Trends in Neurosciences

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Review

Neuroinflammation associated with ultrasound-mediated permeabilization of the blood–brain barrier

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The blood–brain barrier (BBB) continues to represent one of the most significant challenges for successful drug-based treatments of neurological disease. Mechanical modulation of the BBB using focused ultrasound (FUS) and microbubbles (MBs) has shown considerable promise in enhancing the delivery of therapeutics to the brain, but questions remain regarding possible long-term effects of such forced disruption. This review examines the evidence for inflammation associated with ultrasound-induced BBB disruption and potential strategies for managing such inflammatory effects to improve both the efficacy and safety of therapeutic ultrasound in neurological applications.

Highlights

The blood–brain barrier (BBB) plays both a physical and a physiological 'gatekeeping' role in maintaining brain homeostasis.

In recent years, there has been increasing interest in understanding the role of the BBB in neurological disorders that were traditionally considered to be neuron-centric, for instance, Parkinson's disease (PD) and Alzheimer's disease (AD).

Alternative drug delivery techniques such as focused ultrasound (FUS) are emerging as powerful tools to bypass the BBB and facilitate the treatment of neurological conditions.

To enable widespread clinical use of these techniques, there is an urgent need to investigate and address the associated safety concerns, for example, the consequences of sterile inflammation that may be induced by BBB disruption.

Challenges for the treatment of CNS diseases

A significant proportion of the global population is diagnosed annually with some form of neurological disorder or disease – 16.5% of global deaths can be attributed to CNS diseases [1]. There have consequently been many efforts to develop effective CNS-acting compounds and biomolecules. Unfortunately, despite being one of the more heavily funded areas of research in the pharmaceutical industry, CNS drug discovery and development are associated with a low rate of return. Although there is an abundance of promising *in vivo* animal data from pharmacokinetic and pharmacodynamic studies, very few drug candidates show comparable efficacy in human trials [2,3]. Among the reasons for this are key differences in anatomy and physiology between humans and animal models, including the BBB [4].

Conventional methods of structural modification of small compounds have produced only modest improvements in terms of BBB penetration [5]. Consequently, in recent years, there has been increased interest in drug delivery methods to the brain based on local permeabilization of the BBB using FUS, especially in combination with MBs. These methods have shown considerable promise, and several first-in-human clinical trials have reported successful outcomes[i,ii,iii] [6–8]. There are, however, important safety concerns relating to mechanical disruption of the BBB, specifically in relation to the metabolic and physiological pathways required for brain homeostasis. If the permeability of the BBB is modulated to increase drug extravasation into the brain, it is imperative to understand the potential consequences of that disruption, especially in neurological conditions in which the BBB may already be compromised.

This paper aims to provide an overview of the evidence for ultrasound-induced neuroinflammation, its implications, and strategies by which adverse effects could potentially be mitigated to maximize the benefit–risk ratio in clinical applications.

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A brief overview of the BBB

The BBB provides both a physical and a physiological barrier between the brain parenchyma and the bloodstream (Figure 1). It is composed primarily of microvascular endothelial cells supported by pericytes and astrocytic foot processes [9]. The BBB prevents the entrance of exogenous toxins and agents from the bloodstream into the brain parenchyma and maintains the separation between the CNS and the PNS. Given the ability of the BBB to selectively determine the passage of biomolecules and chemicals, its roles in homeostasis, in multiple diseases, and in accurate evaluation of drug efficacy are topics of great interest for clinicians and researchers.

Potential side effects of BBB disruption

A growing body of data demonstrates an important role for the BBB in mediating CNS diseases [10–13]. In developing drug delivery methodologies that disrupt the BBB, it is therefore important

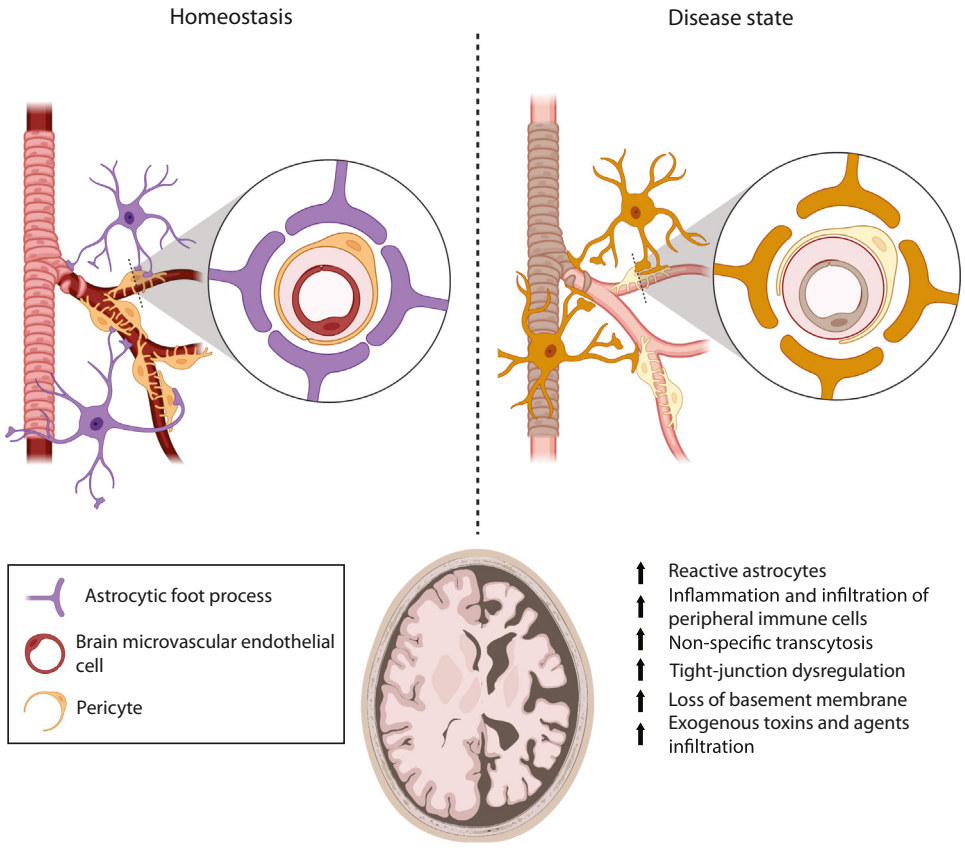

Figure 1. Overview of the cellular composition of the brain vasculature. The blood–brain barrier (BBB) is composed of brain endothelial cells supported by pericytes and endfoot processes of astrocytes. The microvascular endothelial cells form continuous tight junctions with one another, and the astrocytes and pericytes support the vascular network together with the basement membrane that lines the basolateral aspect of the endothelium. In homeostasis, the BBB prevents harmful toxins and agents from entering the CNS. This is essential because neurons are especially sensitive to microenvironmental changes. In many neurological diseases, the ability of endothelial cells to form tight junctions is compromised, the ability of pericytes to effectively support the vascular network is impaired, and reactive astrocytes signal and interact with microglia, the resident brain 'macrophages'. There is an increase in local inflammation that leads to further leakage and dysregulation of tight-junction complexes, that can allow chemotaxis of peripheral immune cells. In extreme circumstances, the increased permeability can be so severe that it allows exogenous agents to enter the brain parenchyma, which can be devastating for neurons and the relevant neuronal networks near the disrupted BBB. Image created with BioRender.

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to consider the downstream effects of modulating the BBB (Figure 1). For example, BBB breakdown is known to coincide with peripheral immune cell infiltration and the inflammation of the brain parenchyma in diseases such as multiple sclerosis (MS) [14,15]. There is also some evidence of BBB involvement in the progression of other neurological diseases such as lysosomal storage disorders [16–19]. For intensively studied neurological diseases such as Parkinson's disease (PD) and Alzheimer's disease (AD), there is now experimental evidence showing that the BBB may play an active role early in their etiology [20–22]. In PD, the accumulation of α-synuclein has been shown to be the dominant pathophysiology that leads to clinical manifestations observed in patients. Although there have been previous reports of neurovascular impairment in PD patients, recent data have shown that α-synuclein overexpression in mice is associated with compromised BBB integrity [23]. In AD research, the accumulation of amyloid-β plaques and neurofibrillary tangles has been a central theme over the past few decades. In recent years, however, there has also been increased interest in the effects of neurovascular factors [24]. For example, it has been proposed that a compromised BBB could allow passage of exogenous toxins and agents into the brain, leading to inflammatory responses that could cause plaque and tangle formation as a byproduct [25]. There is also evidence of a correlation between BBB degradation in AD tissue and bacterial and viral infiltration leading to an innate immune response cascade [26–28]. At the time of writing, these represent areas of considerable uncertainty and debate. For example, it remains to be established whether BBB dysfunction is a causative agent in the disease processes or a symptom of disease progression. These questions, nevertheless, have important implications for drug discovery, design, and development, as well as for preclinical *in vivo* drug evaluation. Leukocyte infiltration through the BBB is known to drive pathophysiology in neuroimmune diseases [29], and non-specific transcytosis and tight-junction dysregulation are upregulated in response to changes in the microenvironment surrounding neurons, for example, during stroke [30]. The emerging use of alternative drug delivery techniques that modify BBB integrity must therefore be balanced against the fact that many of the patients being treated may already have neurovascular complications and/or clinical symptoms that are driven by BBB dysfunction as much as by neuronal dysregulation [11]. It is therefore crucial to investigate the mechanism(s) behind BBB opening via ultrasound-mediated cavitation and the consequences of this manipulation, especially for the treatment of non-terminal diseases for which patients may receive multiple treatments over several years.

Ultrasound and MB-mediated BBB opening

Initial studies

FUS was first used therapeutically for tissue ablation. In this type of procedure, an FUS transducer – operating at a center frequency between 0.5 and 10 MHz – generates a region of sufficient intensity to cause tissue denaturation. Typically, the focal region of an FUS transducer is ~16 mm^3, which enables good spatial control of energy deposition. FUS can rapidly destroy tissue via a range of both mechanical and thermal effects. A common side effect of high-intensity FUS is cavitation, in other words, the formation and subsequent oscillation of bubbles as a result of changes in tissue temperature and pressure. The presence of these bubbles can be beneficial, for example, in accelerating the rate of heating and promoting mechanical erosion [31,32]. Cavitation is, however, a stochastic process, and it was found that similar benefits could be achieved at much lower ultrasound intensities by injecting a suspension of pre-existing MBs into the target tissue. This was particularly important in early preclinical studies of BBB opening using FUS to mitigate the risk of collateral damage [33]. MB-mediated BBB opening was reported as a possible alternative drug delivery technique as well as a theranostic some two decades ago, when FUS was used with contrast agents and magnetic resonance imaging (MRI) to open and detect BBB opening in rabbits [34]. Subsequent studies in mice, rats, and rabbits have focused on the observed bioeffects, including vascular wall damage, ischemia, and tissue necrosis [35]. The findings suggested that limiting

parameters such as the acoustic pressure amplitude and pulse duration may be crucial in producing therapeutic effects with minimal adverse reactions [36,37]. There have also been multiple follow-up studies to investigate the mechanism(s) behind BBB opening [38]. Technical details regarding the physics of ultrasound and MBs can be found in Box 1.

Therapeutic applications

Over the past two decades, the therapeutic potential of FUS+MBs has been explored for a range of neurological conditions (excluding cancer) in preclinical models including the delivery of quercetin-modified sulfur nanoparticles to minimize endoplasmic reticulum (ER) stress in AD [39], BDNF-expressing retrovirus also for the treatment of AD [40], curcumin and neurotrophic factors for treatment of PD [41,42], and to increase laronidase uptake as part of enzyme replacement therapy (ERT) in an animal model of mucopolysaccharidosis type I disease [43]. In clinical trials, FUS+MBs with MRI guidance have been shown to enable localized BBB opening in amyotrophic lateral sclerosis (ALS) [6][i], AD[ii], and PD[iii] patients. Several studies, however, have highlighted potential risks associated with FUS+MBs. These include neuroinflammation, which is discussed in more detail in the next section.

Ultrasound-induced neuroinflammation

Identification of sterile inflammation as a possible bioeffect of FUS+MB-mediated BBB opening

At the low frequencies (<1 MHz) required for efficient transmission of ultrasound through the skull, the probability of inertially driven bubble collapse is higher due to the prolonged rarefactional period. *In vitro* and modeling studies suggest that this can lead to significant and permanent biological damage in the local tissue [44,45]. In addition, studies on rats over recent years have indicated that FUS+MBs can induce sterile inflammation [46]. In some applications, stimulation of an immune response may be beneficial; for example, it has been suggested that FUS+MBs may contribute to killer T cell activation and infiltration in tumors [43,47]. As discussed later, similar approaches have been suggested for brain-specific tumors such as glioblastoma multiforme (GBM), an aggressive brain cancer with very poor prognosis [48]. Whether or not there are also detrimental effects, however, is something that requires more extensive and rigorous investigation.

Since the identification of FUS as a promising alternative delivery technique for CNS therapeutics, there has been extensive assessment of its safety. Table S1 in the supplemental material online

Box 1. Focused ultrasound (FUS) and microbubbles (MBs)

Ultrasound is widely used in diagnostic imaging because it is non-ionizing and facilitates real-time imaging of anatomical structures within the body. A linear or curvilinear array of transducers is used to transmit and receive short pulses at frequencies between 2 and 18 MHz. The received signals provide information about the nature and location of internal structures. Although some features within the body can easily be distinguished by ultrasound, this is not the case for blood vessels, and consequently, gas MBs have been used for over two decades as a contrast agent to improve imaging of the vasculature.

Because they are filled with gas, MBs are highly compressible and hence respond strongly to the mechanical perturbations imposed by a sound field. The fluctuating pressure causes the MBs to volumetrically oscillate and reradiate the incident energy at multiple frequencies. This nonlinear response can be detected by an ultrasound transducer and is fundamental to both MB imaging and real-time control of BBB opening. In therapeutic applications, the oscillations of the MBs are thought to mechanically stimulate BBB opening and thus locally enhance drug uptake.

The attenuation of ultrasound in most tissues increases with frequency via a power law relationship and leads to increased heat deposition due to viscous absorption. Thus, for FUS+MBs, lower frequencies (~1 MHz) than those used in imaging are used to limit off-target heating of the surrounding tissue, especially of bony structures such as the skull. Therapeutic applications also typically use longer pulses than those used in imaging to increase the probability of generating the desired biological effect.

provides a summary of selected studies using FUS+MBs for BBB opening. Before 2017, a primary focus of the research on FUS for BBB opening was to identify acoustic parameters that minimize visible red blood cell (RBC) extravasation, as assessed by histological analyses in mice, rats, and rabbits [36,49–54]. In recent years, studies, primarily in rats, have begun to address FUS-induced CNS inflammation in more detail over time periods between 24 h and 6 weeks post-ultrasound treatment, and there has been discussion of sterile inflammation as a response to BBB opening [55]. An area that requires further investigation, however, is the relationship between inflammation and the acoustic exposure parameters. There has been considerable investigation of how the selection of acoustic parameters affects the degree of BBB permeabilization and how this relates to extravasation of differently sized molecules [51,56], but whether there is a corresponding modulation of sterile inflammatory effects remains to be examined.

Mechanisms

FUS+MB exposure has been shown to permeabilize the BBB through the disruption of tight-junction protein complexes between endothelial cells, that is thought to be facilitated by oscillations of MBs along the endothelial surfaces [57,58]. Localized disruption allows blood-borne components such as circulating therapeutics or albumin to diffuse into the brain parenchyma. In addition to the formation of paracellular holes, neurovascular units may also be stimulated by the oscillating MBs. In rodent studies, this has been shown to stimulate a neuroinflammatory cascade, which upregulates the expression of chemokines, cytokines, and other relevant trophic factors [59–61] (Figure 2).

Several studies have suggested that permanent tissue damage is avoidable when the appropriate ultrasound settings and MB dose are used (Table S1 in the supplemental material online). In view of these findings, subject-specific, preoperative planning could be considered as a possible path to reduce tissue damage. In addition, active monitoring of the MB response allows potential real-time feedback and control of the treatment by modifying the peak negative pressure and/or pulsing regime of the FUS, as exemplified in a recent preclinical study in non-human primates [62]. However, even when using the minimum acoustic settings to cause BBB permeabilization, it is conceivable that a sterile inflammatory response can still occur, although this requires further investigation. Studies in rats have shown that sterile inflammation following FUS-mediated BBB permeabilization is mediated through the NF-κB pathway, and there is evidence that endothelial activation (high ICAM-1 expression) and a cytokine cascade including the production of tumor necrosis factor, a potent inflammatory cytokine, remain elevated even 24 h after sonication [63]. Studies have explored whether prophylactic treatment (i.e., anti-integrin α4β1/VLA-4) might mitigate possible immune infiltration or responses [64–66], but this has not been investigated specifically in the context of FUS+MB treatment for CNS diseases.

In recent years, significant efforts have been made to understand the mechanisms underpinning the observable bioeffects following BBB opening at the cellular and molecular levels [67–69]. In particular, several studies have investigated specific immunomodulatory pathways [46,60,61,63,70] in microglia and astrocytes. Interestingly, there has been less investigation of the role played by endothelial cells and pericytes in potentially inducing the inflammatory cascade post-FUS+MB treatment. This is despite evidence suggesting that these cells and their interactions are crucial for the process [71–76]. Better understanding of the initial physiological responses produced by endothelial cells and pericytes (with and without astrocyte and microglia activity) will be crucial in assessing the cell type-specific effects of FUS+MBs as well as the cell–cell crosstalk that ultimately generates tissue-level neuroinflammation.

It has also been shown that in rats, an innate immune response can be activated for up to 6 days after FUS+MB exposure, as evidenced by infiltration of $CD68^{+}$ monocytes/macrophages [46].

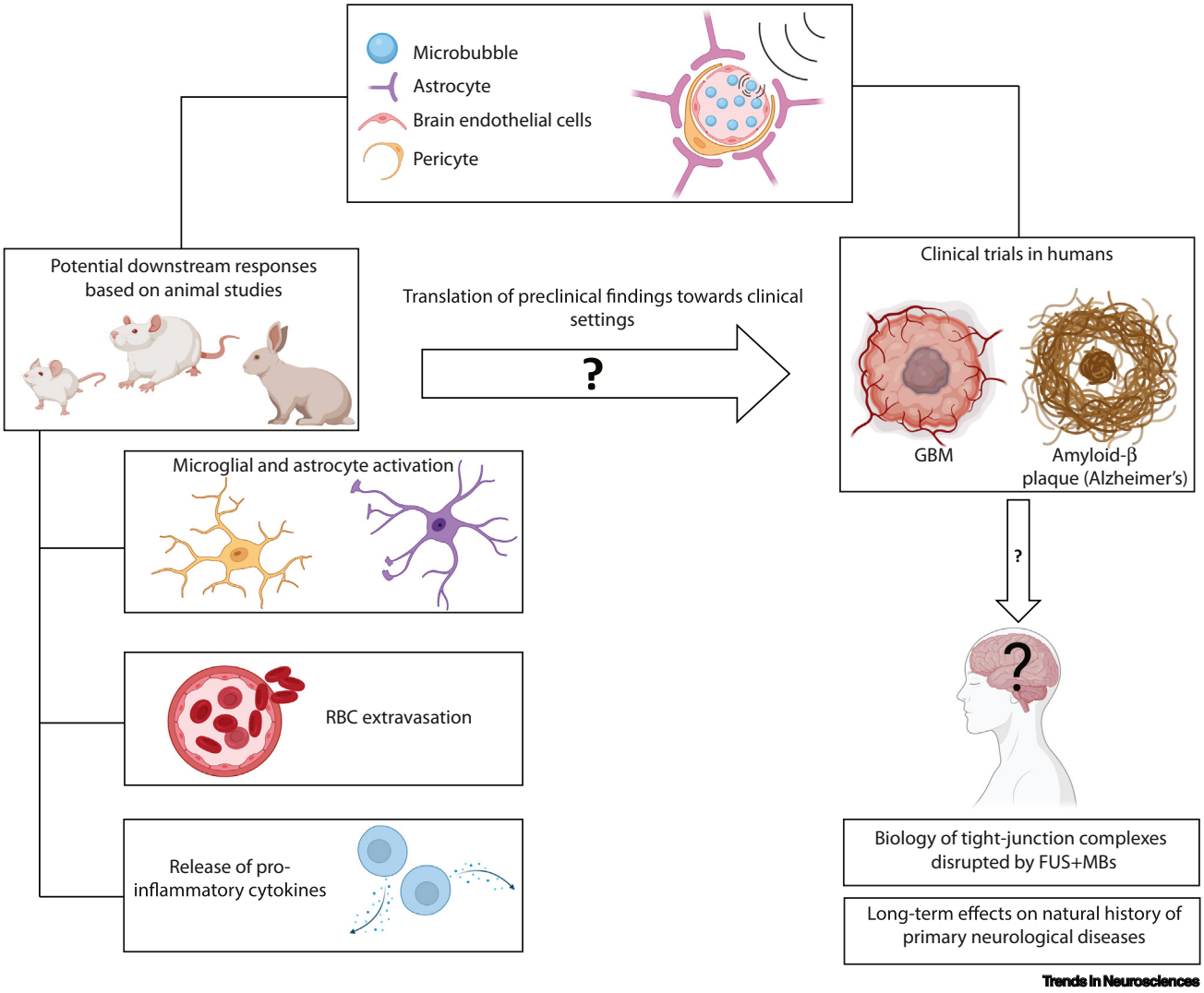

Figure 2. Biological effects of focused ultrasound plus microbubbles (FUS+MBs) via disruption of the blood–brain barrier (BBB). The FUS+MB combination has shown considerable promise as a drug delivery strategy, but further understanding of the downstream effects is required. Depending on the ultrasound exposure conditions, several biological effects have been observed in preclinical rodent models, including activation of microglia and astrocytes increasing with increasing acoustic pressure [60], extravasation of RBCs which can be minimized through appropriate adjustment of acoustic parameters and/or MB size [51,56], and release of cytokines in brain regions contralateral to the hemisphere treated with FUS+MBs [46]. It remains to be seen whether these effects occur in humans. To date, clinical studies of FUS+MB applications have focused on the treatment of GBM[iv,vi], dissolution of protein aggregates in Alzheimer's disease[ii] [7], and alleviation of symptoms in Parkinson's disease[iii,xi] [8]. Further work will be necessary to examine potential longer-term biological effects, particularly as the range of clinical applications is broadened, and clinical trials involve repeated treatments or younger populations[ix,xiii,xiv,xv]. Abbreviations: GBM, glioblastoma multiforme; RBC, red blood cell. Image created with BioRender.

Infiltration of the CNS by peripheral monocytes/macrophages is a hallmark of tissue damage that cannot be managed through microglial activation alone, and can be indicative of impending fibrosis [77], and potentially has long-term implications. Even in cases where BBB integrity is restored within 24 h post-sonication, the neuroinflammatory response does not always subside [70,78]. In addition, FUS+MB exposure has been shown to reduce P-glycoprotein (Pgp, encoded by the *ABCB1* gene) expression; this may allow increased retention of therapeutics in the parenchyma which could have immediate therapeutic benefits, but the downstream physiological effects should be further investigated because Pgp expression and regulation are closely

associated with proinflammatory and anti-inflammatory cytokine expression and release [79,80]. In addition, although there have been preliminary studies showing that FUS does not necessarily lead to tight-junction complex damage [81], it remains to be determined how non-homeostatic changes to the microenvironment may induce or facilitate biological changes in the integrity of the tight junctions or cellular membranes.

Clinical studies on the neuroinflammatory effects of FUS+MB treatment

Table 1 presents an overview of recent clinical trials using FUS+MBs in a range of CNS conditions, together with details of any inflammatory (or anti-inflammatory) pathways where these are

Table 1. Overview of clinical trials using FUS+MBs for the treatment or diagnosis of CNS disease

Clinical trial (NIH reference)	Clinical trial phase	Condition of interest	Study description and role of inflammation as a modulator or side effect, including in associated preclinical work.
NCT02343991[vi]	Phase not applicable	Brain tumors	Evaluate whether FUS can increase the passage of tumor-specific biomarkers into the vasculature and improve the quality of liquid biopsy [106]
NCT02253212[iv]	Phase I/II	Glioblastoma (recurrent)	Evaluate BBB opening tolerated by patients before the delivery of chemotherapeutics; discusses anticancer immune response in the context of other organ-specific cancers (e.g., breast cancer) and studies in other species (e.g., mouse models) [107]
NCT02986932[ii]	Phase I	Alzheimer's disease	Reduction of pathological protein aggregate in AD; no mention of inflammation as a modulator or in post-treatment evaluation [7]
NCT03321487[i]	Phase not applicable	Amyotrophic lateral sclerosis	Evaluation of BBB opening in primary motor cortex; MRI imaging shows transient disruption via gadolinium perfusion [6]; the authors reported no significant inflammation 30 days post-procedure
NCT03551249[vii]	Phase not applicable	Glioma	Establishing the safety profile for patients using FUS+MBs as a first line of therapy (standard chemotherapy); no mention of inflammation as an immunomodulator in glioma treatment
NCT03608553[iii]	Phase I	Parkinson's disease dementia	Performed BBB opening in the parieto-occipito-temporal brain regions of the patients; no adverse effects reported [8]; no mention of inflammation
NCT03616860[viii]	Phase I	Glioma	Evaluating FUS+MB treatment to increase the quality of liquid biopsy via increasing tumor biomarker perfusion into the vasculature through transient BBB opening [108]; no mention of inflammation as a possible modulator
NCT03671889[v]	Phase II	Alzheimer's disease	Evaluation of focal, transient BBB opening in the hippocampus; found indications of perivenous blood/meningeal permeability post-barrier disruption which may be indicative of a tissue healing process (in the context of inflammation) [100]
NCT03782194[ix]	Phase not applicable	Anxiety, obsessive compulsive disorder, post-traumatic stress disorder	Investigate whether FUS pulsation can influence amygdala function to improve emotion regulation
NCT04118764[x]	Phase not applicable	Alzheimer's disease	Prospective study done with non-human primates in which eosinophil count increased; low acoustic pressure leads to minimal inflammatory cell density [109]
NCT04370665[xi]	Phase not applicable	Parkinson's disease	Delivering imiglucerase using the ExAblate MRgFUS system and Definity to open the BBB; no mention of inflammation
NCT04526262[xii]	Phase not applicable	Alzheimer's disease	Evaluated plaque removal and cognitive functions post-FUS+MB treatment (repeated opening) [110]; no mention of inflammation specific to the study
NCT04620460[xiii]	Phase not applicable	Schizophrenia	Investigate whether FUS (no MBs) pulsation can modulate cortical function; no mention of immunomodulation as a mechanistic target
NCT04804709[xiv]	Phase I	Progressive diffuse midline glioma (DMG)	Evaluate whether FUS+MB delivery of panobinostat through transient BBB opening is safe (Phase I); no discussion of immunomodulation as a possible mechanism
NCT05089786[xv]	Phase II	Treatment-resistant neurologic and psychiatric indications	To evaluate whether FUS (no MBs) can improve clinical measurements in neurological and psychiatric disorders; no discussion of inflammation

explicitly mentioned. In non-neurological conditions, FUS+MB exposure has been shown to stimulate immune responses that may be beneficial (e.g., in metastatic cancer) or directly induce anti-inflammatory effects at the target site [82–85]. Recent studies have also shown that immunomodulation can be successfully used to treat GBM, and that FUS+MBs can be effective in inducing targeted immune effects and in delivering immunotherapeutics with promising results [86,87]. It remains to be determined, however, whether an immunomodulatory approach is appropriate for the treatment of non-oncological CNS diseases [88–94]. Although most current clinical trials report no significant inflammation after FUS+MB treatment (Table 1), the evaluation of potential neuroinflammation in many of these studies is limited because, for example, they lack molecular biomarker data in regard to cytokine levels in cerebrospinal fluid (CSF) or tissue biopsies.

Strategies for mitigating sterile inflammation in ultrasound-mediated therapy

As mentioned earlier, the use of FUS+MBs has shown promising results for the treatment of GBM [95–97]. For as long as uncertainties remain over its long-term safety, however, the case for using FUS+MBs in non-terminal CNS conditions is less clear [98]. Several studies have investigated the immediate and short-term consequences of FUS treatment in humans, but these have focused primarily on functional measures designed to observe whether there were rises in biomarkers of concern [99,100]. To the best of our knowledge, long-term follow-up studies of the treated patients are still lacking. Such studies are inevitably difficult to perform because of the complexity of neurological diseases and of confounding factors that may influence interpretation of clinical data. Examining long-term, post-procedure effects in animal models could provide one step towards addressing these complex issues.

There are data showing that ultrasound-induced BBB disruption can induce inflammatory responses even at low acoustic intensities [46,61,101,102]; ultimately, the clinical applicability of FUS+MBs will depend upon understanding the underlying mechanisms and the immediate, as well as long-term, effects of both single and multiple FUS+MB treatments. For example, it is crucial to determine whether repeated treatments produce adverse effects unrelated to the natural history of the neurological disease being treated. This is particularly important when identifying treatments for genetic and hereditary disorders in which many in the diagnosed population are pediatric patients.

Non-mechanical modulation of the BBB has also been shown to induce neuroinflammatory effects, indicating these are not specific to FUS+MBs. For example, the use of D-mannitol, an osmotic agent that has been widely used for modulating intracranial pressure, has been reported to increase proinflammatory cytokines [103]. The opening of the BBB can induce a response from both astrocytes and microglial cells [60], such as a cascade of chemokines that encourage homing and chemotaxis of peripheral immune cells that are circulating in the neurovasculature, especially near the meninges [98]. A potential advantage of FUS+MBs over D-mannitol is that the effects of FUS+MBs can be much more easily localized to specific regions of the brain and their corresponding vasculature.

A key consideration for the use of FUS+MBs in neurological disorders is the degree to which adverse reactions post-procedure can present and whether there are pre- or post-operative measures to minimize such effects. Some studies have shown that corrective measures can be taken post-treatment to inhibit an immune response by using drugs such as dexamethasone [104]. In a similar manner, another group recently reported that the type of anesthetic used before FUS+MB disruption of the BBB can influence gene expression in the brain [105]. Such differences may not have immediate implications post-procedure but are likely to be crucial in understanding how cells respond long after the initial acute disruption of the BBB.

Concluding remarks and future perspectives

This review has sought to examine the current literature on the role of the BBB in mediating sterile inflammation following exposure to FUS+MBs. The number of studies that have evaluated sterile inflammation associated with FUS+MBs, either *in vitro* or *in vivo*, is relatively small; although the technology shows great promise, there is a need to accelerate our understanding of the downstream physiological responses (Figure 2). This need is becoming increasingly pressing as the range of applications of FUS+MB-mediated BBB permeabilization increases and is extended to non-terminal conditions. Further work will be necessary to elucidate the pathways associated with such reactive inflammatory responses when the BBB is disrupted (see Outstanding questions). Addressing this knowledge gap will hopefully encourage further discourse on potential improvements to FUS+MB-mediated treatments for neurological conditions to maximize their benefit–risk ratio.

Outstanding questions

Can some of the hallmarks of neurological disease be attributed to neurovascular dysfunction as much as to neuronal dysregulation?

What are the biological effects of mechanical modulation of the BBB produced by FUS and MBs?

Clinically, FUS+MBs have so far been applied primarily as a treatment for terminal conditions such as GBM. If, however, they are applied in the future to non-terminal CNS diseases, what are the potential long-term adverse effects that should be considered by clinicians and researchers?

How would a course of several FUS+MB treatments affect the long-term integrity of the BBB?

Should there be a strategic algorithm or pipeline in place for determining appropriate use of FUS+MBs?

How can the potential adverse effects of neuroinflammation arising from FUS+MB-mediated disruption of the BBB be minimized? Can pre- or post-operative strategies be developed to contain or mitigate such effects?

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Declaration of interests

The authors declare no conflicts of interest.

Supplemental information

Supplemental information associated with this article can be found, in the online version, at https://doi.org/10.1016/j.tins.2022.03.003.

Resources

[i]https://clinicaltrials.gov/ct2/show/NCT03321487
[ii]https://clinicaltrials.gov/ct2/show/NCT02986932
[iii]https://clinicaltrials.gov/ct2/show/NCT03608553
[iv]https://clinicaltrials.gov/ct2/show/NCT02253212
[v]https://clinicaltrials.gov/ct2/show/NCT03671889
[vi]https://clinicaltrials.gov/ct2/show/NCT02343991
[vii]https://clinicaltrials.gov/ct2/show/NCT03551249
[viii]https://clinicaltrials.gov/ct2/show/NCT03616860
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[xi]https://clinicaltrials.gov/ct2/show/NCT04370665
[xii]https://clinicaltrials.gov/ct2/show/NCT04526262
[xiii]https://clinicaltrials.gov/ct2/show/NCT04620460
[xiv]https://clinicaltrials.gov/ct2/show/NCT04804709
[xv]https://clinicaltrials.gov/ct2/show/NCT05089786

References

1. Feigin, V.L. *et al.* (2017) Global, regional, and national burden of neurological disorders during 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet Neurol.* 16, 877–897
2. Pound, P. and Ram, R. (2020) Are researchers moving away from animal models as a result of poor clinical translation in the field of stroke? An analysis of opinion papers. *BMJ Open Sci.* 4, e100041
3. Cummings, J. (2018) Lessons learned from Alzheimer disease: clinical trials with negative outcomes. *Clin. Transl. Sci.* 11, 147–152
4. O'Brown, N.M. *et al.* (2018) Bridging barriers: a comparative look at the blood–brain barrier across organisms. *Genes Dev.* 32, 466–478
5. Danon, J.J. *et al.* (2019) Challenges and opportunities in central nervous system drug discovery. *Trends Chem.* 1, 612–624
6. Abrahao, A. *et al.* (2019) First-in-human trial of blood–brain barrier opening in amyotrophic lateral sclerosis using MR-guided focused ultrasound. *Nat. Commun.* 10, 4373

7. Lipsman, N. *et al.* (2018) Blood–brain barrier opening in Alzheimer's disease using MR-guided focused ultrasound. *Nat. Commun.* 9, 2336
8. Gasca-Salas, C. *et al.* (2021) Blood–brain barrier opening with focused ultrasound in Parkinson's disease dementia. *Nat. Commun.* 12, 779
9. Abbott, N.J. *et al.* (2006) Astrocyte-endothelial interactions at the blood–brain barrier. *Nat. Rev. Neurosci.* 7, 41–53
10. Song, K. *et al.* (2020) Oxidative stress-mediated blood–brain barrier (BBB) disruption in neurological diseases. *Oxid. Med. Cell. Longev.* 2020, 4356386
11. Montagne, A. *et al.* (2015) Blood–brain barrier breakdown in the aging human hippocampus. *Neuron* 85, 296–302
12. Sweeney, M.D. *et al.* (2019) Vascular dysfunction – the disregarded partner of Alzheimer's disease. *Alzheimers. Dement.* 15, 158–167
13. Nation, D.A. *et al.* (2019) Blood–brain barrier breakdown is an early biomarker of human cognitive dysfunction. *Nat. Med.* 252, 270–276
14. Shimizu, F. *et al.* (2018) Blood–brain barrier dysfunction in immuno-mediated neurological diseases. *Immunol. Med.* 41, 120–128
15. Spencer, J.I. *et al.* (2018) Vascular pathology in multiple sclerosis: reframing pathogenesis around the blood–brain barrier. *J. Neurol. Neurosurg. Psychiatry* 89, 42–52
16. Jeyakumar, M. *et al.* (2003) Central nervous system inflammation is a hallmark of pathogenesis in mouse models of GM1 and GM2 gangliosidosis. *Brain* 126, 974–987
17. Begley, D. *et al.* (2008) Lysosomal storage diseases and the blood–brain barrier. *Curr. Pharm. Des.* 14, 1566–1580
18. Bellettato, C.M. and Scarpa, M. (2018) Possible strategies to cross the blood–brain barrier. *Ital. J. Pediatr.* 44, 131
19. Edelmann, M.J. and Maegawa, G.H.B. (2020) CNS-targeting therapies for lysosomal storage diseases: current advances and challenges. *Front. Mol. Biosci.* 7, 291
20. Profaci, C.P. *et al.* (2020) The blood–brain barrier in health and disease: important unanswered questions. *J. Exp. Med.* 217, e20190062
21. Ivanidze, J. *et al.* (2020) Molecular imaging of striatal dopaminergic neuronal loss and the neurovascular unit in Parkinson disease. *Front. Neurosci.* 14, 528809
22. Lochhead, J.J. *et al.* (2020) Structure, function, and regulation of the blood–brain barrier tight junction in central nervous system disorders. *Front. Physiol.* 11, 914
23. Elabi, O. *et al.* (2021) Human α-synuclein overexpression in a mouse model of Parkinson's disease leads to vascular pathology, blood brain barrier leakage and pericyte activation. *Sci. Rep.* 11, 1120
24. Lin, Z. *et al.* (2021) Blood–brain barrier breakdown in relationship to Alzheimer and vascular disease. *Ann. Neurol.* 90, 227–238
25. Singhrao, S.K. and Harding, A. (2020) Is Alzheimer's disease a polymicrobial host microbiome dysbiosis? *Expert Rev. Anti. Infect. Ther.* 18, 275–277
26. Vigasova, D. *et al.* (2021) Multi-pathogen infections and Alzheimer's disease. *Microb. Cell Fact.* 20, 25
27. Poole, S. *et al.* (2013) Determining the presence of periodontopathic virulence factors in short-term postmortem Alzheimer's disease brain tissue. *J. Alzheimer's Dis.* 36, 665–677
28. Carrasco, L. *et al.* (2017) Alzheimer's disease and fungal infection. In *Handbook of Infection and Alzheimer's Disease* (Vol. 5) (Miklossy, J., ed.), pp. 281–294, IOS Press
29. Kipnis, J. and Filiano, A.J. (2017) 2017 The central nervous system: privileged by immune connections. *Nat. Rev. Immunol.* 18, 83–84
30. Storelli, F. *et al.* (2021) Abundance of P-glycoprotein and other drug transporters at the human blood–brain barrier in Alzheimer's disease: a quantitative targeted proteomic study. *Clin. Pharmacol. Ther.* 109, 667–675
31. Anon (2000) Mechanical bioeffects in the presence of gas-carrier ultrasound contrast agents. *J. Ultrasound Med.* 19, 120
32. Coussios, C. *et al.* (2007) Role of acoustic cavitation in the delivery and monitoring of cancer treatment by high-intensity focused ultrasound (HIFU). *Int. J. Hyperthermia* 23, 105–120
33. Hynynen, K. and Jolesz, F.A. (1998) Demonstration of potential noninvasive ultrasound brain therapy through an intact skull. *Ultrasound Med. Biol.* 24, 275–283
34. Hynynen, K. *et al.* (2001) Noninvasive MR imaging-guided focal opening of the blood–brain barrier in rabbits. *Radiology* 220, 640–646
35. Hynynen, K. *et al.* (2003) The threshold for brain damage in rabbits induced by bursts of ultrasound in the presence of an ultrasound contrast agent (Optison). *Ultrasound Med. Biol.* 29, 473–481
36. McDannold, N. *et al.* (2005) MRI-guided targeted blood–brain barrier disruption with focused ultrasound: histological findings in rabbits. *Ultrasound Med. Biol.* 31, 1527–1537
37. Hynynen, K. *et al.* (2005) Local and reversible blood–brain barrier disruption by noninvasive focused ultrasound at frequencies suitable for trans-skull sonications. *Neuroimage* 24, 12–20
38. Sheikov, N. *et al.* (2004) Cellular mechanisms of the blood–brain barrier opening induced by ultrasound in presence of microbubbles. *Ultrasound Med. Biol.* 30, 979–989
39. Liu, Y. *et al.* (2020) Microbubbles in combination with focused ultrasound for the delivery of quercetin-modified sulfur nanoparticles through the blood brain barrier into the brain parenchyma and relief of endoplasmic reticulum stress to treat Alzheimer's disease. *Nanoscale* 12, 6498–6511
40. Wang, F. *et al.* (2021) Ultrasound combined with microbubbles loading BDNF retrovirus to open blood–brain barrier for treatment of Alzheimer's disease. *Front. Pharmacol.* 12, 615104
41. Zhang, N. *et al.* (2018) Localized delivery of curcumin into brain with polysorbate 80-modified cerasomes by ultrasound-targeted microbubble destruction for improved Parkinson's disease therapy. *Theranostics* 8, 2264
42. Lin, C.Y. *et al.* (2020) Ultrasound-responsive neurotrophic factor-loaded microbubble–liposome complex: preclinical investigation for Parkinson's disease treatment. *J. Control. Release* 321, 519–528
43. Hsu, Y.-H. *et al.* (2017) Transcranial pulsed ultrasound facilitates brain uptake of laronidase in enzyme replacement therapy for mucopolysaccharidosis type I disease. *Orphanet J. Rare Dis.* 121, 1–9
44. Mancia, L. *et al.* (2019) Modeling tissue-selective cavitation damage. *Phys. Med. Biol.* 64, 225001
45. Miller, M.W. *et al.* (1996) A review of in vitro bioeffects of inertial ultrasonic cavitation from a mechanistic perspective. *Ultrasound Med. Biol.* 22, 1131–1154
46. Kovacs, Z.I. *et al.* (2017) Disrupting the blood–brain barrier by focused ultrasound induces sterile inflammation. *Proc. Natl. Acad. Sci. U. S. A.* 114, E75–E84
47. Joiner, J.B. *et al.* (2020) Focused ultrasound for immunomodulation of the tumor microenvironment. *J. Immunol.* 205, 2327–2341
48. Cohen-Inbar, O. *et al.* (2016) Focused ultrasound-aided immunomodulation in glioblastoma multiforme: a therapeutic concept. *J. Ther. Ultrasound* 4, 2
49. Hynynen, K. *et al.* (2006) Focal disruption of the blood–brain barrier due to 260-kHz ultrasound bursts: a method for molecular imaging and targeted drug delivery. *J. Neurosurg.* 105, 445–454
50. Choi, J. *et al.* (2008) Noninvasive and transient blood–brain barrier opening in the hippocampus of Alzheimer's double transgenic mice using focused ultrasound. *Ultrason. Imaging* 30, 189–200
51. Choi, J. *et al.* (2010) Microbubble-size dependence of focused ultrasound-induced blood brain barrier opening in mice in vivo. *IEEE Trans. Biomed. Eng.* 57, 145–154
52. Jordão, J.F. *et al.* (2010) Antibodies targeted to the brain with image-guided focused ultrasound reduces amyloid-β plaque load in the TgCRND8 mouse model of Alzheimer's disease. *PLoS One* 5, e10549
53. Burgess, A. *et al.* (2011) Targeted delivery of neural stem cells to the brain using MRI-guided focused ultrasound to disrupt the blood–brain barrier. *PLoS One* 6, e27866
54. Tung, Y.-S. *et al.* (2011) The mechanism of interaction between focused ultrasound and microbubbles in blood–brain barrier opening in mice. *J. Acoust. Soc. Am.* 130, 3059

55. Todd, N. *et al.* (2020) Secondary effects on brain physiology caused by focused ultrasound-mediated disruption of the blood–brain barrier. *J. Control. Release* 324, 450–459
56. Chen, H. and Konofagou, E.E. (2014) The size of blood–brain barrier opening induced by focused ultrasound is dictated by the acoustic pressure. *J. Cereb. Blood Flow Metab.* 34, 1197–1204
57. Sirsi, S. and Borden, M. (2009) Microbubble compositions, properties and biomedical applications. *Bubble Sci. Eng. Technol.* 1, 3–17
58. Beekers, I. *et al.* (2020) Opening of endothelial cell–cell contacts due to sonoporation. *J. Control. Release* 322, 426–438
59. McMahon, D. *et al.* (2020) Microbubble formulation influences inflammatory response to focused ultrasound exposure in the brain. *Sci. Rep.* 10, 21534
60. Pascal, A. *et al.* (2020) Histologic evaluation of activation of acute inflammatory response in a mouse model following ultrasound-mediated blood–brain barrier using different acoustic pressures and microbubble doses. *Nanotheranostics* 4, 210–223
61. Kovacs, Z.I. *et al.* (2018) Focused ultrasound with microbubbles induces sterile inflammatory response proportional to the blood brain barrier opening: attention to experimental conditions. *Theranostics* 8, 2245–2248
62. Kamimura, H.A. *et al.* (2018) Feedback control of microbubble cavitation for ultrasound-mediated blood–brain barrier disruption in non-human primates under magnetic resonance guidance. *J. Cereb. Blood Flow Metab.* 39, 1191–1203
63. McMahon, D. and Hynynen, K. (2017) Acute inflammatory response following increased blood–brain barrier permeability induced by focused ultrasound is dependent on microbubble dose. *Theranostics* 7, 3989–4000
64. Breuer, J. *et al.* (2018) Blockade of MCAM/CD146 impedes CNS infiltration of T cells over the choroid plexus. *J. Neuroinflammation* 15, 236
65. Jurberg, A.D. *et al.* (2021) VLA-4 as a central target for modulating neuroinflammatory disorders. *Neuroimmunomodulation* 28, 213–221
66. Savino, W. *et al.* (2021) Integrin-directed antibody-based immunotherapy: focus on VLA-4. *Immunother. Adv.* 1, 1–11
67. Sheikov, N. *et al.* (2008) Effect of focused ultrasound applied with an ultrasound contrast agent on the tight junctional integrity of the brain microvascular endothelium. *Ultrasound Med. Biol.* 34, 1093–1104
68. Chang, J.W. *et al.* (2020) Ultrasound stimulation suppresses LPS-induced proinflammatory responses by regulating NF-κB and CREB activation in microglial cells. *Cereb. Cortex* 30, 4597–4606
69. Chen, S. *et al.* (2022) A review of bioeffects induced by focused ultrasound combined with microbubbles on the neurovascular unit. *J. Cereb. Blood Flow Metab.* 42, 3–26
70. Sinharay, S. *et al.* (2019) In vivo imaging of sterile microglial activation in rat brain after disrupting the blood–brain barrier with pulsed focused ultrasound: [^{18}F]DPA-714 PET study. *J. Neuroinflammation* 16, 155
71. Smyth, L.C.D. *et al.* (2018) Unique and shared inflammatory profiles of human brain endothelia and pericytes. *J. Neuroinflammation* 15, 138
72. Yamamoto, S. *et al.* (2015) Inflammation-induced endothelial cell-derived extracellular vesicles modulate the cellular status of pericytes. *Sci. Rep.* 5, 8505
73. Hurtado-Alvarado, G. *et al.* (2014) Pericytes: brain-immune interface modulators. *Front. Integr. Neurosci.* 7, 80
74. Jeong, A.K. *et al.* (2006) Brain endothelial hemostasis regulation by pericytes. *J. Cereb. Blood Flow Metab.* 26, 209–217
75. Rustenhoven, J. *et al.* (2017) Brain pericytes as mediators of neuroinflammation. *Trends Pharmacol. Sci.* 38, 291–304
76. Rudziak, P. *et al.* (2019) Role and molecular mechanisms of pericytes in regulation of leukocyte diapedesis in inflamed tissues. *Mediators Inflamm.* 2019, 4123605
77. Dorrier, C.E. *et al.* (2021) CNS fibroblasts form a fibrotic scar in response to immune cell infiltration. *Nat. Neurosci.* 24, 234–244
78. Zhao, R. *et al.* (2018) Phosphatidylserine-microbubble targeting-activated microglia/macrophage in inflammation combined with ultrasound for breaking through the blood–brain barrier. *J. Neuroinflammation* 15, 334
79. Aryal, M. *et al.* (2017) Effects on P-glycoprotein expression after blood–brain barrier disruption using focused ultrasound and microbubbles. *PLoS One* 12, e01166061
80. Torres-Vergara, P. and Penny, J. (2018) Pro-inflammatory and anti-inflammatory compounds exert similar effects on P-glycoprotein in blood–brain barrier endothelial cells. *J. Pharm. Pharmacol.* 70, 713–722
81. Kugelman, T.L. *et al.* (2020) Safe focused ultrasound-mediated blood–brain barrier opening and repair is not mediated by tight junction degradation. *Cell Rep.* Published online June 3, 2020. https://doi.org/10.2139/ssm.3599080
82. Sheybani, N.D. and Price, R.J. (2019) Perspectives on recent progress in focused ultrasound immunotherapy. *Theranostics* 9, 7749
83. Sheybani, N.D. *et al.* (2020) Combination of thermally ablative focused ultrasound with gemcitabine controls breast cancer via adaptive immunity. *J. Immunother. Cancer* 8, e001008
84. Yuan, J. *et al.* (2021) Therapeutic ultrasound-enhanced immune checkpoint inhibitor therapy. *Front. Phys.* 9, 102
85. Alkins, R. *et al.* (2016) Early treatment of HER2-amplified brain tumors with targeted NK-92 cells and focused ultrasound improves survival. *Neuro. Oncol.* 18, 974
86. Beccaria, K. *et al.* (2021) Blood–brain barrier opening with low intensity pulsed ultrasound for immune modulation and immune therapeutic delivery to CNS tumors. *J. Neurooncol.* 151, 65–73
87. Malo, C.S. *et al.* (2018) Immunomodulation mediated by anti-angiogenic therapy improves CD8 T cell immunity against experimental glioma. *Front. Oncol.* 8, 320
88. Dantzer, R. (2018) Neuroimmune interactions: from the brain to the immune system and vice versa. *Physiol. Rev.* 98, 477
89. Bennett, F.C. and Molofsky, A.V. (2019) The immune system and psychiatric disease: a basic science perspective. *Clin. Exp. Immunol.* 197, 294–307
90. Tansey, M.G. and Romero-Ramos, M. (2019) Immune system responses in Parkinson's disease: early and dynamic. *Eur. J. Neurosci.* 49, 364–383
91. O'Reilly, M.L. and Tom, V.J. (2020) Neuroimmune system as a driving force for plasticity following CNS injury. *Front. Cell. Neurosci.* 14, 187
92. Tchessalova, D. *et al.* (2018) Neuroimmune activation drives multiple brain states. *Front. Syst. Neurosci.* 12, 39
93. Louveau , A. *et al.* (2015) Structural and functional features of central nervous system lymphatic vessels. *Nature* 523, 337–341
94. Absinta, M. *et al.* (2017) Human and nonhuman primate meninges harbor lymphatic vessels that can be visualized noninvasively by MRI. *eLife* 6, e29738
95. Meng, Y. *et al.* (2021) MR-guided focused ultrasound enhances delivery of trastuzumab to Her2-positive brain metastases. *Sci. Transl. Med.* 13, eabj4011
96. Chen, K.-T. *et al.* (2020) Neuronavigation-guided focused ultrasound (NaviFUS) for transcranial blood–brain barrier opening in recurrent glioblastoma patients: clinical trial protocol. *Ann. Transl. Med.* 8, 673
97. Wei, H.J. *et al.* (2021) Focused ultrasound-mediated blood–brain barrier opening increases delivery and efficacy of etoposide for glioblastoma treatment. *Int. J. Radiat. Oncol.* 110, 539–550
98. Poon, C. *et al.* (2021) Neutrophil recruitment and leukocyte response following focused ultrasound and microbubble mediated blood–brain barrier treatments. *Theranostics* 11, 1655
99. D'Haese, P.-F. *et al.* (2020) β-Amyloid plaque reduction in the hippocampus after focused ultrasound-induced blood–brain barrier opening in Alzheimer's disease. *Front. Hum. Neurosci.* 14, 593672
100. Mehta, R.I. *et al.* (2021) Blood–brain barrier opening with MRI-guided focused ultrasound elicits meningeal venous permeability in humans with early Alzheimer disease. *Radiology* 298, 654–662
101. Kovacs, Z.I. *et al.* (2018) MRI and histological evaluation of pulsed focused ultrasound and microbubbles treatment effects in the brain. *Theranostics* 8, 4837

102. Ji, R. *et al.* (2021) Cavitation-modulated inflammatory response following focused ultrasound blood–brain barrier opening. *J. Control. Release* 337, 458–471
103. Burks, S.R. *et al.* (2021) Blood–brain barrier opening by intracarotid artery hyperosmolar mannitol induces sterile inflammatory and innate immune responses. *Proc. Natl. Acad. Sci. U. S. A.* 118, e2021915118
104. McMahon, D. *et al.* (2020) Investigating the effects of dexamethasone on blood–brain barrier permeability and inflammatory response following focused ultrasound and microbubble exposure. *Theranostics* 10, 1604
105. Mathew, A.S. *et al.* (2021) Transcriptomic response of brain tissue to focused ultrasound-mediated blood–brain barrier disruption depends strongly on anesthesia. *Bioeng. Transl. Med.* 6, e10198
106. Mainprize, T. *et al.* (2019) Blood–brain barrier opening in primary brain tumors with non-invasive MR-guided focused ultrasound: a clinical safety and feasibility study. *Sci. Rep.* 9, 321
107. Carpentier, A. *et al.* (2016) Clinical trial of blood–brain barrier disruption by pulsed ultrasound. *Sci. Transl. Med.* 8, 343re2
108. Meng, Y. *et al.* (2021) MR-guided focused ultrasound liquid biopsy enriches circulating biomarkers in patients with brain tumors. *Neuro. Oncol.* 23, 1789–1797
109. Pouliopoulos, A.N. *et al.* (2021) Safety evaluation of a clinical focused ultrasound system for neuronavigation guided blood–brain barrier opening in non-human primates. *Sci. Rep.* 11, 15043
110. Park, S.H. *et al.* (2021) Extensive frontal focused ultrasound mediated blood–brain barrier opening for the treatment of Alzheimer's disease: a proof-of-concept study. *Transl. Neurodegener.* 10, 44