pone.0042817
85. Arena CB, Garcia PA, Sano MB, Olson JD, Rogers-Cotrone T, Rossmeisl JH, Jr, et al. Focal blood-brain-barrier disruption with high-frequency pulsed electric fields. Technology. 2014;2(3):206–13. http://dx.doi.org/10.1142/S2339547814500186
86. Rossmeisl JH, Jr, Garcia PA, Pancotto TE, Robertson JL, Henao-Guerrero N, Neal RE, et al. Safety and feasibility of the Nanoknife system for irreversible electroporation ablative treatment of canine spontaneous intracranial gliomas. J Neurosurg. 2015;123(4):1008–25. http://dx.doi.org/10.3171/2014.12.JNS141768
87. Zupanic A, Kos B, Miklavcic D. Treatment planning of electroporation-based medical interventions: Electrochemotherapy, gene electrotransfer and irreversible electroporation. Phys Med Biol. 2012;57(17):5425. http://dx.doi.org/10.1088/0031-9155/57/17/5425
88. Corovic S, Lackovic I, Sustaric P, Sustar T, Rodic T, Miklavcic D. Modeling of electric field distribution in tissues during electroporation. BioMed Eng Online. 2013;12(1):1–27. http://dx.doi.org/10.1186/1475-925X-12-16
89. Rossmeisl JH, Garcia PA, Robertson JL, Ellis TL, Davalos RV. Pathology of non-thermal irreversible electroporation (N-TIRE) induced ablation of the canine brain. J Vet Sci. 2013;14(4):433–40. http://dx.doi.org/10.4142/jvs.2013.14.4.433
90. Garcia PA, Kos B, Rossmeisl Jr., JH, Pavliha D, Miklavcic D, Davalos RV. Predictive therapeutic planning for irreversible electroporation treatment of spontaneous malignant glioma. Med Phys 2017; e-pub 6/8/17; doi:10.1002/mp.12401
91. Marčan M, Pavliha D, Kos B, Forjanič T, Miklavčič D. Web-based tool for visualization of electric field distribution in deep-seated body structures and planning of electroporation-based treatments. BioMed Eng Online. 2015;14(Suppl 3):S4. http://dx.doi.org/10.1186/1475-925X-14-S3-S4
92. Garcia PA, Davalos RV, Miklavcic D. A numerical investigation of the electric and thermal cell kill distributions in electroporation-based therapies in tissue. PLoS One. 2014;9:e103083. http://dx.doi.org/10.1371/journal.pone.0103083
93. Kranjc M, Bajd F, Sersa I, Woo EJ, Miklavcic D: Ex Vivo and in silico feasibility study of monitoring electric field distribution in tissue during electroporation based treatments. PLoS One. 2012;7:e45737. http://dx.doi.org/10.1371/journal.pone.0045737
94. Lynn JG, Zwemer RL, Chick AJ, Miller AE. A new method for the generation and use of focused ultrasound in experimental biology. J Gen Physiol. 1942;26:179–93. http://dx.doi.org/10.1085/jgp.26.2.179
95. Barnard JW, Fry WJ, Fry FJ, Krumins RF. Effects of high intensity ultrasound on the central nervous system of the cat. J Comp Neurol. 1955;103:459–84. http://dx.doi.org/10.1002/cne.901030304
96. Fry WJ, Brennan JF, Barnard JW. Histological study of changes produced by ultrasound in the gray and white matter of the central nervous system. Ultrasound Med Biol. 1957;3:110–30.
97. Astrom KE, Belle, Ballantine HT, Heidensleben E. An experimental neuropathological study of the effects of high-frequency focused ultrasound on the brain of the cat. J Neuropathol Exp Neurol. 1961;20:484–520. http://dx.doi.org/10.1097/00005072-196120040-00002
98. Meyers R, Fry WJ, Fry FJ, Dreyer LL, Schultz DF, Noyes RF. Early experiences with ultrasonic irradiation of the pallidfugal and nigral complexes in hyperkinetic and hypertonic disorders. J Neurosurg. 1959;16:32–54. http://dx.doi.org/10.3171/jns.1959.16.1.0032
99. Hickey RC, Fry WJ, Meyers R, Fry FJ, Bradbury JT. Human pituitary irradiation with focused ultrasound. An initial report on effect in advanced breast cancer. Arch Surg. 1961;83:620–33. http://dx.doi.org/10.1001/archsurg.1961.01300160132016
100. Tung YS, Marquet F, Teichert T, Ferrera V, Konofagou EE. Feasibility of noninvasive cavitation-guided blood-brain barrier opening using focused ultrasound and microbubbles in nonhuman primates. Appl Phys Lett. 2011;98:163704. http://dx.doi.org/10.1063/1.3580763

101. Fry FJ, Goss SA. Further studies of the transkull transmission of an intense focused ultrasonic beam: Lesion production at 500 kHz. Ultrasound Med Biol. 1980;6:33–8. http://dx.doi.org/10.1016/0301-5629(80)90061-7
102. Leinenga G, Langton C, Nisbet R, Gotz J. Ultrasound treatment of neurological diseases-current and emerging applications. Nat Rev Neurol. 2016;12:161–74. http://dx.doi.org/10.1038/nrneurol.2016.13
103. Ishihara Y, Calderon A, Watanabe H, Okamoto K, Suzuki Y, Kuroda K. A precise and fast temperature mapping using water proton chemical shift. Magn Reson Med. 1995;34:814–23. http://dx.doi.org/10.1002/mrm.1910340606
104. Xu Z, Ludomirsky A, Eun LY, Hall TL, Tran BC, Fowlkes JB, et al. Controlled ultrasound tissue erosion. IEEE Trans Ultrason Ferroelectr Freq Control. 2004;51(6):726–36. http://dx.doi.org/10.1109/TUFFC.2004.1308731
105. Hynynen K. Focused ultrasound for blood-brain disruption and delivery of therapeutic molecules to the brain. Expert Opin Drug Delive. 2007;4(1):27–35.
106. Zhi R, Cohen ZR, Harnof S, Tal S, Faibel M, Nass D, et al. Magnetic resonance image-guided, high-intensity focused ultrasound for brain tumor therapy. Neurosurgery. 2006;59(5):949–56.
107. Hynynen K, Jolesz FA. Demonstration of potential noninvasive ultrasound brain therapy through an intact skull. Ultrasound Med Biol. 1998;24:275–83. http://dx.doi.org/10.1016/S0301-5629(97)00269-X
108. Sun J, Hynynen K. The potential of transskull ultrasound therapy and surgery using the maximum available skull surface area. J Acoust Soc Am. 1999;105:2519–27. http://dx.doi.org/10.1121/1.426863
109. Coluccia D, Fandino J, Schwyzer L, O'Gorman R, Remonda L, Anon J, et al. First non-invasive thermal ablation of a brain tumor with MR-guided focused ultrasound. J Ther Ultrasound. 2014;2:17. http://dx.doi.org/10.1186/2050-5736-2-17
110. Marquet F, Teichert T, Wu Y-S, Downs M, Wang S, Chen C, et al. Real-time transcranial monitoring of safe blood-brain barrier opening in non-human primates. PLoS One. 2014;9:e84310. http://dx.doi.org/10.1371/journal.pone.0084310