

Review

Focused Ultrasound (FUS) and Pediatric Brain Tumors: Current Status and Future Directions

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Abstract

Diffuse intrinsic pontine glioma (DIPG), or as it is newly redefined, diffuse midline glioma (DMG), remains one of the most horrific diagnoses in pediatric oncology. Aggressive and inaccessible to standard treatments, it is generally considered incurable. Focused ultrasound technology has developed over the last several decades as a noninvasive means to target various types of tumors in both adults and children. Recent advances, particularly in low-intensity focused ultrasound (LIFU), have opened new avenues for enhancing drug delivery and modulating the tumor microenvironment in these challenging tumors. This review provides a comprehensive overview of preclinical and clinical research developments in the use of LIFU for pediatric DMGs. We highlight key findings from animal models demonstrating improved blood–brain barrier (BBB) permeability, increased chemotherapeutic and nanoparticle delivery, and potential immunomodulatory effects of LIFU. Emerging clinical studies, including early-phase safety and feasibility trials, are also discussed, with attention to technical parameters, imaging guidance strategies, and biomarkers of response. The review concludes by addressing the challenges of translating LIFU into routine clinical practice, including device optimization for pediatric anatomy, regulatory hurdles, and the need for standardized treatment protocols. Collectively, these recent advances underscore the promise of LIFU as a minimally invasive, image-guided adjunct to current and future therapies for pediatric DMGs, warranting continued research and collaborative clinical efforts.

Keywords: diffuse midline glioma; focused ultrasound; pediatric oncology; drug delivery; blood–brain barrier



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1. Introduction

Brain tumors are the second most common childhood malignancy and the most common solid tumors in children, as well as the leading cause of cancer-related mortality [1]. Among pediatric central nervous system (CNS) tumors, diffuse midline gliomas account for approximately 10% to 15% and occur at an incidence of 0.35 per 100,000 children per year [2,3]. These tumors are WHO grade 4 gliomas that arise in the pons, thalamus and other midline structures, and they most commonly affect children between the ages of 5 and 9 [3].

Historically, treatment strategies in neuro-oncology have centered around three primary modalities: surgery, radiation therapy, and chemotherapy. Although progress in molecular classification and subtyping has significantly advanced our understanding of tumor biology and informed the development of targeted therapies, effective treatment remains limited. One of the major barriers to therapeutic advancement is the presence of the blood–brain barrier, which severely restricts drug delivery to the central nervous system [4].

Focused ultrasound (FUS) is a noninvasive therapeutic modality with a range of applications in both malignant and non-malignant conditions. While its clinical use has expanded in adult populations, it received FDA approval for use in pediatric patients only in 2020 [5]. FUS encompasses several approaches, including high-intensity focused ultrasound (HIFU), low-intensity focused ultrasound (LIFU), and sonodynamic therapy, all of which non-invasively transmit acoustic energy across the skull. These techniques rely on the transmission of mechanical energy at specific frequencies to target tissue, with differing biological effects based on frequency and intensity [6]. HIFU, typically operating at 650 kHz, induces thermal ablation of targeted tissue, whereas LIFU at lower frequencies (e.g., 220 kHz) can modulate neuronal activity and transiently open the BBB, particularly when combined with microbubble contrast agents (Figure 1) [1].

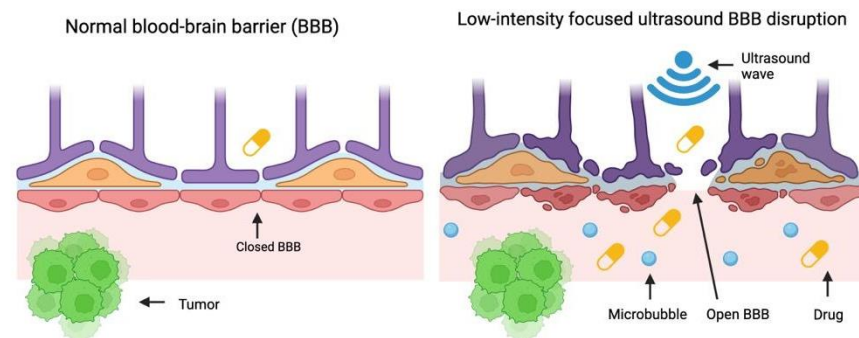


Figure 1. Mechanism of low-intensity focused ultrasound.

The ability of LIFU to transiently and reversibly disrupt the BBB presents a promising strategy to enhance drug delivery to infiltrative CNS tumors such as DMGs. Over the past two decades, numerous preclinical studies have demonstrated the feasibility and safety of this approach, with increasing evidence supporting its utility in enhancing delivery of chemotherapeutic agents, antibodies, and nanoparticle-based therapies to brain tumors. More recently, this work has begun to translate into early-phase clinical trials, reflecting growing interest in its potential clinical application.

This review provides an up-to-date overview of recent and ongoing preclinical and clinical studies using LIFU in the treatment of pediatric diffuse intrinsic pontine glioma/diffuse midline glioma, highlighting its evolving role in the neuro-oncologic treatment landscape and outlining current challenges and future directions.

2. Methodology

A scoping review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews (PRISMA-ScR) guidelines (Figure 2). To evaluate past and present preclinical studies regarding focused ultrasound, a comprehensive literature review was conducted using publications retrieved from PubMed using the keywords “low-intensity focused ultrasound”, “pediatric DIPG focused ultrasound” and “LIFU DMG”. Articles included peer-reviewed original research and technical reports regarding experiments which examined FUS-mediated BBB disruption in DIPG/DMG animal models during the time period 2021–2025. A systematic

review was also performed of active clinical studies from clinicaltrials.gov. Clinical trials that were excluded were those involving sonodynamic therapy as the focus of this review was low-intensity focused ultrasound. Subsequent investigation of any notable results of these trials thus far was ascertained through further PubMed search individualized to each NCT identification number.

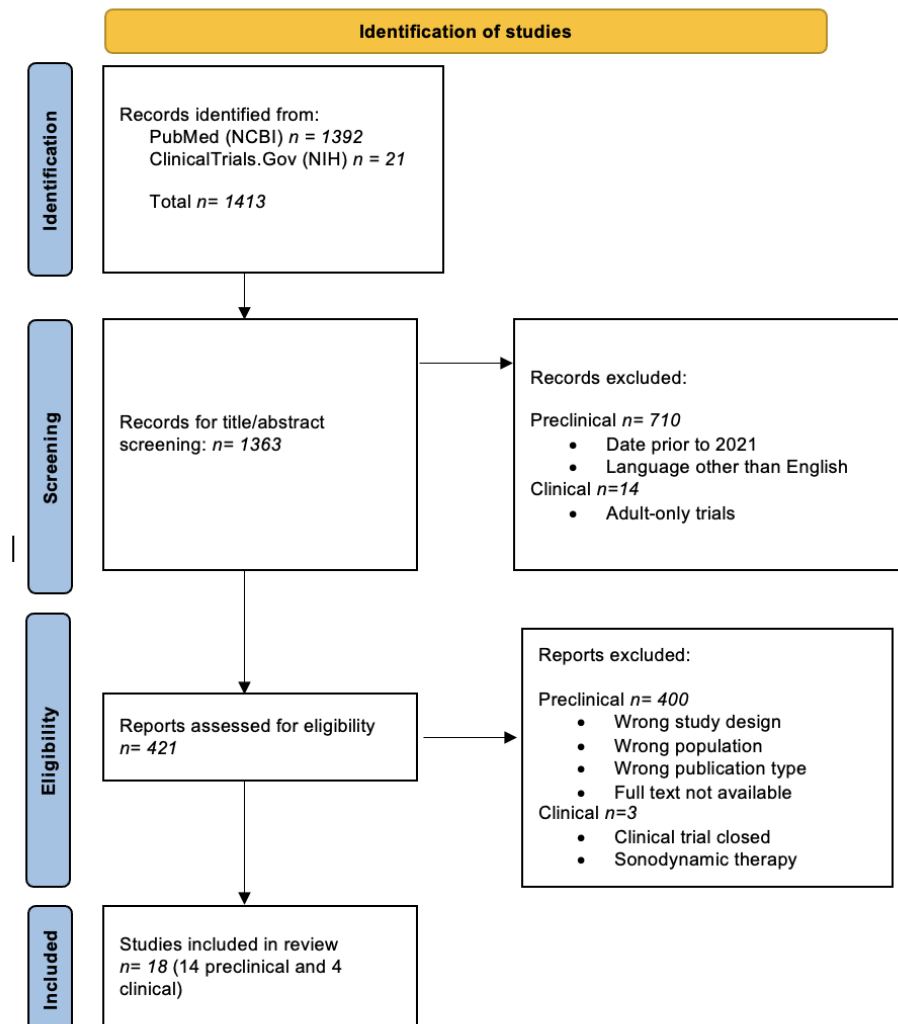


Figure 2. PRISMA flowchart for study identification.

From the selected preclinical and clinical sources, the following was collected for each: study design and model type, FUS modality (LIFU), therapeutic adjunct (chemotherapy, sonosensitizers), technical parameters, safety outcomes, feasibility, technical challenges, as well as early efficacy or biological endpoints, where available.

3. Results

In total, 14 preclinical analyses and four clinical trials of FUS in pediatric DMG in the selected time frame were found. DIPG models demonstrated overall that FUS-mediated BBB opening significantly enhances various therapeutic agent accumulation within tumors, for a wide variety of different chemotherapies, radiation, immunotherapies, and biologic modifiers. And several ongoing early-phase clinical trials are evaluating the safety and feasibility of these approaches in pediatric patients with DMG. Of note, due to high heterogeneity of the included studies, this review mainly carries out narrative synthesis rather than meta-analysis.

4. Preclinical Studies Evaluating LIFU and DMG

4.1. Chemotherapy

Doxorubicin

Doxorubicin, a compound of the anthracycline class, is well known as an extremely powerful antineoplastic drug with one of the broadest spectrums of activity, and has been well-studied for use in DIPG [7]. An initial preclinical study by Sewing et al. demonstrated that high-grade glioma and DIPG cells exhibit sensitivity to doxorubicin, while normal human astrocytes are relatively spared [8]. However, its clinical use in central nervous system tumors has been limited by its inability to cross the BBB due to its large size and hydrophilicity [5]. To circumvent these limitations, Filieri et al. highlighted the rationale for FUS-mediated BBB disruption to facilitate localized doxorubicin delivery [9].

In 2021, Kim et al. used a closed-loop, trans-skull MR-guided FUS hyperthermia system optimized at ~1.7 MHz to deliver controlled, localized heating through the skull, minimizing off-target effects [10]. Safety experiments showed that hyperthermia at 41.5 ± 0.5 °C for 10 min could be maintained consistently without tissue injury. Using this data, the group tested thermosensitive liposomal doxorubicin (TSL-Dox) in GL261 glioma-bearing mice and F98 glioma-bearing rats. Hyperthermia was induced using FUS at 42 °C for 10–20 min post-injection. They found that FUS-mediated hyperthermia significantly improved drug delivery, with about a 3.5-fold increase in doxorubicin uptake in mice and a 5-fold increase in rats compared to TSL-Dox alone. Fluorescence microscopy confirmed that in the absence of hyperthermia, doxorubicin remained localized to the vessel wall, while FUS heating promoted deeper penetration into tumor tissue. To investigate this mechanism, the investigators used physiologically based pharmacokinetic modeling and dynamic contrast-enhanced MRI which both demonstrated that thermal stress increased vascular permeability and transport capacity, with higher permeability values observed in FUS-treated tumors. These results indicated that the benefit of hyperthermia came from both the triggered release of doxorubicin and the changes in tumor vascularity. Importantly, in a survival study conducted with the GL261-bearing mice, the combination of TSL-Dox and FUS hyperthermia led to a significant extension of median survival compared to the TSL-Dox group alone.

Subsequently, Choi et al. investigated a novel two-step FUS protocol in male Sprague Dawley rats implanted with intracranial 9L gliosarcoma tumors [11]. Their design included three study groups: FUS without microbubbles, FUS with microbubble-mediated BBB disruption, and a combined sequential strategy consisting of FUS stimulation followed by microbubble-mediated blood–brain barrier disruption. Using MRI guidance, researchers found that the sequential approach significantly increased blood–tumor permeability and doxorubicin delivery compared to either method alone. T1-weighted contrast-enhanced MRI showed a 2.65-fold increase in signal intensity in the tumor region compared to the contralateral control, and a 1.45-fold increase compared to microbubble-mediated BBB disruption. Dynamic contrast-enhanced MRI further demonstrated a 2.08-fold increase in permeability in tumor regions, which was also 1.25-fold higher than BBB disruption alone. In terms of drug accumulation, the sequential protocol produced a 1.91-fold increase in doxorubicin concentration within the tumor compared to control tissue, outperforming the 1.44-fold increase seen with BBB disruption alone. Importantly, histopathological examination revealed no evidence of tissue injury across groups, supporting the safety of this multi-step approach.

4.2. Temozolomide (TMZ)

Already commonly utilized in the treatment of pediatric brain tumors, due to its bioavailability, ability to cross the BB, and induction of cytotoxicity, TMZ makes for an intriguing combination with FUS. However, its efficacy is frequently affected by resistance mechanisms, particularly overexpression of the DNA repair enzyme O6-methylguanine-DNA methyltransferase (MGMT), which directly reverses TMZ-induced DNA lesions and thereby allows tumors to survive despite treatment. To overcome this issue, Yang et al. developed lipid-polymer hybrid nanoparticles (LPHNs) functionalized with cyclic RGD peptides (cRGD) for targeted delivery to tumors with CRISPR/Cas9 plasmids targeting the MGMT gene in orthotopic glioblastoma-bearing nude mice implanted with human T98G glioblastoma cells [12]. FUS with microbubbles was then used to transiently and safely open the BBB, enabling efficient nanoparticle delivery into the tumor microenvironment. Imaging studies confirmed that this approach enhanced intratumoral drug accumulation. The triple combination of FUS, CRISPR-loaded LPHNs, and TMZ produced robust tumor suppression, smaller tumor volumes on serial MRI, and a marked improvement in survival, extending median lifespan to 43 days compared with 22–30 days in other groups.

4.3. Epirubicin

Epirubicin, another derivative of the anthracycline class, is hindered in clinical application for CNS tumors by the restrictive nature of the BBB. To address this limitation, Shen et al. created a DNA nanocarrier loaded with epirubicin and tested its delivery in combination with FUS and microbubble-mediated BBB disruption [13]. The epirubicin loaded DNA nanocarriers were injected into Sprague Dawley rats implanted with C6 rat glioma cells and FUS was applied to target tumor regions. Quantitative fluorescence imaging and histopathologic analysis confirmed a 4.4-fold increase in drug accumulation within tumor cells. Additionally, tumor growth was suppressed by nearly 87% at day 23 compared to controls, and survival was significantly prolonged, with median survival extended to about 36.5 days and 30% of animals survived beyond 40 days.

4.4. Olaparib

Olaparib, a PARP1 inhibitor that blocks PAR synthesis and impairs DNA repair, was studied by Hart et al. as a radiosensitizer in DMG [14]. They investigated whether FUS-mediated BBB opening could improve olaparib delivery to the pons and enhance its therapeutic effects in combination with radiotherapy. In vitro, patient-derived DMG cell lines (HSJD-DIPG-007 and HSJD-DIPG-011) showed that olaparib suppressed PAR synthesis, enhanced radiosensitivity, and delayed tumor regrowth, with prolonged low-dose exposure proving more effective than short high-dose exposure. In vivo, FUS with microbubbles increased pontine olaparib concentration 5.36-fold. In an orthotopic HSJD-DIPG-007 xenograft model, treatment arms including radiotherapy reduced primary tumor growth, and the combination of FUS, olaparib, and radiation therapy produced the strongest local suppression. However, overall survival was not improved due to rapid tumor dissemination and metastatic spread.

4.5. Dordaviprone

Dordaviprone (also known as ONC201) is a mitochondrial ClpP (caseinolytic protease P) activator that induces mitochondrial dysfunction and apoptosis through disruption of proteostasis and increased production of reactive oxygen species (ROS) [9]. It recently received FDA accelerated approval for progressive DMGs after positive responses in clinical trials.

Woldegerima et al. investigated whether FUS could enhance Dordaviprone delivery by transiently disrupting the BBB in a syngeneic diffuse midline glioma (DMG) mouse model [15]. BBB opening was confirmed with contrast-enhanced T1-weighted MRI, and treatment effects were validated through molecular and imaging analyses. In mice treated with ONC201+FUS, Western blots showed significantly decreased levels of NADH-ubiquinone oxidoreductase 1 alpha subcomplex 12 (NDUFA12), a pharmacodynamic biomarker of ONC201 response, and elevated ROS compared to mice treated with ONC201 alone. Mice in the combination group also demonstrated reduced tumor burden on imaging compared to those receiving ONC201 monotherapy, supporting the utility of FUS in augmenting ONC201 efficacy.

4.6. Radiation Therapy

Radiation therapy (RT) has been a mainstay of neuro-oncology in general, and its combination with FUS to potentiate therapeutic outcomes has been an area of great interest. Recent studies over the last two years have provided additional insight into the possible clinical benefits of this combination.

Chen et al. used a mouse glioblastoma model and compared three groups: RT alone (2 Gy), RT and FUS (2 Gy), and RT (5 Gy) [16]. FUS was delivered immediately before radiation, targeting tumor regions. The study found that mice receiving FUS prior to RT (2 Gy) exhibited significantly longer survival compared to those receiving RT (2 Gy) alone or no treatment, though survival was comparable to the RT (5 Gy) group.

Fletcher et al. used both healthy rats and rats bearing F98 glioma tumors [17]. In healthy rats, the combination of FUS and RT at 8 and 15 Gy induced ablative lesions detectable by MRI within 72 h, persisting up to 21 days. In the F98 glioma model, FUS combined with RT (4 Gy), FUS alone, and combination therapy were compared. The FUS-RT group showed tumor volume reductions of 45–57% compared to controls. However, survival benefits were minimal. Histological analysis showed significant increases in apoptosis and vessel-associated ceramin in the FUS-RT group compared to FUS or RT alone.

Tazhibi et al. looked at the feasibility of brainstem FUS in combination with clinical doses of RT through a murine model of DMG [18]. The brainstems of male B6 (Cg)-Tyrc-2/J albino mice were intracranially injected with mouse DMG cells (PDGFB⁺, H3.3K27M, p53^{-/-}). A clinical RT dose of 39 Gy in 13 fractions (39 Gy/13fx) was delivered using the Small Animal Radiation Research Platform (SARRP) or XRAD-320 irradiator. FUS was administered via a 0.5 MHz transducer, with BBBO and tumor volume monitored by magnetic resonance imaging (MRI). Results showed good tolerance of FUS-mediated BBBO in combination with RT, with no impact on cardiorespiratory rate, motor function, or tissue integrity. Local control was obtained, though disease progression occurred after 3–4 weeks.

Gallitto et al. explored the use of napabucasin, an NAD(P)H quinone dehydrogenase 1 (NQO1)-bioactivatable reactive oxygen species (ROS)-inducer, as a potential therapeutic radiosensitizer in DMG18 [19]. Through the use of patient-derived DMG cultures, focused ultrasound (FUS) and convection-enhanced delivery (CED) were used to overcome the BBB and optimize targeted drug delivery. In combination with radiotherapy, this improved local control.

4.7. Immunotherapy

Monoclonal Anti-CD47 Antibody

CD47 is an immune checkpoint molecule overexpressed on tumor cells that interacts with singes on macrophages to inhibit phagocytosis. Anti-CD45 monoclonal antibodies

block this interaction, enhancing macrophage-mediated clearance of cancer. Sheybani et al. demonstrated that timing of FUS delivery was critical for maximizing antibody uptake [20]. They used Zirconium-89-labeled anti-CD47 monoclonal antibodies in a glioma model and utilized PET imaging and ex vivo biodistribution to show that post-FUS antibody delivery led to significantly enhanced antibody accumulation in gliomas, compared to pre-FUS or controls. Treated mice showed slowed tumor progression and longer survival with reduced antibody dosage, indicating more efficient and targeted delivery.

4.8. Cetuximab

Cetuximab is a monoclonal antibody that inhibits cell proliferation and angiogenesis by targeting the epidermal growth factor receptor (EGFR) frequently found on tumor cells. Porret et al. tested cetuximab, an EGFR-targeting monoclonal antibody, using orthotopic U251 glioblastoma xenografts in nude mice [21]. FUS was applied at peak plasma levels to maximize delivery. FUS significantly improved early delivery and homogenization of cetuximab in the brain, including tumor regions. However, long-term accumulation or retention of the antibody in tumors was not statistically different, and there was no significant difference in survival between FUS-treated and control groups.

4.9. Immune Checkpoint Inhibitors

Immune checkpoint inhibitors, such as Anti-PD1 and anti-CTLA4 antibodies, are commonly utilized in solid tumor treatments to reactivate the T-cell response against tumors. Anti-PD1 antibodies block PD-1 receptors, restoring cytotoxic activity. Anti-CTLA-4 antibodies enhance T-cell activation by blocking CTLA-4, which normally suppresses T-cell activation. However, like many other current therapeutics, they are limited by the BBB for use in brain tumors. Lee et al. tried these immunotherapies with FUS in orthotopic GL261 glioma bearing C57BL/6 mice [22]. They applied a closed-loop FUS system to precisely open the BBB before administering anti-PD1 and anti-CTLA4 antibodies. Flow cytometry and immunohistochemistry revealed increased CD8+ T-cell infiltration, reduced tumor growth, and improved survival compared to immune checkpoint blockade alone.

4.10. Biological Modifiers

Gambogic Acid

Gambogic acid is a natural compound that induces apoptosis by activating caspases and disrupting mitochondrial function. Dong et al. explored the use of gambogic acid-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles for glioma therapy in U87 and U251 glioma-bearing mice [23]. FUS was applied at defined intervals post-injection to target tumor zones, and uptake was tracked using fluorescent dye-labeled particles. FUS treatment enhanced nanoparticle delivery to tumor cells by over 3-fold, and histological analysis confirmed increased apoptosis and tumor inhibition. FUS-treated mice had significantly smaller tumors and improved survival compared to controls.

4.11. Commentary

Across drug classes—including chemotherapies, radiosensitizers, immunotherapies, and biologics (Table 1)—common patterns emerge in terms of drug uptake, tumor control, and survival outcomes, while also highlighting specific challenges that warrant further investigation.

Table 1. Preclinical Focused Ultrasound Studies for DMG/DIPG.

Reference	Treatment	Dose	Animal model	Ultrasound Intensity or Frequency	BBB Permeability Measure	Results	Median Survival	Adverse Events
Kim et al. 2021 [10]	Doxorubicin	8 mg/kg	GL261 glioma-bearing mice and F98 glioma-bearing rats	1.7 MHz, 10 min at 41.5 °C	T2-weighted MR images	FUS-induced hyperthermia significantly enhanced the delivery of thermosensitive liposomal doxorubicin to tumor, improved vascular permeability, and prolonged survival of glioma-bearing rodents.	16 days (vs. 9 days in the TSL-Dox alone)	Hemorrhage in the brain near the skull surface when hyperthermia was applied at 42.5 °C for 10 min in mice. Lower hyperthermia (41.5 °C) was safe in rodents, with no damage seen.
Choi et al., 2025 [11]	Doxorubicin	5.67 mg/kg	Male Sprague Dawley rats implanted with intracranial 9L gliosarcoma tumors	1 MHz, 120 s duration, acoustic pressure of 0.5, 1, 2, or 0.72 Mpa	T1-weighted or Dynamic contrast-enhanced MRI, Evans blue dye staining	FUS stimulation without microbubbles followed by BBB disruption with microbubbles significantly increased blood-tumor permeability and doxorubicin delivery compared to BBB disruption alone. Dynamic contrast-enhanced MRI showed a 2.65-fold increase in signal intensity and a 2.08-fold increase in permeability in tumor regions.	N/A	None detected
Yang et al. 2021 [12]	Temozolomide (TMZ)	50 mg/kg daily for 5 days	Orthotopic glioblastoma-bearing nude mice implanted with human T98G glioblastoma cells	1.84 W, 3–5 min duration	Evans blue dye staining	FUS improved accumulation of lipid-polymer hybrid nanoparticles (LPHNs) in tumor regions and enabled CRISPR/Cas9-mediated MGMT knockdown, restoring sensitivity to TMZ. This combination therapy inhibited tumor growth and significantly extended survival in glioma-bearing mice.	43 days (vs. 22–30 days in other groups)	At higher FUS/MB doses: erythrocyte extravasation and hemorrhage during BBB opening.
Shen et al., 2022 [13]	Epirubicin	2 mg/kg	Nude mice bearing intracranial glioma xenografts	0.971 MHz, 60 s duration, 0.52 MPa	In vivo fluorescence imaging using an IVIS imaging system	FUS treatment significantly increased epirubicin accumulation in glioma tissue (4.4-fold enhancement over controls). In vivo imaging and histology confirmed deep tumor penetration and prolonged survival (50% increase in median survival).	36.5 days (vs. 25 days in other groups)	None detected

Table 1. Cont.

Reference	Treatment	Dose	Animal model	Ultrasound Intensity or Frequency	BBB Permeability Measure	Results	Median Survival	Adverse Events
Hart et al., 2023 [14]	Olaparib	10 or 100 mg/kg	Patient-derived xenograft (PDX) DMG mouse model.	1 MHz, 1.6 Hz pulse repetition frequency, 400 kPa pressure, 120 s duration	Evans blue dye staining	Effects of PARP1 inhibition were evaluated in vitro using viability, clonogenic, and neurosphere assays. In vivo olaparib extravasation and pharmacokinetic profiling following FUS-BBBO was measured by LC-MS/MS. Survival benefit of FUS-BBBO combined with olaparib and RT was assessed.	N/A	None detected
Woldegerima et al., 2024 [15]	Dordaviprone (ONC201)	N/A	Syngeneic diffuse midline glioma mouse	N/A	Gadolinium-enhanced MRI	FUS enhanced ONC201 delivery, leading to greater biomarker response, increased ROS, and reduced tumor burden compared to ONC201 alone.	N/A	None detected
Chen et al., 2023 [16]	Radiotherapy (RT)	Whole brain, 2 and 5 Gy at a rate of 3.3 Gy/min	Mouse glioblastoma (GBM) model	500 kHz, 0.4–0.56 mechanical index (MI), duty cycle of 1%, burst period of 1 s, 120 s duration	MRI	Mice receiving FUS prior to RT (2 Gy) exhibited significantly longer survival compared to those receiving RT (2 Gy) alone or no treatment, though survival was comparable to the RT (5 Gy) group.	N/A	None detected across 24 RT-FUS sessions; one grade-3 radiation necrosis attributed to re-irradiation (RT)
Fletcher et al., 2024 [17]	Radiotherapy (RT)	4, 8, 15 Gy	Healthy rats and rats bearing F98 glioma tumors	220 kHz, 102–444 kPa	Contrast enhanced T1-weighted MRI	In healthy rats, the combination of FUS and RT at 8 and 15 Gy induced ablative lesions detectable by MRI within 72 h, persisting up to 21 days. In the F98 glioma model, FUS combined with 4 Gy RT reduced tumor volumes by 45–57% compared to controls. However, survival benefits were minimal. Histological analysis showed significant increases in apoptosis and vessel-associated ceramin in the FUS-RT group compared to FUS or RT alone.	28 days (vs. 27 days in control and RT only groups)	Transient edema on MRI; rare minor T2 change (1/13 rats); no motor/neurologic deficits. FUS+RT at high dose: MRI-visible lesions and histologic scarring; authors caution on MB dose/pressure escalation.

Table 1. Cont.

Reference	Treatment	Dose	Animal model	Ultrasound Intensity or Frequency	BBB Permeability Measure	Results	Median Survival	Adverse Events
Tazhibi et al., 2024 [18]	Radiotherapy (RT)	39 Gy in 13 fractions	Non–tumor-bearing mice and syngeneic DMG murine model	0.5 MHz, peak-negative pressure 0.3 Mpa, 5 Hz repetition time, 120 s duration	MRI	Demonstrated that repeated brainstem FUS during RT is safe, feasible, and well-tolerated; progression still occurred post-RT	54 days (vs. 28 days in control)	None detected
Gallitto et al., 2025 [19]	Napabucasin + Radiotherapy (RT)	2 Gy in 5 fractions; Napabucasin 80 µM	Patient-derived DMG cultures; orthotopic DMG mouse	N/A	Gadolinium contrast enhancement on MRI	Napabucasin acted as a potent radiosensitizer; CED delivery improved survival in vivo.	46 days (vs. 33 days RT alone and 26 days napabucasin alone)	None detected
Sheybani et al. 2021 [20]	Monoclonal anti-CD47 antibody (mcD47)	8 or 32 mg/kg every 3 days for 3 doses	Orthotopic murine glioma model using GL261 cells implanted in C57BL/6 mice	1.1 MHz, 0.5% duty cycle, 0.4 MPa, 2 m duration	Contrast-enhanced MR imaging	Post-FUS delivery of Zirconium-89 labeled mCD47 led to significantly enhanced antibody accumulation in gliomas, compared to pre-FUS administration. This treatment sequence suppressed tumor growth and prolonged survival using less antibody than prior methods.	Increased 14 day survival by 40%	None detected
Porret et al., 2023 [21]	Cetuximab	N/A	Orthotopic U251 glioblastoma xenografts in nude mice	1 MHz, 0.5% duty cycle, 60 s duration	Evans blue dye staining	FUS significantly increased early deliver and homogenization of cetuximab in the brain, including tumor regions. However, it did not enhance long-term accumulation or retention of the antibody in tumors. There was no significant difference in survival between FUS-treated and control groups.	N/A	None detected

Table 1. Cont.

Reference	Treatment	Dose	Animal model	Ultrasound Intensity or Frequency	BBB Permeability Measure	Results	Median Survival	Adverse Events
Lee et al., 2022 [22]	Immune checkpoint inhibitors (anti-PD-1 and anti-CTLA-4 antibodies)	100 mg/kg	Orthotopic GL261 glioma bearing C57BL/6 mice	1.64 MHz, 10-ms pulse length, 1-Hz pulse repetition frequency, 120 s duration, 70-kPa peak negative pressure	Dynamic contrast-enhanced MRI	Closed-loop controlled FUS precisely opened the BBB, enhancing delivery of immune checkpoint inhibitors to brain tumors. This combination therapy increased infiltration of cytotoxic T cells, reduced tumor growth, and improved survival compared to immune checkpoint blockade alone.	N/A	Minimal inflammatory effects outside tumor; one animal death due to anesthesia (not FUS-related)
Dong et al. 2022 [23]	Gambogic Acid	50 µL (1.5 µmol/L)	U87 and U251 glioma-bearing mice	900 W, 0.6% duty cycle, 2 m duration, 0.63 Mpa	Fluorescence signal was detected with a live animal imaging system (IVIS)	FUS enhanced delivery of Gambogic acid-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles to glioma cells, increasing drug uptake and tumor inhibition.	59 days (vs. 38–56 days in other groups)	Higher intensities (0.76–0.88 MPa) caused erythrocyte extravasation and apoptosis in adjacent tissue

Among chemotherapeutics, anthracyclines such as doxorubicin and epirubicin—which typically fail to penetrate the BBB—demonstrated significantly enhanced intratumoral accumulation when paired with FUS. In Kim et al.'s study, thermosensitive liposomal doxorubicin (TSL-Dox) combined with FUS hyperthermia achieved 3.5- to 5-fold increases in drug uptake and prolonged survival in glioma-bearing rodents [10]. Similarly, Shen et al. reported a 4.4-fold increase in epirubicin delivery with DNA nanocarriers and microbubble-assisted FUS, resulting in nearly 87% tumor growth inhibition and extended survival [13]. These effects are not limited to traditional cytotoxics. Yang et al. combined FUS with a CRISPR/Cas9-loaded nanoparticle targeting MGMT, a resistance mediator in temozolomide (TMZ) therapy [12]. This approach sensitized glioblastoma cells to TMZ and doubled median survival compared to TMZ monotherapy. Likewise, ONC201 delivery was markedly improved by FUS in a syngeneic DMG model, leading to enhanced ROS generation and tumor reduction [15]. Collectively, these studies demonstrate that FUS not only facilitates delivery of impermeable drugs, but also enables combinatorial strategies—such as gene editing or thermosensitive release—unfeasible under standard conditions.

The studies combining low-intensity focused ultrasound (LIFU) with radiotherapy (RT) have shown promise in improving local tumor control, but survival benefits remain limited—highlighting a critical translational gap. While FUS evidently enhances local effects of RT—via improved drug delivery, BBB permeability, and localized apoptosis—the limited systemic impact, presence of infiltrative disease, and short follow-up periods may explain the modest survival outcomes. Although LIFU-enhanced RT may achieve precise, localized tumor ablation or radiosensitization, these effects do not address micrometastatic disease or diffuse infiltrative tumor cells, which are characteristic of high-grade gliomas. Consequently, local tumor regression may not translate into meaningful survival gains if systemic or distant tumor burden remains unaddressed. Moreover, the timing, dose, and fractionation of radiotherapy relative to LIFU exposure may significantly influence outcomes. For instance, suboptimal synchronization between BBB opening and drug/radiation delivery could reduce therapeutic efficacy. In some studies, BBB disruption may have occurred outside the window of maximal radiosensitization or immune activation, limiting synergistic effects. Additionally, the total radiation dose used in certain models may have been insufficient to produce durable responses, especially when not paired with effective systemic agents. A more durable effect may require integrating FUS-enhanced RT with systemic or immunomodulatory therapies in future studies.

Immunotherapy, while transformative in other cancers, has underperformed in brain tumors due to limited immune infiltration and poor BBB penetration of monoclonal antibodies. FUS appears to directly address both issues. In glioma models, Sheybani et al. demonstrated that post-FUS delivery of radiolabeled anti-CD47 antibodies significantly improved antibody accumulation and survival, even at reduced doses [20]. Similarly, Lee et al. showed that FUS-enhanced delivery of anti-PD1 and anti-CTLA4 antibodies led to increased CD8⁺ T-cell infiltration, reduced tumor burden, and prolonged survival compared to checkpoint inhibitors alone [22].

Overall, focused ultrasound (FUS) in combination with therapeutic agents or radiotherapy resulted in observable BBB opening in 6 out of 7 studies, confirmed by imaging modalities such as gadolinium-enhanced MRI or histological tracers. Reported signal enhancement or tracer penetration typically ranged from approximately 20% to over 50%, indicating consistent, though variable, permeability effects depending on parameters and delivery method. In terms of therapeutic efficacy, survival benefit ranged from negligible to significant, with increases of up to ~50% in median survival observed in models treated with temozolomide, doxorubicin, epirubicin, ONC201, and radiotherapy. In contrast, cetuximab administration showed enhanced delivery without a corresponding survival

improvement. Importantly, adverse effects were minimal in most studies, with no detectable neurotoxicity reported in 5 of 7 investigations. The only observed complications included mild thermal damage at higher hyperthermia thresholds (≥ 42.5 °C) and radiation necrosis in re-irradiation contexts, not directly attributable to FUS. These composite findings support the safety and potential efficacy of FUS-mediated delivery in glioma models and underscore the need for standardized reporting of key outcome measures to facilitate future meta-analyses.

Important to note is that when extrapolating data from adult glioblastoma models to pediatric diffuse midline gliomas (DMG), several critical differences must be considered. Pediatric DMGs, such as those harboring the H3K27M mutation, have distinct molecular profiles, epigenetic landscapes, and tumor microenvironments compared to adult GBMs. These tumors often arise in anatomically and functionally sensitive regions like the brainstem, where therapeutic windows are narrower and safety margins are limited. Moreover, the developing pediatric brain differs significantly in vascular permeability, immune response, and tissue composition, all of which can influence how focused ultrasound interacts with both tumor and surrounding tissue. The pharmacokinetics of therapeutics delivered via blood–brain barrier disruption may also differ in children, impacting efficacy and safety. As such, while adult GBM models offer valuable proof-of-concept data, caution is warranted when interpreting these results in the pediatric context. There remains an urgent need for preclinical models that faithfully replicate the biological and anatomical characteristics of pediatric DMG to guide the safe and effective translation of focused ultrasound technologies.

5. Clinical Trials Involving Focused Ultrasound for Pediatric DMG

As FUS has exhibited promising safety and efficacy in preclinical studies, extension into the clinical field has continued to expand over recent years.

Translating transcranial applications of FUS from pre-clinical models such as rodents to humans requires multiple parameter optimization steps. Often, non-human primate models are employed for this parameter optimization due the similarities in skull thickness and brain size, however significant parameter optimization is also required during clinical trials. The main parameter that needs to be changed from pre-clinical to clinical applications is the ultrasound frequency, as a lower frequency is required for efficient transcranial FUS insertion without significant energy loss due to attenuation. FUS applications in human clinical trials use frequencies such as 0.25 MHz [24], which are significantly lower than pre-clinical models. Other parameters such as power and time are still being optimized in pre-clinical models and can be leveraged to affect the resulting BBB opening and drug/gene delivery.

Experience from adult LIFU protocols, both in malignant and non-malignant conditions, provides a valuable reference point. Adult studies have established dosing thresholds, thermal safety limits, and targeting strategies under image guidance, which can inform the development of pediatric protocols. Early clinical experiences—though limited—suggest the feasibility of LIFU in selected pediatric cases when protocols are appropriately modified. Drawing on adult data while accounting for pediatric-specific anatomical and developmental considerations can provide a pragmatic and evidence-based roadmap for expanding LIFU applications in younger populations, as is currently being seen with four ongoing LIFU clinical trials in pediatrics (Table 2).

Table 2. Focused Ultrasound for Pediatric DMG/DIPG clinical trials.

Title	NCT	Status	Location	Size	Device	Drug
Non-Invasive Focused Ultrasound (FUS) With Oral Panobinostat in Children With Progressive Diffuse Midline Glioma (DMG)	NCT04804709	Active, not recruiting	New York, NY	3	Focused Ultrasound with neuro-navigator-controlled sonication	Panobinostat
A Feasibility Study Examining the Use of Non-Invasive Focused Ultrasound (FUS) With Oral Etoposide Administration in Children With Progressive Diffuse Midline Glioma (DMG)	NCT05762419	Recruiting	New York, NY	10	Focused ultrasound with neuro-navigator-controlled sonication	Etoposide
Blood Brain Barrier (BBB) Disruption Using Exablate Focused Ultrasound With Doxorubicin for Treatment of Pediatric Diffuse Intrinsic Pontine Gliomas (DIPG)	NCT05630209	Recruiting	Washington, DC and Miami, FL	10	Exablate	Doxorubicin
Blood Brain Barrier (BBB) Disruption Using Exablate Focused Ultrasound With Doxorubicin for Treatment of Pediatric DIPG	NCT05615623	Recruiting	Toronto, ON	10	Exablate	Doxorubicin

The first ever clinical trial of FUS for pediatric DIPG/DMG at Columbia University (NCT04804709) started in 2020 and used the Delsona device to open the BBB in conjunction with oral Panobinostat [25]. As a phase 1 trial, the primary outcome was safety, with secondary outcome measures looking at 6-month progression free survival and blood–brain barrier/tumor imaging changes. Eligible participants were aged 4 to 21 with confirmed pontine or thalamic DMG, able to tolerate anesthesia, and able to swallow capsules. Key exclusions included active infections, coagulopathies, prior cerebrovascular events, or contraindications to panobinostat or MRI contrast. The trial enrolled five patients (three accrued), and FUS successfully achieved BBB opening in most procedures. Only mild, transient side effects were reported, with one case of Grade 1 dermatologic toxicity. However, the study was terminated early due to the withdrawal of panobinostat from the market. Despite early closure, the findings demonstrated that BBB disruption with FUS was feasible and well tolerated. The trial is now re-opened and recruiting, with the use of oral etoposide as replacement (NCT05762419) [26].

The other two ongoing clinical trials (NCT05630209 and NCT05615623) utilize Insightec’s Exablate Model 4000 to disrupt the blood–brain barrier [27]. All three institutions involved—Children’s National Hospital, Nicklaus Children’s Hospital, and Sunnybrook Research Institute, are also using doxorubicin as the chemotherapy delivered concurrently to the tumor site during the temporary BBB opening. As Phase 1/2 studies, the goal is to evaluate the safety, feasibility, and preliminary efficacy of using magnetic resonance–guided focused ultrasound (MRgFUS) to transiently open the blood–brain barrier (BBB) and enhance the delivery of doxorubicin in pediatric patients with diffuse intrinsic pontine glioma (DIPG). Both studies, which are currently recruiting, include children aged 5 to 18 years who have completed radiation therapy and have a life expectancy of at least six months. Key inclusion criteria also require stable steroid use and confirmed DIPG diagnosis, while exclusion criteria include prior maximum anthracycline exposure, bleeding disorders, and contraindications to doxorubicin, ultrasound contrast agents, or MRI. The primary end-

points are the safety and feasibility of BBB opening, with secondary endpoints focusing on imaging outcomes and survival metrics. Results have not yet been reported as the trials are ongoing.

6. Future Research

With continued advancements in both preclinical and clinical focused ultrasound research, several promising avenues for future investigation in the treatment of pediatric diffuse midline gliomas. Preclinical studies have shown that low-intensity focused ultrasound can transiently disrupt the blood–brain barrier, enhancing the delivery and bioavailability of chemotherapeutic agents like etoposide and doxorubicin that otherwise exhibit poor CNS penetration [28,29]. This represents a critical opportunity, as systemic therapy for DMG has historically been limited by the inability to achieve therapeutic drug concentrations within the tumor [30].

Expanding the repertoire of systemically administered agents tested in combination with FUS is an important next step. This includes not only traditional chemotherapies but also targeted drugs and new agents being developed [31]. A rational approach to drug selection will be essential, with emphasis on agents whose pharmacokinetics are compatible with the temporal window of BBB permeability following FUS. Integration of radiolabeling or other molecular imaging techniques to confirm increased intratumoral concentrations in real time will be vital for validating the efficacy of delivery and for guiding dose optimization in early-phase clinical trials.

In parallel, the combination of FUS with other treatment modalities offers an exciting area of exploration. Radiotherapy remains the standard of care in DMG, and FUS may enhance the radiosensitivity of tumor tissue through mechanisms such as vascular disruption or modulation of DNA repair pathways. Moreover, increasing attention is being paid to the potential immunomodulatory effects of FUS. Studies have shown that FUS-mediated BBB opening leads to increased infiltration of immune cells, including microglia and macrophages, into the CNS in both healthy and tumor-bearing brains [30,32]. This important role of the immune system on patterns of progression in malignancy could be leveraged by combination approaches with immune checkpoint inhibitors, oncolytic viruses, or adoptive cell therapies—therapies that have previously been limited by the immunologically “cold” nature of DMG [33].

Future research should prioritize in-depth characterization of the immune microenvironment in DMG before and after FUS, using single-cell sequencing, spatial transcriptomics, and immunohistochemistry to elucidate dynamic changes in immune cell composition and function [31,34]. These studies may uncover biomarkers predictive of treatment response and inform the timing and sequencing of immunotherapy relative to FUS application.

To advance the translational application of LIFU in pediatrics as FUS platforms and targeting precision continue to improve, it is essential to consider both regulatory and anatomical dimensions with greater specificity [35]. From a regulatory standpoint, the U.S. FDA offers several pathways that may facilitate the clinical translation of LIFU technologies in children. The Humanitarian Device Exemption (HDE) allows for the approval of devices intended to treat rare conditions (affecting fewer than 8000 individuals per year in the U.S.), which may be particularly relevant given the low incidence of some pediatric neurological disorders. Additionally, the Early Feasibility Study (EFS) program supports initial clinical evaluations of novel devices in a small number of patients, allowing for protocol refinements based on early findings. These frameworks provide flexible mechanisms for generating safety and feasibility data in pediatric populations, which could be used to support expanded indications in the future.

Anatomically, children present unique challenges for LIFU application. Important considerations include the heterogeneity of gray and white matter, distribution of vasculature, and thickness and unevenness of skulls (skull thickness is generally lower in younger children, especially infants) which must be considerations when designing FUS devices as this can significantly affect acoustic transmission and focal accuracy [36]. Historically, transcranial FUS has been limited by variations in skull thickness that result in distortion of the ultrasound beams, but developments of hemispherical phased-array transducers and software to correct for phase aberrations have been overcoming these challenges since the 1990s and continue to progress [6]. The presence of open fontanelles in infants also offers a potential acoustic window, which may facilitate more precise targeting without skull interference, but also raises concerns about vulnerability to thermal or mechanical damage. Brain maturation further complicates treatment planning, as the developmental characteristics of children's brain tissue—such as higher water content and ongoing myelination—pose unique challenges for energy delivery and safety. Current technical bottlenecks include precise real-time targeting through small or irregular acoustic windows, managing heat deposition in delicate tissues, and developing age-specific models for treatment planning. Some limitations, such as achieving consistent energy penetration across a wide range of pediatric skull anatomies, remain unresolved and require further innovation in transducer design and adaptive focusing algorithms. These factors underscore the need for age-specific calibration of dosing parameters, targeting algorithms, and safety margins. Future clinical trials will need to address these challenges.

Finally, both immediate and long-term monitoring for potential off-target effects, especially in the sensitive structures of the brainstem, is vital [37]. Real-time FUS treatment monitoring is crucial for ensuring safety and for advancing clinical adoption. In MR-guided FUS, thermal monitoring is possible with MR thermometry, which would help avoid tissue overheating and thermal damaging effects. However, with clinical systems that are located outside the MRI, this type of monitoring is not possible. Acoustic monitoring of microbubble activity (cavitation) is therefore an essential component of US-guided FUS clinical systems, as it enables the real-time assessment of microbubble activity and the avoidance of damaging violent microbubble collapse, known as inertial cavitation. However, cavitation monitoring still requires additional development for reliable transcranial microbubble monitoring, and further research on the cavitation thresholds for safe and effective BBB opening needs to take place before widespread clinical adoption. In parallel, longitudinal assessments of neurocognitive function and quality of life will be essential to ensure that FUS-based interventions do not compromise neurologic integrity, particularly in a developing pediatric brain.

Overall, the integration of FUS into multi-modal treatment strategies for pediatric DMG holds substantial promise. The next generation of preclinical models and clinical trials must be thoughtfully designed to leverage this technology's full potential while maintaining a rigorous focus on safety, efficacy, and translational feasibility.

7. Conclusions

Diffuse midline gliomas, including DIPG, continue to present an urgent clinical challenge in the realm of pediatric oncology, with limited effective treatment options and dismal prognoses. While focused ultrasound is a technology that has existed since the 1940s, recent advances represent a paradigm shift in the approach to drug delivery for these inoperable brainstem tumors, offering a noninvasive method to transiently disrupt the blood–brain barrier and enhance therapeutic efficacy [38,39]. Preclinical studies have consistently demonstrated the potential of LIFU to increase intratumoral delivery of chemotherapeutics, with recent advances in different medications being utilized. Further clinical trial openings

will help to elucidate further on this approach, which is both feasible and safe in pediatric populations, but long-term outcomes and survival benefits remain unknown.

Continued interdisciplinary collaboration and well-designed clinical trials will be essential to fully translate the promise of LIFU into a viable adjunct for treating DMGs in the pediatric population, as most trials are currently focused on adults [40,41]. As the field evolves, focused ultrasound may emerge as a cornerstone technology in overcoming the longstanding therapeutic barriers of pediatric neuro-oncology [2,42]. Moreover, integrating LIFU with targeted therapies or immunotherapeutics may further amplify its clinical benefits, particularly in a precision medicine framework tailored to the molecular profiles of individual tumors.

Nonetheless, several challenges must be addressed before LIFU can become a routine clinical modality. These include optimizing sonication parameters for maximal drug delivery with minimal off-target effects, developing robust imaging and biomarker tools to monitor treatment response, and ensuring equitable access to this technology across diverse healthcare settings. Understanding the biological response of tumor and normal brain tissue to repeated BBB disruption also remains a critical area of ongoing investigation, as a significant limitation remains that preclinical data has thus far been isolated to specific cell lines and are not yet validated in multiple models.

In addition to technical and biological considerations, the feasibility of widespread clinical adoption of low-intensity focused ultrasound (LIFU) is also influenced by economic and logistical factors. Currently, the cost of LIFU systems remains high due to the complexity of the technology and limited market availability. This includes not only the equipment itself but also the infrastructure required for real-time monitoring with MRI. Furthermore, specialized training is essential for clinicians and technical staff to ensure safe and effective operation, which adds to the initial implementation burden. Compared to traditional treatments such as surgery or radiotherapy, which are already integrated into hospital workflows, LIFU may face higher upfront costs, though its noninvasive nature could reduce long-term expenses related to hospital stays, complications, and rehabilitation. To improve cost-effectiveness, strategies such as shared device models across institutions, development of simplified user interfaces, and standardized training programs could help reduce barriers to entry. Future health economic analyses are needed to quantitatively compare the cost-benefit profiles of LIFU and conventional therapies, particularly in pediatric populations where long-term outcomes and quality of life are especially critical.

The potential applications of LIFU extend beyond DMGs, suggesting a broader relevance for other central nervous system malignancies and neurodegenerative disorders where BBB permeability limits therapeutic efficacy. In this context, the current momentum surrounding LIFU research signals a transformative moment in neuro-oncology, one that holds promise not only for extending survival but also for improving the quality of life in a population of patients who have long faced limited hope.

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