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Engineering focused ultrasound for glioblastoma

Marcus S. Bell [a,b,*], Chase M. Walton [a], Mark J. Williams [d], Thomas Eckert [c], Joshua C. Brown [f], Nathan C. Rowland [d], Ozgur Sahin [e], Ben A. Strickland [d]

[a] College of Medicine, Medical University of South Carolina, Charleston, SC, 29401, USA
[b] Department of Neuroscience, Medical University of South Carolina, Charleston, SC, 29401, USA
[c] School of Medicine, University of South Carolina, Columbia, SC, 29209, USA
[d] Department of Neurosurgery, Medical University of South Carolina, Charleston, SC, 29401, USA
[e] Department of Biochemistry & Molecular Biology, Medical University of South Carolina, Charleston, SC, 29401, USA
[f] McLean Hospital, Department of Psychiatry, Harvard Medical School, Belmont, MA, 02478, USA

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ABSTRACT

Background: Focused ultrasound (FUS) is a rapidly advancing noninvasive energy delivery technology with the capacity to precisely modulate the tumor microenvironment (TME) through acoustic waves. Glioblastoma (GBM) is characterized by profound TME immune suppression and treatment resistance and has emerged as a key subject to treatment with FUS therapy.
Objective: This review examines the technical evolution of FUS and its expanded applications in GBM, including subtypes of low- and high-intensity FUS and their mechanistic contributions to therapeutic effect.
Methods: A comprehensive literature review was conducted using PubMed, Scopus, and Google Scholar to identify preclinical and clinical studies utilizing FUS in the context of GBM. Articles were included if they discussed FUS mechanisms (thermal, mechanical), bioeffects (immunomodulation, barrier permeability, cell death), or combinatory approaches (e.g., drug delivery, CAR T cells, sonodynamic therapy).
Results: A literature search yielded 312 studies; 95 met inclusion criteria (67 preclinical, 14 clinical trials, 14 reviews) with defined FUS parameters and biological endpoints. FUS enables spatiotemporal control of thermal and mechanical effects in GBM. Modulation of duty cycle, acoustic pressure, and exposure time allows FUS to operate across therapeutic regimes. Preclinical data support using FUS for targeted drug delivery, immune cell repolarization, and synergistic effects with immunotherapies. Clinical trials demonstrate the safety and feasibility of several FUS platforms.
Conclusions: FUS offers a tunable multimodal platform with the potential to overcome core resistance mechanisms in GBM. Recurrent glioblastoma could be effectively treated by integrating FUS as an adjunct therapy alongside emerging immunotherapies and targeted drug delivery systems.

1. Introduction

Focused ultrasound (FUS) is a non-invasive energy delivery technology that converges acoustic waves into a focal point of varying size relative to frequency, transducer design, and the properties of penetrated tissue [1,2]. This is achieved through constructive interference of ultrasonic waves emanating from a powered transducer [3]. Transcranial FUS can be delivered to the deep brain to produce selective effects without incisions or ionizing radiation [3,4]. The initial therapeutic effect of FUS typically occurs at the focal point exclusively, but broader effects can ensue such as during neuromodulation, wherein activating a neural hub can have brain-wide network effects (Fig. 1A and B) [5,6]. Evidence is growing for FUS's use in non-brain cancers as well as in neurological and psychiatric conditions [5], spurring clinical trials of medically urgent neuro-oncology applications [7]. The many possible effects of focused ultrasound in the brain are tunable. They include blood-brain barrier (BBB) opening, enhanced drug tissue-penetrance, molecular reprogramming, and deep brain stimulation [3,7–9]. Herein we review the technical foundations of FUS and its evolving applications in glioblastoma (GBM). We explore its role in immunomodulation, tumor-associated macrophage (TAM) repolarization, drug delivery across the blood–brain tumor barrier (BBTB), sonodynamic activation,

* Corresponding author. 68 Presidents St, Charleston, SC, 29425, USA.
E-mail address: bellmar@musc.edu (M.S. Bell).

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and the enhancement of Chimeric Antigen Receptor T (CAR T) cell delivery and activity. These applications are considered within the broader context of reprogramming the glioblastoma microenvironment as an adjunct to current treatment and as a potential primary treatment for recurrent disease.

1.1. Focused ultrasound design evolution

FUS therapy uses convergent acoustic waves to achieve precise, noninvasive effects within deep tissues (Fig. 1A and B) [22]. A modern FUS system comprises: (1) a piezoelectric transducer, wherein some arrays allow shaping and steering of ultrasound beams; (2) an integrated control platform that modulates parameters such as frequency, intensity, pulse length, and duty cycle; and in some cases, real-time imaging guidance (e.g., MRI) for treatment planning and monitoring (Fig. 1D) [23]. Early FUS work relied primarily on high-intensity continuous waves for thermal ablation, but were limited by collateral heating and lacked robust image guidance [10]. Integration of MRI guidance and refined acoustic delivery enabled more accurate targeting and the adoption of pulsed exposures to minimize off-target damage (Fig. 1A and B) [10]. MR-guided systems incorporate MR-thermometry feedback to guide focusing fidelity at the target site for ablation. The combination of imaging capabilities with microbubble-assisted techniques expanded applications beyond ablation to include safe, transient BBB opening and improved drug penetration [11]. Low-intensity or pulsed FUS has demonstrated the capacity to alter tumor vasculature and immune cell behavior through mechanical rather than purely thermal mechanisms [12]. Current strategies incorporate real-time acoustic feedback utilizing acoustic emissions from microbubbles or native tissue for lower-intensity applications that do not generate a thermal signal (e.g., cavitation monitoring). Integration with immunotherapies delivers spatiotemporally precise biomechanical stimuli that synergistically enhance drug delivery and tissue sensitization [13–15].

1.2. Focused ultrasound mechanics

Transducers can be arranged in single or multi-transducer arrays which prioritize simplicity or features like electronic beam steering or

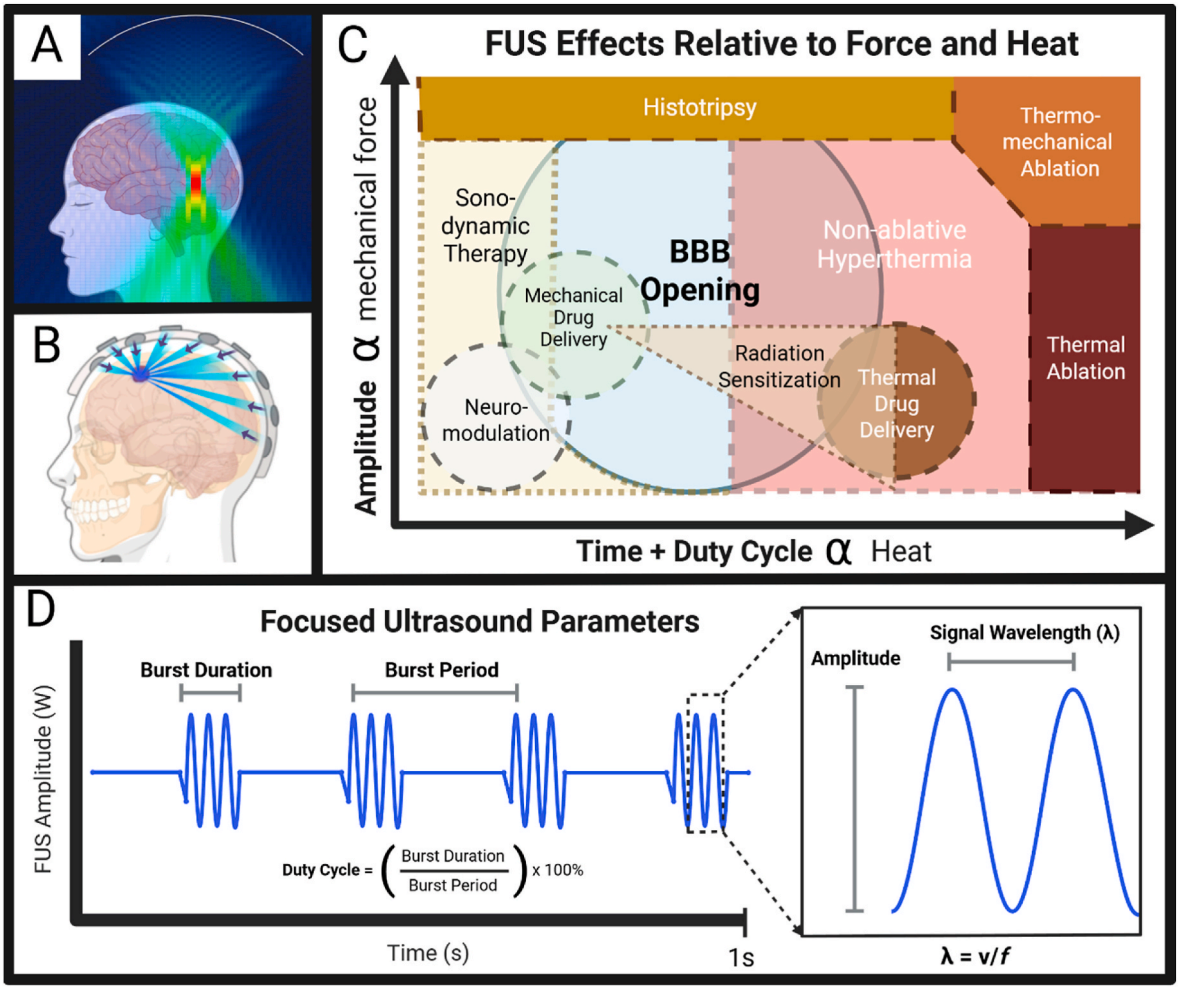

Fig. 1. Core principles of Focused Ultrasound. (A) A simulation of single-transducer focused ultrasound shows how constructive acoustic forces converge from a concave transducer to exhibit maximal force delivery at the focal point in a deep brain target. **(B)** A schematic of multi-transducer beam convergence targeting a specific intracranial focal point. **(C)** A conceptual framework illustrating FUS bioeffects across gradients of acoustic amplitude (mechanical force) and duty cycle (thermal deposition) [10–21]. High-amplitude, low-duty cycle regimes yield mechanical effects such as histotripsy and sonodynamic therapy, while high-duty cycle exposures induce thermal effects like hyperthermia and thermal ablation. Histotripsy, thermal ablation, and thermomechanical ablation are regarded as HIFU. Low-to-intermediate conditions are considered LIFU and support neuromodulation, BBB opening, drug delivery, radiation sensitization, and sonodynamic therapy. **(D)** Depiction of key FUS waveform parameters. Ultrasound energy is delivered in bursts, defined by burst duration (active "on" time), burst period (total cycle length), and duty cycle, calculated as the ratio of burst duration to burst period. Within each burst, the signal frequency (carrier frequency) determines the wavelength ($\lambda = v/f$), and the amplitude modulates force deposition. These programmable parameters drive mechanical and thermal bioeffects. Created in https://BioRender.com.

aberration correction for the skull, respectively [22,23]. Applied acoustic pressure at the focal point depends on amplifier output power, transducer electroacoustic efficiency, and focal geometry, with a portion of electrical power dissipated as heat rather than converted to acoustic output [16]. Focal point size and distance are determined by transducer convexity, aperture size, signal frequency, and the relative phases applied to elements of a multi-element array. Multi-element arrays enable dynamic electronic steering to adjust focal targeting without physical repositioning [17]. Signal wavelength is also proportional to focal point size [23,16,17]. Longer wavelengths, i.e. lower frequencies, while less narrow in focal area, can penetrate deeper into tissue and across the skull [17]. FUS effects can be tuned via heat and mechanical force manipulation, and therapies can be subdivided based on their location within a thermomechanical graph (Fig. 1C) 18–21,24–30. Ultrasound amplitude/intensity, session length, and duty cycle determine heat exposure. Under iTRUSST guidelines, low-intensity focused ultrasound (LIFU) induces low-to-moderate tissue temperature rise (0–6 °C or up to tissue temperature of 37–43 °C) and mechanical index (MI; in situ peak negative pressure divided by the square root of frequency) < 0.8 with microbubbles or < 1.9 without exogenous agents [31]. LIFU includes sonodynamic therapy, mechanical and thermal drug delivery, radiation sensitization, non-ablative hyperthermia, and, most notably, BBB opening [2,5,26]. High intensity focused ultrasound (HIFU) induces tissue temperature rise above 43 °C for thermal ablation or mechanical index >1.9 for histotripsy [1,18–20,31]. HIFU includes histotripsy, which causes mechanical tissue destruction through inertial cavitation, and thermal ablation, which causes heat-induced cell death (tissue temperature >43 °C, typically 50–>60 °C). Thermo-mechanical ablation occurs when both thermal and mechanical thresholds are elevated." Unless otherwise specified, applications involving BBB or BBTB disruption (including BBB opening, drug delivery, and sonobiopsy) utilize intravenously administered microbubbles, while ablative and neuromodulatory applications typically do not.

1.3. Focused ultrasound in the management of glioblastoma

A prime candidate disease for transcranial FUS therapy is glioblastoma (GBM) [7,32,33]. GBM is an incurable and common adult brain cancer with a median survival of 15 months [34]. The current standard of GBM care is prompt surgical resection followed by adjuvant chemotherapy and radiation [35,36]. Yet, these treatments provide only a mild survival benefit and recurrence is inevitable [34]. Recurrent GBM's broad resistance to therapies has resulted in no standard treatment, with nearly all patients being diverted into clinical trials [34]. Following strong preclinical evidence, several clinical trials have been initiated starting in 2017 to treat GBM using FUS; several have entered phase 2 and 3 [9]. The greatest breakthroughs, which include drug delivery, blood-brain barrier opening and radiation sensitization, have paved the path to investigate more nuanced FUS effects such as sonodynamic therapy, sonobiopsy, and selective immunomodulation [32]. These could impact both diagnosis and progression-free survival [9].

FUS has the potential to overcome several mechanisms of therapy resistance observed in glioblastoma. GBM treatment resistance primarily stems from its profoundly immunosuppressive tumor microenvironment (TME) [37]. A quintessential "cold" tumor, GBM's immune-suppression is heavily influenced by tumor-associated macrophages (TAMs). TAMs make up nearly 50 % of dry tumor mass [34,38]. They include both brain-resident microglia and infiltrating monocyte-derived macrophages [39]. TAMs have a predominantly immunosuppressive phenotype that secrete a myriad of cytokines which promote T cell anergy, dysfunction, and depletion [34,38–41]. After tumorigenesis, infiltrating macrophages quickly dominate the TAM population [38,39]. The tumor milieu then polarizes them to an immune-suppressive phenotype [42], Repolarizing TAMs has been effective in treating other cancers preclinically by rescuing the immunosuppressed TME [43,44]; recent studies have even suggested that FUS with microbubbles can drive pro-inflammatory polarization of TAMs in GBM [33,45]. Conversely, LIFU without microbubbles conversely may lower microglial activation, suggesting tunable control over LIFU-mediated immunological outcomes [33].

GBM's leaky but selective blood-brain tumor barrier (BBTB) is another primary therapy resistance mechanism [39,46]. The BBTB is sustained by angiogenic tumor signaling and a hypoxic, acidic microenvironment [47–49]. These create a complex extracellular matrix with irregular vasculature which repel drugs before they arrive at the surface of the dense solid tumor [49]. FUS with microbubbles may temporarily fenestrate vasculature, disrupt tight junctions, and mechanically press drugs deep into the target tissue [7,50–52]. Immunotherapies may also experience boosted effectiveness from FUS [53–55]. Chimeric Antigen Receptor T (CAR T) cells are an example of improved function aided by FUS. They are typically thwarted in GBM by denial of entry into the tumor and suppression by the immunosuppressive microenvironment [37,56]. GBM's major treatment barriers would be lifted if FUS with microbubbles can repolarize the TME and push immunotherapies across the BBTB [32,38,39,41,56–58].

2. Methods

2.1. Literature search strategy

A comprehensive literature review was conducted between January and October 2025 to identify studies investigating FUS applications in glioblastoma. Three electronic databases (PubMed, Scopus, and Google Scholar) were systematically searched using combinations of the following terms: "focused ultrasound," "FUS," "MRgFUS," "neuronavigation-guided FUS," "glioblastoma," "GBM," "high-grade glioma," "blood-brain barrier," "BBB opening," "drug delivery," "sonodynamic therapy," "sonobiopsy," "radiation sensitization," "5-ALA," "temozolomide," "bevacizumab," and "clinical trials."

2.2. Study selection

Studies were included if they were published in English between 2018 and 2025, reported on FUS applications in GBM using either preclinical models or clinical populations, and provided data on acoustic parameters or biological endpoints. Exclusion criteria comprised studies in non-CNS tumors without GBM relevance, conference abstracts lacking full-text availability, and duplicate publications. Clinical trial data was supplemented by searching ClinicalTrials.gov (accessed November 2025) using "focused ultrasound" AND ("glioblastoma" OR "GBM" OR "high-grade glioma"), with all registered trials included regardless of recruitment status.

2.3. Data extraction and synthesis

Extracted data included study design, FUS modality (low-intensity versus high-intensity), primary therapeutic application, acoustic parameters when available, and key outcomes. For clinical trials, we additionally recorded NCT numbers, trial phase (distinguishing between investigational device exemption [IDE] Phase 0 studies and traditional investigational new drug [IND] phases), enrollment targets, and primary endpoints. Studies were categorized by their primary FUS application: BBB opening with drug delivery, sonodynamic therapy, sonobiopsy, radiation sensitization, or combination approaches.

3. Results

3.1. Characteristics of included studies

The analysis identified 17 active clinical trials investigating FUS in glioblastoma treatment, demonstrating remarkable growth from 3 trials initiated in 2018 to 17 active trials by 2025. These trials represent

diverse regulatory pathways: 8 trials (47 %) operate under IDE as Phase 0 early feasibility studies, while 9 trials (53 %) follow traditional IND pathways (1 Phase 1, 1 Phase 2, 1 Phase 3, and 6 Phase 1/2 combination studies).

3.2. Overview of literature search

The systematic search of ClinicalTrials.gov yielded 17 registered FUS trials for glioblastoma spanning 2018–2026. Chronological analysis revealed steady growth: 3 trials initiated in 2018 (NCT05281731, NCT03616860, NCT03739905), 2 in 2019 (NCT04988750, NCT04446416), 3 in 2020 (NCT05879120, NCT04440358, NCT03626896), 3 in 2021 (NCT06039708, NCT03551249, NCT04998864), 5 in 2022 (NCT05370508, NCT05362409, NCT06498971, NCT04417088, NCT06329570), and 1 in 2023 (NCT04845919).

3.3. Distribution of FUS applications

Among the 17 clinical trials, BBB opening with drug delivery represented the dominant application (11 trials, 65 %), reflecting the critical need to overcome drug delivery barriers in GBM treatment. All BBB opening trials inherently include drug delivery enhancement, with notable examples including NCT03616860 (ExAblate system, Phase 2) and NCT03551249 (Phase 3). Sonodynamic therapy emerged as a promising modality with 3 trials (18 %), including the pivotal NCT05362409 investigating 5-ALA-mediated SDT. Combined approaches included 2 trials (12 %) investigating BBB opening with radiation sensitization (NCT04988750, NCT06498971), while specialized applications included 1 sonobiopsy trial (NCT05281731, 6 %) and 1 standalone radiation sensitization trial (NCT06039708, 6 %). One trial (NCT03739905) served as a control study for BBB opening in Alzheimer's disease patients, providing comparative data for liquid biopsy applications.

4. Discussion

4.1. Clinical trial landscape

There have been five mechanistically distinct clinical deployments of FUS for GBM to date across over a dozen different trials (Fig. 2) [59–72].

Sonobiopsy: Sonobiopsy is the noninvasive, intravenous retrieval of GBM tissue-derived molecules liberated from the tumor into the bloodstream [72]. This can be achieved through multiple mechanisms, including nonthermal BBB opening that enhances tumor cell shedding into circulation, or through controlled ablation that directly fragment and release tumor material as investigated in NCT04940507 [72,73]. Microbubbles are co-administered with this treatment in preclinical models to maximize tumor escape and venous retrieval. While maximal safe resection remains the standard-of-care intervention for GBM supported by Class I evidence [74], sonobiopsy offers immediate clinical utility during post-surgical treatment. During adjuvant chemoradiotherapy, FUS could simultaneously enhance drug delivery through BBB opening and enable serial tumor reprofiling via sonobiopsy. This would allow identification of treatment-emergent mutations and resistance mechanisms, informing adaptive therapy selection without repeat craniotomy, which early clinical experience has demonstrated.

BBB Opening: BBB opening is the most clinically advanced LIFU application for GBM [59,66,67,67,70,75]. Typically achieved via mechanical, nonthermal LIFU, it spans a broad thermo-mechanical parameter range [75,76]. Intravenous microbubbles are co-administered to enhance opening via stable cavitation, which is a reversible oscillation of gas-filled bubbles that creates transient fenestrations within the vascular endothelium without tissue destruction [11]. In GBM, FUS circumvents the BBB/BBTB by inducing localized, transient junctional disruption and enhanced transcytosis, improving

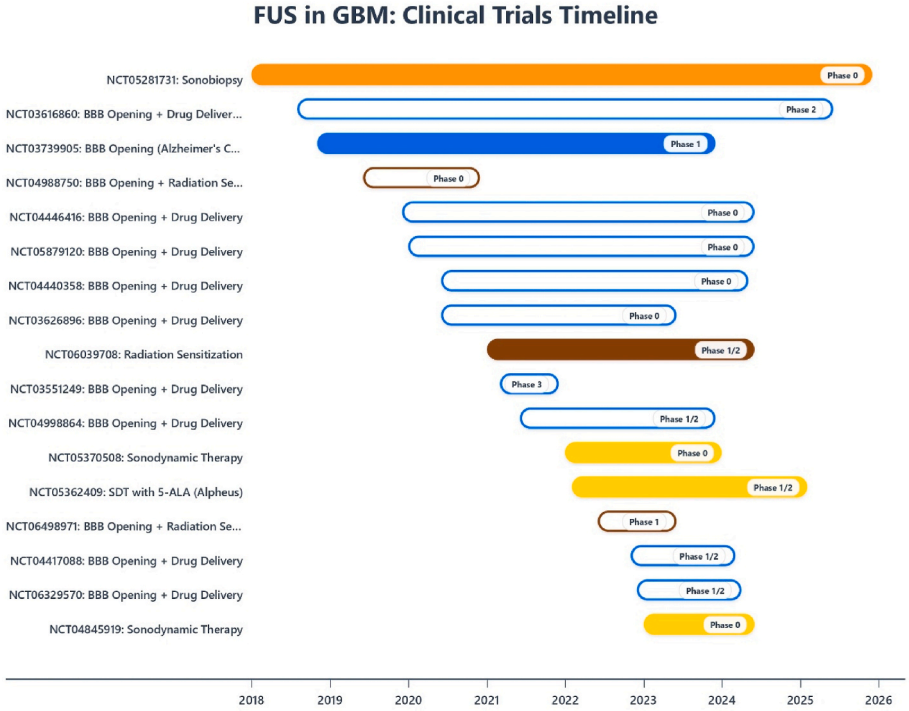

Fig. 2. Timeline of clinical trials using focused ultrasound (FUS) for glioblastoma treatment. Swimmer plot displaying the initiation and projected durations of FUS-based trials categorized by intervention type. Trials follow either investigational device exemption (IDE, labeled "Phase 0″ for early feasibility) or investigational new drug (IND, labeled by phase) regulatory pathways. All blood-brain barrier (BBB) opening trials inherently include drug delivery enhancement. Bars represent trial activity periods as of November 2025. Color scheme: Orange = sonobiopsy; Blue = BBB opening with drug delivery; Brown = radiation sensitization; Yellow = sonodynamic therapy; Outlined bars = combination therapies (brown outline = BBB opening + radiation sensitization).

intratumoral drug distribution beyond areas of natural leakiness [75,76,76].

Radiation Sensitization: Radiosensitization is the second most clinically advanced FUS application for GBM [70,77,78]. Primarily heat-mediated, it can also result from sub-ablative mechanical stress that enhances DNA damage at lower thermal thresholds [79]. Among three active trials, one incorporates BBB-opening with microbubbles parameters [77], while two isolate FUS settings specific to sensitization [70,78].

Sonodynamic Therapy: FUS enhances drug delivery by increasing tissue penetration and prolonging bioretention [3,29,46,52]. Mechanisms span thermal (e.g., hyperthermia-induced vascular permeability) and nonthermal (e.g., microbubble-mediated cavitation) domains. Clinical trials have demonstrated feasibility of FUS-mediated drug delivery in GBM. The Carthera SonoCloud, an implantable device, achieved successful BBB opening with carboplatin in phase 1/2 trials [80]. Insightec transcranial MRgFUS systems have combined BBB opening with chemotherapeutic agents in multiple trials [81,82]. Preclinical studies have advanced ultrasound-responsive nanoparticle systems, including thermosensitive liposomes, phase-transition nanodroplets, and polymeric nanoparticles engineered with tumor-targeting ligands [13,14,25,83,84]. Clinically, FUS drug delivery trials typically combine BBB disruption with other endpoints [5]. Clinically, all ongoing FUS drug delivery trials also designate BBB disruption as a primary or secondary endpoint. Sonodynamic therapy involves vibrationally-sensitive compounds that become active upon sonication [85]. One such drug, 5-aminolevulinic acid (5-ALA), is metabolized into protoporphyrin IX which accumulates in tumor cells and produces reactive oxygen species (ROS) following FUS exposure [60,86]. A first-in-human trial (NCT05362409) using the Alpheus CV01 device demonstrated safety and preliminary efficacy of hemispheric low-intensity diffuse ultrasound combined with oral 5-ALA for recurrent high-grade glioma, with no treatment-limiting toxicities and favorable survival outcomes [87,88]. Early neoadjuvant applications in newly diagnosed GBM showed imaging evidence of cytotoxic effects and increased apoptosis markers following a single treatment [89]. Sonodynamic therapy is among the most recent FUS modalities to enter clinical translation and adds to the precision of FUS-induced drug delivery [90].

Challenges and Safety Considerations: Several challenges remain for FUS implementation in GBM. Skull-induced acoustic aberrations require patient-specific phase correction algorithms [91–93], and tumor heterogeneity may cause inconsistent responses to standardized parameters [39,49]. FUS-mediated BBB opening is generally well-tolerated, with transient headache, edema, and rare microhemorrhage resolving within 24–48 h [94,95]. In one glioblastoma safety trial, grade 4 edema occurred in 11 % of participants but resolved with steroids [96]. However, serious adverse events remain possible. A recent case documented severe brain injury with persistent deficits following low-intensity FUS, attributed to inertial cavitation [97]. Thermal ablation risks perilesional edema and damage to eloquent structures. Real-time cavitation

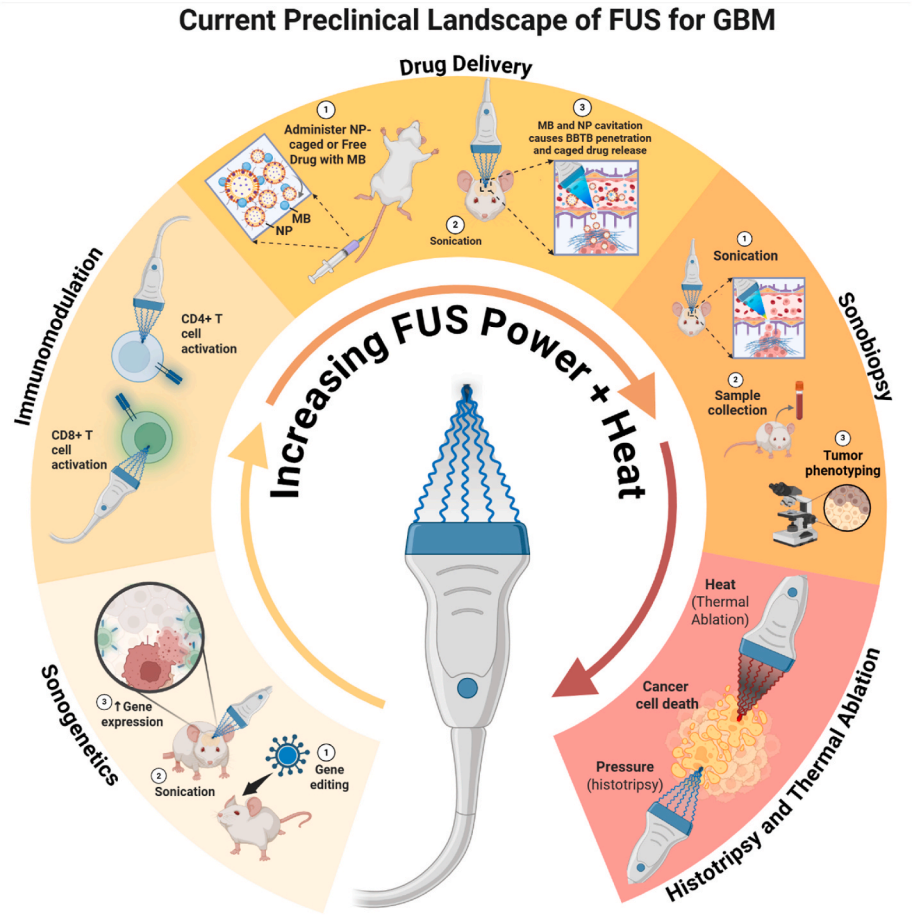

Fig. 3. Current preclinical landscape of FUS applications in GBM. Schematic illustrates major experimental uses of FUS in GBM models, organized along a gradient of increasing acoustic power and thermal deposition. At lower intensities, FUS has been used for immunomodulation by enhancing $CD4^+$ and $CD8^+$ T cell infiltration and reprogramming TAMs. Sonogenetic approaches combine FUS with viral delivery to drive transgene expression in defined cell populations. Sonobiopsy applies mechanical FUS to release tumor-derived material into circulation for downstream phenotyping. At higher powers, FUS induces thermal ablation or mechanical tissue fractionation (histotripsy), leading to targeted tumor cytoreduction. Abbreviations: FUS- Focused ultrasound; GBM- Glioblastoma; MB- Microbubble; NP- Nanoparticle; TAM- Tumor-associated macrophage. Created in https://BioRender.com.

monitoring and MRI thermometry are essential [8]. Long-term safety data remain limited as ongoing trials seek to establish optimal parameters and identify suitable patient populations.

4.2. Current preclinical work

While these early-phase trials have established the safety and technical viability of FUS in human GBM, emerging preclinical studies are now pushing further to interrogate cellular mechanisms, immune modulation, and next-generation therapeutic integrations. Expanded applications for FUS in GBM include sonobiopsy, sonogenetics, histotripsy, thermal ablation, and immunomodulation (Fig. 3) [9,98–106].

Sonogenetics (virus and gene delivery): Currently most exciting in the preclinical setting, sonogenetics has the potential to append optogenetics and chemogenetics in the basic scientist's arsenal of genetic engineering tools. For neuroscientists, intracranial viral injections leading to transfection and expression of sonodynamic membrane ion channels could be layered with Cre or Flp systems to isolate circuit function [101,102]. Following viral transfection, FUS provides noninvasive neuromodulation without requiring implanted fiber optic ferrules as needed for optogenetics. In application to GBM, the selective transfection of tumor cells to express sonodynamic proteins intracellularly using GBM- or patient-specific markers could increase tumor destruction in response to extremely low-threshold ultrasound stimulation. Sonogenetic CAR T cells were recently developed with FUS inducing anti-GBM CAR expression [103].

Histotripsy and Thermal Ablation: Histotripsy delivers high-amplitude, short-duration pulses to mechanically fractionate tissue via cavitation without thermal deposition [21]. In extracranial models, it promotes immune activation through DAMP release and antigen exposure [24,104]. In GBM-bearing mice, early studies show localized tumor destruction with limited peripheral damage and preliminary evidence of immune cell infiltration [24,104,105]. Translation to humans is limited by skull-induced waveform distortion and the need for precise cavitation control, as with all forms of FUS. Thermal ablation uses continuous-wave or high-duty-cycle FUS to induce coagulative necrosis via sustained heating [3]. In GBM models, it achieves localized cytoreduction and enhances glioblastoma therapy when paired with sonosensitizers [106].

Immunomodulation: LIFU with microbubbles or HIFU alters the GBM immune microenvironment through both mechanical and thermal mechanisms [9]. Conversely, LIFU without microbubbles may lower immune activation states [107]. Mechanical stimuli, such as cavitation from LIFU plus microbubbles, promote proinflammatory macrophage polarization via moderate DAMP signaling and enhance T cell infiltration [50,108]. In contrast, thermal FUS can have dose-dependent effects: mild hyperthermia may amplify immune activation, while higher or prolonged heat exposure can induce immunosuppressive phenotypes associated with immune suppression through overwhelming DAMP exposure [109]. Any modality of FUS used for GBM should avoid off-target immunomodulation that may potentiate the heavily immunosuppressive TAM signaling.

4.3. Future directions

Much can be drawn from the current advancements of clinical trials and recent understanding in basic science about the biophysics of FUS. Primarily, tunable effects can either overlap or be administered independently. Secondly, individual cell types respond differently to the same ultrasound parameters. Thus, in GBM, the heterogenous TME can be dissected and interrogated with varying FUS parameters to deliver highly selective direct or indirect anti-tumor activity. We propose that FUS has the greatest potential to selectively treat GBM in the realms of TAM repolarization, CAR T delivery, and multi-modal FUS including nanoparticle delivery.

Researchers now have the early beginnings of a heat and power map designating the differential effects of FUS as a function of burst period, burst length, intensity, wavelength, and treatment time [9,32,45,108]. In addition, simultaneous application of distinct FUS settings has been used to achieve differential imaging and functional effects [110]. Decoding and combining FUS parameters focused on both BBTB disruption with microbubbles and macrophage repolarization, for example, could induce synergistic effects in treating GBM. As is becoming evident from clinical trials, FUS paradigms can also be safely layered with existing treatments which may only work combinatorially [9]. Therefore, we propose using FUS to reverse treatment resistance as a multi-modal, feasible, low-risk, high-reward therapeutic repository for combating glioblastoma [9].

TAM Repolarization (Fig. 4)*:* FUS has repolarized TAMs in extracranial cancers and peripheral immunosuppressive macrophages, and similar effects have been observed in GBM models. Mechanically predominant, nonthermal FUS settings (e.g., low-intensity pulsed ultrasound with microbubbles) are associated with proinflammatory macrophage activation and increased pro-inflammatory cytokine release. Theoretically, this would lead to increased tumor penetration and anti-tumor activity by both native and CAR T cells. In contrast, high thermal or high-amplitude exposures can induce immunosuppressive phenotypes, promoting immune tolerance and local tissue damage. Importantly, FUS without microbubbles can also modulate immune responses. Recent work demonstrated that low-intensity FUS alone (250 kHz, MI = 0.9) significantly reduced microglial activation (IBA-1 staining) in mouse brain injury models through mechanosensitive ion channel activation (Piezo/TRP channels) [107]. This anti-inflammatory effect contrasts with the pro-inflammatory M1 polarization induced by microbubble-mediated FUS. These opposing effects may be complementary: microbubble-FUS could initially activate TAMs and enhance drug delivery, while subsequent microbubble-free FUS could modulate excessive inflammation. The net immunological effect likely depends on FUS parameters, timing, and baseline tumor inflammation. Future studies should evaluate whether combining both methods optimizes pro-inflammatory tumor destruction versus neuroprotection in GBM.

CAR T Cell Delivery and Activation (Fig. 4)*:* CAR T cells have limited efficacy in GBM due to poor infiltration and TME-mediated anergy [56]. FUS-mediated BBTB opening with microbubbles can increase intratumoral trafficking and bioretention. Adjunctive sonodynamic therapies may enhance CAR T cell activity by modifying the TME or enabling local release of stimulatory agents. Engineering CAR T cells to express FUS-responsive constructs (e.g., sonosensitizers or mechanosensitive channels) could further improve their tumor reactivity [109]. Mechanical or thermal modulation of the TME may restore T cell function post-infiltration [45], extending CAR T persistence and efficacy.

FUS-Mediated Nanoparticle Delivery: FUS can localize and activate a broad range of nanocarriers [111]. Albumin-based nanoparticles possess intrinsic BBB penetrance and can be mechanically disassembled at the tumor site [112]. Lipid nanoparticles (LNPs) can be triggered acoustically for site-specific drug release and decorated with tumor-targeting ligands to enhance specificity [83]. Polymeric nanoparticles are even more tunable, allowing decoupling of drug payload, targeting, and FUS sensitivity [111]. Sonodynamic elements can be incorporated into nanoparticle platforms to deliver simultaneous drug payload and mechanochemical tumor sensitization. Beyond microbubbles, other ultrasound-responsive nanocarriers have been developed for drug delivery. Perfluorocarbon (PFC) nanodroplets use phase-shift nanoemulsions as both drug carriers and ultrasound contrast agents [113]. These nanodroplets, typically 200–750 nm in diameter, remain stable in circulation but undergo acoustic droplet vaporization (ADV) when exposed to focused ultrasound, transitioning from liquid to gaseous microbubbles. This phase transition can be triggered at specific locations to release encapsulated drugs while simultaneously enhancing local permeability through cavitation effects. Drug-loaded PFC nanodroplets stabilized by biodegradable block copolymers (e.g., PEG-PLLA, PEG-PCL) have demonstrated successful tumor-targeted delivery in

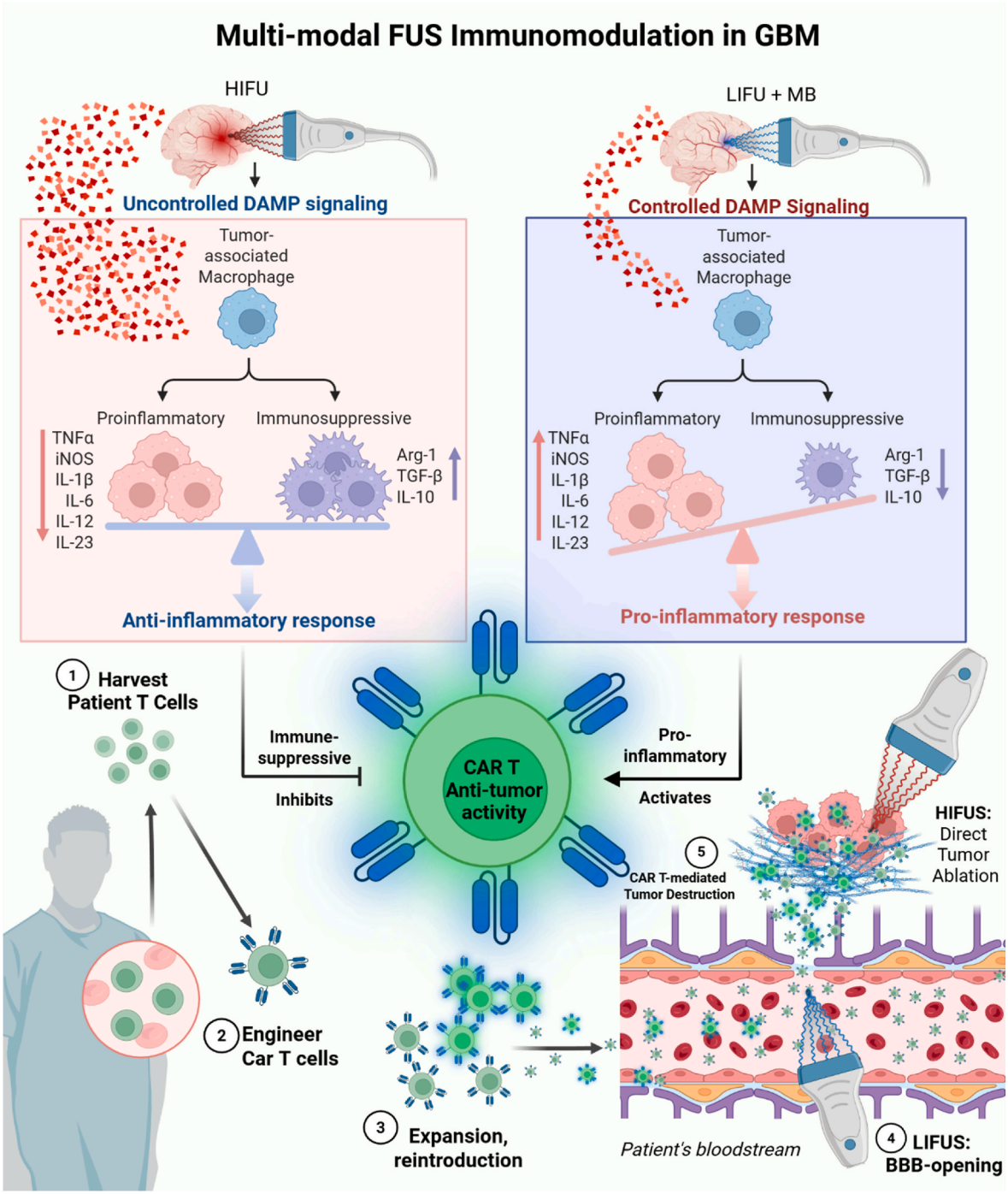

Fig. 4. Conceptual model: Focused ultrasound as a multimodal immunomodulatory adjunct to CAR T cell therapy in glioblastoma (GBM). Patient-derived T cells are engineered to express CARs, expanded, and reinfused. LIFU may induce controlled DAMP signaling, transiently opens the BBTB, and promotes proinflammatory TAM polarization, enhancing CAR T cell infiltration and anti-tumor activity. HIFU may induce *uncontrolled* DAMP signaling, skewing TAMs toward an immunosuppressive phenotype, potentially limiting T cell efficacy, but can also ablate tumor tissue directly. **Abbreviations:** FUS- Focused Ultrasound; LIFU- Low-intensity FUS; HIFU- High-intensity FUS; CAR-T- Chimeric Antigen Receptor T cell; BBTB- Blood-Brain Tumor Barrier; GBM- Glioblastoma; MB- Microbubbles; TAM-Tumor-Associated Macrophage; DAMP- Danger-Associated Molecular Pattern. Created in https://BioRender.com.

preclinical cancer models, with the added benefit of reduced premature drug release compared to conventional micelles [113]. Acoustically activatable liposomes offer another strategy, where temperature-sensitive formulations respond to mild ultrasound-induced hyperthermia [113]. These systems have been commercialized (ThermoDox®) and combine radiofrequency thermal ablation with triggered drug release. The thermal mechanism allows precise spatiotemporal control of drug delivery at physiologically tolerable temperatures, complementing the mechanical effects observed with cavitation-based techniques. Recent advances include acoustically activatable liposomes with enhanced stability and targeting capabilities [114].

Multi-modal FUS (Fig. 4)*:* FUS can be tuned across a wide parameter space to engage thermal, mechanical, and sonochemical mechanisms in parallel (Fig. 1C). Multi-modal strategies permit combinatorial targeting of the tumor, vasculature, and immune microenvironment in a single session, as seen in clinical trials (Table 1). Mechanically mediated BBB

Table 1
Summary of focused ultrasound clinical trials for glioblastoma treatment.

NCT Number	Trial Name/ Description	Intervention Type	Phase	Duration
NCT05281731	Sonobiopsy	Sonobiopsy	Phase 0	2018–2025
NCT03616860	BBB Opening + Drug Delivery (ExAblate)	BBB Opening + Drug Delivery	Phase 2	2018–2025
NCT03739905	BBB Opening (Alzheimer's Control)	BBB Opening	Phase 1	2018–2023
NCT04988750	BBB Opening + Radiation Sensitization	BBB + Radiation Sensitization	Phase 0	2019–2020
NCT04446416	BBB Opening + Drug Delivery	BBB Opening + Drug Delivery	Phase 0	2019–2024
NCT05879120	BBB Opening + Drug Delivery	BBB Opening + Drug Delivery	Phase 0	2020–2024
NCT04440358	BBB Opening + Drug Delivery	BBB Opening + Drug Delivery	Phase 0	2020–2024
NCT03626896	BBB Opening + Drug Delivery	BBB Opening + Drug Delivery	Phase 0	2020–2023
NCT06039708	Radiation Sensitization	Radiation Sensitization	Phase 1/2	2021–2024
NCT03551249	BBB Opening + Drug Delivery	BBB Opening + Drug Delivery	Phase 3	2021
NCT04998864	BBB Opening + Drug Delivery	BBB Opening + Drug Delivery	Phase 1/2	2021–2023
NCT05370508	Sonodynamic Therapy	Sonodynamic Therapy	Phase 0	2022–2024
NCT05362409	SDT with 5-ALA (Alpheus)	Sonodynamic Therapy	Phase 1/2	2022–2025
NCT06498971	BBB Opening + Radiation Sensitization	BBB + Radiation Sensitization	Phase 1	2022–2023
NCT04417088	BBB Opening + Drug Delivery	BBB Opening + Drug Delivery	Phase 1/2	2022–2024
NCT06329570	BBB Opening + Drug Delivery	BBB Opening + Drug Delivery	Phase 1/2	2022–2024
NCT04845919	Sonodynamic Therapy	Sonodynamic Therapy	Phase 0	2023–2024

Table 1. Timeline of clinical trials using focused ultrasound for glioblastoma treatment. Summary table of FUS-based clinical trials for glioblastoma, organized chronologically by start date. Trials follow either investigational device exemption (IDE, labeled as "Phase 0″ for early feasibility studies) or investigational new drug (IND, labeled by traditional phase) regulatory pathways. All blood-brain barrier (BBB) opening trials inherently include drug delivery enhancement. Intervention types include: sonobiopsy (liquid biopsy enhancement), BBB opening with drug delivery, sonodynamic therapy (SDT), radiation sensitization, and combination approaches. Data current as of November 2025. Abbreviations: BBB- blood-brain barrier; FUS- focused ultrasound; GBM-glioblastoma; IDE-investigational device exemption; IND-investigational new drug; SDT-sonodynamic therapy; 5-ALA- 5-aminolevulinic acid.

opening with microbubbles, thermally driven radiosensitization, and sonodynamic immune activation can be co-delivered with minimal off-target toxicity. Layering FUS atop conventional treatments may unmask synergies previously inaccessible due to pharmacokinetic or delivery limitations.

Adjuvant-FUS Therapies: FUS may enable re-evaluation of previously ineffective agents by altering local tissue pharmacodynamics [67]. Controlled acoustic exposure can enhance drug uptake, prolong intratumoral retention, and selectively sensitize tumor cells [3,29,106]. Retrospective analysis of failed GBM trials may identify agents with latent efficacy under FUS-enabled conditions. Then, rationally designing dual-parameter FUS regimens tailored to individual drugs could lead to salvage of candidates previously discarded.

FUS systems are becoming increasingly programmable with real-time feedback and high spatial fidelity. The field is moving toward precision acoustic immunotherapy. Progress in FUS parameterization, immune modulation, and delivery vehicles could enable functional redefinition of glioblastoma from a surgically managed tumor to a pharmacophysically addressable disease. Future glioblastoma therapies may employ both new drugs and smarter delivery of known agents activated and controlled by the physics of focused ultrasound.

CRediT authorship contribution statement

Marcus S. Bell: Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Chase M. Walton:** Writing – review & editing. **Mark J. Williams:** Writing – review & editing. **Thomas Eckert:** Writing – review & editing. **Joshua C. Brown:** Writing – review & editing, Supervision. **Nathan C. Rowland:** Writing – review & editing, Supervision. **Ozgur Sahin:** Writing – review & editing, Supervision. **Ben A. Strickland:** Writing – review & editing, Supervision, Conceptualization.

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Declaration of competing interest

The authors have nothing to declare.

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