Neuro-Oncology Advances
8(S4), vdag080, 2026 | https://doi.org/10.1093/noajnl/vdag080

Focused ultrasound for enhancing cell-based immunotherapies in neuro-oncology

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Abstract

Cancers of the brain remain notoriously difficult to treat because of the unique and complex barriers imposed by the central nervous system and tumor microenvironment. Current therapeutic paradigms remain largely insufficient to achieve durable responses or cures. Cellular immunotherapies (CIs), including chimeric antigen receptor (CAR) T-cells and natural killer cell therapies, represent a new class of "living drugs" that harness endogenous or adoptively transferred immune cells to target and destroy cancer. Although transformative in hematologic malignancies, CIs have faced substantial barriers in solid tumors, particularly in the brain. Focused ultrasound (FUS), a noninvasive, nonionizing, and highly tunable platform for transcranial acoustic intervention, may help address these challenges through blood-brain barrier opening, vascular activation, immunologic reprogramming, and sonogenetic control. In this review, we systematically survey the current literature at the intersection of FUS and CIs in cancer, with emphasis on brain tumors and highlight key evidence supporting combinatorial strategies, immuno-imaging approaches for CI surveillance, and emerging translational opportunities—including a first-in-human clinical investigation in glioblastoma. We conclude with perspectives on how FUS may help enable safer, more effective, and more precise CI paradigms in neuro-oncology.

Key Points

- Cellular immunotherapies, such as CAR-T-cell therapy, are in a period of rapid ascension within neuro-oncology.
- Focused ultrasound offers a uniquely versatile, noninvasive strategy for overcoming some of the key limitations facing cellular immunotherapy in brain tumors.
- Multiple preclinical studies have demonstrated the potential of FUS to cooperate with cellular immunotherapies and early phase clinical investigation of FUS and T-cell therapy is now underway.

Brain malignancies, encompassing both primary brain tumors and metastatic lesions, represent a diverse group of neoplasms that pose significant therapeutic challenges due to their complex biology, critical anatomical location, and limited treatment responsiveness.[1] The standard of care for brain tumors typically involves a multimodal approach that includes maximal safe surgical resection when feasible, followed by ionizing radiotherapy and/or systemic therapy tailored to the tumor's histology and molecular profile.[2,3] Treatment plans are guided by tumor location, patient performance status, and the extent of intracranial and systemic disease, with increasing integration of chemotherapies, targeted therapies, immunotherapy, and clinical trial enrollment where appropriate.[3] Treatment options largely remain invasive, carrying substantial risks, including neurological deficits and treatment-related toxicity, while conferring modest benefit. These limitations have driven growing interest in cellular immunotherapies (CIs), which hold the promise of transforming brain tumor care by delivering systemically active,

Received February 14, 2025; accepted March 29, 2026.

barrier-penetrating "living" therapies that can selectively eradicate malignant cells while preserving the integrity of normal brain tissue.[4,5]

Indeed, CIs represent a rapidly advancing area of investigation for the treatment of a multitude of malignancies, including brain cancer. The paradigm for CIs has historically involved isolation of peripheral immune cells; genetic engineering ex vivo by viral transduction or nonviral methods for tumor targeting, cytotoxicity, and/or persistence evaluation; and re-administration (e.g. adoptive transfer) to patients with the goal of achieving the precise localization and attack of cancer cells.[5] Recently emerging approaches are also enabling direct in vivo immune cell engineering and allogeneic transfers. https://www.nature.com/articles/s41577-024-01022-8, https://www.cell.com/trends/pharmacological-sciences/fulltext/S0165-6147(24)00052-X. There are several broad categories of CIs, including but not limited to, chimeric antigen receptor (CAR) T-cell therapy, engineered T-cell receptor therapy, tumor-infiltrating lymphocyte (TIL) therapy, natural killer (NK) cell therapy, and engineered macrophage therapy.[5–7] Perhaps the most burgeoning among these categories is CAR-T-cell therapy, in which T cells are armed with a synthetic receptor (i.e. CAR), which can recognize a specific antigen expressed on the cell surface, irrespective of major histocompatibility complex presentation, resulting in vigorous T-cell activation and potent anti-tumor responses.[8,9] CAR-T cells have exhibited unparalleled success in B-cell malignancies and other select hematological malignancies, but their efficacy has been thus far limited in solid tumors—in particular, brain tumors—due to several unique and complex biophysical and biochemical barriers that will be discussed in detail in the next section of this review. In brain tumors, these barriers include antigen escape, immune cell exhaustion, a stromal and immunosuppressive tumor microenvironment (TME), CI-specific toxicity, the blood-brain barrier (BBB), and diminished chemotaxis.

Numerous studies have explored combinatorial approaches—ranging from pharmacologic to surgical interventions—in conjunction with CIs to address these challenges. While these strategies have yielded critical insights and gains, this review centers on focused ultrasound (FUS)—a multimodal tool for noninvasive acoustic therapy—as a uniquely promising strategy for precisely modulating the tumor-immune microenvironment and enhancing the performance of CIs in brain tumors. FUS directs highly concentrated acoustic energy into tissue targets to yield tunable mechanical and thermal bioeffects with submillimeter spatial precision.[10] Notably, in the brain, this can be achieved through an intact skull, substantially minimizing patient risk and burden in contrast to traditional surgical approaches. This review highlights evidence and opportunities for cooperation between FUS and CIs, highlighting ways that FUS may serve to enhance the safety and efficacy profile of CIs with specific emphasis on T- and NK-based cell therapies. We herein comprehensively survey the state of the field for FUS in relation to key CI barriers in neuro-oncology. We also provide an overview of the critical advancements in immuno-imaging that are lending to more effective longitudinal surveillance of CI performance. Finally, we offer perspectives toward crystallizing a future in which FUS and CIs can be rationally aligned for safer and more effective amelioration of brain malignancies.

Canonical Barriers to CI

To understand the multimodal nature of allyship between FUS and CIs, one must first understand the diverse bottlenecks restricting CI efficacy and their current solutions. Indeed, achieving efficacy with CIs in solid tumors remains elusive. Solid tumors, whether located in the brain or periphery, present many challenges lacking in most blood-based cancers. The formation of a solid tumor mass is accompanied by a host of pro-tumorigenic factors that aid in its survival while serving as major barriers to successful CI intervention.[11] Amongst these barriers are vascular barriers, chemotactic barriers, stromal barriers, immunosuppressive signaling in the TME, antigen downregulation and escape, T-cell exhaustion, and immune-related toxicities resultant from high-dose therapy necessitated by these barriers (Figure 1). In this section, we briefly describe each of these barriers and their known impacts on CI performance.

Antigen Downregulation and Escape

Antigen escape is a foundational problem with targeted CIs in both brain and peripheral tumors. Single-antigen targeting creates selective pressure that favors the outgrowth of antigen-low or antigen-negative clones. Indeed, univalent CI constructs have a natural tendency to select for cell populations expressing the antigen of choice, while antigen-low or antigen-negative populations are free to expand (Figure 1C).[12] This problem was exemplified in a recent clinical trial utilizing epidermal growth factor receptor variant III (EGFRvIII)-targeted CAR-T cells against recurrent glioblastoma (GBM) (NCT02664363). A comparison of tumor specimens from pre to post CAR-T-cell infusion revealed decreased levels of EGFRvIII expression in 5 out of the 7 patients.[13] Several approaches have been taken to circumvent this problem, including the engineering of CAR constructs containing 2 single-chain fragment variables, allowing for bivalent, or dual antigen, targeting.[14] While there is limited published literature on the ability of FUS to impact antigen escape, it can be postulated that FUS could alter antigen availability or expression depending upon the acoustic exposure conditions, consistent with demonstrations in extracranial literature.[15] However, more research needs to be done to explore this phenomenon.

Cellular Exhaustion

For CIs to elicit a productive and sustained anti-tumor response, they must be able to maintain their effector function within the TME. However, tonic antigen signaling can lead to a hypoactive state known as exhaustion. T-cell exhaustion is considered a negative feedback mechanism for T-cell activation and is characterized by a decrease in effector function, loss of cytokine production, and sustained expression of inhibitory receptors, including PD-1, TIM-3, and LAG-3 (Figure 1F).[16] Furthermore, exhaustion is often accompanied by the downregulation of cytokines, which are critical to T-cell-mediated responses against tumors,

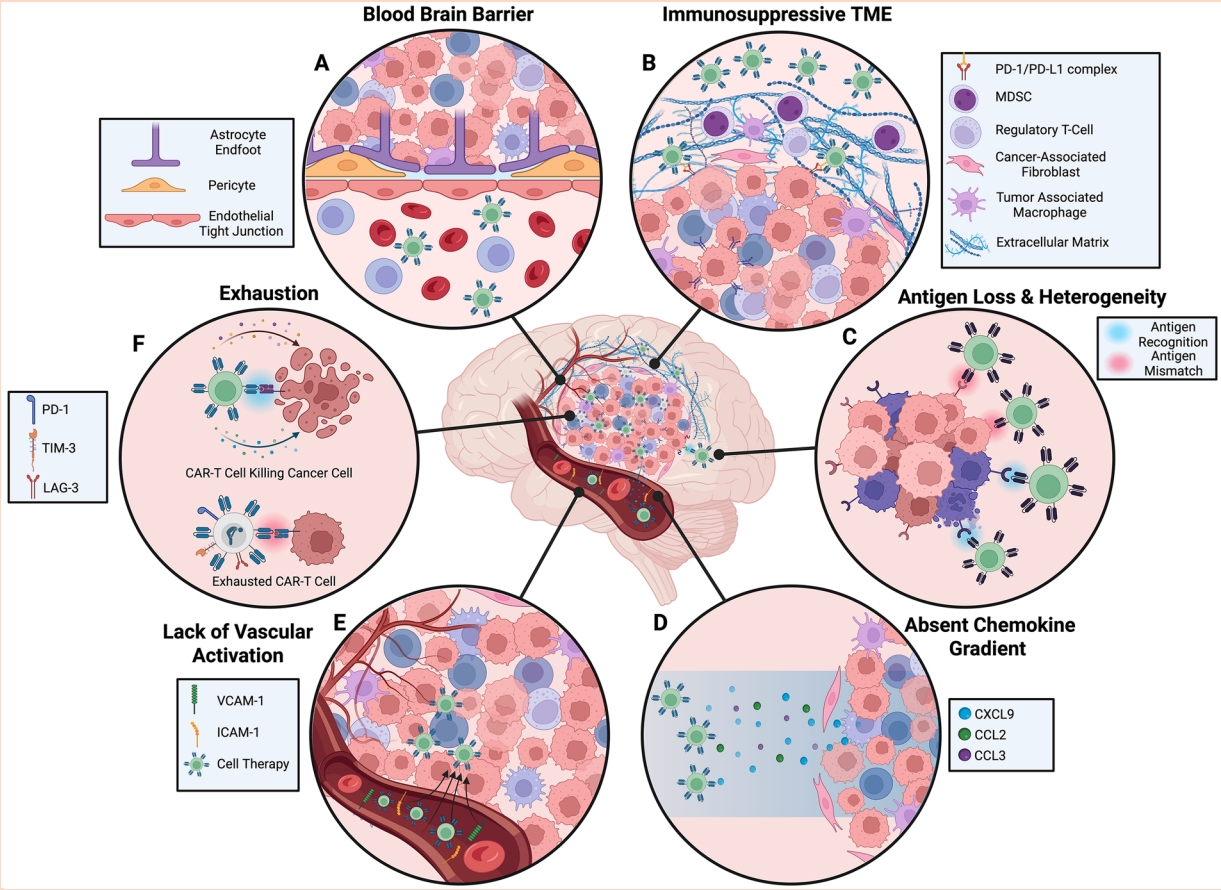

Figure 1. Barriers to effective cell immunotherapy in the brain. (A) The blood-brain barrier (BBB) is a network of selectively permeable blood vessels, most notably characterized by tight junctions lining the endothelium. The BBB restricts delivery of most macromolecular therapeutics and can pose limitations to transport of cellular immunotherapies. (B) The tumor microenvironment (TME) of solid cancers manifests a variety of pro-tumor and immunosuppressive characteristics. Examples of pro-tumor and immunosuppressive qualities are dense ECM, prevalence of myeloid-derived suppressor cells, tumor-associated macrophages, and immunological signaling axes that contribute to T-cell exhaustion such as the PD-1/PD-L1 complex. (C) Antigen loss is a primary mechanism of cell therapy evasion whereby key target proteins are downregulated or selected against. Heterogeneous antigen expression among tumor cell populations can also contribute to incomplete eradication. (D) Tumors often lack the chemokine gradients required for immune cell homing. Examples of common chemokines involved in immune cell trafficking include CXCL9, CCL2, and CCL3. (E) Chronic stimulation can lead to CAR-T cell exhaustion, which can be characterized by the expression of a combination of immune checkpoint molecules including PD-1, TIM-3, and LAG-3. Created in BioRender. Sheybani (2025) https://BioRender.com/j57v297. MDSC, myeloid-derived suppressor cells; CAR, chimeric antigen receptor; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1.

including TNF-α, IFN-γ, and IL-2.[16] A major area of focus for combatting exhaustion has been through T-cell engineering—for example, by modification of the CAR constructs themselves. A recent study compared CAR-T cells targeted against the integrin protein $\alpha_v\beta_3$ with CD-28 vs 4-1BB costimulatory domains in the settings of diffuse intrinsic pontine glioma and GBM. Interestingly, $\alpha_v\beta_3$.28ζCAR-T cells exhibited superior tumor cell cytotoxicity in vitro, whereas $\alpha_v\beta_3$.BBζ CAR-T cells achieved a more favorable in vivo response, despite a slower expansion profile.[17] Other efforts to combat exhaustion include the coadministration of checkpoint inhibitors such as αPD-1 or αCTLA-4 in combination with CIs. A recent clinical case study of 10 adult patients with high-grade gliomas demonstrated successful blockade of PD-1 on both endogenous and intraventricularly administered CAR-T cells in the cerebral spinal fluid (CSF) after intravenous injection of pembrolizumab.[18] This study demonstrated that blockade of PD-1 on IL13alphaR2-targeted CAR-T cells enhanced CAR-T cell in vitro cytotoxicity against PD-L1-overexpressing patient-derived xenografts. However, exhaustion-specific markers, such as FOXP3, TIM3, and LAG3, and T-cell functional capacity were not assessed. These results indicate that checkpoint blockade may be a promising strategy for combatting PD-1-mediated T-cell exhaustion in CI settings. FUS offers potential as a promising strategy for combatting exhaustion via targeted checkpoint inhibitor delivery or direct modulation of the TME, which will be discussed in the following sections.

Stromal Barriers

Solid tumors have an arsenal of pro-tumorigenic mechanisms used to promote their proliferation and survival. Among these mechanisms is the formation of a tumor stroma capable of immunosuppressive biochemical signaling and generation of biophysical barriers preventing the successful penetrance of CIs. The extracellular matrix of solid tumors, generally composed of a mixture of collagen, proteoglycans, and heparin sulfate, is often dense and highly disorganized (Figure 1B).[19] Tumor cells do not synthesize these matrix components directly but rather stimulate their production in neighboring cell types. The collagen networks in many solid tumors are highly misaligned with little to no structure. This is exceptionally important in the context of brains tumors as tumor ECM stiffness has been correlated with increasing tumor grade in gliomas.[20] Lack of fiber alignment can impede T-cell trafficking, as it has been found that cytotoxic T-cells traffic more efficiently on aligned collagen fibers compared to misaligned ones.[19] Dense tumor ECM can compress blood vessels, generating regions of hypoxia, which can further potentiate the hostile immunosuppressive TME.[21] Additionally, this compression can lead to high interstitial fluid pressures capable of hindering the accumulation of therapeutic agents, including those at the cellular scale.[22]

A variety of approaches have been studied for the potential to overcome or eliminate stromal barriers in the context of CIs. A study by Caruana et al engineered CAR-T cells to express the enzyme heparinase. The expression of this enzyme enhanced the ability of T cells to degrade the tumor matrix, which promoted their infiltration and anti-tumor activity.[23] Another recent study developed CAR-T cells targeted to the supporting stroma instead of the tumor cells themselves. Using CAR-T cells directed against fibroblast activation protein (FAP), expressed on cancer-associated fibroblasts (CAFs), this study revealed that elimination of FAP^+ CAFs resulted in a loss of the structural integrity of the desmoplastic matrix. Treatment with FAP-targeting CAR-T cells prior to the administration of tumor-targeted mesothelin CAR-T cells and PD-1 blockade resulted in significant tumor growth control.[24] The capacity of FUS to break down these barriers as a stromal rewiring or gross debulking strategy offers yet another lens for promising combinations with CIs to be discussed.

Immunosuppressive TME

The highly immunosuppressed nature of most TMEs represents another major barrier broadly facing immunotherapies in both brain and peripheral settings—and CIs are no exception (Figure 1B). Macrophages are a major component of the tumor-immune environment, particularly in brain tumors, wherein up to 30% of tumor mass can consist of tissue-resident microglia and bone marrow-derived macrophages.[25] Tumor-associated macrophages (TAMs) can inhibit T-cell-mediated anti-tumor activity through a variety of mechanisms, including the secretion of amino acid-depleting enzymes such as indoleamine 2,3-dioxygenase and through cytokine secretion. Additionally, TAMs can aid in the recruitment of regulatory T-cells (Tregs) to further bolster the immunosuppressive TME.[26] Tregs are a principal component of the pro-tumorigenic landscape, as they have been known to suppress the cytotoxic activity of CIs through the production of immunosuppressive cytokines, competitive consumption of IL-2, and CTLA-4-induced antigen-presenting cell (APC) suppression.[27] Myeloid-derived suppressor cells (MDSCs) represent another canonically immunosuppressive cell subtype that has garnered increasing interest for its profound role in CAR-T-cell inhibition. Using titrated dosing regimens, MDSCs can also be transiently depleted using chemotherapies such as gemcitabine, cyclophosphamide, and 5-fluorouracil.[28] Indeed, promising results obtained from preclinical murine GBM studies have been translated into a clinical trial investigating combinations of low-dose capecitabine and bevacizumab for immunomodulatory reduction of MDSCs in patients with recurrent GBM (NCT02669173).[29,30] The immunomodulatory effects of FUS and potential synergies with myeloid-targeted therapy offer a prime opportunity for targeting this barrier, as discussed in the following sections of this review.

Immune-Related Toxicities

Regardless of whether a tumor is located in the periphery or the central nervous system (CNS), toxicity remains a major barrier to the successful implementation of CIs in the clinical setting. For example, CAR-T-cell-induced toxicity can manifest in several forms, including cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS). CRS typically occurs within hours to days post-cell infusion, with patients experiencing a spectrum of symptoms including fatigue, fever, chills, headache, dyspnea, tachycardia, and even life-threatening organ failure.[31,32] CRS is caused by the release of proinflammatory cytokines (e.g. IFN-γ, IL-1, IL-2, IL-10), which are released by CAR-T cells upon their engagement with cancer cells. The standard of care treatment for CRS is tocilizumab, an IL-6 receptor inhibitor.[32] ICANS is a serious side effect of CAR-T-cell treatment that affects a patient's nervous system, with symptoms including headaches, agitation, confusion, tremors, seizures, and a host of other neurological complications.[33] ICANS has similar pathological origins to that of CRS in that cytokines released by CAR-T cells are thought to act on monocytes and macrophages that further release IL-1, IL-6, and inducible nitric oxide synthase.[31] Furthermore, these cytokines can lead to overactivation of brain endothelial cells that result in a loss of BBB integrity and subsequent neuroinflammation.[31] The severe nature of CI toxicity emphasizes a critical gap that FUS could fill for achieving dose de-escalation and improved targeted cellular delivery. These opportunities will be highlighted in the following sections of this review.

Vascular Barriers

In the context of CNS malignancies, a vascular barrier that bears specific mention is the necessarily restrictive BBB (Figure 1A). The BBB is a unique, densely vascularized

network that limits the transport of macromolecules—inclusive of many pharmacologic agents—into the brain. The pharmacologic and immunologic distinctions associated with crossing the BBB begin with consideration of its highly regulated vascular endothelium, sealed by endothelial tight junction protein complexes.[10] While activated T-cells can cross the BBB, their trafficking to and within the CNS is complex and can include other routes such as via the CSF and choroid plexus.[9] FUS offers a unique and tunable strategy for modulation of both the BBB, as will be discussed, and glymphatics.[10,34,35] However, an intrinsic characteristic of the tumor vasculature that often impedes immune cell trafficking is its consistent state of vascular anergy. This state is characterized by the downregulation of cellular adhesion molecules, including vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1), which are required for immune cell extravasation (Figure 1E).[36] There are a variety of factors that can lead to this lack of vascular activation, but it is believed that the secretion of proangiogenic factors, including VEGF and fibroblast growth factors, is key contributing entity.[37] The structural nature of the tumor endothelium also impedes immune cell homing, as tumors often have physically distorted and tortuous blood vessels that yield heterogeneous leakiness and irregular blood flow.[38] As will be discussed, FUS may help address these vascular barriers by transiently modulating BBB permeability and promoting endothelial activation, chemokine gradients, or adhesion molecule expression that can support immune cell extravasation.

Chemotactic Barriers

While adhesion molecule silencing and irregular vascular structure can pose challenges to immune cell migration into the TME, these barriers often act in concert with others. Effector cells rely on cues provided by chemotactic cytokine gradients within the circulation to home to sites of inflammation. Chemokines, including CCL2, CCL3, CCL5, and CXCL9 (alongside CXCL10 and CXCL11), are highly expressed in T-cell-inflamed tumors, where they play key roles in promoting T-cell recruitment and infiltration, alongside recruitment of other immune subsets.[39] In the context of chemotactic signaling, mismatch between cytokines secreted by tumor cells and the receptors present on CIs also represents a major concern for proper immune cell trafficking (Figure 1D). Efforts have been made to circumnavigate these barriers, such as the engineering of CAR-T cells to express cytokine receptors to match those secreted by tumor cells. For example, a study by Jin et al took advantage of radiation-induced IL-8 release by modifying CD70-targeted CAR-T cells to express the IL-8 receptor (CXCR1 or CXCR2). The modified CARs demonstrated enhanced migration and persistence within the tumor along with induction of complete tumor regression in mouse models of GBM, ovarian cancer, and pancreatic cancer.[40] To be discussed further, FUS can elicit the expression of pro-inflammatory chemokines to guide immune cell trafficking, offering another potential pathway for effective combination therapy.

Overview of FUS Strategies in Neuro-Oncology

Among its many advantages, FUS is a uniquely versatile intervention strategy. Indeed, acoustic exposure conditions can be tuned to promote a wide range of impacts to targeted tissues, ranging from thermal to mechanical and tissue-destructive to nondestructive, which could in turn elaborate improved responses to CIs in brain tumors in various ways. This section offers a broad overview of FUS mechanisms of action that have been investigated or otherwise hold promise for investigation with CIs in neuro-oncologic indications. These include applications of FUS for mechanical or thermal BBB permeabilization, ablative or sonodynamic tumor destruction, and sonogenetic control of CIs.

FUS for Transient BBB Permeabilization

Among FUS modalities relevant to neuro-oncology, microbubble (MB)-enhanced FUS for transient BBB opening (BBBO) is one of the most clinically advanced. Using micro- to millisecond, low-duty-cycle pressure pulses delivered in the presence of intravenously administered MBs, FUS can reversibly increase BBB permeability in a spatially localized manner, and human studies have established its safety and feasibility for neuro-oncologic drug delivery.[10,41–44] First demonstrated by Hynynen et al[41] in 2001, FUS BBBO combines low-intensity ultrasound pulses with circulating MBs–ultrasound contrast agents, typically of a polydisperse composition, to transiently disrupt the BBB for therapeutic delivery and other bioeffects (Figure 2). MBs act as in vivo acoustic amplifiers, enabling the amount of FUS pressure necessary for BBBO to be greatly reduced and, in turn, limiting the risk of skull heating.[29] BBBO arises from MB-mediated mechanical effects initiated within the local vasculature: when MBs oscillate (i.e. cavitate), they induce vessel distension and invagination, transiently disrupting endothelial tight junctions and promoting endothelial sonoporation among other mechanisms.[45] Techniques such as passive cavitation detection, passive acoustic mapping, and passive cavitation imaging can be used to monitor cavitation activity, guide safe BBBO, and in some cases, infer therapeutic delivery.[46–51] To date, technical advances in FUS BBBO technology and a growing variety of commercially available clinical systems have enabled hundreds of patients worldwide to safely undergo this procedure for applications ranging from drug-free interventions to chemotherapy and antibody delivery.[52–55] As discussed further below, this paradigm is now being extended to CI in the active phase 1 University of Virginia study (NCT07343986) evaluating a combination of FUS BBBO and bispecific antibody-armed T-cells for newly diagnosed GBM.

FUS for Sublethal Heating

In addition to MB-assisted FUS for BBBO, FUS hyperthermia has been demonstrated as an alternative method for BBB

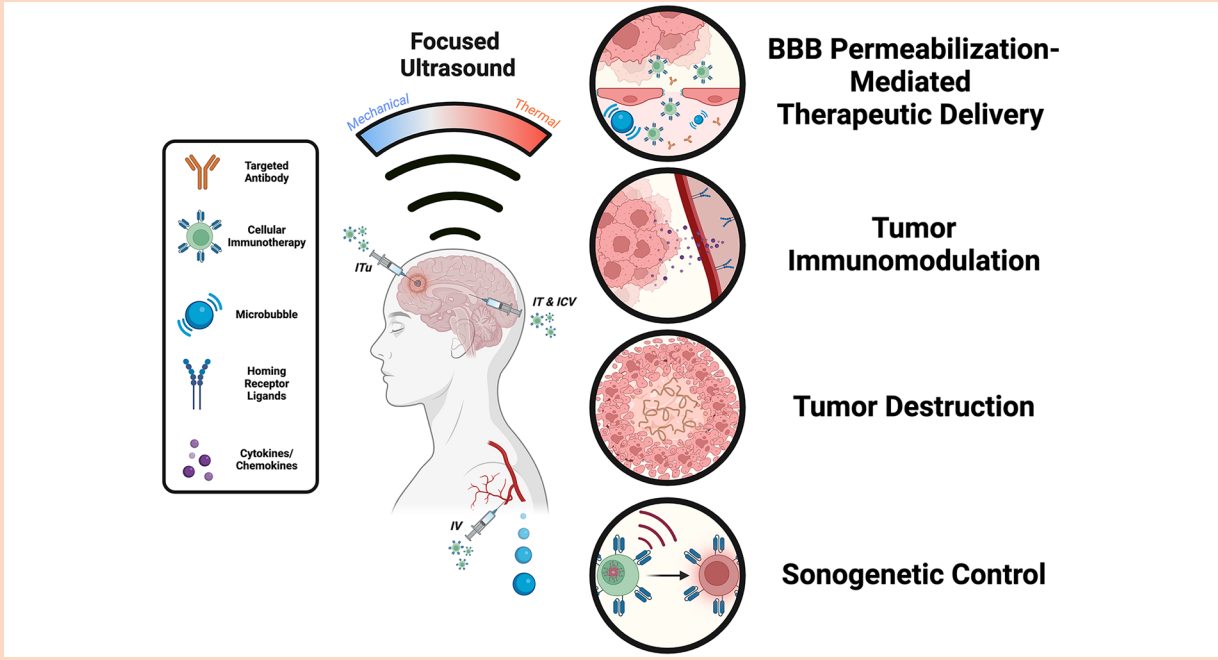

Figure 2. Focused ultrasound as a versatile tool for improvement of cellular immunotherapy strategies. FUS modalities range from mechanical (e.g. microbubble-mediated BBB permeabilization for improved therapeutic delivery) to thermal (e.g. warming or ablation of tissues). These modes of action can also yield immunomodulatory effects or be engineered for sonogenetic control of immune cells. All modalities can be achieved noninvasively and contribute unique avenues for improved CI delivery and/or function. These approaches may intersect with administration of CIs by multiple different common routes, including intravenous (IV), intrathecal (IT), intracerebroventricular (ICV), and intratumoral (ITu) or intracavitary injection. Created in BioRender. Sheybani (2025) https://BioRender.com/2ey58de. FUS, focused ultrasound; BBB, blood-brain barrier.

permeabilization that may cooperate with immunotherapeutic and CI delivery. The bioeffects of hyperthermia extend well beyond the vasculature, however. Hyperthermia is characterized by sustained heating to sub-lethal temperature levels (~43°C).[56] Many previous studies have leveraged FUS hyperthermia as a radiosensitizing technique for the treatment of primary brain tumors.[57] FUS hyperthermia has also been investigated in combination with several immunotherapeutic and cell-based strategies (the latter will be discussed in the context of sonogenetics). McDannold et al[58] demonstrated that FUS hyperthermia can induce BBBO in the brains of rabbits. Additionally, Kim et al[59] leveraged a closed-loop FUS hyperthermia regimen to safely elevate K^{trans} permeabilization values in orthotopic GL261 brain tumors, promoting vascular transport changes that ultimately improved thermosensitive drug delivery. Of note, FUS hyperthermia has also been shown to induce immunogenic thermal stress, as in the example of upregulated heat shock protein (HSP)-70 expression in tumors, upregulation of pro-inflammatory chemokines and cytokines, increased cytotoxic activity of NK and $CD8^+$ T cells in addition to DC maturation.[29,60] As will be surveyed, BBB permeabilization and hyperthermia are the most active areas of investigation to date for the use of FUS in conjunction with CIs.

FUS for Targeted Tumor Destruction

It is widely known that the degree of tumor burden at the time of CI administration can play a significant role in resultant efficacy and toxicity.[61] Although the tissue-destructive bioeffects of FUS have not been a topic of investigation with CIs to date, they represent a promising pillar for FUS neuro-oncology going forward. FUS modalities that enable tumor destruction—the debulking and immune activation features of which may synergize with CIs—include histotripsy, thermal ablation (T-FUS), and sonodynamic therapy (SDT).

Targeted FUS thermal ablation, achieved through continuous wave application of high-amplitude sound waves to small tissue volumes, can promote heat-induced cellular destruction (Figure 2).[57] To date, preclinical and clinical transcranial thermal ablation studies have spanned a wide range of oncologic and nononcologic indications.[62,63] Notably, FUS thermal ablation is FDA-approved for incisionless ablation in the setting of select movement disorders.[64] In 2009, McDannold et al[65] disseminated initial findings from the deployment of transcranial FUS for noninvasive thermal ablation of GBM in 3 patients. Since that time, combinatorial strategies utilizing FUS thermal ablation—inclusive of combination with CIs—have been understudied in neuro-oncology despite the strong precedent set by other ablative modalities such as laser interstitial thermal therapy.[66] This may partly reflect current limitations of clinical transcranial FUS thermal ablation platforms, including treatment-envelope constraints and the time required to sonicate larger tumor volumes, which may reduce their practicality for large-volume brain tumor ablation.

Histotripsy, a mechanical ablation modality, offers yet another emerging approach that is still nascent in

neuro-oncology indications. This nonthermal technique utilizes high-pressure amplitudes to drive nonlinear oscillation (i.e. cavitation) of endogenous gas bubbles within tissues, rendering them into acellular debris through precise mechanical fractionation.[67] To date, multiple studies have established neuronavigation- and MRI-guided histotripsy for transcranial applications.[68,69] Moreover, the feasibility of ablating primary and secondary brain tumors with histotripsy has been shown in preclinical studies.[70,71] Though no published studies to date have combined histotripsy with CIs, the powerful immunostimulatory effects induced by histotripsy in extracranial studies suggest that it could synergize well with CIs.[15]

In contrast with thermal or mechanical ablation techniques, SDT utilizes low-intensity sound waves and sonosensitizers such as 5-aminolevulinic acid (5-ALA) to achieve cell-selective cytotoxicity. Upon ultrasound activation, the sonosensitizer undergoes excitation and triggers oxidative stress within tumor cells, leading to apoptosis and necrosis without causing gross tissue destruction.[72,73] This nonablative approach—which is under active clinical investigation in high-grade adult and pediatric glioma settings (e.g. NCT06039709, NCT04559685, NCT05123534)—allows for spatially targeted tumor cell killing while preserving surrounding healthy structures, and it can be further tuned by varying sensitizer chemistry, dosing, and ultrasound parameters. Importantly, SDT not only mediates direct tumor cell death but also promotes immunogenic cell death, offering opportunities for synergy with CIs.[74]

Combination of FUS with CIs

The topic of CIs has seen unprecedented growth across the sectors of basic discovery and clinical translation over the last several decades. This growth has been mirrored in the FUS domain, which is just beginning to reach an inflection point in studies at this nexus. Having outlined the mechanistic spectrum of FUS, we next summarize how these modalities have been combined with adoptive CIs. From publications available to date, we can glean critical insights but also recognize knowledge gaps that must be considered toward refining goals and designing optimal FUS + CI combinations. This section surveys the published literature examining FUS and CIs to date, focusing on 3 key areas: preclinical studies involving NK cells, CAR-T cells, and the emerging frontier of sonogenetics for acoustically controllable CIs. A summary of studies is provided in Table 1.

FUS and Natural Killer Cells

Several studies have utilized FUS in combination with NK cells in both brain and peripheral tumor models, with results that could shed light on the relationship between cellular delivery and therapeutic benefit. One such study performed MB-assisted FUS (0.5 MPa, 1 MHz) with NK-92-MI cells in an ovarian flank tumor model. Even though more NK cells were found in the FUS-recipient tumors 24 h later, this increase was insufficient to confer differences in tumor outgrowth—suggesting either that increased NK cell penetrance was transient or that efficacy was limited by other TME-related factors.[75] In this study, differences in cell trafficking were postulated to be ICAM-1-mediated, but no differences in cell adhesion molecule expression could be pinpointed. Notably, CX3CL1 was upregulated in the combination group 24 h post-FUS, suggesting more work is needed to cement FUS-induced chemokine release as a mediator of NK cell migration.

Similar results were found in a human colorectal adenocarcinoma flank model when comparing 0.25 and 0.5 MPa pulsed FUS (pFUS). Only the higher pressure group yielded enhanced accumulation of ferumoxytol labeled NK-92 cells at 1, 6, and 24 h post-FUS, while no differences were observed in the lower pressure group.[76] MR imaging revealed enhanced NK cell accumulation in contralateral untreated tumors when the opposing tumors were sonicated at 0.5 MPa. In the absence of outgrowth data, this finding still aligns with others suggesting an abscopal impact of FUS on distal sites beyond the primary treated tumor. The biological mechanisms underlying these effects still require further investigation.

An increasingly important consideration when combining FUS with CIs is the timing of FUS intervention relative to cell administration, in addition to the route of administration. While the latter has yet to be deeply explored, the former has been the topic of recent work. A series of studies published by Alkins et al investigated this relationship in the context of HER2-amplified breast cancer brain metastasis (BCBM) and chimeric HER2-targeted NK-92 cells. They found that sonicating right after NK cell transfer drastically enhanced cell migration. The average ratio of NK-92 cells to tumor cells was 1:100 when cells were injected immediately before FUS, whereas transfer after sonication or without FUS achieved ratios of 2:1000 and 1:1000, respectively.[77] Building off these results, a follow-on study investigated the effects of repeated BBBOs on CAR-NK cell migration in the same BCBM model. Treatments were broken up into a front-loaded arm where 5 sonications were applied in the first week, 2 in the second week, and 1 in the third week. The second arm distributed the treatments twice weekly over 4 weeks.[78] Only the front-loaded arm saw a significant reduction in tumor outgrowth and survival benefit, underscoring the importance of therapeutic sequencing. It is also worth noting that the aforementioned survival benefit was only observed in half of the animal cohort, wherein the authors postulated that tumor starting volume may have been a contributing factor. This highlights yet another broadly important consideration in the design of FUS + CI paradigms.

FUS and CAR-T Cells

Although only a limited number of studies have explored the combination of FUS with CAR-T cells for brain malignancies, this is undeniably a topic of growing excitement and interest. While the emerging data covered here center on CAR-T cells, these studies offer important insights and highlight the potential of FUS to enhance T-cell therapies more broadly. A study by Sabbagh et al[79] investigated the role of low-intensity pulsed ultrasound for BBBO and EGFRvIII-targeted CAR-T cells in the setting of EGFRvIII-enriched U87 gliomas. When luciferase-expressing CAR-T cells were administered immediately before MBs and

Table 1. Summary of preclinical studies to date combining FUS and CIs

Study title	Cell therapy	Ultrasound parameters	Microbubble type and dose (if applicable)	Cell line (location)	Animal model	Key observations	Ref
Focused ultrasound improves NK-92MI cells infiltration into tumors	NK-92MI	1.0 MHz, 0.5 MPa, 10% duty cycle; 10 ms every second for 1 min	UsphereTrans+ microbubbles ($1\text{-}4\times10^{10}$ particles/mL; 100 µL)	SKOV3 human ovarian cancer cells (flank)	NCG mice	Improved cell delivery to SKOV3 tumors using BLI, no difference in outgrowth between NK-92MI vs FUS + NK-92MI	76
Low-dose focused ultrasound induces enhanced tumor accumulation of natural killer cells	NK cells purified from PMBC	510 kHz, 0.25 and 0.5 MPa; 10 ms every second for 1 min	Optison (100 µL)	LS-174T human colorectal adeno-carcinoma (flank)	NSG mice	Significant accumulation of ferumoxytol labeled NK cells via MRI in 0.5 MPa group with no detectable accumulation in 0.25 MPa group	77
Focused ultrasound delivers targeted immune cells to metastatic brain tumors	HER2-specific NK-92-scFv(FRP5)-zeta cells	551.5 kHz, 10ms pulses, 1 Hz PRF, 120s duration; 0.33 MPa average in situ (range: 0.32-0.35 MPa)	Definity (1:10 dilution in saline; 0.2 mL/kg)	Human HER2 expressing MDA-MB-231 breast tumor cells (intracranial)	Male athymic nude rats	Significant accumulation of iron-labeled NK cells at tumor site with BBBO; presence of NK cells in the circulation at time of FUS yielded signifi-cantly greater effector-to-tumor cell ratio	78
Early treatment of HER2-amplified brain tumors with targeted NK-92 cells and focused ultrasound improves survival	HER2-specific NK-92-scFv(FRP5)-zeta cells	551.5 kHz, 10 ms pulses, 2 Hz PRF, 120 s duration; PNP not reported* **Acoustic power modulated to predetermined ultraharmonic signatures consistent with previously published work*	Definity (1:10 dilution in saline; 20 µL/kg)	Human HER2 expressing MDA-MB-231 breast tumor cells, (intracranial)	Male athymic nude rats	Early intensive cell delivery with BBBO resulted in improved survival (vs biweekly treatments), yielding long term survival in 50% of subjects	79
Opening of the blood-brain barrier using low-intensity pulsed ultrasound enhances responses to immunotherapy in preclinical glioma models	EGFRvIII-targeted human CAR-T cells	1 MHz, 1 Hz PRF, 25,000 cycle pulse length, 2.5% duty cycle, 0.3 MPa, 120 duration	200 µL lipid based, Lumason lipid microbubbles	EGFRvIII-U87 human glioblas-toma, (intracranial)	NSG Mice	Significant survival benefit with addition of LIPU immediately before CAR-T administration	80
Ultrasound frequency-controlled microbubble dynamics in brain vessels regulate the enrichment of inflammatory pathways in the blood-brain barrier	EGFRvIII-targeted murine CAR-T cells	0.5 and 1.5 MHz, 10 ms bursts, 2 Hz PRF, 2 min duration; 150 and 175 kPa	Optison, Definity (5×10^{8} MBs/ml; 1×10^{7} MB per sonication)	SB28-EGFRvIII murine glioma, (intracranial)	C57BL/6 mice	Enhanced infiltration of CAR-T cells after sonication with 1.5 MHz frequency	82

Table 1 Continued

Study title	Cell therapy	Ultrasound parameters	Microbubble type and dose (if applicable)	Cell line (location)	Animal model	Key observations	Ref
Control of the activity of CAR-T cells within tumours via focused ultrasound	Primary human T cells isolated from PBMCs; transduced with inducible Cre and *lox*-stop CAR reporter	1.5 MHz 8-element annular array transducer; temperature-controlled feedback loop, local temperature elevation to 43 °C with FUS	N/A	Nalm-6 and PC3 tumors (flank)	NSG mice	Mitigated on-target off-tumor activity and enhanced tumor growth control T cells bearing FUS-inducible CAR vs noninducible CAR-T cells	85
Engineering sonogenetic EchoBack-CAR-T cells	Human EchoBack-hGD2 CAR-T cells, EchoBack-PSM CAR-T cells	1.1 MHz single element; temperature-controlled feedback loop, local temperature elevation to 43 °C with FUS	N/A	U87-MG, PC-3 tumors (flank, intracranial)	NSG mice	Sustained CAR expression, enhanced cytotoxicity and reduced exhaustion in Echoback CAR-T cells	86
Closed-loop sonothermogenetic control of CART cells for metronomic brain cancer therapy	Human and murine αHER2 CAR-T cells	1.7 MHz; heating for 10 minutes at 41.5 °C or 42.5 °C with custom closed-loop feedback controller systems	N/A	Human HER2 expressing MDA-MB-468 breast cancer cells, SB28 murine glioma cells (intracranial)	NSG mice; albino C57BL/6 mice	10-fold increase in CAR-T cell luminescent activity and enhanced tumor control with hyperthermia	87
Tumour priming by ultrasound mechanogenetics for CAR T therapy	Human synNOTCH CAR-T cells	1 MHz, repetition at 1 min intervals every 2 min over a 30 min period; 1.23 MPa	N/A	PC-3-CaDox cells (flank)	NSG mice	Doxycycline-gated AND-logic genetic circuit integration to drive local CD19 antigen expression via FUS-induced calcium signaling; priming system enabled tumor control with CAR-T cells	88

FUS, focused ultrasound; CIs, cellular immunotherapies; BBBO, blood-brain barrier opening; NK, natural killer; CAR, chimeric antigen receptor; scFv, single-chain fragment variable.

sonication (0.3 MPa, 1 MHz), a significant increase in cerebral luminescence signal was noted at 24- and 72-h post-injection compared to unsonicated controls; this was corroborated by significant survival benefit in the FUS group. These imaging timepoints extend beyond the acute permeability window typically measured after FUS BBBO, which is often assessed by contrast-enhanced MRI or tracer leakage and generally resolves within hours to ~24-48 h in humans, with parameter-dependent preclinical closure windows varying within this range.[80,81] Because T-cells are inherently capable of trafficking across the BBB,[82] synergy between FUS and CIs observed past this acute permeability window may reflect not only enhanced trans-BBB access but also FUS-induced vascular or inflammatory effects, retention, or expansion of transferred cells. The mechanistic impact of FUS on CAR-T trafficking, persistence, and expansion remains to be fully defined.

While the data combining FUS with CIs supports the trend of increasing effector cell access to tumors, there remains a deep sensitivity of these effects to acoustic parameters and MB formulations. To this end, tuning the immunobiological impacts of FUS in preclinical brain tumor models that faithfully recapitulate human disease is imperative. A recent study by Guo et al exemplifies all the above. Investigators studied the effect of FUS BBBO frequency and pressure on the accumulation of murine EGFRvIII CAR-T cells in a myeloid-enriched syngeneic glioma model, SB28. CAR-T cells were intravenously injected prior to FUS, and full tumor volumes were sonicated at 0.5 and 1.5 MHz and at 150 and 175 kPa, respectively, in the presence of lipid-shelled MBs. Intratumoral accumulation of CAR-T cells was enumerated using flow cytometry 48 h post-treatment. The higher frequency FUS groups exhibited significantly greater CAR infiltration compared to control, while lower frequency FUS was significant by some but not all methods.[83] Interestingly, 86% of tumor-infiltrating CAR-T cells expressed lymphocyte function-associated antigen-1 and P-selectin glycoprotein ligand-1 (PSGL-1), which are receptors involved in leukocyte extravasation. However, this trend was consistent across all groups and not elevated in any of the FUS groups. Even though there was a significant trend in the accumulation of CAR-T cells in syngeneic gliomas with the addition of FUS, corroborating previously highlighted data in xenografts, the path to therapeutic benefit in light of mechanisms such as myeloid immunosuppression and T-cell exhaustion remains complex. Taken together, preclinical evidence to date supports enhanced local CI accumulation with FUS. Meanwhile, consistent therapeutic benefit remains model- and regimen-dependent. The enabling mechanisms of FUS-induced cell migration and the ability to surmount other TME-level barriers remain to be determined.

FUS and Sonogenetically Controlled T Cells

The emergence of advanced synthetic biology and genetic engineering platforms has opened new possibilities for spatially precise, remotely actuated control of CIs via sonogenetics. In this paradigm, thermo- or mechanosensitive biological logic gates are engineered to convert acoustic stimuli into tightly regulated, programmable molecular outputs (Figure 2). A study by Pan et al[84] took advantage of the mechanosensitive ion channel Peizo1 to induce the translation of a CAR receptor in Jurkat and primary T-cells when exposed to ultrasound waves and MBs. Analogously, studies have been done using thermal FUS stimuli (e.g. hyperthermia) to control CAR-T-cell activity through HSPs. By wiring CAR expression to a heat-responsive genetic switch, investigators have leveraged sublethal heating to exert precise control over a central determinant of engineered cell safety and specificity.[85] Indeed, Wu et al[86] demonstrated the feasibility of controlling both the genetic makeup and cellular function of CAR-T cells using heat generated by short pulses of FUS, via a CAR cassette under the control of an HSP promoter. With the goal of combating antigen engagement-induced CAR downregulation, the same investigators also generated a next-generation sonosensitive CAR-T cell utilizing a synthetic positive feedback loop that induces CAR receptor expression upon stimulation with FUS hyperthermia—termed "EchoBack-CAR-T cells."[87] These next-generation CAR-T cells displayed long-lasting CAR expression after FUS stimulation and exhibited enhanced toxicity against GBM and prostate cancer models. Building on these advancements, other groups have performed similar studies using thermally sensitive aHER2 CAR-T cells expressing the reporter gene, firefly luciferase (ffLuc). MRgFUS hyperthermia induced a 10-fold increase in bioluminescence signal after sonication in addition to significant CAR-T-induced growth control in both a xenograft model of breast cancer brain metastases and immunocompetent GBM models.[88] While many of these studies focus on using FUS as a method of controlling CAR-T cells directly, recent work has instead leveraged FUS as a means to tune antigen expression within the tumor population. Yoon et al integrated FUS mechanical stimulation with the calcium response of cancer cells and a doxycycline-gated AND-logic gated circuit to induce the localized expression of CD19 within a tumor population. This served as a local site to activate CAR-T cells, allowing for superior tumor suppression.[89] These studies highlight how sonogenetic strategies could enable spatiotemporally controlled or metronomic reactivation of CAR programs or costimulatory circuits, offering yet another relevant FUS-enabled opportunity for overcoming CI dysfunction and exhaustion.

Looking Ahead: Opportunities for FUS

FUS-Mediated Immunologic Reprogramming

Although direct combinations of FUS with CIs remain limited, there is a growing body of evidence that supports FUS as a potent tool for immunostimulation capable of reshaping the TME in ways that could fundamentally synergize with CIs. In naive brain settings, FUS-mediated BBBO has been shown to elicit transient sterile inflammatory responses characterized by the upregulation of cytokines, chemokines, and stress-related genes. One study investigated changes induced by FUS BBBO at the transcriptional level in dorsal hippocampal rat microvasculature. Significant increases in the transcription of *Ccl2*, *Ccl3*, *Cxcl1*, *Cxcl11*, *Il1b*, and *Il6* were noted 6 h post-FUS, with a return to baseline by 24 h.[90]

It is important to note the transient nature of this response, as chronic inflammation could prove detrimental.[90] Similar studies have been conducted at the transcriptional level in rodent models. Within 30 min following exposure to FUS and MBs, mRNA for cell trophic factors, including *Ccl12*, *Cxcl1*, and *Cxcl3* were detected with a 5-fold increase compared to untreated control brains.[91] Expression of HSP70, along with proinflammatory factors TNF-α, IL1-α, IL1-β, IL-18, and IFN-γ, was also detected 12-24 h following sonication.[92] This indicates that FUS's temporary induction of a sterile inflammatory response could lend to establishing favorable chemokine gradients, which could be leveraged in the biochemical attraction of CIs to their target.

The extent of the inflammatory response can in part be controlled by modulating barrier opening parameters, including MB formulation, dose, peak negative pressure, frequency, and other acoustic parameters that directly influence cavitation levels. In a study using naïve CD-1 IGS mice, researchers observed an upregulation of 12 distinct gene sets at higher bubble doses and mechanical index (a standard acoustic exposure metric) used in BBBO, some of which include sets for TNFα signaling via NFκB, inflammatory response, and hypoxia.[92] Similar results were observed in a rat model where higher MB doses—10× the clinical standard—had a significant impact on the expression of several inflammatory genes and upregulated NFκB pathway genes.[93] As FUS represents a highly modular and tunable toolbox of interventions, efforts have been made to modulate degrees of neuroinflammation by varying levels of effective mechanical energy deposition (i.e. MB cavitation dose). A study by Ji et al[94] found a linear trend between cavitation dose and the expression levels of *Ccl2* and *Ccl7* in naïve murine brains, with a return to baseline by 72 h. A recent study by Guo et al[83] investigated the effects of frequency and pressure on the transcriptome of mouse brain endothelial cells. Interestingly, at 8 h following FUS treatment at 175 kPa, transcripts for *Cxcl1*, *Cxcl2*, *Ccl2*, *Ccl7*, *Ccl12*, *Ccr1*, and *Ccr2* were significantly higher in the 1.5 MHz group when compared to 0.5 MHz. The 1.5 MHz group also saw upregulation of *Ccl3*, *Ccl9*, and several proinflammatory cytokines, including *TNF* and *Il6*, not observed in the 0.5 MHz group.[83] While most of these data were based on gene expression with limited confirmation at the protein level, they provide promising evidence for the ability of FUS to modulate neuroinflammation in a manner concordant with improved vascular activation and other local T-cell recruitment cues.

In the context of high-grade brain tumors, which bear a canonically "cold" immunophenotype, the immunostimulatory effects of FUS may be leveraged to augment immune cell infiltration, antigen presentation, and migratory signal expression. As previously described, the lack of productive chemokine gradients can be a major barrier preventing effective migration of cell therapies into solid tumors.[39] The ability of FUS to induce chemokine release in naïve brain has been echoed in preclinical brain tumor models. In 1 study, this effect was analyzed at the protein level via Luminex, assaying for a variety of proinflammatory cytokines, chemokines, and trophic factors at several time intervals post FUS-mediated BBBO in a GL261 murine GBM model.[95] It was found that FUS-treated tumors exhibited increases in these proteins with peaks occurring at 24 h post-FUS. This expression occurred earlier at 6 h when tumors were exposed to repeated FUS sessions (3 in total). In addition to migratory signal modulation, FUS in the setting of brain tumors has been demonstrated to augment immune cell infiltration and activation state of APCs. In a study that examined the immune landscape of FUS BBBO in a GL261 murine GBM model, higher numbers of dendritic cells were observed in tumors along with cervical lymph nodes following FUS BBBO.[96] Notably, the frequency of mature (CD86+) DCs was upregulated at a higher FUS pressure (0.6 MPa vs 0.4 MPa), again corroborating the sensitivity of a key bioeffect to acoustic pressure. In the context of FUS-mediated DC modulation, similar results were observed in a murine melanoma brain metastasis model. DCs in the meninges of FUS-treated tumor-bearing mice displayed higher levels of CD86 in addition to heightened antigen loading in tumor-resident DCs.[97] The ability of FUS to augment DC-mediated antigen presentation may be vital to supporting an effective adaptive immune response and could play an important role in enhancing the efficacy of CIs.

Importantly, observations of FUS-mediated immune modulation should be interpreted with translational caution, as acoustic parameters, such as mechanical index and cavitation dose, are influenced by species-, skull-, and platform-specific factors and therefore may not be directly comparable across preclinical and clinical settings. Accordingly, findings to date offer relative descriptors of exposure and bioeffect rather than directly translatable thresholds across settings.

FUS-Mediated Vascular Activation

A related dimension of FUS-induced immunologic modulation involves its effects on the vasculature. Indeed, for CIs to be effective against brain tumors, immune cells must be able to extravasate through the vasculature and into the tumor parenchyma. The efficiency of this process relies heavily on cell adhesion receptor interactions, including those mediated by VCAM-1, ICAM-1, and E-selectin. A mechanism by which tumor cells can inhibit immune infiltration is via suppressing the production of TNF-α, INF-γ, and CCL5—effectively blunting the gradients that drive immune cell homing.[98] A bypass mechanism used to avoid this homing problem is direct injection of CIs into the tumor, resection cavity, or intraventricular space. However, these interventions require highly invasive procedures and can be limited by the anatomical location of the tumor.[99] Toward leveraging systemic CI transfers and promoting circulating T-cell recruitment, several studies have investigated whether FUS can induce adhesion molecule expression on the endothelium in the brain tumor setting, with varying results. Through a closed-loop acoustic-emission control strategy, Lee et al[100] dynamically tuned FUS BBBO conditions to drive dose-dependent ICAM-1 upregulation in GL261 GBMs. However, similar impacts on vascular activation were not observed in the setting of melanoma brain metastasis. Despite transcriptomic changes in inflammatory molecules in response to FUS BBBO, flow cytometry staining on tumor endothelial cells for ICAM-1, and VCAM-1 at 6 and 24 h post-sonication revealed no differences.[97] The contrast in findings between these studies could be due to several

factors, including FUS exposure conditions, MB composition, and the characteristics of the models being used. However, these differences offer intriguing insights and considerations for tuning the degree of vascular activation by modulating key FUS parameters. This phenomenon has been demonstrated in the naïve mouse brain, where researchers compared 2 distinct frequencies (0.5 vs 1.5 MHz) and pressures (150 kPa vs 175 kPa) on ICAM-1 expression. Immunofluorescent (IF) staining 8 h post-FUS revealed a significant increase in ICAM-1 expression at 1.5 MHz.[83] Consistent with these findings, another study in a healthy rat brain model showed that FUS BBBO significantly upregulated ICAM-1 and VCAM expression, as confirmed by IF, and this was associated with a 2-fold increase in mesenchymal stem cell homing.[101]

Evidence for mechanical FUS-induced vascular activation extends beyond the brain, and these insights could bear relevance for neuro-oncology paradigms. We offer limited examples given that this topic extends beyond the scope of this review. Multiple studies have examined the effect of pFUS in the absence of MBs on vascular activation in the mouse periphery. By applying pFUS in a mouse muscle hamstring model, researchers found an upregulation of VCAM-1 and ICAM-1 on muscle vasculature in addition to the production of several cytokines, including IL1-α, IL1-β, TNF-α, INF-γ, MIP-1α, MCP-1, and GM-CSF, on days 0 and 1 post-FUS.[102] Similar results were obtained when applying pFUS to a murine kidney to enhance the homing of bone marrow mesenchymal stromal cells.[103] pFUS increased expression of VCAM-1 and ICAM-1 by 3× on day 1 and by 2× on day 2 post-FUS. Taken together, these studies illustrate that FUS can modulate vascular activation in a context- and parameter-dependent manner, highlighting future opportunities to tune endothelial receptivity and improve immune cell homing.

FUS-Enabled Precision Therapeutic Delivery

The challenges facing CIs in brain cancer are multifaceted, ranging from restrictive tumor access to upregulation of exhaustion mechanisms, all of which become exacerbated in advanced disease.[104] Combinatorial pharmacologic strategies have gained momentum as a means of overcoming these barriers. Numerous studies have paired CIs with immunotherapies (e.g. checkpoint inhibitors) or other agents, though such combinations carry the risk of amplifying immune-related or off-target toxicities.

For example, checkpoint inhibitors such as αPD-1 have been tested in combination with CAR-T cells in the setting of GBM. In a preclinical GBM model, Song et al combined αPD-1 with EGVRvIII-targeted CAR-T cells and observed that, while there were more TILs in the combination group, the ability of these CAR-T cells to control primary volume was not enhanced. However, combination with αPD-1 conferred a significant survival benefit.[105] Several clinical trials have been conducted combining CAR-T cells with ipilimumab, pembrolizumab, durvalumab, and atezolizumab in B-cell lymphoma and, more recently, in GBM.[106] Indeed, a recent trial (NCT03726515) combined EGFRvIII CAR-T cells with pembrolizumab in GBM, affirming safety and biological activity despite a lack of survival benefit.[107] Critically, this finding underscores that despite their great potential, immunotherapy combinations are still plagued by the same issues traditionally facing systemic drug delivery to the CNS, for example low targeted (intratumoral) accumulation and deleterious side effects.[108]

Therein lies a rich set of opportunities for FUS, which is now well-established as a safe and reliable tool for enhancing delivery of therapeutics to the TME, particularly in brain tumors. A straightforward opportunity for increasing accessibility of CIs is improvement of efficacy associated with systemic doses and de-intensification of dosing therein. Systemic dose reduction has been shown with FUS BBBO in the example of CD47 blockade in murine GBMs. Repeat BBBO-mediated antibody delivery resulted in enhanced tumor growth control and significantly improved survival. Most importantly, these results were achieved using an antibody dose that was ~18-fold lower than a comparable monotherapy regimen conferring similar survival benefit.[109]

Multiple preclinical studies have shown similar findings using FUS BBBO to deliver αPD-1 to brain tumors. Significant survival benefits were observed with the addition of FUS to αPD-1 therapy in 2 separate studies in the GL261 GBM model.[79,100] One such study used the closed-loop acoustic emissions-based feedback system previously described and demonstrated improved antibody delivery, confirmed via IF staining. Furthermore, combination treatment induced memory T-cell formation that aided in tumor rejection upon rechallenge.[100] In addition to antibody-based therapies, FUS has been shown to enhance the delivery of chemotherapeutics and other small molecule therapies to brain tumors.[10] For example, in a cohort of 4 GBM patients as well as preclinical GBM models, FUS-mediated BBBO augmented the concentration of doxorubicin by 2- and 3.9-fold in human and mouse brains, respectively, within 2 days of sonication.[110] In the same study, pembrolizumab was codelivered with doxorubicin, and similar results were obtained—with FUS yielding approximately 6- and 2-fold increases in antibody in mouse and human brains, respectively. This increase in drug accumulation was accompanied by a significant survival benefit in murine models.[110] Extension of combination drug approaches to CI paradigms is promising but inherently limited by compounding of off-target and/or immune-related toxicities. Promisingly, these findings suggest that FUS could augment targeted delivery in the context of adjuvants to CI and facilitate systemic dose de-escalation, thereby improving the safety profile of combination strategies.

Immuno-Imaging Approaches for CI Surveillance

CIs, unlike small molecules or antibodies, are living drugs that rely on the orchestration of complex immunological signaling networks to find their way to targets and elicit tumoricidal effects. For this category of therapy, in particular, resolution of spatiotemporal kinetics is paramount. Recent advances with optimal imaging, positron emission tomography (PET), single-photon emission computed tomography

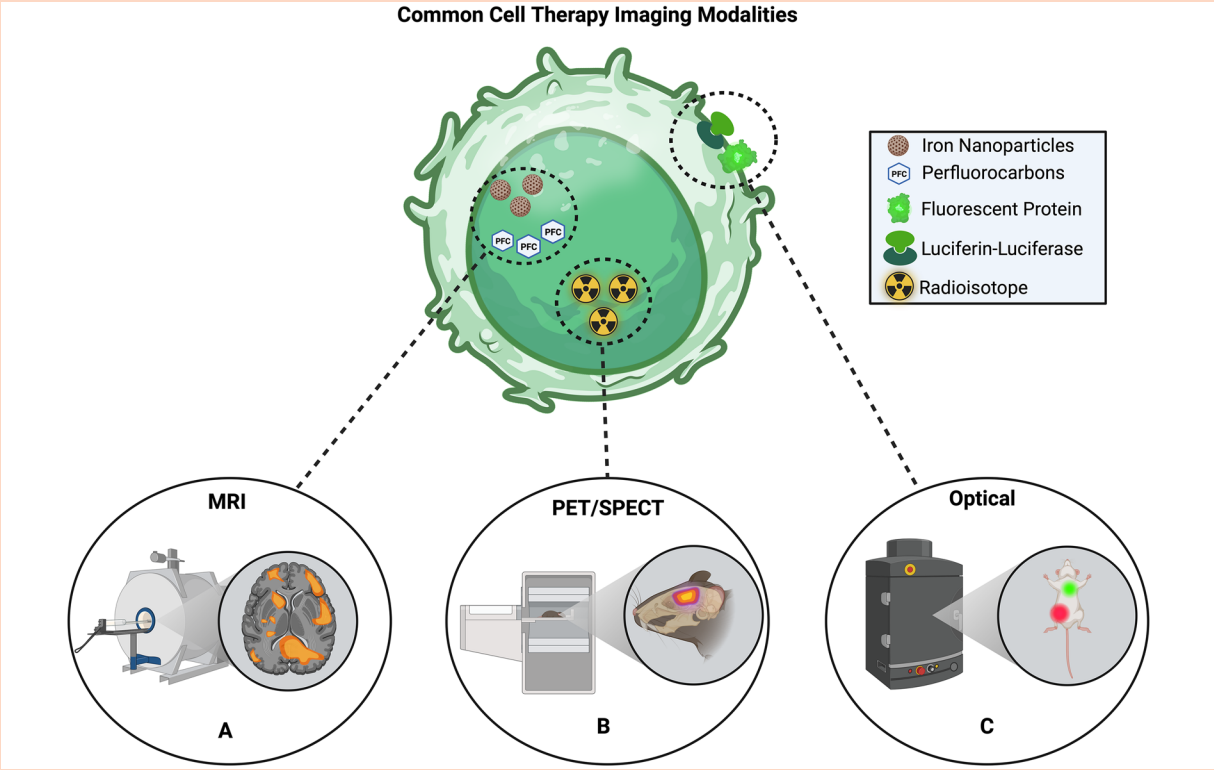

Figure 3. Common imaging modalities for surveilling cell therapies. (A) MRI can be used to track cell immunotherapies in vivo, for example, through integration with intracellular perfluorocarbon-labeled and iron-labeled nanoparticles. (B) Positron emission tomography (PET) and single-photon emission computed tomography (SPECT) can also provide molecular sensitivity for molecularly tracking cell immunotherapies in vivo, for example, via radioisotopes conjugated to delivered cells. (C) Optical imaging of cell immunotherapies offers an accessible tracking approach which relies on engineering of immune cells to express fluorescent or bioluminescent reporter proteins. Created in BioRender. Sheybani (2025) https://BioRender.com/8x5sejk.

(SPECT), and MRI have enabled researchers to noninvasively, longitudinally surveil in vivo distribution and activity of CIs—thereby generating critical context for underpinnings of and limitations on efficacy in distinct cancer settings (Figure 3). In this section, we briefly survey a selection of molecular imaging modalities in relation to CIs and detail accompanying pros and cons to inform their hopeful future utilization in FUS-enabled CI paradigms. Given our goal of highlighting clinically actionable strategies, we limit this immuno-imaging overview to macroscopic modalities that offer direct or near-term relevance for patient-level CI surveillance.

Optical Imaging

An accessible modality for in vivo CI tracking, albeit primarily preclinical, is optical imaging—wherein CIs are engineered to express a bioluminescent and/or biofluorescent reporter (Figure 3C). In this section, we focus on the former. Sabbagh et al demonstrated the capability of tracking EGFRvIII-targeted CAR-T cells in murine GBMs after FUS-mediated BBBO. The investigators successfully constructed CAR-T cells bearing a ffLuc reporter. In both FUS-treated and control groups, ffLuc-CAR-T cells preferentially trafficked to the liver and lungs, with a small fraction trafficking to the brain—evident by in vivo bioluminescence imaging.[79] One day following BBBO and administration of ffLuc-CAR-T cells, a significantly increased bioluminescence signal was observed in sonicated brains on imaging. Corroborating imaging findings, mice treated with ffLuc-CAR-T cells and FUS exhibited significantly increased survival benefit.[79]

The theme of CI tracking has been analogously demonstrated in NK cells. In a paper by Yang et al,[75] MB-enhanced FUS was performed to achieve vascular disruption in murine ovarian tumors and combined with lipophilic carbocyanine $DiOC_{18}$ NK-92-MI cells. After FUS treatment, in vivo bioluminescence imaging revealed a significant increase in NK-92MI cells trafficking to the tumor. Moreover, the combination of MB-enhanced FUS with NK-92MI cells inhibited tumor growth compared to controls. Although this study was not performed in a neurological setting, it further exemplifies the predictive utility of surveilling post-FUS CI kinetics and timeline of localization within tumors.

Recent advances in genetic engineering have also enabled the use of dual-luciferase reporters. By employing 2

luciferase enzymes that emit distinct wavelengths, this approach permits simultaneous imaging of 2 populations—for example, monitoring metastatic burden via bioluminescent tumor cells while concurrently tracking bioluminescent CIs.[111] Although dual-luciferase systems are limited by modest spatial resolution and the need for complex spectral unmixing, they offer a key advantage: reporter expression is heritable, enabling longitudinal assessment of CI proliferation through signal dilution. This contrasts with surface-labeling methods (discussed below), where the signal diminishes rapidly as cells divide.[112]

PET/SPECT

PET and SPECT remain amongst the most powerful tools for imaging CIs, owing to their exceptional sensitivity and ability to quantify whole-body biodistribution. The imaging process begins with effectively labeling the CI of interest with an appropriate radioisotope (Figure 3B). To this end, it is crucial to consider that the labeling process must not impact cell effector binding or function—and the activity must stay within the cell for the lifetime of the study.[112] While radionuclides with shorter half-lives (e.g. F-18) are generally safer to work with and result in less radiation exposure, imaging times are consequentially shorter, with the opposite being said for longer half-life radionuclides (e.g. Zr-89).

The use of PET or SPECT imaging for CI tracking is particularly noteworthy given the advantages of high spatial resolution and sensitivity. Over the years, a plethora of studies have investigated trafficking and cell therapies using radiolabeled cells.[113] In 1 study, hPSMA-targeted CAR-T cells were radiolabeled with F-18 and deployed in a murine metastatic prostate cancer model. Delayed tumor growth was correlated with an increase in radioactive signal in tumors. CAR-T cell signal via PET was colocalized with bioluminescence imaging using CBR-luc-tagged CAR-T cells, which further validated findings and affirmed that radioactivity was still associated with cells after in vivo injection.[114] Another study used Zr-89-oxine labeling to track Lym-1-targeted CAR-T cells over the course of several days instead of hours. Intravenously administered CAR-T cells became trapped in the lungs for 3-5 h and then migrated to the spleen for up to 2-3 days. In this study, investigators postulated this entrapment as a possible mechanism for CAR-T inactivation prior to reaching the tumor, again underscoring the power of biological insights that can be drawn from CI imaging.[115]

Many cell radiolabeling procedures involve the sequestration of isotope within the cellular cytoplasm, where it persists for the duration of the imaging study.[112] A recent study by Pruller et al successfully demonstrated the use of an Indium-111-labeled membrane-targeted peptide for tracking cells with SPECT imaging. The peptide, called [^{111}In]In-DTPA-CTP, was able to bind to 5T33 murine myeloma cells to enable surveillance of cell trafficking to the lungs immediately after injection.[116] The versatility of PET and SPECT can be hugely enabling for the effective monitoring of CIs in combination with FUS (as we have already seen with other FUS-immunotherapy combinations)—informing questions related to delivery, distribution, persistence, and eventually even serving as a predictive tool for risk stratification and treatment adaptation.[109]

Magnetic Resonance Imaging

Another high-resolution approach for visualizing CIs—one that importantly avoids radioactive exposure—is MR imaging. As a widely used, multiparametric modality, MRI offers rich structural and functional information, and emerging neuroimaging techniques are expanding its potential for noninvasively tracking CI localization and activity in brain tumors (Figure 3A). There are many studies successfully combining MR-specific reporter strategies with CIs, specifically CAR-T cell therapy. In 1 study by Dubois et al,[117] perfluorocarbon (PFC)-labeled CAR-T cells targeted against CD19 were used to treat mice bearing CD19$^+$ human B cell acute lymphoblastic leukemias coexpressing tdTomato and ffLuc reporters, lending to a robust multimodal imaging approach. CAR-T-cell localization to the tumor site was visualized using ^{19}F-MRI and bioluminescence imaging. Encouragingly, PFC$^+$ and PFC$^-$ CAR-T cells showed similar tumor killing efficacy, showcasing the opportunity for a true theranostic approach. Multiple groups have also utilized ultra-small superparamagnetic iron oxide nanoparticle (USPIO)-conjugated CAR-T cells as a tool for CI surveillance on MRI.[118,119] One study by Xie et al[118] demonstrated successful visualization of EGFRvIII-targeted and IL-13α2-targeted CAR-T cell localization in a U87 GBM mouse model. The researchers identified no negative impact of USPIO labeling on CAR-T cell efficacy, and both EGFRvIII and IL-13α2-CAR-T-cell therapies yielded improved survival outcomes compared to controls. Additionally, a study by Kiru et al strongly corroborates the findings from Xie et al in its investigation of USPIO-conjugated CAR-T cells targeting B7-H3 in an osteosarcoma model. No difference was determined in the tumor killing efficacy of USPIO$^+$ and USPIO$^-$ CAR-T cells, and the localization of the USPIO$^+$ CAR-T cells could be visualized using MRI.[119] Like the studies using USPIOs to visualize CAR-T-cell localization in vivo, Maria et al demonstrated the feasibility of performing this technique with NK cells. In this study, an MB-assisted FUS paradigm enhanced USPIO-labeled NK cell trafficking. Albeit in a colorectal adenocarcinoma xenograft, this study offers a demonstration of CI tracking via MRI that can be translated to intracranial disease.[76] Indeed, similar results were achieved by Alkins et al,[77] whereby an increase in iron-labeled NK-92 cells was observed in FUS-treated tumors via T2* MR imaging. Taken together, these studies showcase the utility of MRI for surveilling CIs in conjunction with FUS treatment and demonstrate a promising future avenue for effective longitudinal tracking of CIs in vivo—which will significantly streamline future efforts to rationally align and adapt CIs in combination with FUS.

Outlook and Conclusions

The convergence of FUS and CIs in neuro-oncology is an emerging frontier that is gaining rapid traction as both fields coevolve. While a modest number of studies have directly combined these modalities to date, the accelerating pace of innovation in FUS technology, advanced imaging, and next-generation cell therapies suggests that this landscape will expand substantially in the coming years.

Importantly, the clinical integration of FUS and CIs is no longer prospective. The field has now entered active clinical translation, exemplified by an ongoing "first-in-human" phase 1 clinical trial at the University of Virginia (NCT07343986), which is evaluating low-intensity FUS BBBO in combination with anti-CD3 × anti-EGFR bispecific antibody-armed autologous activated T cells (EGFR-BATs) in patients with newly diagnosed, MGMT-unmethylated, IDH-wildtype GBM. In addition to assessing the safety and feasibility of this combinatorial approach, the trial incorporates immuno-PET imaging of ^{89}Zr-oxine-labeled EGFR-BATs to quantify radiolabeled cell uptake in brain and tumor regions, thereby providing a longitudinal window into whether FUS can enhance intratumoral trafficking of adoptively transferred cells. As this and other emerging early-stage studies mature, careful mechanistic and safety correlative data will be essential for defining how best to sequence, monitor, and optimize FUS-enabled CI approaches in neuro-oncology.

To this end, our systematic review of the available literature reveals several trends that should guide the next wave of investigation. In preclinical studies, FUS has demonstrated the capacity to enhance CI delivery into the TME across diverse brain and peripheral tumor models. Determining which CIs are truly constrained by access bottlenecks—and in which tumor contexts—will be essential for strengthening the translational rationale for FUS as a precision CI delivery adjunct. Notably, improved delivery does not uniformly translate into therapeutic benefit, underscoring the need for real-time and longitudinal in vivo imaging strategies to confirm on-target cell trafficking, persistence, and function. The multipronged capabilities of FUS—as a delivery tool, immunomodulator, and means of controlled tissue perturbation—position it as a uniquely versatile platform for overcoming key limitations of CIs, but realizing this potential will require a clearer mechanistic understanding of when and how these effects can be most productively leveraged.

The limitations of current experimental systems must also be acknowledged. For example, many human CAR T-cell studies rely on immunodeficient NSG mice, which facilitate human cell engraftment but lack key components of the myeloid and adaptive immune landscape that are germane to developing a complete picture of FUS-CI interactions. Accordingly, future studies will benefit from the complementary use of immunocompetent, syngeneic, humanized, and clinically relevant large-animal models to better distinguish FUS-driven effects on CI delivery from broader effects on immune function, persistence, and therapeutic durability.

Beyond improving model systems, the field must also advance beyond feasibility and observational studies toward more rigorous mechanistic investigation. Future work should define how FUS shapes antitumor immunity at the molecular and cellular levels and how these effects intersect with CI design, phenotype, and functional durability. Several key variables remain insufficiently resolved, including the sequencing, route, and dosing of CI administration relative to FUS; the influence of acoustic exposure conditions and MB formulations; the extent to which FUS alters CI trafficking, intratumoral retention, and persistence; and the relative contributions of direct delivery enhancement vs broader tumor-immune reprogramming to therapeutic outcome. Greater clarity will also be needed regarding how these relationships vary across tumor type, anatomic site, vascular and immune context, and CI platform.

Beyond questions of therapeutic efficacy and mechanism, patient safety remains paramount. The potential for adverse immune reactions—ranging from neuroinflammation to aberrant or off-target immune activation—underscores the need for rigorous characterization of how FUS shapes the CNS immune landscape. Defining these effects will be critical for anticipating, mitigating, and ultimately preventing toxicities as combination strategies advance through the translational pipeline. Closely linked to safety is the question of durability. Indeed, the long-term safety profile of repeated FUS exposures remains largely uncharted in immuno-oncology contexts, with sparse longitudinal data available to guide clinical decision-making. Carefully designed preclinical and early-stage clinical studies (e.g. NCT07343986) that dissect the biological outcomes of combining FUS with CIs across acute, subacute, and chronic time windows will be essential for refining treatment parameters and patient selection criteria. A foundation of mechanistic safety data will be indispensable for ensuring that current and future investigations maximize therapeutic promise while maintaining the highest standards of patient safety.

A final, especially underdeveloped but promising, frontier is the potential synergy between ablative FUS and CIs. Although no published studies have yet interrogated this combination in the brain, the rapid maturation of thermal and mechanical ablation technologies suggests that this space is also poised for meaningful growth. Ablative FUS may offer unique opportunities to reshape the tumor immune microenvironment, generate immunogenic cell death, and broaden the therapeutic window for engineered immune cell products.

Together, these considerations highlight a rich set of scientific and translational opportunities. As mechanistic clarity deepens and optimized treatment paradigms emerge, the convergence of FUS and CIs holds exceptional promise for advancing precision immunotherapy in neuro-oncology.

Keywords

brain cancer | CAR-T-cell therapy | cell therapy | drug delivery | focused ultrasound | image-guided therapy | immuno-imaging | immunomodulation | immunotherapy | neuro-oncology | NK cell therapy | sonogenetics

Author Contributions

Conceptualization, T.S., A.T.T., and N.D.S.; writing—original draft preparation, T.S. and A.T.T.; writing—review and editing, T.S., A.T.T., and N.D.S.; supervision, N.D.S.; funding acquisition, N.D.S. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest Statement

None declared.

Funding

N.D.S. is supported by the National Institutes of Health DP5OD031846, R21CA292273; U.S. Department of Defense HT9425-25-1-0411, HT9425-24-1-0940; the Ben and Catherine Ivy Foundation Translational Adult Glioma Grant Award; grants from UVA Brain Institute, UVA School of Engineering and Applied Sciences, UVA Comprehensive Cancer Center, and the UVA Focused Ultrasound Immuno-Oncology Center. T.S. is supported by NIH Cancer Training Grant T32CA009109. A.T.T. is supported by The Olive B. and Franklin C. Mac Krell Jefferson Fellowship and a UVA SEAS Endowed Fellowship. This article appears as part of the supplement "Role of Focused Ultrasound in Brain Tumor Management," sponsored by Focused Ultrasound Foundation and Centre of Excellence in Focused Ultrasound at Sunnybrook Health Sciences Centre.

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 Neuro-Oncology Advances 2026 8(S4) iv60–iv78
https://doi.org/10.1093/noajnl/vdag080
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