Supplementary Material- Methods

Magnetic Resonance Imaging (MRI) Protocol

MRI images were obtained at 1.5T (Intera, Philips Healthcare, Andover, MA, USA) with patients imaged in an 8-channel head coil. Standard sequences obtained in all patients included pre- and post-contrast T1-weighted images in three planes, pre- and post-contrast 3DT1-weighted gradient recall echo, T2-weighted turbo spin echo in the sagittal and transverse planes, T2*-weighted gradient echo in the transverse plane, T2-weighted fluid attenuated inversion recovery (FLAIR) in the transverse plane, and diffusion tensor imaging (DTI).

Volumetric tumor quantification was determined using image analysis software (OsiriX MD, Pixmeo Sarl, Geneva, Switzerland). Volumes were defined from post-contrast T1-weighted MR images, as all tumors demonstrated contrast enhancement. Manually defined regions of interest (ROIs) were generated for individual contiguous MR image slices, and volumes calculated with ROI volume software.

HFIRE Therapeutic Planning

A. Tissue segmentation and finite element analyses (FEA)

Critical structures such as the cranium, brain parenchyma, tumor, ventricles, and major vasculature were reconstructed and segmented from stacked MR images (Osirix MD). Two small cylinders (18AWG) of 5mm in length were used to represent the exposed electrodes in the tissue.

i. Electric field distribution

The 3D segmented model was then imported into COMSOL Multiphysics (Version 5.2, COMSOL Inc., Palo Alto, CA, USA) for meshing and FEA (Fig. 2C). The number of elements per mesh varied on a patient-specific basis ranging from 9,338-24,520 elements. FEA solutions per probe-pair were found in less than 1 minute on a 3.4 GHz Intel Core i7-6700 processor. The electric field distribution during HFIRE pulses can be described by the following differential equation:

$$[\nabla \cdot (\sigma_d \nabla \varphi) = 0] \quad \text{(Equation 1)}$$

Where σ_d represents the electrical conductivity of the tissue, which will have dynamic properties dependent of the magnitude of the electric field, and φ is the voltage applied. This dynamic behavior has been observed across several tissues by different groups.[1-4]

The sigmoidal curve representing σ_d is mainly composed of a baseline conductivity σ_0, a change in conductivity established by the conductivity of tissue that has been electroporated σ_{EP}, and a transition zone. In this first study the change in electrical conductivity was calculated from previous results of IRE in brain tissue as $\sigma_{EP} = 2.5*\sigma_0$.[2] This curve was modeled in COMSOL by defining σ_d as a step function located at 600V/cm (reversible EP threshold) and going from σ_0 to σ_{EP}. In "smoothing" settings, the transition zone for the function was given a range of 100V/cm and the continuous derivative value for the function was assigned to 2. Full treatment simulation was carried by solving for 1 electrode-pair each time, one electrode boundary was set to $\varphi = V_0$ while the other pairing-electrode boundary was set to $\varphi = 0$. All external boundaries were treated as thermally and electrically insulative.

Solutions for FEA of electric field distributions predicted all ablations to be contiguous when assuming 1000V/cm to be the threshold for cell death. For Dog 1, contiguous ablations with a volume of 0.39 and 0.49 cm^3 were predicted assuming a lethal threshold of 500V/cm for electrode-pairs 1 and 2, respectively. In Dog 2, it was intentionally planned for subtotal resection of the tumor surrounding the rostral aspect of the dorsal sagittal sinus (DSS-ROI) to occur to evaluate the effects of HFIRE on situ tumor. Therefore, complete ablation of the *in situ* tumor remaining in the DSS-ROI, which had volume of 1.83 cm^3, was performed. This required four electrode insertions, with each initial insertion followed by a more superficial ablation performed by retracting the electrodes of 5-6 mm towards the brain surface along the same electrode trajectory, for a total of 8 ablations (T1-T8) in the DSS-ROI. A conservative approach was used in order to avoid ablation of healthy brain parenchyma and considered ablations with lower lethal thresholds (500-700V/cm) based on previously reported IRE and HFIRE thresholds for brain tissue.[5,6] At 500V/cm the DSS-ROI was fully covered with some ablation extending beyond the ROI margin (~4mm) and at 700V/cm 94% of the DSS-ROI was expected to be covered. For Dog 2, ablations 1-8 were expected to result in similar ablation volumes to those predicted for patient 1, while for ablations 9 and 10 volumes of 1.21 and 1.29 cm^3, respectively. In Dog 2, after encountering intraoperative hemorrhage during tumor resection, resection of the tumor surrounding the dorsal sagittal sinus (DSS-ROI) was completed using an ultrasonic surgical aspirator, which preclude morphological evalution of ablation T1-T8 . For Dog 3, one contiguous ablation with a volume of 0.20 cm^3 was predicted assuming lethal threshold of 500V/cm.

ii) Joule heating

Although HFIRE's primary mechanism of action is non-thermal, if inappropriately delivered thermal damage from Joule heating may occur. Temperature increments from resistive losses were calculated by solving the following Joule heating equation:

$$[\nabla \cdot (k\nabla T) + \frac{\sigma|\nabla\varphi|^2 \cdot d}{\tau} = \rho c_p \frac{\delta T}{\delta t} \quad] \text{(Equation 2)}$$

where, specific to brain tissue, T is the temperature (started at 37°C), k is the thermal conductivity, c_p is the heat capacity, and ρ is the density. $\sigma|\nabla\varphi|^2$ is the Joule heating term, which was altered to consider the energy delivered per second using a duty cycle approach. This method is represented by the ratio of pulse duration d and pulse interval τ and is used to significantly reduces computational time.[7] Values used for variables relevant to equations 1 and 2 can be found in **Table S1**.

Table S1: Physical properties used for FEA of HFIRE in canine patients with brain tumors

Parameter	Symbol	Value	Units	Reference
Baseline electrical conductivity	σ_0	0.12	[S/m]	[8]
Conductivity of electroporated tissue	σ_{EP}	0.30	[S/m]	[2]
Brain tissue density	ρ	1060	[kg/m^3]	[9]
Brain tissue heat capacity	c_p	3680	[J/(kg*K)]	[10]
Brain tissue thermal conductivity	k	0.565	[W/(m*K)]	[11]
Duty cycle	d/τ	0.001	-	-
Electrode electrical conductivity	σ_e	2.22E6	[S/m]	[7]
Electrode density	ρ_e	7900	[kg/m^3]	[7]
Electrode heat capacity	c_p	500	[J/(kg*K)]	[7]
Electrode thermal conductivity	k_p	15	[W/(m*K)]	[7]

Results indicated that delivery of a 100us burst at a frequency of 1Hz would not result in significant thermal damage even when increasing σ_{EP} to extreme values such as 1S/m.[12] Maximum predicted temperature for clinical procedures (σ_{EP} = 0.3) would never reach 43°C (T_{max} = 39.7°C), while for extreme cases (σ_{EP} = 1) some tissue (<0.24cm^3) reached temperatures about 43°C but this exposure did not last longer than 3 min (T_{max} = 47.0°C). It is important to note these maximum temperatures occurred at the electrode-tissue boundary and they were only near these values for less than 1 minute. Calculations performed indicated that in order to reach

these extreme temperatures amperage must consistently approach of 5.76A, and all clinical values recorded were consistently below 5A.

Supplementary Content-Methods References

1. Neal RE, Garcia PA, Robertson JL, Davalos RV. Experimental characterization and numerical modeling of tissue electrical conductivity during pulsed electric fields for irreversible electroporation treatment planning. *IEEE Trans Bio-Med Eng*. 2012;59(4):1076-85.
2. Garcia PA, Rossmeisl JH, Davalos RV. Electrical conductivity changes during irreversible electroporation treatment of brain cancer. *2011 Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)*. 2011:739-42.
3. Pavlin M, Miklavcic D. Theoretical and experimental analysis of conductivity, ion diffusion and molecular transport during cell electroporation--relation between short-lived and long-lived pores. *Bioelectrochemistry*. 2008;74(1):38-46.
4. Ivorra A, Rubinsky B. In vivo electrical impedance measurements during and after electroporation of rat liver. *Bioelectrochemistry*. 2007;70(2):287-95.
5. 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.
6. Garcia P, Rossmeisl J, Neal R, Ellis TL, Olson JD, Henao-Guerrero N, et al. Intracranial nonthermal irreversible electroporation: In vivo analysis. *J Membr Biol*. 2010;236(1):127-36.
7. Garcia PA, Rossmeisl JH, Jr., Neal RE, 2nd, Ellis TL, Davalos RV. A parametric study delineating irreversible electroporation from thermal damage based on a minimally invasive intracranial procedure. *Biomed Eng Online*. 2011;10:34.
8. Hasgall P, Neufeld E, Gosselin M, Klingenböck A, Kuster N. IT'IS Database for thermal and electromagnetic parameters of biological tissues. Version 2.4, July 30th, 2013. *Zurich, Switzerland: IT'IS Foundation. www. itis. ethz. ch/database [accessed January 9, 2015]*.
9. Asami K, Takahashi Y, Takashima S. Dielectric-properties of mouse lymphocytes and erythrocytes. *Biochim Biophys Acta*. 1989;1010(1):49-55.
10. Sano MB, Henslee EA, Schmelz EM, Davalos RV. Contactless dielectrophoretic spectroscopy: Examination of the dielectric properties of cells found in blood. *Electrophoresis*. 2011;32(22):3164-71.

11. Alberts B, Bray D, Lewis J, et al. Molecular biology of the cell (3rd edn). *Trends in Biochemical Sciences*. 1995;20(5):210-210.
12. Yarmolenko PS, Moon EJ, Landon C, Manzoor A, Hochman DW, Viglianti BL, et al. Thresholds for thermal damage to normal tissues: an update. *Int J Hyperthermia*. 2011;27(4):320-43.

Supplementary Material- Videos

Video 1: Dog 3, Pre-HFIRE- Baseline neurological examination of Dog 3 on Day 1, prior to HFIRE treatment, demonstrating proprioceptive positioning and hopping deficits in the left pelvic limb.

Video 2: Dog 3, Post-HFIRE- Neurological examination of Dog 3 on Day 3, 12 hours after HFIRE treatment, illustrating a clinical status that remained unchanged from baseline.