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Supplementary Information for

Localized blood-brain barrier opening in infiltrating gliomas with MRI-guided acoustic emissions-controlled focused ultrasound

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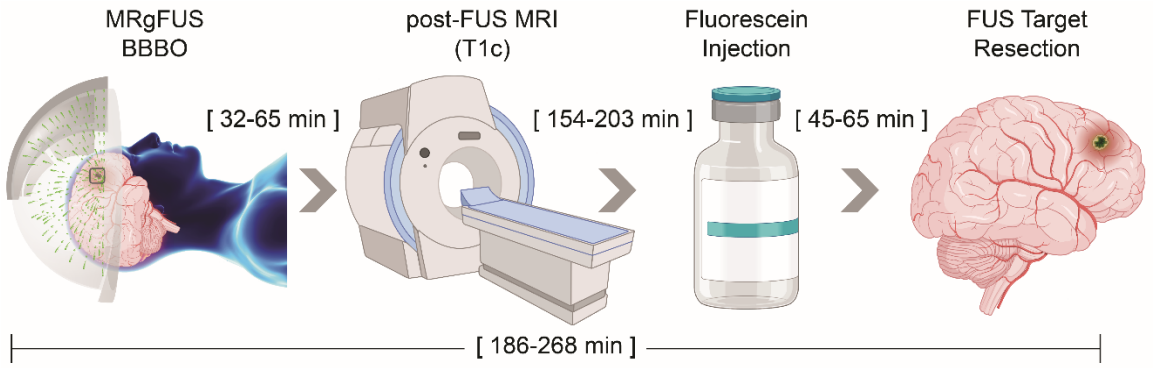

Fig. S1. Study timeline and associated intervals: The timeline indicates the stages of the study protocol and the time intervals between the key components: MB-FUS treatment (left), the post-FUS contrast-enhanced (T1c) MRI scan (second from left), the fluorescein injection in the operating room (third from left), and the stereotactically localized tumor resection (right). The time interval range is given in brackets [min = minutes]. Created with BioRender and Adobe® Illustrator.

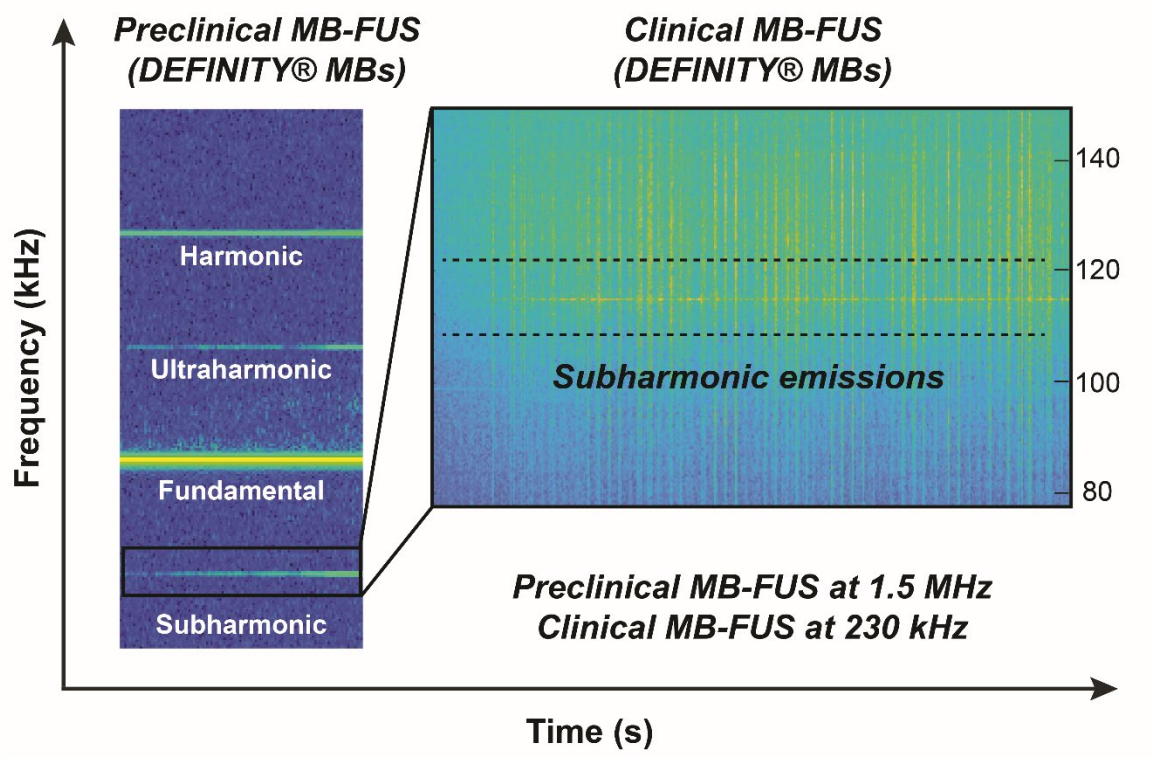

Fig. S2. Acoustic emissions data from MB-FUS treatment in an *in vivo* murine model system: Spectrograms were recorded as a function of time from a hydrophone during MB-FUS treatments in mice after intravenous injection of perflutren lipid microspheres (DEFINITY®) at 5 µL/kg. Consistent acoustic emissions patterns show the strong emission band at the fundamental frequency (relating directly to the center frequency of the transducer) and the characteristic harmonic bands above (e.g., ultraharmonics) and below (e.g., subharmonic) the fundamental frequency (left panel). Given prior evidence that the subharmonic band (enlarged inset from clinical treatments; right) corresponds to stable oscillations of circulating MBs, the clinical acoustic emissions monitoring system was tuned to monitor and record subharmonic emissions (right panel) during the course of each MB-FUS treatment.

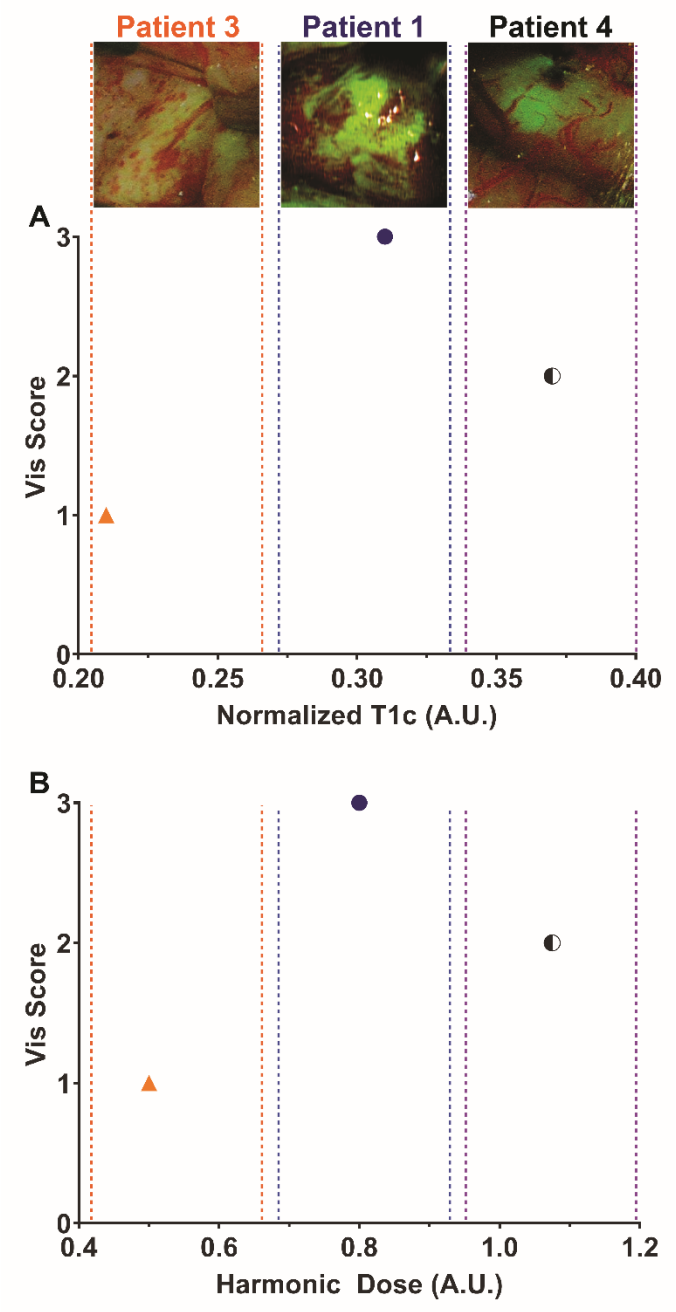

Fig. S3. The intraoperative visualization grade correlates with both the new T1c signal and harmonic dose: The surgeon-defined visualization grade of intra-operative fluorescence during stereotactically-localized resection (top images) of the MB-FUS treated region varied with the (A) new T1c intensity and (B) harmonic dose level. The scores were graded based on clear visualization (score = 3), moderate visualization (score = 2), minimal visualization (score = 1), or no visualization (score = 0).

Table S1. Study inclusion and exclusion criteria.

Inclusion criteria

1. Male or Female between 21-85 years of age.
2. Able and willing to give informed consent.
3. Subjects with suspected infiltrating glioma on preoperative brain imaging scans including non-enhancing MRI tumor components in non-eloquent regions that are within the planned surgical resection volume.
4. Surgical area targeted for ExAblate® treatment (e.g., prescribed Region of Treatment) ≤30 cm^3; planned surgical resection volume may exceed the targeted treatment volume.
5. Karnofsky Performance Score 70-100.
6. Able to communicate sensations during the ExAblate® BBB opening procedure.

Exclusion criteria

1. MRI or clinical findings of:
    - Active or chronic infection(s) or inflammatory processes.
    - Acute or chronic hemorrhages, specifically any lobar microbleeds, and no siderosis, amyloid angiopathy, or microhemorrhages.
    - Intracranial thrombosis, vascular malformation, cerebral aneurysm, or vasculitis.
    - Evidence of tumor-related calcification, cyst, or hemorrhage.
    - Midline shift of >10mm or evidence of subfalcine, uncal, or tonsillar herniation on preoperative imaging.
2. More than 30% of the skull area traversed by the sonication pathway is covered by scars, scalp disorders (e.g., eczema), or atrophy of the scalp.
3. Clips, shunts, or any metallic implanted objects in the skull or the brain or the presence of unknown MR unsafe devices anywhere within the body.
4. Significant cardiac disease or unstable hemodynamic status.
5. Uncontrolled hypertension (systolic > 150 and diastolic BP > 100 on medication).
6. Receiving anticoagulant (e.g., warfarin) or antiplatelet (e.g., aspirin) therapy within one week of focused ultrasound procedure or drugs known to increase risk of hemorrhage (e.g., Avastin) within one month of focused ultrasound procedure.
7. History of liver disease, bleeding disorder, coagulopathy, or a history of spontaneous hemorrhage.
8. Abnormal coagulation profile (Platelets < 100,000), PT (>14) or PTT (>36), and INR > 1.3
9. Lacunar lesions or evidence of increased risk of bleeding.
10. Known cerebral or systemic vasculopathy.
11. Significant depression and at potential risk of suicide.
12. Known sensitivity/allergy to gadolinium or other intravascular contrast agents.
13. Active seizures despite medication treatment (defined as >1 seizure per month) which could be worsened by disruption of the blood-brain barrier.
14. Evidence of worsening neurological function.
15. Dexamethasone dose ≥ 24mg daily or equivalent steroid dose.
16. History of drug or alcohol disorder, which has a higher risk for seizures, infection and/or poor executive functioning.
17. Positive HIV status, which can lead to the increased entry of HIV into the brain parenchyma leading to HIV encephalitis.
18. Potential blood-borne infections which can lead to increased entry to brain parenchyma leading to meningitis or brain abscess.

19. Any contraindications to MRI scanning, including:
    - Large subjects not fitting comfortably into the scanner
    - Difficulty lying supine and still for up to 3 hours in the MRI unit or claustrophobia
20. Untreated, uncontrolled sleep apnea.
21. Impaired renal function with estimated glomerular filtration rate <30 mL/min/1.73m^2.
22. Respiratory: chronic pulmonary disorders (e.g., severe emphysema, pulmonary vasculitis), or other causes of reduced pulmonary vascular cross-sectional area, patients with a history of drug allergies, asthma or hay fever, and multiple allergies where the benefit/risk of administering DEFINITY® is considered unfavorable by the study physicians in relation to the product labeling for DEFINITY®.
23. Currently in a clinical trial involving an investigational product or non-approved use of a drug or device.