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Focused Ultrasound-Mediated Blood-Brain Barrier Opening to Enhance Temozolomide Delivery in Glioblastoma: A Systematic Review of Preclinical and Early Clinical Evidence

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Abstract

Introduction

Glioblastoma (GBM) is the most aggressive primary malignant brain tumor in adults, with persistently poor survival despite chemoradiation and temozolomide (TMZ). Limited intratumoral drug delivery imposed by the blood-brain barrier (BBB), along with intrinsic and acquired resistance mechanisms, constrains TMZ efficacy. Focused ultrasound (FUS)-mediated BBB opening (BBBO) has emerged as a noninvasive strategy to transiently and locally enhance central nervous system drug delivery.

Purpose

To systematically synthesize preclinical and early clinical evidence evaluating the feasibility, safety, and therapeutic impact of FUS-enhanced TMZ delivery in GBM, and to identify key methodological and translational gaps informing future study design.

Methods

A systematic search of PubMed, Cochrane Library, ClinicalTrials.gov, Embase, and Scopus was conducted through October 2025. Eligible studies combined FUS-mediated BBBO with systemic TMZ in preclinical GBM models or human patients and reported pharmacologic, therapeutic, or safety outcomes.

Results

Nine studies (6 preclinical, 3 clinical) met inclusion criteria. All studies employed microbubbles to facilitate BBBO. Preclinical studies consistently demonstrated increased intratumoral TMZ delivery, improved tumor control, and prolonged survival with FUS plus TMZ compared with TMZ alone. Two studies directly quantified enhanced intratumoral drug exposure. Clinical studies demonstrated reproducible, MR-guided BBBO with favorable safety profiles and no procedure-related neurological complications. However, survival and pharmacokinetic endpoints were not evaluated.

Conclusion

Focused ultrasound-mediated BBBO is a feasible and well-tolerated strategy to augment TMZ delivery in GBM. While preclinical evidence supports enhanced intratumoral drug exposure and therapeutic benefit, clinical efficacy remains unproven. Future studies should prioritize standardized protocols, direct pharmacokinetic validation, and adequately powered trials incorporating molecular stratification and clinically meaningful endpoints.

Introduction

Glioblastoma (GBM) is the most common and aggressive primary malignant brain tumor in adults and remains associated with poor outcomes despite decades of therapeutic advances. Under the current World Health Organization (WHO) classification, GBM is defined as an IDH-wildtype diffuse astrocytic glioma with Grade IV histology (necrosis or microvascular proliferation) or, in their absence, at least one of the following molecular features: TERT promoter mutation, EGFR amplification, or combined gain of chromosome 7 with loss of chromosome 10 [1, 2]. Clinically, GBM is distinguished by its diffuse infiltration, rapid progression, and near universal recurrence [2–4]. Median overall survival remains approximately 12–15 months, with fewer than 10% of patients surviving beyond five years, even with maximal multimodal therapy [3, 5].

Temozolomide (TMZ) remains the primary systemic therapy for GBM due to its oral bioavailability and ability to cross the blood-brain barrier (BBB) [3–6]. Standard of care follows the Stupp protocol, consisting of maximal safe surgical resection and radiotherapy with concurrent and adjuvant TMZ [3, 5, 7]. While TMZ provides a modest survival benefit, its therapeutic impact is limited by biological resistance and limited intratumoral delivery [3–6]. Promoter methylation of the O6-methylguanine-DNA methyltransferase (MGMT) gene is a well-established predictor of TMZ responsiveness and highlights the importance of DNA mechanisms in mediating resistance [3]. However, even in MGMT-methylated tumors, heterogeneous BBB permeability and intact peritumoral vasculature restrict penetration, resulting in subtherapeutic concentrations in infiltrative tumor regions. Escalation of TMZ dosing is limited by dose-dependent myelosuppression and hematologic toxicity [3].

Focused ultrasound (FUS)-mediated BBB opening (BBBO) has emerged as a noninvasive strategy to enhance central nervous system (CNS) drug delivery [4–6, 8]. When applied with gaseous microbubbles, low-intensity FUS (LIFU) induces transient, localized, and reversible disruption of endothelial tight junctions without permanent tissue injury [4–6, 8]. This effect is reproducible and temporally controlled, allowing targeted delivery to the tumor and peritumoral regions [2, 8, 9]. TMZ, given its short half-life and intrinsic BBB penetration, represents an attractive candidate for delivery augmentation. Preclinical studies suggest that FUS combined with TMZ increases intratumoral drug accumulation, improves tumor control, and prolongs survival in animal models. Clinical studies have demonstrated the feasibility and safety of repeated MR-guided FUS-mediated BBB disruption in patients with GBM receiving TMZ. However, these investigations have primarily focused on procedural outcomes, with limited evaluation of pharmacokinetics or clinical efficacy [2, 4, 5, 8, 9].

Current literature evaluating FUS-enhanced TMZ delivery remains heterogeneous. Direct intratumoral quantification of TMZ has been inconsistently performed, tumor characteristics are variably reported, and clinical studies have limited survival or pharmacokinetic endpoints. These gaps must be addressed before broader clinical adoption. Therefore, we conducted a systematic review of clinical and preclinical studies investigating FUS-mediated enhancement of TMZ delivery in GBM. Our objectives were to synthesize evidence on feasibility, safety, pharmacokinetics, and therapeutic outcomes; to critically appraise methodological quality and sources of bias; and to identify translational barriers to guide the design of future experimental and clinical studies.

Methods

Literature Search

A comprehensive literature search was performed in PubMed, Cochrane Library, Embase, Scopus, and ClinicalTrials.gov from inception through December 2025. These databases were selected to capture both preclinical and clinical studies evaluating FUS-mediated enhancement of TMZ delivery in GBM.

The search strategy combined MeSH terms and free-text keywords including “glioblastoma,” “GBM,” “focused ultrasound,” “MR-guided focused ultrasound (MRgFUS),” “low-intensity focused ultrasound (LIFU),” “SonoCloud,” “blood-brain barrier opening,” and “temozolomide.” Boolean operators (“AND,” “OR”) were applied to integrate these terms. Complete search strings are provided in Appendix A. Citation snowballing was conducted through references of included studies, which were screened to identify additional publications. All retrieved citations were exported to Rayyan (Qatar Computing Research Institute, Doha, Qatar) for deduplication and systematic screening.

Eligibility Criteria

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines [10]. Eligibility criteria were defined a priori in accordance with PRISMA guidelines [10]. Studies were eligible if they met all of the following criteria: (1) evaluated focused ultrasound (FUS) or related modalities (including MR-guided FUS, LIFU, SonoCloud, or implanted emitters) in combination with systemic temozolomide (TMZ), where ultrasound was used to enhance drug delivery via BBBO or formulation-based strategies; (2) Reported at least one in vivo outcome, including pharmacokinetic measures (e.g., intratumoral drug concentration), tumor response (radiographic, volumetric, or histologic), survival outcomes, or safety and adverse events; (3) Employed preclinical in vivo animal models or human clinical study designs, including phase 0-III trials, case series, or case reports; and (4) Were published from inception onward, with no language restrictions, and translations performed when necessary.

Studies were excluded if they (1) did not include concomitant utilization of FUS and administration of TMZ; (2) were limited to in vitro experiments without corresponding in vivo therapeutic or safety outcomes; (3) consisted solely of review articles, editorials, commentaries, or conference abstracts without full-text data; or (4) investigated non-GBM tumors unless GBM-specific data were separately extractable. Preclinical studies using established glioma models and clinical studies defined under both pre-2021 and 2021 WHO classifications were eligible [1].

Study Selection and Data Extraction

Two reviewers independently screened titles and abstracts for eligibility. Full-text articles were subsequently reviewed to confirm inclusion. Discrepancies were resolved by consensus.

Data were extracted using a standardized template developed prior to full extraction. Extracted variables included study design, model or patient characteristics, ultrasound modality and acoustic parameters, microbubble use, TMZ formulation and dosing, comparator groups, pharmacokinetic measurements, tumor response, survival outcomes, safety findings, and molecular tumor characteristics when reported. Extracted data were independently verified for accuracy and completeness prior to qualitative synthesis. The full data-extraction framework is provided in Supplementary Table S1.

Quality Assessment

Methodological quality and risk of bias were assessed independently by both reviewers using validated tools. Preclinical animal studies were evaluated using the SYRCLE risk-of-bias tool, with attention to selection, performance, detection, attrition, and reporting biases. Adherence to ARRIVE reporting guidelines was also considered to assess transparency and reproducibility. Clinical studies were appraised using the CONSORT reporting framework. Given the limited number of eligible studies and substantial heterogeneity reported variables, quality assessment was used to contextualize findings rather than to generate summary scores or exclude studies. Results were synthesized qualitatively.

Data Synthesis

Due to methodological heterogeneity and the small number of included studies, meta-analysis was not performed. Findings were synthesized narratively, with emphasis on feasibility, safety, pharmacokinetics, and therapeutic outcomes, as well as sources of heterogeneity and translational limitations.

Results

Study Selection and Characteristics

The systematic literature search identified 198 records and following removal of duplicates, 103 unique records were screened by title and abstract. Full-text review was performed for eligible studies, resulting in nine studies meeting the inclusion criteria and included in the qualitative synthesis (Fig. 1).

Six out of nine studies were preclinical and employed a range of GBM models, including rodent glioma cell lines and one patient-derived xenograft model. The remaining three studies were early-phase clinical studies that enrolled patients with GBM, receiving standard-of-care chemoradiation and TMZ [7]. All included studies evaluated FUS-mediated BBBO in combination with systemic TMZ. Ultrasound platforms varied and included LIFU, MRgFUS, benchtop systems, and contrast-enhanced pulsed ultrasound approaches. Microbubbles were used in all studies to facilitate BBB disruption. TMZ was administered systemically in all studies, with dosing regimens and routes of administration varying by experimental model or clinical protocol. Comparator groups in

preclinical studies included TMZ-only, sham ultrasound, or untreated controls. The clinical studies were single-arm feasibility investigations without randomized comparator groups. Study-level characteristics, including model or patient population, ultrasound modality and parameters, TMZ formulation and dosing, comparator groups, and reported outcomes, are summarized in Supplementary Table S1.

Feasibility and Safety of Focused Ultrasound-Mediated BBB Opening

FUS-mediated BBBO was technically feasible in all studies when performed with circulating microbubbles. Successful BBB disruption was achieved in both preclinical GBM models and clinical settings using a range of ultrasound platforms and acoustic parameters.

Preclinical Feasibility and Safety

Among the six preclinical studies, FUS reliably induced localized BBBO without evidence of significant tissue injury at optimized acoustic settings. Histologic, immunohistochemical, and imaging-based assessments demonstrated preserved tissue architecture, absence of widespread edema or hemorrhage, and no consistent activation of astroglial or microglial markers following treatment in studies employing low- to moderate-pressure sonication [9]. Several investigations confirmed BBB disruption using contrast-enhanced MRI or tracer extravasation while reporting no acute neurological or systemic toxicity.

One preclinical study systematically evaluated the effects of escalating acoustic pressures and reported pressure-dependent toxicity at higher energy levels. At peak negative pressures exceeding approximately 10–11 Mpa, localized brain edema, necrosis, hemorrhage, and increased expression of pro-inflammatory cytokines were observed [12]. In contrast, lower pressure settings achieved BBBO without detectable tissue injury.

Clinical Feasibility and Safety

The three included clinical studies evaluated MRgFUS-mediated BBB disruption in patients with newly diagnosed GBM receiving standard TMZ therapy. Across multiple treatment cycles and target volumes, BBBO was successfully achieved in the majority of planned sonications. Procedural success was confirmed by contrast enhancement on MRI without evidence of treatment failure. Notably, recent clinical data provide the first comparative evidence that adding FUS to adjuvant TMZ significantly extends median overall survival (31.3 months) compared to matched controls [11].

No procedure-related neurological deficits, intracranial hemorrhage, or persistent radiographic edema were reported. Observed adverse events were limited and attributed to TMZ rather than ultrasound exposure. In one study, asymptomatic petechial changes were noted in a small number of treatment sessions but resolved before subsequent sonications and were not associated with clinical sequelae [13].

Together, these findings demonstrate that FUS-mediated BBBO is technically feasible and can be performed with an acceptable short-term safety profile in both preclinical models and early-phase clinical studies when low-pressure, sub-harmonic-controlled acoustic settings are used, as referenced in Table 1.

Therapeutic efficacy of Focused Ultrasound-Enhanced Temozolomide Delivery

Preclinical Therapeutic Outcomes

All six preclinical studies reported improved therapeutic outcomes when FUS-mediated BBBO was combined with TMZ compared with TMZ-only or control conditions. Reported endpoints included tumor growth inhibition, volumetric or radiographic tumor response, and overall survival.

Several studies demonstrated significant reductions in tumor burden. In rodent glioma models, FUS plus TMZ was associated with decreased tumor volumes on serial imaging and histologic assessment compared with TMZ alone. One study reported a statistically significant reduction in mean tumor volume (73.6 ± 14.3 mm^3) in the ultrasound-treated group compared with TMZ-only controls (115.5 ± 20.2 mm^3), corresponding to a tumor inhibition rate of 36.2% [14].

Survival outcomes were consistently improved across preclinical investigations. Reported benefits included increases in median survival of approximately 15% and increases in mean survival of up to 37.7% relative to control groups [15]. In multiple studies, long-term survivors were observed exclusively in groups receiving combined FUS and TMZ, whereas no such survivors were reported among comparator cohorts. These effects were observed across different GBM models, including one patient-derived xenograft model.

Formulation-based strategies further augmented therapeutic effects in several studies. Liposomal TMZ, liposome-encapsulated MGMT inhibitors, and ultrasound-sensitive carrier systems were associated with greater tumor suppression and prolonged survival compared with free TMZ formulations under otherwise similar experimental conditions [12, 16, 17].

Clinical Observations

While initial clinical studies were designed to assess feasibility and safety without survival endpoints, the most recent multicenter phase 1/2 trial (BT008NA) evaluated FUS-enhanced TMZ delivery in newly diagnosed GBM patients. Although therapeutic efficacy was a secondary outcome in this single arm design, the trial reported a median OS of 31.3 months and a median PFS of 13.5 months [11].

Radiographic assessments performed during treatment demonstrated stable disease at sonicated sites in all treated patients over the study periods, as assessed by standard MRI criteria [9]. However, while the BT008NA trial used a matched external control group to suggest a significant survival benefit, the single-arm design of these studies means findings should still be interpreted with caution and confirmed through randomized controlled trials.

Pharmacokinetic and Drug Delivery Findings

Direct Intratumoral Quantification

Two preclinical studies directly measured intratumoral TMZ exposure following FUS treatment. In one study, high-performance liquid chromatography was used to quantify TMZ concentrations in tumor tissue, demonstrating significantly higher intratumoral levels in animals receiving liposomal TMZ combined with FUS compared with those receiving free TMZ or liposomal TMZ without ultrasound [17]. Intratumoral concentrations were 1.86 ± 0.06 μg/g for the combined treatment group, compared with 0.78 ± 0.04 μg/g for liposomal TMZ alone and 0.61 ± 0.02 μg/g for free TMZ at the same post-treatment time point.

In a separate study, intratumoral drug delivery was measured through the stable TMZ metabolite 5-amino-imidazole-4-carboxamide [18]. FUS combined with TMZ resulted in a 2.26-fold increase in intratumoral AIC peak area compared with TMZ alone, confirming enhanced local drug exposure following BBB disruption.

Surrogate Measures of Drug Delivery

Other preclinical studies inferred enhanced drug delivery using indirect measures. One investigation measured TMZ concentrations in CSF and plasma, reporting an increased CSF fluid-to-plasma ratio following FUS compared with TMZ alone. Additional studies assessed drug delivery through increased accumulation of fluorescently labeled liposomes within tumor tissue following ultrasound-mediated BBBO or through downstream pharmacodynamic effects such as MGMT protein depletion. While these measures suggest increased CNS exposure, they do not directly quantify TMZ concentrations and therefore provide limited insight into drug delivery enhancement.

Clinical Pharmacokinetic Assessment

No clinical study evaluated TMZ concentrations in tumor tissue, peritumoral brain, or cerebrospinal fluid following FUS-mediated BBBO. Pharmacokinetic endpoints were not incorporated into the clinical protocols, and enhanced drug delivery was inferred solely from procedural confirmation of BBB disruption on MRI.

Mechanistic and Molecular Insights

Across included studies, FUS-mediated BBBO was achieved using microbubble-assisted cavitation, with mechanistic evaluation primarily centered on vascular permeability, cavitation dynamics, and downstream pharmacodynamic markers. Direct assessment of molecular tumor characteristics and mechanistic correlates varied substantially across studies.

Ultrasound-Microbubble Interactions and Cavitation Monitoring

Preclinical and clinical studies uniformly employed circulating microbubbles to facilitate BBBO. Several investigations incorporated real-time monitoring of acoustic emissions to confirm cavitation activity and guide sonication parameters [9, 16]. In studies utilizing feedback-controlled cavitation systems, individualized acoustic dosing was applied to achieve BBB disruption while limiting excessive energy deposition [11,16].

One clinical study analyzing acoustic emission profiles reported that wideband emissions accounted for a disproportionate share of total cavitation energy relative to their frequency of occurrence (84% of the total cavitation dose originated from wideband emission bursts, despite these accounting for only 21% of all bursts), while stable cavitation signals were comparatively less prominent [13]. This observation was derived from post hoc analysis of recorded acoustic data and was not linked to therapeutic outcomes within the study.

Molecular and Cellular Assessments

Molecular characterization of tumor tissue was inconsistently reported across preclinical studies. Most animal investigations relied on established GBM cell lines without molecular annotation of MGMT promoter methylation, IDH mutation status, or other clinically relevant genomic features. One preclinical study specifically examined MGMT promoter-unmethylated, TMZ-resistant GBM models and evaluated MGMT protein depletion as a pharmacodynamic marker following combined FUS and liposomal drug delivery [16].

Assessment of glial or inflammatory activation following ultrasound-mediated BBBO was reported in select studies [18]. Immunohistochemical analyses evaluating astroglial (GFAP) and microglial (Iba-1) markers demonstrated no consistent evidence of sustained activation following treatment at optimized acoustic parameters.

Clinical Molecular Annotation

In two clinical studies, molecular tumor characteristics were partially reported. Both studies enrolled patients with IDH-wildtype GBM, and one study documented MGMT promoter methylation status for all participants [9]. In the BT008NA study, FUS facilitated a “sono-liquid biopsy” by enriching plasma cell-free DNA (cfDNA) for longitudinal molecular surveillance [11]. No clinical investigation stratified outcomes by molecular subtype or evaluated differential effects of BBBO based on tumor genomics.

Table 1
Summaries of demographic and basic data of included studies.

Category	Question	Papachristodoulou et al.	Song et al.	Wei et al.	Shin et al.	Park et al.	Dalin
Screening	Does the study involve glioblastoma (human or animal model)?	Animal	Animal	Animal	Animal	Human (patients with grade IV malignant GBM).	In vit (hum gliom and a
Screening	Was focused ultrasound (FUS, MRgFUS, LIFU, SonoCloud, or similar) used?	Yes	Yes	Yes	Yes	Yes	Yes
Screening	Was temozolomide (TMZ) administered?	Yes, both in vitro (clonogenic assays) and in vivo (oral gavage in mice following LIFU and liposome administration)	Yes, TMZ was administered both as a free drug and as a liposomal formulation (TMZ-lipo).	Yes, TMZ was administered orally.	Yes. TMZ was administered intraperitoneally.	Yes, all patients received standard adjuvant TMZ chemotherapy.	Yes, T admi
Screening	Does the study measure at least one therapeutic or pharmacologic outcome (drug concentration, tumor response, survival, or safety)?	Yes, LIFU-mediated liposomal delivery (LP-O6BTG-C18 + TMZ) significantly inhibited tumor growth (to 15 mm^3 vs. 40 mm^3 for TMZ only) and prolonged median survival to 28 days (vs. 20 days for TMZ only) in TMZ-resistant GBM models, achieved through MGMT depletion (0.18 rel. densitometry) and confirmed by 0% hemolysis.	Yes, FUS-mediated BBBO achieved significantly higher intratumoral delivery of TMZ-lipo and reduced peripheral organ toxicity ($p < 0.01$). This combination nearly completely suppressed tumor progression in vivo and resulted in superior survival and apoptosis rates (e.g., TUNEL: 78.98% ±4.53 vs. 57.73%±1.86 for free TMZ in in vitro BBB model) compared to TMZ-lipo alone or free TMZ.	Yes, FUS enhanced TMZ delivery, increasing the CSF/Plasma ratio (38.6% vs. 22.7% for TMZ only). This combination yielded the lowest tumor progression ratio (5.06 ± 3.78 vs. 9.16 ± 6.79 for high-dose TMZ only) and extended mean survival to 26.3 days (vs. 19.1 days for control), showing a 15% increase in median survival, with no significant brain tissue damage or edema detected by MRI/histology.	Yes, FUS significantly increased intratumoral TMZ concentration (2.26-fold increase in AIC metabolite peak area, $p < 0.05$) and substantially reduced tumor volume at week 9 ($p < 0.05$) in a patient-derived xenograft model, leading to significantly prolonged survival ($p < 0.05$) without evidence of glial activation, tissue damage, or edema.	Yes, MRgFUS-mediated BBBD was safe and feasible over multiple cycles in GBM patients receiving TMZ, confirming disruption in 92.4% of targets (134/145 total). No clinical adverse events, hemorrhage, or edema related to the procedure were observed, and all patients maintained radiologically stable disease during the study period.	Yes, ((OA)- lipos achie TMZ enca effici to 43 and i in vit cytot again cells comp conv lipos FUS-I deliv succ accu fluor lipos glion howe press exce MPa signi vivo t inclu edem necr hemo and e pro-inflar cytok TNF-a unde the n of pr optim
Study Design	What type of study is this (animal preclinical / clinical trial / case series / case report)?	Preclinical animal study.	Preclinical animal study.	Preclinical animal study.	Preclinical animal study	Clinical trial.	Precl study and a mode

Category	Question	Papachristodoulou et al.	Song et al.	Wei et al.	Shin et al.	Park et al.	Dalin
Study Design	What was the sample size or number of animals/patients?	The numbers varied: groups included 3–7 mice per arm for survival/tumor volumetric studies, n = 3 for serum/pharmacokinetics, and n = 3 for hemolysis assay.	Male Sprague-Dawley rats (200–250 g) for the tumor model. For efficacy and survival studies, the rats were divided into five groups with n = 6 in each group (total of 30 rats for the main treatment groups).	A total of 85 rats were used: 30 normal and 55 tumor-bearing animals were assigned to different experimental and control groups.	The sample sizes varied by experiment: n = 3 to n = 4 for drug concentration analysis, n = 5 per group for Western blot and bioluminescence imaging/efficacy analyses, and n = 3 per group for BBB permeability assays. .	Six human patients were enrolled. One patient dropped out after three cycles, so final evaluable subjects were five for full protocol completion.	For in safet mice group used asse numb other expe (mole histo seem least replic samp speci samp (e.g., mode detai ment inject 50,00 cells mous
Study Design	What was the country or institution where it was performed?	Switzerland	China	Taiwan	South Korea	South Korea	Russ
Intervention Details	What type of ultrasound device or modality was used (MRgFUS, LIFU, SonoCloud, implanted emitter, etc.)?	LIFU	FUS	FUS	FUS	MRgFUS	FUS
Intervention Details	Were microbubbles used?	Yes	Yes	Yes	Yes	Yes	Yes
Intervention Details	What were the ultrasound parameters (frequency, power, duration, pulse, etc.)?	Pressure: 0.28–0.55 MPa (below inertial cavitation threshold) Burst length: 10 ms Repetition frequency: 1 Hz Total sonication duration: 180 s Cavitation control: Feedback loop for individualized cavitation dose	Frequency: 1 MHz Acoustic pressure: 800 kPa (in vivo) Pulse repetition frequency (PRF): 1 Hz Cycle number: 10,000 Sonication duration: 1 minute	Frequency: 500 kHz Acoustic power: 3 W Peak negative pressure: 0.6 MPa Burst length: 10 ms Pulse repetition frequency (PRF): 1 Hz Exposure time: 60 s	Frequency: 0.515 MHz Peak negative pressure: 0.25 MPa Burst length: 10 ms Repetition rate: 1 Hz Sonication duration: 2 minutes per session Treatment schedule: Applied on days 1, 3, and 5 during TMZ administration	Frequency: 220 kHz Power: Ramp sonication up to 40 W Optimal power: ~50% of inertial cavitation threshold Pulse train: 10 pulses Pulse length: 2.6 ms Inter-pulse interval: 30.4 ms Session duration: 90 s per sonication Repetitions: 2–4 sonications per target	Acou press teste MPa Pulse repet frequ (PRF) Duty Expo 60 s Temp rise: Main withi
Intervention Details	How was temozolomide administered (dose, timing, route)?	TMZ dose: 10 mg/kg Route of administration: Oral gavage Timing: Initiated 2 days after LIFU/liposome treatment; continued for 5 consecutive days	TMZ dose: 20 mg/kg Route of administration: Intravenous (tail vein) injection Timing: Administered daily for 3	TMZ dose: 50, 75, or 100 mg/kg per day (group-dependent) Route of administration: Oral gavage Timing: Administered	TMZ dose: 30 mg/kg Route of administration: Intraperitoneal injection Timing: Administered once daily for 5 consecutive days starting on day 8 post-xenograft implantation; TMZ injected	TMZ dose: Not specified (standard clinical protocols) Route of administration: Oral (standard for temozolomide)	TMZ Lipos TMZ using TMZ synth batch dosir speci Route admi

Category	Question	Papachristodoulou et al.	Song et al.	Wei et al.	Shin et al.	Park et al.	Dalin
			consecutive days; tissues collected 2 hours after injection and ultrasound treatment for biodistribution analysis	for 5 consecutive days; FUS performed twice (days 1 and 3) immediately after TMZ administration	immediately after FUS in treatment groups	Timing: MRgFUS-mediated BBB disruption performed on day 1 or 2 of each 4-week chemotherapy cycle for 6 cycles, following concurrent radiotherapy	Intrav inject TMZ- lipos vein) Timir Lipos TMZ admi follov medi disru
Comparator	What was the control or comparison group (TMZ only, sham ultrasound, no treatment, etc.)?	TMZ alone; LIFU + TMZ; LIFU + TMZ + liposomal MGMT inactivator; vehicle controls; and sham or untreated controls (no LIFU)	PBS control; microbubbles + ultrasound (sham drug); free TMZ; liposomal TMZ alone; and liposomal TMZ + ultrasound-mediated BBBO	Sham controls (no drug, no FUS); TMZ alone at low, medium, and high doses (no FUS); high-dose TMZ control; and FUS + TMZ (mid-dose TMZ with two FUS sessions)	Control (no treatment); TMZ-only; and TMZ + FUS; additional groups in selected assays included tumor-only (no FUS, no TMZ) and FUS-only	None; single-arm feasibility and safety study in which all patients received MR-guided FUS BBB disruption plus standard adjuvant temozolomide following surgery and chemoradiation	Conv versu acid- lipos free T versu lipos TMZ; versu FUS p level perfu (no F versu micro BBBC biodi analy
Outcomes	Did the study measure intratumoral TMZ concentration?	Not directly measured; liposome delivery and MGMT depletion at the tumor site were used as pharmacodynamic surrogates	Directly measured; TMZ-lipo + ultrasound achieved 1.86 ± 0.06 μg/g, compared with 0.78 ± 0.04 μg/g for TMZ-lipo alone and 0.61 ± 0.02 μg/g for free TMZ at 2 hours post-injection	Not directly measured; TMZ concentrations were assessed in cerebrospinal fluid and plasma only	Directly quantified using LC–MS/MS measurement of the stable TMZ metabolite 5-amino-imidazole-4-carboxamide (AIC); TMZ + FUS group demonstrated a 2.26-fold increase in AIC peak area (3.28 × 10^5AU) compared with TMZ-only (1.45 × 10^5AU; $p < 0.05$)	Not measured in tumor tissue or brain; explicitly acknowledged as a study limitation	Not d meas vivo; accu of flu TMZ- lipos gliom was demo follov disru
Outcomes	What was the tumor response (radiographic, histologic, or volumetric)?	Serial MRI demonstrated significant tumor growth inhibition in the LIFU + liposome + TMZ group compared with controls; histologic analyses confirmed targeted drug delivery and absence of tissue damage	Volumetric tumor growth monitored by T2-weighted MRI; histologic assessment with H&E, TUNEL, and caspase-3 staining demonstrated increased apoptosis and tumor necrosis in ultrasound-treated groups	Serial T2-weighted MRI showed significantly reduced tumor progression in the FUS + TMZ group; histology revealed reduced gliosis and chronic inflammation compared with extensive necrotic tumor beds in TMZ-only animals	Volumetric/radiographic response assessed using bioluminescence imaging as a surrogate for tumor volume; histologic confirmation of tumor presence with H&E and ZEB-1 staining	Radiographic stability observed in all patients during the study period, assessed by MRI using RANO criteria, with no evidence of progression or new enhancing lesions at sonicated sites	No di volun radio tumo respo repor tumo asse indire fluore lipos accu withi tissu
Outcomes	What were the survival outcomes (PFS, OS)?	Kaplan-Meier analysis demonstrated significantly prolonged survival in the LIFU + liposome + TMZ group compared with all comparator groups; PFS not separately reported	Overall survival significantly prolonged in the TMZ-lipo + ultrasound group compared with all other groups by Kaplan-Meier analysis; PFS not explicitly reported	Mean survival increased by 37.7% (from 19.1 to 26.3 days) and median survival increased by 15% (from 20 to 23 days) in the TMZ + FUS group compared with controls	Kaplan-Meier analysis showed significantly prolonged survival in the TMZ + FUS group compared with TMZ-only and control groups; exact median survival durations not specified	Progression-free survival and overall survival were not reported; longer follow-up was required to assess survival endpoints	Progr free s and o survi not re
Outcomes	Were there any adverse events or	No significant adverse events reported; no acute	No significant adverse events	No significant brain tissue	No major safety concerns reported; no	No clinical or radiological	Press depe

Category	Question	Papachristodoulou et al.	Song et al.	Wei et al.	Shin et al.	Park et al.	Dalin
	safety issues reported?	hematologic toxicity (normal blood counts) and no major tissue or vascular damage on histologic assessment	or organ toxicity detected; histologic analysis of heart, liver, spleen, lung, and kidney showed no abnormalities compared with controls	damage, edema, or other adverse events observed at selected FUS parameters, as assessed by MRI and histology	significant astroglial or microglial activation (GFAP, Iba-1 immunostaining) and no edema or tissue damage on T2-weighted MRI	adverse events related to MRgFUS BBB disruption over six treatment cycles; one unrelated hematologic adverse event attributed to temozolomide	toxici obse FUS p (10– result brain necro lesio locali hemo and e pro- inflar cytok (TNF 1-alp CCL2 docu by M histo
Outcomes	Were there functional or neurocognitive findings?	Not reported.	Not reported.	Not reported.	Not reported.	No cognitive, emotional, or neurological deficits observed following MRgFUS-mediated BBB disruption, including procedures near eloquent brain regions	Not r
Other	Was there any mention of MGMT, IDH, or TERT mutation status (for clinical studies)?	MGMT promoter-unmethylated (TMZ-resistant) gliomas specifically studied, with MGMT protein depletion measured; IDH and TERT status not reported (preclinical study)	MGMT, IDH, and TERT mutation status not reported (preclinical C6 glioma model)	MGMT, IDH, and TERT mutation status not reported (preclinical rat glioma model)	MGMT methylation and IDH/TERT mutation status not evaluated; TMZ resistance mechanisms acknowledged as a limitation for future work	MGMT methylation status and IDH mutation status reported for all patients; all tumors were IDH-wildtype, with variable MGMT methylation status	MGM and T muta statu repor (prec study
Other	Who funded the study, and were there conflicts of interest?	Public research grant (national funding agency)	Public research grant (national funding agency)	Public research grant (national funding agency)	Public research grant (national funding agency)	Mixed public and industry funding	Publi resea (natio fundi agen
Other	What were the main conclusions of the study?	LIFU combined with liposomal MGMT inactivator reverses TMZ resistance, significantly inhibits tumor growth, and prolongs survival in preclinical GBM models, showing promise for clinical translation.	Ultrasound-mediated BBBO enhances intratumoral liposomal TMZ concentration, leading to significantly inhibited tumor growth and prolonged animal survival without adverse effects, confirming its potential for improving GBM outcomes.	FUS-induced BBBO significantly increased TMZ concentration in the brain/CSF, suppressed tumor growth, and extended animal survival compared to TMZ alone, supporting its clinical potential for brain tumor treatment.	Early FUS administration enhances intratumoral TMZ delivery by 2.26-fold, effectively suppressing tumor growth and prolonging survival in a PDX model, suggesting its therapeutic advantage in early-stage GBM treatment.	MRgFUS-mediated BBBD is feasible, accurate, and safe for repeated use in GBM patients receiving adjuvant TMZ chemotherapy, potentially enabling the delivery of other restricted therapeutic agents.	OA-b lipos highl sensi FUS, effec micro assis delive accu of lip TMZ tissu press be ca optim avoid tissu abov
Other	Limitations	The study is a murine proof-of-concept reliant on LIFU-mediated BBBO,	Its conclusions are constrained by	Translation is limited by a rat model,	The work does not assess TMZ resistance mechanisms or immune	The study cannot confirm clinical benefit	Clinic trans const

Category	Question	Papachristodoulou et al.	Song et al.	Wei et al.	Shin et al.	Park et al.	Dalin
		without direct intratumoral TMZ quantification or evaluation of repeated treatments, so its findings require validation in more representative models and clinical trials.	the use of a single rat glioma cell line and an in vitro BBB model, a lack of direct tumor TMZ measurements, assessment at only one short post-treatment time point, and short-term toxicity evaluation that cannot capture delayed or chronic effects.	imperfect FUS targeting geometry, absence of direct tumor TMZ measurements (relying instead on CSF/plasma ratios), a non-optimized two-exposure regimen, and dependence of survival benefit on the specific model and drug used.	effects, uses only immunodeficient nude mice treated very early after engraftment, and tests only a single FUS cycle, leaving unresolved how tumor stage, vascularization, immunity, and repeated treatments affect outcomes.	because drug delivery was not quantified, and follow-up was short, and its small sample size with one dropout, plus the logistical and emotional burden of repeated frame-based MRgFUS, limit generalizability and feasibility.	the n furth optim stabi base lipos persi chall achie thera intrat drug safet high press reliar narro cell li mous mode abse direc tumo quan

Discussion

In this systematic review, we sought to synthesize preclinical and clinical evidence evaluating FUS-mediated enhancement of TMZ delivery in GBM. Across nine studies, FUS-mediated BBBO was consistently feasible when performed with microbubbles across ultrasound platforms and treatment paradigms. Our findings support FUS-mediated BBBO as a drug delivery modifier, rather than as an independent antitumor therapy.

Six preclinical investigations demonstrated improved tumor control and survival when FUS was combined with TMZ relative to TMZ alone [12, 14–18]. Earlier clinical studies were explicitly designed as feasibility and safety evaluations, and the BT008NA trial expanded this scope to include survival outcomes [11]. Preclinical efficacy signals were observed across multiple models, including rodent glioma lines and one patient-derived xenograft, suggesting that enhanced delivery may translate into measurable therapeutic effects (Shin et al.). Clinical studies extend these findings by demonstrating reproducible MR-guided BBBO over repeated treatment cycles without procedure-related neurological toxicity. Notably, the BT008NA trial provides the first comparative clinical evidence of a survival benefit, reporting a median overall survival of 31.3 months and a median progression-free survival of 13.5 months, which represented significant improvements compared to matched external controls.

FUS-mediated BBBO directly addresses a recognized limitation of systemic TMZ therapy: inadequate and heterogeneous intratumoral drug penetration. Even though TMZ is relatively small and lipophilic, both preclinical and clinical evidence indicate that an intact or partially preserved BBB in infiltrative tumor regions restricts effective drug exposure [14, 15]. This paradigm may be especially relevant for tumors abutting eloquent cortex, distributed brain networks, or deep white matter tracts, where re-resection or escalation of radiotherapy carries increased morbidity. In addition, patients with MGMT promoter unmethylated tumors represent a biologically compelling population, as limited responsiveness to TMZ is driven in part by insufficient effective intratumoral drug exposure, which cannot be overcome through systemic dose escalation alone. By transiently increasing vascular permeability, FUS enables localized enhancement of drug delivery without increasing systemic dosing. Importantly, several studies demonstrate that delivery augmentation alone is insufficient without favorable tumor biology. Combining FUS with a liposomal MGMT inhibitor was necessary to overcome resistance in MGMT-unmethylated GBM models, indicating that BBBO facilitates, but does not replace, biological susceptibility [16]. Similarly, liposomal TMZ combined with FUS produced greater tumor suppression than free TMZ, highlighting the interaction between formulation strategy and delivery enhancement [17]. Together, these findings support a conceptual framework in which FUS functions as a delivery-enabling technology.

Despite consistent demonstration of BBB disruption, direct pharmacokinetic validation of enhanced TMZ delivery remains limited. Only two preclinical studies directly quantified intratumoral drug exposure following FUS and both studies reported higher intratumoral drug concentrations following BBBO. In contrast, other studies relied on surrogate markers. Wei et al. measured TMZ concentrations in CSF and plasma, noting increased CSF-to-plasma ratios but acknowledging that these measures may underestimate true intratumoral exposure. Dong et al. and Dalinina et al. inferred delivery enhancement through fluorescent liposome accumulation and downstream tumor response rather than direct drug quantification. While these approaches suggest increased CNS exposure, they do not define the magnitude, spatial distribution, or persistence of intratumoral drug levels. Addressing this challenge, the BT00NA trial's sono-liquid biopsy enabled molecular surveillance and demonstrated correlation with clinical progression which provides a valuable, albeit indirect, window into treatment response and disease dynamics, helping to bridge the gap left by the absence of direct intratumoral TMZ quantification [11].

Although TMZ concentrations in tumor tissue, peritumoral brain, or CSF were measured in the two early clinical studies, the BT008NA trial reported the first statistically significant comparative survival benefit, with increased PFS and median OS, and an improved hazard ratio compared to matched external controls [11]. Consequently, while BBBO was reliably confirmed radiographically, the magnitude of FUS-enhanced drug delivery in human GBM still lacks direct measurement, but the emerging clinical efficacy signals provide initial evidence of therapeutic benefit.

Substantial technical heterogeneity exists across the reviewed studies. Preclinical investigations employed a wide range of ultrasound frequencies, acoustic pressures, pulse structures, and cavitation-monitoring strategies [12, 14, 15], while clinical studies differed in sonication targets and cavitation-dose control

paradigms [9, 13]. Dalinina et al. demonstrated that acoustic pressure exceeding 10–11 MPa resulted in tissue injury, whereas lower pressures achieved BBBO without detectable damage, emphasizing the narrow therapeutic window associated with ultrasound-mediated delivery. These findings delineate a parameter-dependent safety profile rather than an intrinsic limitation of the technique.

Biological heterogeneity further limits generalizability. Most preclinical studies relied on established rodent glioma models without molecular annotation, limiting relevance to human GBM subtypes [14, 15, 17]. Only Papachristodoulou et al. explicitly examined MGMT-unmethylated, TMZ-resistant tumors, and only Shin et al. incorporated a patient-derived xenograft model. In clinical studies, Park et al. (2020) reported MGMT methylation and IDH mutation status, but outcomes were not stratified by molecular subtype. These limitations may introduce dilution of outcomes, potentially masking a true biologic effect despite technically successful BBBO.

Several priorities for future clinical translation exist. First, incorporation of pharmacokinetic endpoints such as intratumoral drug concentration or validated surrogate measures should be considered essential for trials evaluating FUS-enhanced drug delivery. The preclinical studies by Song et al. and Shin et al. provide clear methodological templates for such assessments. Second, standardized reporting of ultrasound parameters and cavitation metrics is necessary to enable reproducibility and cross-study comparison, as variability in acoustic dosing and monitoring complicates interpretation across existing studies [12, 13]. Finally, future trials should integrate molecular tumor profiling and clinically meaningful endpoints, including PFS, OS, neurocognitive outcomes, and quality of life. The emerging survival data from the BT008NA trial provide a strong rationale for larger, randomized controlled trials to definitively establish the therapeutic benefit of focused ultrasound-mediated BBBO for enhancing TMZ delivery in GBM [11].

Strengths and Limitations

This systematic review synthesizes preclinical and clinical studies evaluating FUS-mediated enhancement of TMZ delivery in GBM, across the translational continuum. A key strength is the emphasis on pharmacokinetic validation. Only two preclinical studies directly quantified intratumoral TMZ using LC-MS, clearly distinguished from studies using surrogate measures such as CSF concentrations or fluorescent carrier accumulation [12, 14, 15, 17,18]. This distinction enables transparent appraisal of where delivery evidence is robust versus inferential. Inclusion of early clinical studies demonstrates that repeated MR-guided BBBO can be performed safely in patients receiving standard TMZ therapy, linking experimental delivery enhancement with clinical feasibility. [9, 13]. Additionally, the inclusion of the BT008NA study provides a more robust assessment of safety across different institutions and offers the first comparative survival signal in a clinical cohort [11].

Several limitations constrain interpretation. The evidence base remains small and technically heterogeneous, with wide variation in ultrasound platforms, acoustic parameters, microbubble formulations, and treatment protocols, precluding quantitative meta-analysis. [9, 12–15]. Generalizability of both clinical and preclinical studies is limited by predominant use of rodent cell lines and immunodeficient hosts, with inconsistent reporting of molecular tumor characteristics, including MGMT promoter methylation and IDH mutation status [14–18].

Pharmacokinetic validation remains incomplete. No clinical study measured TMZ concentrations in tumor tissue or CSF, leaving magnitude, spatial distribution, and durability of human delivery enhancement undefined [9, 13, 17, 18]. Additional limitations include small sample sizes, short follow-up durations, limited evaluation of long-term outcomes, potential publication bias, and variability in reported endpoints [9, 13, 17, 18]. Addressing these limitations through standardized protocols, molecularly informed trial design, and direct measurement of intratumoral drug exposure will be essential to translate procedural feasibility into clinical benefit.

Conclusion

FUS-mediated BBBO feasibly enhances TMZ delivery in GBM patients. Preclinical studies demonstrate increased intratumoral exposure and improved survival; early trials confirm safety during standard therapy. Substantial heterogeneity persists across platforms and parameters. Future studies should prioritize direct drug measurement, standardized reporting, and adequately powered, molecularly informed trials with meaningful outcomes to determine whether procedural feasibility translates to clinical benefit.

Declarations

Author Contributions

Design or conceptualization of the study- JC, MS, RSD

Acquisition of data- JC, MS, NB, RSD

Analysis or interpretation of the data- JC, MS, NB, BRE, RSD

Drafting of the manuscript- JC, MS, NB, BRE, SS, RSD

Critical review of the manuscript for important intellectual content- SS, JAB, RSD

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Clinical trial number: Not applicable

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Figures

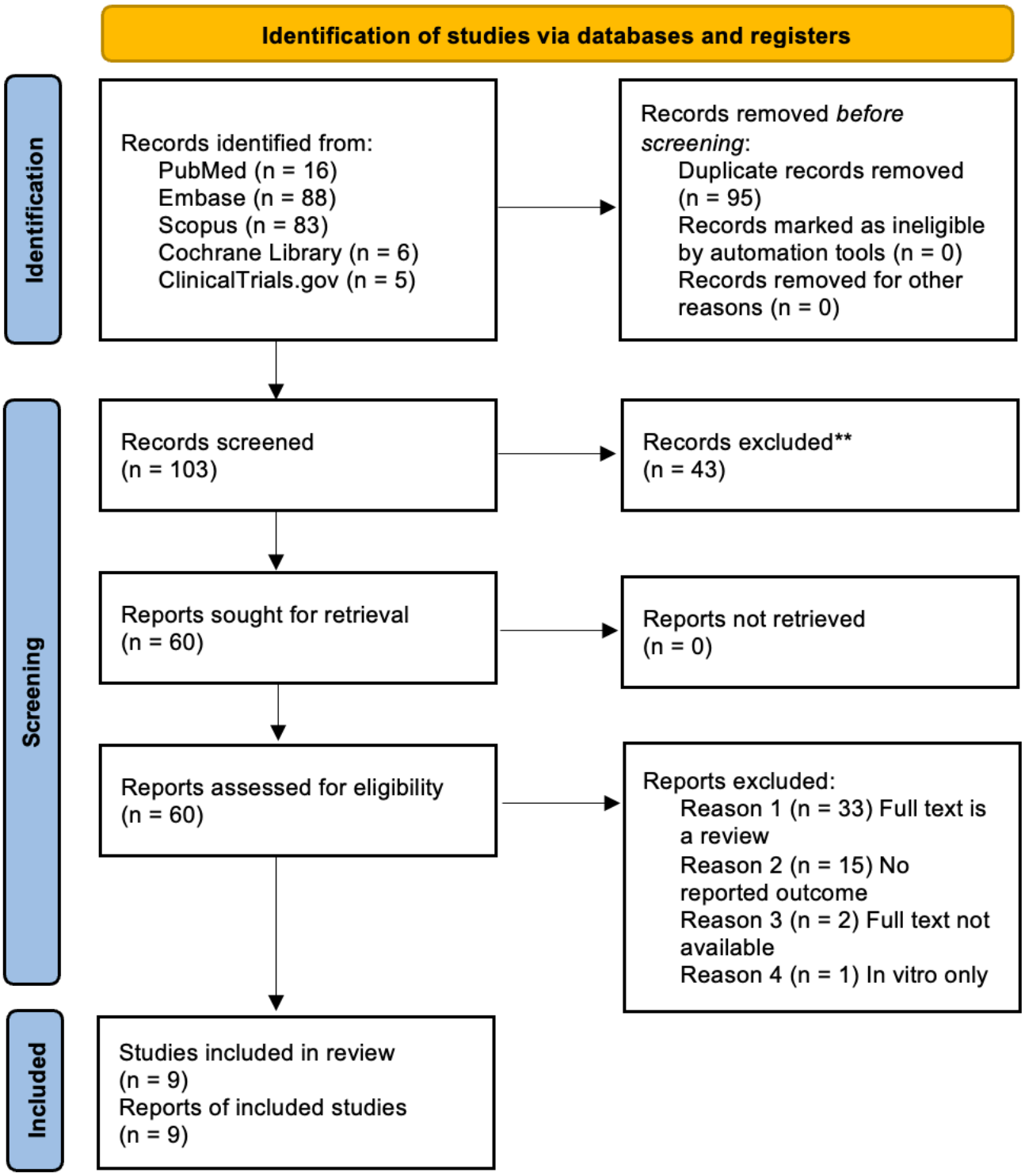


Figure 1

PRISMA 2020 flowchart illustrating the search and selection of studies included in the review. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses [10].

Supplementary Files

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- FUSTMZSS1.21.26Supplementary.pdf