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Combination of Neuronavigation-Guided Focused Ultrasound and Bevacizumab for Patients With Recurrent Glioblastoma: A Pilot Study

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BACKGROUND AND OBJECTIVES: Combining focused ultrasound (FUS)–induced blood-brain barrier opening with bevacizumab (BEV) has demonstrated survival benefits in preclinical models. This study aimed to evaluate the safety and feasibility of repeated FUS-BEV treatments in patients with recurrent glioblastoma and to explore imaging and serum biomarkers in relation to disease status.

METHODS: This was a prospective, single-arm, open-label pilot trial. The primary end point was 6-month progression-free survival (PFS). Disease progression was assessed according to the Response Assessment in Neuro-Oncology criteria by independent radiological review. Radiological response was evaluated using fluid-attenuated inversion-recovery sequences to compare FUS-exposed vs nonexposed regions. Plasma cell–free DNA (cfDNA) concentrations were measured before and after FUS treatment.

RESULTS: Between July 2020 and August 2023, 6 patients received a median of 14.5 sessions of biweekly FUS-BEV (10 mg/kg). The median PFS was 11 months, with a 6-month PFS rate of 66.7%. The only FUS-related adverse event was transient scalp heating (grade 1; 1.9%). A fluid-attenuated inversion recovery normalization effect emerged within 1 month after treatment. Plasma cfDNA increased significantly post-FUS, with total cfDNA rising 2.03 ± 0.76-fold, *EGFR* cfDNA 1.77 ± 0.76-fold, and *HMBS* cfDNA 1.68 ± 0.66-fold.

CONCLUSION: Repeated FUS-BEV treatment is safe and feasible in patients with recurrent glioblastoma. Randomized controlled trials are warranted to confirm its therapeutic efficacy and validate imaging and liquid biopsy biomarkers.

KEY WORDS: Blood-brain barrier, Bevacizumab, Focused ultrasound, Liquid biopsy, Recurrent glioblastoma

Bevacizumab (BEV) is a humanized antivascular endothelial growth factor (VEGF) monoclonal antibody that binds to VEGF secreted by glioblastoma (GBM), thereby inhibiting its proangiogenic function.[1] Despite its incorporation as a standard of care in recurrent GBM (rGBM),[2] the median progression-free survival (mPFS) with BEV-based combination therapies

ABBREVIATIONS: AE, adverse event; **AEF,** acoustic emission feedback; **AEM,** acoustic emission mapping; **BBB,** blood-brain barrier; **BBBO,** BBB opening; **BEV,** bevacizumab; **cfDNA,** cell-free DNA; **EORTC,** European Organization for Research and Treatment of Cancer; **FUS,** focused ultrasound; **GBM,** glioblastoma; **IC,** internal control; **MB,** microbubble; **OS,** overall survival; **rGBM,** recurrent GBM; **SI,** signal intensity.

Supplemental digital content is available for this article at neurosurgery-online.com.

ranges from 3.4 to 4.8 months,[3-6] with 6-month PFS rates between 22.6% and 42%,[3-6] comparable with those achieved with BEV monotherapy (**Supplemental Digital Content 1, Table 1**, http://links.lww.com/NEU/F120). The improvement in PFS without a corresponding overall survival (OS) benefit may not fully capture the true therapeutic effect of BEV in patients with rGBM. The term pseudoresponse describes the reduction in gadolinium enhancement observed in patients with GBM after BEV-induced blood-brain barrier (BBB) normalization through its anti-VEGF effect.[7] However, it has been suggested that BEV-induced vascular normalization—which restricts BEV from continuously crossing the BBB to neutralize VEGF-driven angiogenesis—may also limit its long-term efficacy.[8,9]

Evidence indicates that BEV can disrupt the perivascular cancer stem-cell niche,[10] which is considered a key source of treatment resistance and tumor recurrence.[11] In addition, BEV has been shown to reduce tumor growth in a dose-dependent manner,[12] suggesting a potential true antitumor effect, particularly at higher doses. One approach involved repeated, selective intra-arterial infusions of high-dose BEV (15 mg/kg) after BBB disruption with the hyperosmolar agent mannitol.[13] In their phase I/II clinical trial of patients with newly diagnosed GBM, this strategy yielded encouraging results, with a median OS of 23.1 months, prompting initiation of an ongoing phase III trial. By contrast, we used focused ultrasound (FUS) with systemic microbubbles (MB-FUS) to locally enhance BEV delivery.

Safe and feasible novel devices using BBB opening (BBBO) mediated by MB-FUS have emerged as strategies for repeated, targeted, and transcranial enhancement of drug delivery. These include chemotherapies such as carboplatin (371 Da)[14,15] and temozolomide (194 Da)[16] for patients with GBM and targeted therapies such as trastuzumab (148 kDa)[17] for breast cancer patients with brain metastases.[18,19] Previously, we demonstrated that the MB-FUS approach significantly increased intratumoral BEV concentration and prolonged median OS by 135% in a preclinical GBM model.[20] In addition, elevated levels of circulating brain-derived biomarkers during MB-FUS treatment have been reported, suggesting the potential application of FUS-assisted liquid biopsy (sonobiopsy) of brain diseases.[21]

Based on these rationales, we hypothesized that combining MB-FUS would enhance BEV delivery to the peritumoral regions in patients with surgically resected rGBM. This study was designed to evaluate the feasibility and potential efficacy of repeated FUS-BEV treatments targeted on the surgical cavity and to explore the associated application of sonobiopsy in this patient population.

METHODS

Study Design and Participants

This was a prospective, open-label, single-arm pilot study investigating the combination of BEV and FUS-mediated BBBO using the NaviFUS System in patients with rGBM. The inclusion criteria were as follows: (1) histologically confirmed rGBM with disease progression after radiation and temozolomide therapy, with allowance for reresection; (2) age ≥20 years; (3) an Eastern Cooperative Oncology Group performance status of 0, 1, or 2; and (4) adequate organ function assessed by routine biochemistry, hematology, and electrocardiography. Radiological assessment and time-to-event treatment response were evaluated according to the Response Assessment in Neuro-Oncology criteria for high-grade gliomas.[22] Radiological responses were independently assessed by a senior radiologist blinded to clinical outcomes. In addition, health-related quality of life was assessed at baseline and during follow-up visits using the European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire (QLQ-C30) and EORTC Brain Cancer Module (BN20).

The pilot study was designed to enroll 10 patients, aiming for at least 8 evaluable participants who completed 6 or more cycles of FUS-BEV treatment, with the additional 2 patients accounting for an anticipated 20% dropout rate. This study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice guidelines, and all applicable regulations of the Taiwan Food and Drug Administration. The protocol was approved by the Chang Gung Medical Foundation Institutional Review Board (No. 201901744A0) and registered at ClinicalTrials.gov (NCT04446416). Written informed consent was obtained from all participants before enrollment, following a detailed discussion of the study rationale, risks, and the procedural details. A study representativeness summary is provided in **Supplemental Digital Content 1, Table 2** (http://links.lww.com/NEU/F120). The clinical data from this trial are available upon request to the corresponding author.

FUS Target (Region of Interest [ROI]) Selection and Treatment Planning

After patient enrollment, an individualized FUS treatment plan was developed in 3 key steps (Figure 1):

1. ROI selection: A fundamental requirement of the FUS device (NaviFUS-001) was that each ROI be located at least 3 cm from the inner skull table. ROIs were strategically designed to (1) encompass T1-enhancing tumor regions, (2) cover areas of high T2 signal intensity (SI), and (3) block potential invasive routes along white matter tracts or deep vasculatures.[23,24] Each ROI was envisioned as a cylindrical structure measuring 15 mm in both height and diameter (volume 2.65 cm^3) and covered by a 19-spot scanning pattern (Figure 2). This design resulted in variable numbers of ROIs across patients (**Supplemental Digital Content 1, Figure 1**, http://links.lww.com/NEU/F120), necessitating an organized treatment sequence (step 3).
2. FUS simulation: Neurosurgeons used a dedicated planning software package that integrated each patient's skull computed tomography and brain MRI data. The software computed transcranial pressure distribution and transmission rates for individual array elements, according to beam incidence angles, tissue characteristics, and skull density ratios. For each ROI, optimal probe positions and incidence angles were simulated, with inclusion contingent upon achieving sufficient focal beam acoustic pressure transmission (≥15%).
3. Order of FUS treatment: The treatment sequence was arranged based on 2 primary considerations: (1) prioritizing T1-enhancing tumor regions and (2) selecting 2 ROIs with similar probe

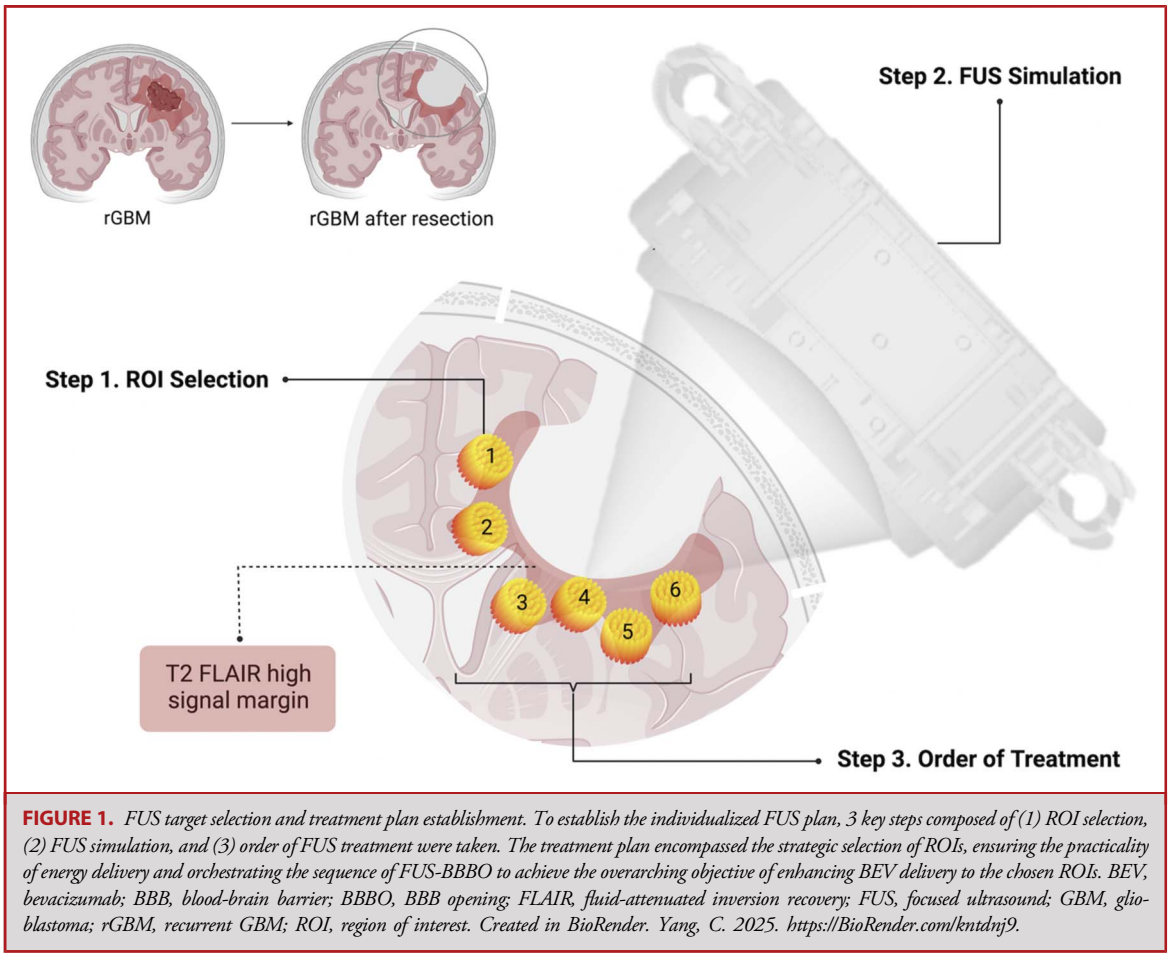

FIGURE 1. *FUS target selection and treatment plan establishment. To establish the individualized FUS plan, 3 key steps composed of (1) ROI selection, (2) FUS simulation, and (3) order of FUS treatment were taken. The treatment plan encompassed the strategic selection of ROIs, ensuring the practicality of energy delivery and orchestrating the sequence of FUS-BBBO to achieve the overarching objective of enhancing BEV delivery to the chosen ROIs. BEV, bevacizumab; BBB, blood-brain barrier; BBBO, BBB opening; FLAIR, fluid-attenuated inversion recovery; FUS, focused ultrasound; GBM, glioblastoma; rGBM, recurrent GBM; ROI, region of interest. Created in BioRender. Yang, C. 2025. https://BioRender.com/kntdnj9.*

positions whenever feasible because each session permitted treatment of 2 ROIs after a single systemic microbubble infusion (Figure 2). The objective was to rotate and reapply ROIs as extensively as possible throughout the biweekly BEV treatment course (**Supplemental Digital Content 1, Figure 2**, http://links.lww.com/NEU/F120).

Neuronavigation-Guided FUS-BBBO

A neuronavigation-guided FUS device designed for clinical use (NaviFUS-001, NaviFUS Corp) was used. Patient preparation and procedural details have been described previously.[25] The treatment protocol consisted of 18 visits over 36 weeks, with a 2-week interval between each session (**Supplemental Digital Content 1, Figure 2**, http://links.lww.com/NEU/F120). At each visit, BEV (10 mg/kg; Roche-Genentech) was administered intravenously within 1 hour before FUS. After neuronavigation registration, a weight-based microbubble contrast agent (SonoVue, 0.1 mL/kg) was infused intravenously. Patients remained awake without rigid skull fixation, enabling real-time feedback during the procedure.

FUS Exposure Control and Monitoring

The FUS system incorporated a 500-kHz, 256-channel phased array. Most array elements operated exclusively in transmission mode, whereas sparsely distributed elements alternated between transmit and receive modes to enable both exposure control (acoustic emission feedback [AEF]) and monitoring (acoustic emission mapping [AEM]). The conceptual workflow of the procedure is shown in Figure 2. A detailed description of AEF regulation and AEM reconstruction is provided in **Supplemental Digital Content 1, Figure 3-12** (http://links.lww.com/NEU/F120).

Treatment Response Assessment

The fluid-attenuated inversion recovery (FLAIR) sequence is considered to provide superior response assessment and prognostic value in patients with rGBM receiving BEV treatment.[26,27] We adopted the method described by Auer et al[28] using contralateral normal brain tissue as an internal control (IC) to assess the therapeutic effect of FUS-BEV. Radiological response was analyzed based on SI on FLAIR imaging within FUS-targeted regions (n = 5 per patient; designated as FUS-BEV) and untreated peritumoral regions (n = 5; designated as BEV-alone). The FUS-BEV/IC and BEV-alone/IC ratios were recorded at visits 1, 2, 4, 6, and 10 (corresponding to baseline, 0.5, 1, 2, and 4 months, respectively).

Liquid Biopsy Methods

Serum samples were collected immediately before and within 2 hours after FUS treatments. Because of retrospective preparation, suitable

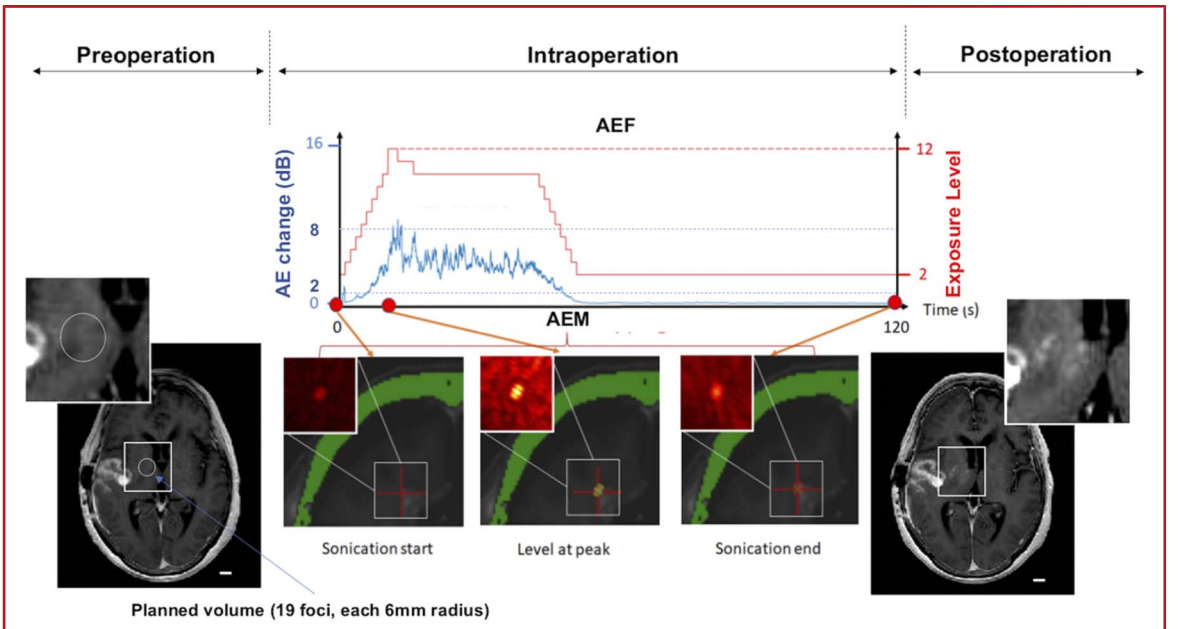

FIGURE 2. *Conceptual flow of the FUS treatment procedure. Before performing FUS-BBBO, CT and MRI were used to obtain detailed 3D skull and cerebral information for preoperative planning and intraoperative neuronavigation guidance. AEF was used to control the acoustic pressure during each treatment, ensuring proper BBBO. The AEF involved real-time monitoring through the 32 receiving-only channels with the backscattered acoustic emissions to regulate the exposure level for BBBO control (details of the AEF implementation are provided in* ***Supplemental Digital Content 1, Methods****, http://links.lww.com/NEU/F120). During FUS treatment, another 32 elements operated in transmit and receive modes in parallel to acquire 32-channel RF data, enabling transcranial reconstruction of the acoustic emission distribution to visualize the focal beam in AEM. Full 32-channel RF data were collected at 3 time points, at (1) sonication start, (2) level at the peak, and (3) sonication end. The AEM algorithm is described in detail in the* ***Supplemental Digital Content 1, Methods****, (http://links.lww.com/NEU/F120). AE, adverse event; AEF, acoustic emission feedback; AEM, acoustic emission mapping; BBB, blood-brain barrier; BBBO, BBB opening; CT, computed tomography; FUS, focused ultrasound; RF, radiofrequency.*

paired serum samples were available for only 4 of the 6 subjects, with 2 excluded from analysis. In these 4 patients, cell-free DNA (cfDNA) levels were quantified and expressed as the ratio of post-FUS to pre-FUS concentrations. This ratio was then correlated with FUS-related parameters, including FUS-beam coverage volume, peak AEF, cumulative AEF (area under the curve [AUC] of the acoustic pressure), and AEM, to explore potential real-time biomarkers during FUS transmission (**Supplemental Digital Content 1, Methods**, http://links.lww.com/NEU/F120).

Statistical Analysis

All statistical analyses were performed after study completion, with the database locked in August 2023. The primary efficacy end point was the 6-month PFS rate (PFS-6). Time-to-event outcomes, including PFS and OS with 95% CIs, were estimated using the Kaplan-Meier method. Safety was assessed according to the MedDRA Primary System Organ Class and Preferred Terms. Adverse events (AEs) were categorized by severity and by their relationship to FUS, BEV, or MB, in accordance with the Common Terminology Criteria for Adverse Events. Radiological outcomes were analyzed using Student's paired *t* test to compare FLAIR SI between baseline and treatment and between FUS-BEV and BEV-alone regions. Pearson's correlation coefficient was applied to evaluate the association between sonication parameters and both radiographic and clinical outcomes and the relationship between FUS parameters and cfDNA ratios. All analyses were conducted using SAS software (SAS Institute, Inc, version 9.4 for Windows 10). A two-sided *P* value <.05 was considered statistically significant.

RESULTS

Between July 2020 and August 2023, 6 patients with rGBM met the evaluable criteria. The 6-month PFS rate was 66.7% (4/6; 95% CI: 19.5-90.4 months), representing a substantial improvement compared with historical data, where 6-month PFS rates range from 29% to 42%[3-5,29,30] (**Supplemental Digital Content 1, Table 1**, http://links.lww.com/NEU/F120). Based on the achievement of the predefined primary end point, this pilot study (NF-2019-01) was concluded early.

All evaluable patients underwent surgical resections, with a mean residual T1-enhancing tumor volume of 8.2 ± 13.8 mL and a T2 hyperintense volume of 52.1 ± 52.8 mL (Table). A median of 6 FUS targets per patient were planned to cover residual tumor and infiltrated regions. In total, 83 treatment sessions were delivered, with a median of 14.5 sessions per patient. Four patients maintained stable disease during the study period, of whom 2

TABLE. Characteristics of Clinical Data for Patients With rGBM From Previous Therapy With RT/TMZ and Who Underwent Reresection in the FUS-BEV Trial (n = 6)

Patient no.	Age (yr), sex	No. of maintenance TMZ cycles	Time since the end of RT (mo)	Treatment location	Tumor volume (cm^3): T1 C+/T2	No. of FUS targets	Treatment sessions	Disease status	PFS since first FUS-BEV[a]	FUS-related AEs
S101	68, M	11	26	Frontal	0.1/80.4	6	17	PD[b]	12	Transient scalp heating
S104	35, M	33	41	Temporal-insular	34.9/69.8	8	8	PD	4	None
S105	35, M	2	3.4	Parietal	0.2/12.4	8	17	PD[b]	10	Transient scalp heating
S107	52, F	6	10.2	Temporal	1.2/6.1	5	18	CR	21	None
S109	65, F	3	7.8	Frontal	1/7.5	5	12	SD	20	None
S110	60, M	2	4.9	Parietal	11.5/136.5	6	11	PD	6	None
Median	56	8.5	9		1.1/41.1	6	14.5		11	
Mean (SD)	52.5 (14.6)	9.5 (12)	15.5 (14.9)		8.2 (13.8)/ 52.1 (52.8)	6.3 (1.4)	13.8 (3.7)		12.2 (7.1)	

AE, adverse event; BEV, bevacizumab; CR, complete remission; FUS, focused ultrasound; GBM, glioblastoma; PD, progressive disease; PFS, progression-free survival; rGBM, recurrent GBM; RT/TMZ, radiation and temozolomide therapy; SD, stable disease.
[a]PFS was presented in months (data censored on August 15, 2023).
[b]Disease progression was found after the end of FUS-BEV study period.

(S101 and S105) eventually developed progression. The median PFS after initiation of FUS-BEV was 11 months (IQR: 5.5-20.25).

We next examined longitudinal changes in the radiological biomarker FLAIR SI. A significantly lower FLAIR-to-IC ratio was observed in FUS-BEV–treated regions compared with BEV-alone areas (Figure 3A and **Supplemental Digital Content 1, Figure 2**, http://links.lww.com/NEU/F120). This effect, referred to as FLAIR normalization (signal hyperintensity returning toward normal), emerged within 1 month of FUS treatments and persisted for at least 4 months (Figure 3B). Moreover, the cumulative number of FUS sonications per ROI correlated positively with PFS (R^2 = 0.695, P = .04; **Supplemental Digital Content 1, Table 3**, http://links.lww.com/NEU/F120).

Across 154 sonications, 3 episodes (1.9%) of transient scalp heat sensation were reported in 2 patients. All events were grade 1 AEs, were tolerable, and did not necessitate discontinuation of the FUS treatments (Table). Both the EORTC QLQ-C30 and BN20 questionnaires demonstrated preserved quality of life before disease progression, indicating no adverse cognitive impact from repeated FUS-BEV sessions (**Supplemental Digital Content 1, Table 4**, http://links.lww.com/NEU/F120).

To assess the impact of FUS-BBBO on circulating biomarkers, we analyzed serum cfDNA from 24 paired samples (pre- and post-FUS) in 4 subjects. A significant increase in total cfDNA concentration was observed post-FUS (2.03 ± 0.76-fold), accompanied by elevated levels of *EGFR*$^+$ cfDNA (1.77 ± 0.76-fold) and *HMBS*$^+$ cfDNA (1.68 ± 0.66-fold; $P < .0001$, $P < .001$, and $P < .0001$, respectively; Figure 4A).

We further explored correlations between cfDNA ratio (post-/pre-FUS) and FUS parameters (Figure 4B-4F). A positive correlation trend was observed between FUS-coverage volume and cfDNA ratio (R^2 = 0.78, P = .12; Figure 4B). Estimated transcranial pressure showed no correlation with either the per-subject (red; R^2 = 0.35, P = .40) or per-treatment (black; R^2 = 0.014, P = .59) level (Figure 4C). Similarly, peak AEF showed no correlation with cfDNA ratio (R^2 = 0.08, P = .71; R^2 = 0.005, P = .74; Figure 4D). However, cumulative AEF (AUC) demonstrated a strong per-subject (R^2 = 0.88, P = .06) and weak per-treatment (R^2 = 0.10, P = .13) correlation with cfDNA ratio (Figure 4E). Finally, AEM intensity showed a significant moderate correlation at the per-subject level (R^2 = 0.44, P = .0004) and a weaker, though significant, correlation at the per-treatment level (R^2 = 0.33, P = .0051; Figure 4F).

DISCUSSION

The Potential of FUS-Enhanced BEV Delivery

The limited survival benefit of BEV has been attributed to the adaptive vessel co-option after prolonged inhibition of interstitial VEGF.[9,31] This adaptation restricts BEV's capacity to continuously penetrate a leaky BBB, thereby reducing its efficacy against cancer stem cells.[8,32] While it may seem counterintuitive to

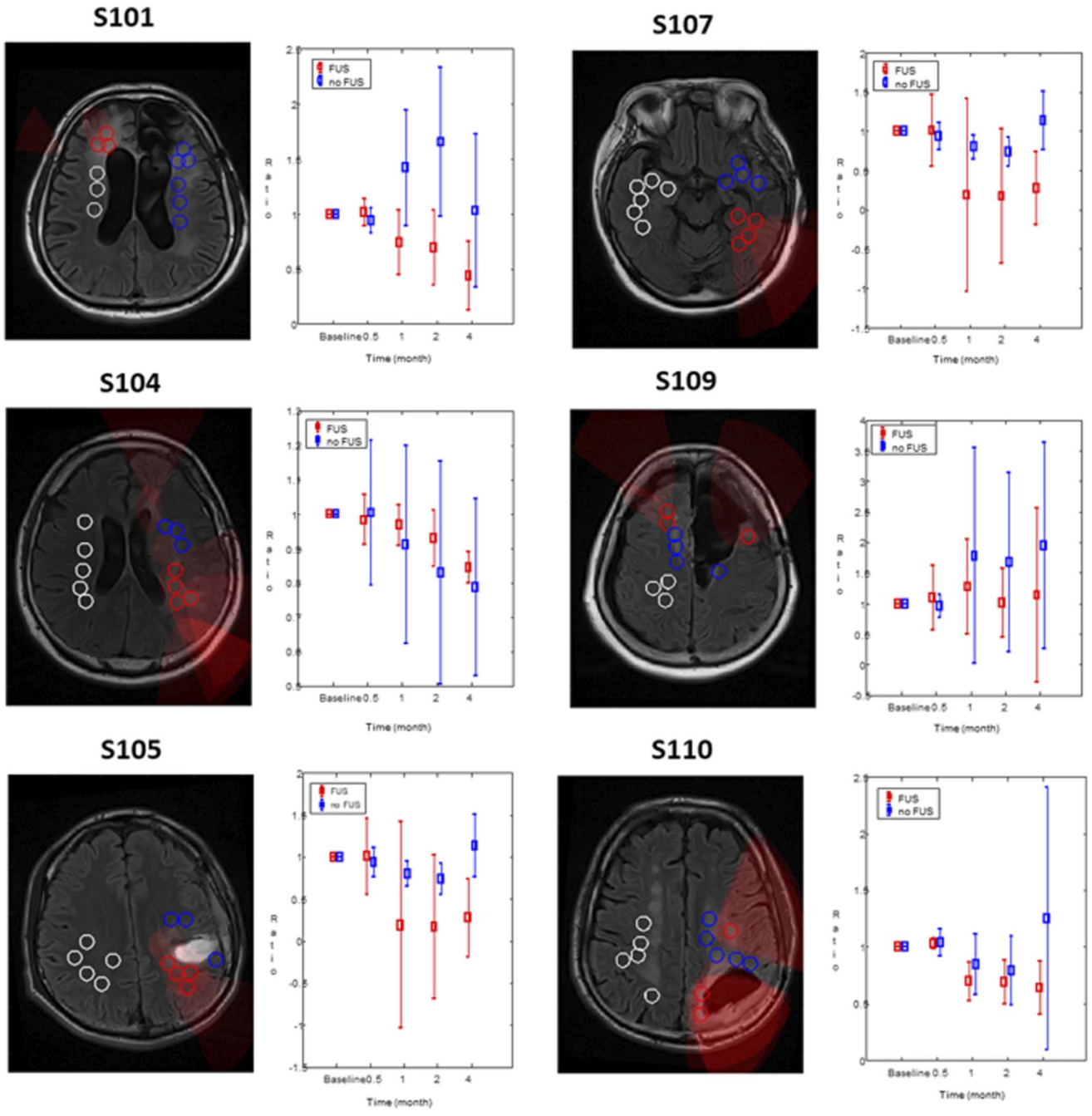

FIGURE 3. *Analysis of FLAIR hyperintensity in FUS-BEV vs BEV-alone peritumoral regions.* **A**, *For each patient, FUS-treated FLAIR hyperintensity regions (red circles, in red shadows) and FUS-nontreated FLAIR hyperintensity regions (blue circles) were selected from the baseline MRI images taken during the planning stage. Normal SI regions in the subcortical white matter (white circles) were selected and used as ICs. The changes in the FUS-BEV/IC and BEV-alone/IC ratios at baseline, 0.5, 1, 2, and 4 months for each subject were analyzed within and between groups, as shown in the box plots. Each box plot shows the median, first and third quartiles, and range.* **B**, *By pooling all data from the 6 patients over 4 consecutive months of FUS-BEV treatments, a significant "FLAIR normalization effect" was observed beginning 1 month after FUS-BEV treatments, with the effect remaining significant through 4-month period. *P < .05. BEV, bevacizumab; FLAIR, fluid-attenuated inversion-recovery; FUS, focused ultrasound; IC, internal control; SI, signal intensity.*

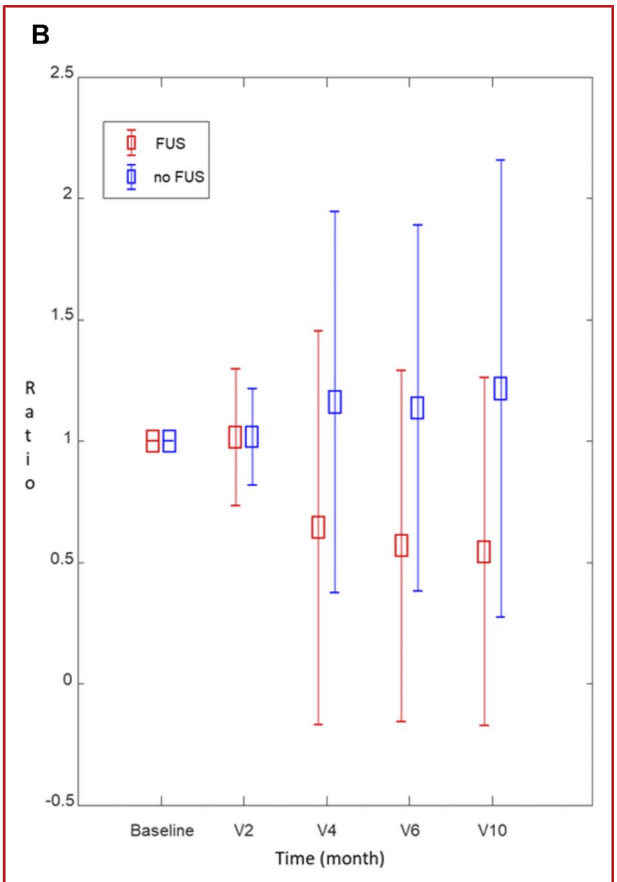

FIGURE 3. *Continued.*

combine FUS-BBBO with BEV—given that BEV itself normalizes the BBB—the addition of FUS enhances BEV penetration into peritumoral, non–contrast-enhancing regions with an otherwise intact BBB. This effect has the potential to disrupt the perivascular cancer stem-cell niche.[10,20] An augmented therapeutic effect through inhibition of cancer stem cell-endothelial cell VEGF signaling has been proposed as one mechanism underlying the promising outcomes observed in a previous phase I/II clinical trial.[13] In that study, focal enhancement of BEV concentration was achieved using selective intra-arterial infusion of high-dose BEV after BBB disruption with a hyperosmolar agent, in combination with the Stupp protocol for newly diagnosed GBM.[13] Moreover, as VEGF exerts immunosuppressive effects, anti-VEGF therapy may also potentiate immune responses.[33] Thus, the combination of BEV with FUS-BBBO presumably extend and amplify immunostimulatory responses[25] although further evidence is required to substantiate this hypothesis.

FLAIR Normalization Effect

A reduction in T2 FLAIR hyperintensity typically coincides with BEV-induced decreases in vascular permeability and vasogenic edema.[7] In this study, we observed a distinct FLAIR normalization effect that emerged after 1 month (2 FUS-BEV sessions). By directly comparing FUS-BEV–treated and BEV-alone regions—both normalized to contralateral IC—we were able to highlight the additional impact of FUS-BBBO. These findings are partially supported by established intravascular and interstitial mechanisms of BEV,[10,31,33] at least on a group-wise level. At the individual patient level, integration of disease status (Table) with longitudinal FLAIR-to-IC ratios (Figure 3A) provided further insight. Notably, only 1 patient (S104), who experienced early disease progression, failed to demonstrate FLAIR normalization (FUS-BEV/BEV-alone ratio >1; Figure 3A) and had a PFS of 4 months. By contrast, the remaining 5 patients exhibited ratios <1 early in treatment and achieved better disease control, which translated to longer PFS (with the exception of subject 110). These observations are consistent with standard imaging assessments, where extended PFS after BEV therapy correlates with radiological evidence of remission or disease stabilization on MRI.[34] Importantly, we confirmed that no patients received corticosteroids during FUS-BEV treatment, excluding steroid effects as a confounding factor in FLAIR signal reduction.

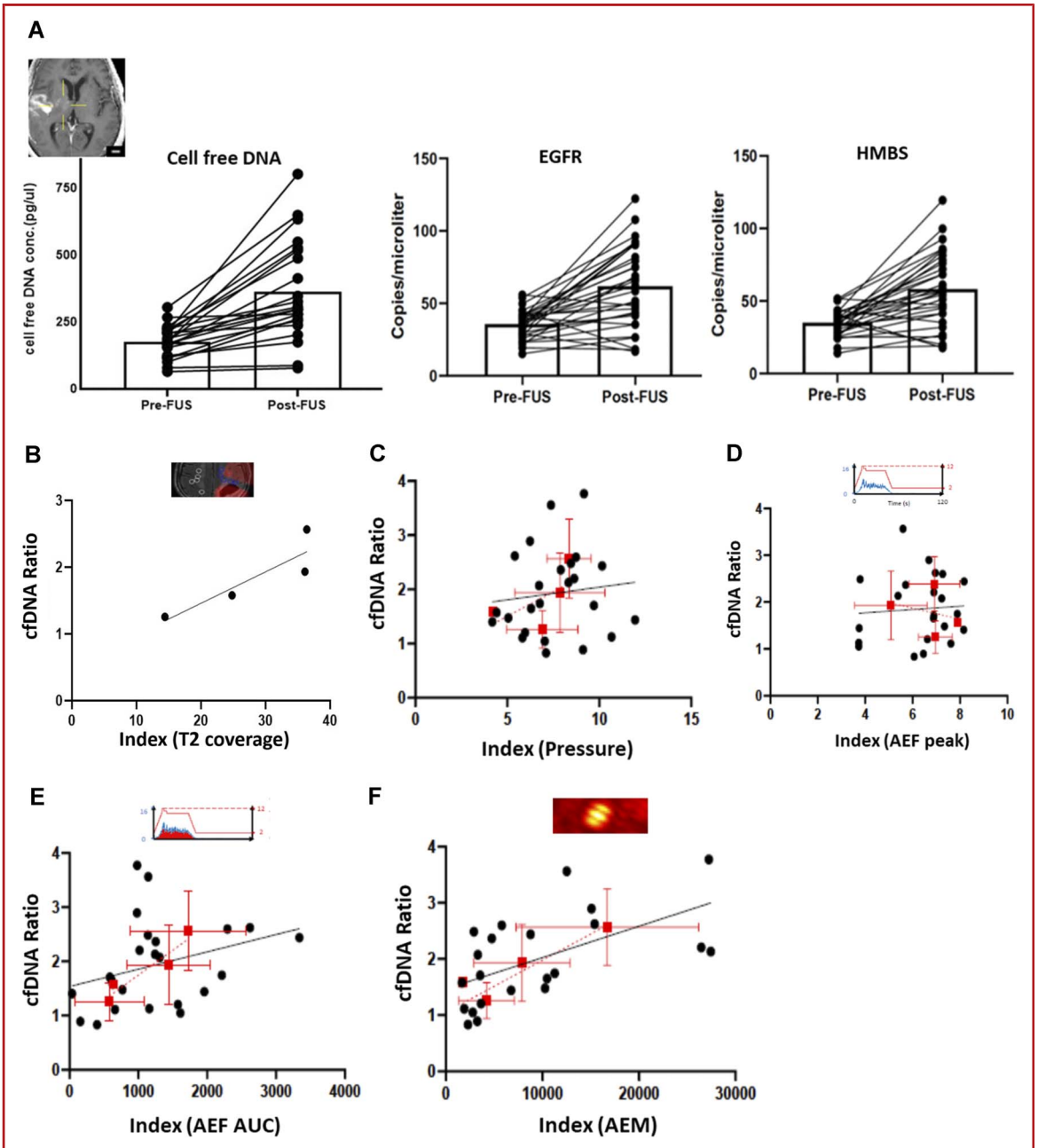

FIGURE 4. *Elevation of cfDNA in peripheral blood sampled before and after FUS treatments.* **A**, *Changes in serum cfDNA concentrations before and after FUS treatment and the correlations between different FUS parameters and post-FUS serum cfDNA levels.* **B-F**, *The AEF peak represents the peak acoustic emission pressure, whereas AEF sum represents the AUC of the acoustic pressure. Correlations between AEF peak, AEF sum, AEM, and cfDNA ratios are shown in* **D**, **E**, *and* **F**, *respectively. Black dots represent individual samples, whereas red squares and error bars denote the mean and SD. The insets illustrate each method, and please refer to* ***Supplemental Digital Content 1*** *(http://links.lww.com/NEU/F120) for further details. AEF, acoustic emission feedback; AEM, acoustic emission mapping; AUC, area under the curve; cfDNA, cell-free DNA; FUS, focused ultrasound.*

Liquid Biopsy and Feedback Monitoring

FUS-aided liquid biopsy has been demonstrated as a feasible approach for detecting the circulating brain-derived biomarkers.[21] Consistent with the findings of Meng et al,[21] we observed significant increases in both tumor-specific and nonspecific cfDNAs in plasma after FUS-BBBO. Moreover, we found that the cfDNA

ratio was positively correlated not only with FUS-targeted volume but also with FUS-penetrated brain tissue volume (data not shown). In our study, the mean cfDNA increase was 1.77 ± 0.76-fold, lower than the 2.6 ± 1.2-fold increase reported by Meng et al.[21] This discrepancy may be explained by differences in treatment parameters: Meng et al reported longer sonication times (111 ± 39 vs 13 ± 2 minutes) and larger BBBO volumes (7.8 ± 6.0 vs 5.3 mL) compared with this study. Because cfDNA release is correlated with both treatment volume and duration, the energy delivered to the targeted region is also likely to be an important determinant. Our system's incorporation of AEF ensured that the cavitation level remained stable within the FUS-targeted region, whereas AEM provided temporospatial energy data in real time (**Supplemental Digital Content 1, Methods**, http://links.lww.com/NEU/F120). As a result, the cfDNA ratio correlated more strongly with cumulative AEF (AUC) and AEM intensity than with the estimated transcranial pressure or peak AEF alone (Figure 4). These findings suggest that circulating cfDNA may serve as a real-time surrogate marker of FUS-BBBO activity. Furthermore, integrating AEF and AEM into FUS monitoring not only enhances safety but also provides visualized, feedback-guided targeting during therapy.

Limitations

This study has several limitations. First, the small sample size and absence of a control group limit the generalizability of our findings. Nonetheless, the results provide preliminary evidence supporting the safety and feasibility of combining FUS with BEV, with fewer than 2% FUS-related AEs and no reported seizures or intracranial hemorrhages. A randomized controlled trial will be necessary to confirm efficacy. Second, FUS targets were restricted to regions located at least 3 cm beneath the inner skull surface, in accordance with manufacturer recommendations, to minimize risks such as skull-brain interface heating. This restriction, in part, reduced the number of eligible patients from 10 to 6. Third, the biological mechanism underlying the FLAIR normalization effect and its clinical implications remain uncertain. Finally, the association between elevated circulating cfDNA levels after sonobiopsy and disease status is yet to be clarified.

CONCLUSION

FUS represents an emerging technology in neuro-oncology. This study demonstrates that a dual mechanism—therapeutic drug delivery and sonobiopsy—can be achieved simultaneously. Importantly, biological and radiological responses appear to correlate with sonication parameters, providing a foundation for optimizing treatment strategies. In the upcoming pivotal trial, we aim to further clarify the clinical, radiological, and survival impact of FUS-BEV in patients with rGBM.

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Disclosures

Hao-Li Liu serves as a technical consultant of NaviFUS Corp, Taiwan, and currently holds several therapeutic ultrasound-related patents. The other authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article.

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Supplemental digital content *is available for this article at neurosurgery-online.com.*

Supplemental Digital Content 1, Methods: The supplementary methods section provides details of technical advancement of NaviFUS setting in this pilot clinical trial. We used a real-time acoustic-emission-feedback controller to regulate microbubble-assisted FUS blood-brain barrier opening, automatically adjusting power based on harmonic features in backscattered signals. Therapeutic long bursts were interleaved with brief pulses for feedback, while acoustic-emission mapping with CT-guided phase correction verified focal energy delivery through the skull. The approach was validated preclinically and paired with a liquid-biopsy workflow that quantifies plasma cfDNA by digital PCR to relate molecular readouts to the ultrasound procedure.

Supplemental Digital Content 1, Table 1: Clinical prospective trials evaluating BEV in patients with new (gray shading) and recurrent (no shading) glioblastomas.

Supplemental Digital Content 1, Table 2: Representativeness of study participants.

Supplemental Digital Content 1, Table 3: Correlation of FUS sonication parameters with radiographic and survival outcomes.

Supplemental Digital Content 1, Table 4: Summary of QLQ-C30 and BN20 scores.

Supplemental Digital Content 1, Figure 1: Individual serial MRI images obtained at baseline (V1), V2, V4, V6, and V10 and with an overlay of the beam path.

Supplemental Digital Content 1, Figure 2: The study protocol of FUS-BEV treatments and MRI follow-up.

Supplemental Digital Content 1, Figure 3: Concept of the AEF controller. Ramp-up phase: initially, the ultrasound energy output of the NaviFUS device was continuously and gradually ramped up while the acoustic emissions were measured. The power level ramp-up process ended when the change in acoustic emissions reached the first designated level of 8 dB. Ramp-down phase: the power level was ramped down until the change in acoustic emissions was less than the second designated level of 2 dB, and the exposure level was then measured throughout the remaining exposure period.

Supplemental Digital Content 1, Figure 4: Geometry of the FUS with a selected target position showing the concept of the coherent backscattered emission beam formation implemented in a 2D spherical FUS array transcranially.

Supplemental Digital Content 1, Figure 5: Signal processing procedure of the AEM algorithm for a transcranial application with the 1-way delay-and-sum beamforming algorithm.

Supplemental Digital Content 1, Figure 6: Consideration of transcranial beam diffraction and phase correction based on the ray-tracing concept.

Supplemental Digital Content 1, Figure 7: A, Experimental setup for pressure-field measurements. **B**, Experimental setup for AEM.

Supplemental Digital Content 1, Figure 8: Correlation between the change in acoustic emissions and the BBB state. **A**, Definition of the BBB status and **B**, correlation between the change in acoustic emissions and the BBB status. Data are presented as mean and SD.

Supplemental Digital Content 1, Figure 9: Results for the AEF controller showing successful BBB opening **A**, without and with **B**, accompanying RBC extravasation. The extravasation in panel B was measured to be 372 μm in diameter and so did not pose any safety-related concerns.

Supplemental Digital Content 1, Figure 10: A and **B**, Hydrophone-measured transcranial pressure distributions. **C**, Passive focal beam distribution estimated from AEM.

Supplemental Digital Content 1, Figure 11: (Upper and middle) Transcranial pressure distributions without and with applying phase correction when the geometrical center is electrically steered 9 mm from the focus. (Lower) The corresponding AEMs for predicting the transcranial pressure steering position.

Supplemental Digital Content 1, Figure 12: Correlation between the measured transcranial pressure at the focus and the AEM peak magnitude.

COMMENTS

This is timely paper from a Taiwanese group with an established interest in using FUS to manipulate the blood brain barrier focally to allow delivery of Avastin for recurrent GBM. This is based on available evidence that indicates that BEV can disrupt the perivascular cancer stem-cell niche which is considered the origin of treatment resistance and tumor recurrence. Other methods to disrupt the BBB such as mannitol administration have been used to deliver greater doses of BEZ with promising results and are undergoing phase III trials.

This was a prospective pilot trial in patients with recurrent GBM to demonstrate the safety and feasibility of repeated FUS-BEV treatments which they did. With this adjuvant treatment they were able to achieve a 6-month PFS rate of 66.7% and a median PFS of 11 months.

They also performed liquid biopsy with evaluation of changes in plasma cell-free DNA (cfDNA) concentrations before and after FUS treatment and found increased levels of plasma cfDNA, EGFR, and HMBS cfDNA after FUS treatment.

MRI FLAIR sequences were used to compare FUS treated with nontreated regions, and the authors observed FLAIR signal normalization 1 month after treatment though the underlying mechanism is unknown.

It is worth noting a limitation of the FUS technique in that targets were restricted to regions located at least 3 cm beneath the inner skull surface, in accordance with manufacturer recommendations, to minimize risks such as skull-brain interface heating. This restriction, in part, reduced the number of eligible patients from 10 to 6.

Though FUS with BEV increases PFS, as yet no corresponding improvement in overall survival has been demonstrated.

I agree with authors' conclusion that repeated FUS-BEV treatment is safe and feasible in selected patients and that randomized trials are needed to confirm its therapeutic efficacy in rGBM patients.

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