Journal of Neuro-Oncology
https://doi.org/10.1007/s11060-020-03425-8

TOPIC REVIEW

Blood–brain barrier opening with low intensity pulsed ultrasound for immune modulation and immune therapeutic delivery to CNS tumors

Kevin Beccaria[1] · Aria Sabbagh[1] · John de Groot[2] · Michael Canney[3] · Alexandre Carpentier[4] · Amy B. Heimberger[1,5]

Received: 21 August 2019 / Accepted: 5 February 2020

Abstract

Introduction Opening of the blood–brain barrier (BBB) by pulsed low intensity ultrasound has been developed during the last decade and is now recognized as a safe technique to transiently and repeatedly open the BBB. This non- or minimally invasive technique allows for a targeted and uniform dispersal of a wide range of therapeutic substances throughout the brain, including immune cells and antibodies.

Methods In this review article, we summarize pre-clinical studies that have used BBB-opening by pulsed low intensity ultrasound to enhance the delivery of immune therapeutics and effector cell populations, as well as several recent clinical studies that have been initiated. Based on this analysis, we propose immune therapeutic strategies that are most likely to benefit from this strategy. The literature review and trial data research were performed using Medline/Pubmed databases and clinical trial registry www.clinicaltrials.gov. The reference lists of all included articles were searched for additional studies.

Results A wide range of immune therapeutic agents, including small molecular weight drugs, antibodies or NK cells, have been safely and efficiently delivered to the brain with pulsed low intensity ultrasound in preclinical models, and both tumor control and increased survival have been demonstrated in different types of brain tumor models in rodents. Ultrasound-induced BBB disruption may also stimulate innate and cellular immune responses.

Conclusions Ultrasound BBB opening has just recently entered clinical trials with encouraging results, and the association of this strategy with immune therapeutics creates a new field of brain tumor treatment.

Keywords Blood–brain barrier · Low intensity pulsed ultrasound · Drug delivery · Immunotherapy

Abbreviations

anti-Aß	Anti-amyloïd beta
APC	Antigen-presenting cell
BBB	Blood–brain barrier
BBBD	Blood–brain barrier disruption
CNS	Central nervous system
CD	Cluster of differentiation
CED	Convection-enhanced delivery
CTL	Cytolytic T cell
DNA	Deoxyribonucleic acid
FISPION	Fluorescently-labeled superparamagnetic iron oxide nanoparticles
FUS	Focused ultrasound
Fab	Fragment antigen-binding
GFAP	Glial fibrillary acidic protein
G-CSF	Granulocyte colony-stimulating factor
HSP70	Heat-shock protein 70
HPLC	High performance liquid chromatography

✉ Amy B. Heimberger
aheimber@mdanderson.org

1 Department of Neurosurgery, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

2 Neuro-Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

3 CarThera, Institut du Cerveau Et de La Moelle épinière (ICM), 75013 Paris, France

4 Department of Neurosurgery, Sorbonne Université, UPMC Univ Paris 06, Assistance Publique-Hôpitaux de Paris (AP-HP), Hôpitaux Universitaires La Pitié-Salpêtrière, Paris, France

5 Department of Neurosurgery, The University of Texas MD Anderson Cancer Center, Unit 422, P.O. Box 301402, Houston, TX 77230-1402, USA

Published online: 28 February 2020

HER2	Human epidermal growth factor receptor-2
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IFNγ	Interferon gamma
IL	Interleukin
IP	Intraperitoneal
MIP3α	Macrophage inflammatory protein-3 alpha
NK	Natural killer
NF-κB	Nuclear factor-kappa B
OS	Overall survival
PFS	Progression-free survival
scFv	Single-chain variable fragment
SPIO	Super-paramagnetic iron oxides
Treg	Regulatory T cell
TNFα	Tumor necrosis factor alpha
US	Ultrasound
VEGF-A	Vascular endothelial growth factor A

Rationale for use of temporary BBB-opening by ultrasound with immune therapeutics

Conventionally, the brain has been considered to be immunologically privileged based on the work of Medawar in 1948, in which allogeneic skin grafts transplanted onto the brains of experimental animals escaped host rejection [1]. This premise was bolstered by the notions that the central nervous system (CNS) lacked antigen-presenting cells, had a tightly imposed blood–brain barrier (BBB) restricting immune access, and lacked lymphatic drainage. Widespread acceptance of the view that the brain is an immunologically privileged site has hampered the enthusiasm and development of immune therapeutics for brain tumors. Over the last several decades, each of these purported barriers has been dismantled or refuted, including identification of antigen-presenting cells in the brain [2], proof that circulating immune cells are capable of penetrating the BBB to perform routine immune surveillance functions in the CNS [3, 4], and demonstration of the presence of an elegant lymphatic system [5–7].

Four key steps are needed to obtain immunological clearance of a cancer: (1) an immunological target; (2) immune activation; (3) trafficking to the tumor microenvironment; and 4) maintenance of the effector function in the setting of tumor-mediated immune suppression. All of these must be operational to obtain cancer control by the immune system. Although there is a large therapeutic portfolio of approved drugs that addresses tumor antigen targets, immune activators, and modulators for controlling immune suppression, there are few modalities that are directed at enhancing the relative number of effectors in the tumor microenvironment and/or the delivery of immune modulators to the tumor. The BBB serves as a specific impediment to the delivery of large molecules and antibodies. Specifically, less than 1% of administered antibodies can usually be detected in the CNS, but they can be engineered to have increased penetration [8].

Gliomas are immunologically unique in that they are enriched in some types of immune cells such as macrophages [9, 10] but are relatively lacking in others such as T and NK cells [11] that are capable of exerting direct tumor cytolytic activity. Even in the setting of active immunotherapy with agents such as immune checkpoint inhibitors, there may not be significant enrichment of these cells. The T cell in particular can be sequestered in the bone marrow in patients with intracranial tumors [12]. As such, unique strategies need to be considered for enhancing their presence in the tumor.

Different strategies have been used to increase the concentration of a therapeutic agent within the CNS, such as: convection enhanced delivery (CED), which provides a continuous infusion of drugs through an intracerebral catheter [13]; intra-arterial chemotherapy with microcatheters and endovascular devices [14]; and deposition of implants in the tumoral cavity [15, 16]. Noninvasive techniques are based on osmotic [17] or chemical [18] disruption of the BBB. Chemical modification of drugs may also enable them to pass through the BBB [19], but this will require reformulation of each drug and is unlikely to be compatible with the entire therapeutic compendium. Physiological approaches using specific transport systems [20] could also be considered. Ultrasound (US)-induced BBB disruption (BBBD), a technology that has been in pre-clinical development for more than 20 years and which has just recently entered clinical trials, has several advantages in being non- or minimally invasive, can be administered in a targeted manner, and may allow for a more uniform dispersal of the therapeutic throughout the tumor microenvironment [21]. In this review article, we summarize pre-clinical studies that have used BBB-opening by ultrasound to enhance the delivery of immune therapeutics and effector cell populations, as well as several recent clinical studies that have been initiated. We also propose strategies that are most likely to benefit from this combinatorial approach.

Ultrasound-induced BBB disruption

Intravenously injected microbubbles administered during the delivery of low intensity pulsed ultrasound can induce transient and safe opening of the BBB in rabbits [21, 22]. The mechanisms underlying US-induced BBBD are not totally elucidated but are based on the expansion and contraction of injected microbubbles, also called cavitation, which implicate four different cellular mechanisms: (1) transcytosis, (2) transendothelial openings (fenestrations, channels formation), (3) opening of the tight junctions and interendothelial

clefts, and (4) free passage of molecules through the permeable endothelium at higher ultrasonic energies [23]. Figure 1 illustrates the primary mechanisms believed to underlie US-induced BBBD. In the initial studies of this strategy, endogenous IgG was observed to cross the BBB secondary to transcellular processes and, to a lesser extent, intercellular shuttling [23]. For the latter, most of the IgG was transported in vesicles after internalization on the luminal surface of endothelial cells (i.e., transcytosis). Very little IgG was detected passing through interendothelial clefts.

The safety of US-induced BBBD has been assessed in preclinical models in both small and large animals and in clinical studies. With optimal US parameters, the histological side effects observed in animal models are limited to potential red blood-cell extravasations, with very few apoptotic or ischemic cells [24], for either single or multiple sonication sessions [25]. Multi-parametric, long-term studies have shown that repeated US-induced disruption of the BBB was clinically well tolerated in primates [26–28]. A large variety of antineoplastic molecules and agents have been shown to have enhanced delivery to the brain after BBBD including small molecular weight drugs [29], monoclonal antibodies [30], enzymes [31], neurotrophic factors [32], DNA [33], and cells [34]. Both tumor control and increased survival times have been achieved in preclinical tumor models of glioma after US-induced opening of the BBB and systemic injection of drugs [35–37].

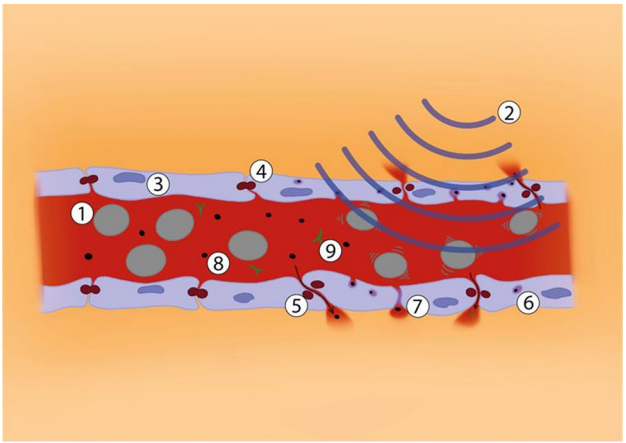

Fig. 1 Mechanisms of blood–brain barrier (BBB) opening with low intensity pulsed ultrasound. Passage of microbubbles (1) in the ultrasound beam (2) makes them vibrate, creating cavitation. This induces modifications of the endothelial cells (3) and opening of the BBB in different ways. Closed tight junctions (4) are temporarily opened, creating intercellular routes (5). Transcellular transport is activated, with formation and movement of vesicles from luminal to abluminal surfaces (6). Vesicles can also merge and form large fenestrations and then form transendothelial channels (7). A large variety of entities, from small drugs (8) to antibodies (9) and cells, can cross the BBB through these intercellular and transcellular ways, and thus be delivered to the brain parenchyma

Clinical translation

Two strategies have been developed in order to open the BBB with ultrasound and have led to multiple clinical trials: extracranially-applied focused ultrasound and implantable ultrasound devices. The first technique was described more than 15 years ago [21] and is currently the most widespread. Clinical systems based on transcranial ultrasound were first developed for thermal ablation applications. These devices allow for opening of the BBB in a focused manner usually spanning several millimeters in the brain parenchyma. With a scanning setup, or adjustment of the phasing of large arrays, larger volumes of brain can be safely sonicated [38]. The skull represents the main obstacle to transcranial application of ultrasound because the bone induces a 30 to 60-times higher acoustic attenuation than soft tissues do, causes rapid heating inside the skull, and it also has a significant effect on the US beam's propagation. By using lower ultrasound frequencies, hemispherical shaped arrays [39], and multi-element phased array ultrasound systems [40], these difficulties were progressively overcome. Preclinical studies led to the development of a low frequency hemispherical multi-element phased-array transducer, a transcranial and totally noninvasive system—the ExAblate® system (InSightec, Haifa, Israel [41, 42]). Several on-going clinical trials are evaluating the safety and feasibility of BBBD with the ExAblate® system in patients with high-grade glioma (NCT03551249, NCT03616860, NCT03712293, NCT02343991), breast cancer brain metastases (NCT03714243), Alzheimer's disease (NCT02986932, NCT03739905, NCT03671889), Parkinson's disease (NCT03608553), and amyotrophic lateral sclerosis (NCT03321487). The first BBB opening in patients with malignant brain tumors using the ExAblate® system in a phase I, single-armed study, has been recently published [43] (NCT02343991). Safe and reversible opening of the BBB was obtained at tumor margins, with a 15–50% increase of contrast enhancement on T1-weighted magnetic resonance imaging (MRI), with resolution 20 h later. Increased temozolomide concentrations were observed in sonicated peritumoral tissue relative to unsonicated peritumoral tissue (3.47×10^{-4} ng/mg versus 0.45×10^{-4} ng/mg, respectively). A custom-designed multichannel hemispherical phased-array ultrasound system, the NaviFUS® System, has been designed by a Taiwanese team and has been recently assessed in a single-armed and dose escalation study in patients with recurrent GBM (NCT03626896). The results have not yet been published. A single element, transcranial, focused ultrasound system that uses neuronavigation to guide treatment has also been approved by the FDA for a pilot clinical trial for Alzheimer's disease (Columbia University). An MRI is not

needed during the procedure and cavitation feedback using a passive cavitation detection device is used to control the treatment [27].

As an alternative approach, CarThera has designed an implantable ultrasound device that can be inserted into the skull during surgical resection. Multiple preclinical studies have assessed this device, the SonoCloud®, and demonstrated that it allows for safe, transient, and repeated opening of the BBB in both small and large animals [26, 44]. In addition, temozolomide, irinotecan, and carboplatin concentrations have been enhanced in the brain parenchyma of rabbits, mice and non-human primates with this device [29, 45]. Both increased survival times and delayed tumor growth have been observed in murine models of glioblastoma treated with these chemotherapeutics using ultrasound BBB opening [37]. As such, the first clinical trial using the SonoCloud® technology has been performed in patients with recurrent glioblastoma treated with intravenous carboplatin (NCT02253212). Opening of the BBB was obtained without severe adverse events, including when sonicating eloquent brain regions [22]. Moreover, both median progression-free survival (PFS) and overall survival (OS) times were increased relative to historical data (4.11 months versus 2–3 months, respectively, for PFS and 12.94 months versus 6–9 months, respectively, for OS). A trend for tumor control in the sonication field was also shown, although these results will have to be confirmed in a larger study with more patients. The SonoCloud-9®, a new version of the SonoCloud® device, consists of nine 1-cm diameter transducers arranged on an implantable grid, so that it can cover a larger resection area and surrounding tissues. A multicenter clinical trial is still ongoing to evaluate the safety of the device in patients with recurrent glioblastoma (NCT03744026). Two other clinical trials are underway in order to evaluate the safety and feasibility of BBBD using the SonoCloud-1® device in patients with melanoma brain metastases (NCT04021420) and in patients with Alzheimer's disease (NCT03119961).

The use of BBB-opening ultrasound for CNS diseases

Clinical applications of BBB-opening ultrasound technology were first considered in the context of Alzheimer's disease. Anti-Aß antibodies were safely delivered to the hippocampus of different strains of plaque-bearing, transgenic mice in two model systems of Alzheimer's disease (APPswe:PSEN1dE9 and PDAPP models), with or without MRI guidance [46]. In a TgCRND8 mouse model of Alzheimer's disease, the levels of both IgG and IgM that were directed against amyloid plaques were significantly increased in specific regions of the brain treated with MR-guided US relative to non-treated cortex. Such increases were observed from 4 h to 4 days after sonication and correlated with decreased plaque burden [47, 48]. Multiple other studies have also shown that this strategy can enhance the delivery of intravenously-injected antibodies to the brain. For example, Dopamine D4 receptor-targeting antibodies were delivered to the hippocampus and basal ganglia in mice after BBB opening, whereas no antibodies were detected in the contralateral and unsonicated brain regions [30]. The same team was able to deliver trastuzumab, a humanized antihuman epidermal growth factor receptor 2 monoclonal antibody, to mouse brain parenchyma, with the amount of the antibody delivered directly correlating with the MRI signal change after gadolinium injection, allowing for imaging assessment of delivery [49]. Anti-Aß antibody delivery was enhanced specifically to reach the hippocampus of mice modeling Alzheimer's disease (TgCRND8 model), which was associated with reduced Aß plaque load [47]. Clinical improvement (of anxiety-like behavior) in a tau transgenic mouse model was shown in mice treated with BBB-opening scanning ultrasound and intravenous injection of a tau-specific single-chain antibody [50]. In this study, the whole brain was submitted to sequential six-second sonications per spot using a scanning setup, with a good clinical tolerance and no damage to the brain tissue [38]. Another study demonstrated that different variations of antibodies (IgG, Fab, scFv, from 29 to 156 kDa) could be delivered to the brain with scanning ultrasound [51]. It is interesting to note that in this later study, the IgG, which was a larger molecule than either Fab or scFv, achieved the highest concentration in the brain. This was due to the longer half-life of the full-sized antibody (IgG), which led to an increase in the circulating serum levels of the IgG.

Use of BBB-opening by ultrasound in preclinical brain metastases models

Tumor growth control has been shown in breast cancer brain metastases models in rats treated with BBB disruption. CNS metastases completely resolved in a subset of rats ($n=4$) after 5 to 7 weekly injections of trastuzumab associated with US-BBBD. In this study, the response to the treatment was not uniform because, in contrast to the "responder rats", the other six rats treated with trastuzumab and ultrasound had tumor growth similar to that in the control groups [52]. The same phenomenon was observed in a second study, in which tumor control growth was obtained in a subset of responder rats after weekly treatments with trastuzumab and pertuzumab in conjunction with BBBD, whereas no control was obtained in the remaining six rats treated with the same protocol. No complete responses were observed in this study [53]. The authors did not provide a clear explanation for these discrepancies in responses, but they

suggested that these could be attributed to variations in tumor vasculature or the disruption technique.

In a rat preclinical model of breast metastases, Alkins et al. observed enhanced delivery of HER2-specific NK cells to the brain after US-induced BBBD and intravenous injection of the effector cells [34]. Accumulation of NK effectors in the brain was significantly enhanced in the whole US beam, including within the healthy brain. The number of NK cells accumulating in the tumors after intravenous injection was approximately 0.34% of the total number of NK cells administered. The ratio of HER2-specific NK cells to tumor cells was increased five-fold with the ultrasound BBBD. The authors hypothesized that the association of US and microbubbles had an effect on both the BBB and the NK cells circulating in the blood flow, thereby inducing diapedesis and migration into the tumor. Cytolytic activity of the NK cells was not altered by the sonication. They also observed a reduction in tumor volume and an increase in survival time after applying multiple sonications for three weeks [54].

BBB opening for delivery of therapeutics for glioma

Delivery of IL-12 to brain tumors (C6 glioma model) was significantly enhanced in rats when intraperitoneal (IP) injection of the drug was associated with US-BBBD (2.87-fold when compared to controls) [55]. Enhanced delivery of IL-12 secondary to BBBD was associated with both significant control of tumor progression and benefit in survival. Liu et al. assessed the delivery of bevacizumab, a humanized monoclonal antibody that binds to the VEGF-A isoform, in a U87 glioma model in mice [56]. Using an acoustic pressure of 0.4 MPa, a 5.73-fold increase in bevacizumab penetration of the brain parenchyma was measured. Both tumor growth control and an increase in median survival time were observed in the group of mice treated with US and bevacizumab (2.35-fold higher than the median survival in the control group and 1.58-fold higher than the median survival in the bevacizumab-alone group). They did not observe such "responders" and "nonresponders" as was observed in studies with trastuzumab and pertuzumab mentioned earlier [52–54]. One difference between this study and these prior studies (with heterogeneous results) was the cancer lineage, which may produce differences in the tumor vasculature as discussed by the other authors [52, 53]. Moreover, Liu et al. [56] treated their animals with bevacizumab, which is an anti-VEGF antibody that has direct action on the vasculature.

BBB disruption induces transient sterile inflammation at high microbubble dosages

Kovacs et al. reported the results of proteomic and transcriptomic changes after focused ultrasound (FUS)-BBB opening in rats [57]. Within 5 min of FUS, BBBD was associated with increased expression of damage-associated molecular patterns leading to a sterile inflammatory reaction through the NF-κB pathways that lasted 24 h. BBBD rapidly induced local production of chemotactic factors, heat-shock protein 70 (HSP70), and proinflammatory cytokines such as TNFα, IL1α, IL1ß, IL18, and IFNγ, lasting 12–24 h. An increase in CD68+ macrophages was also detected, but their immune function was not defined. The local production of cytokines, chemokines, and trophic factors, and the influx of serum products that represent damage signals could explain the microglial and astrocyte activation observed. However, these inflammatory reactions are correlated to ultrasound parameters and a decrease in microbubble dose and drastic calibration of peak negative pressure to avoid inertial cavitation significantly reduces the magnitude of this acute inflammatory response [58–60]. Thus, optimizing ultrasound parameters may allow for a safe opening of the BBB with no or mild inflammatory reaction.

In another study, the investigators performed adeno-associated virus (AAV)1/2 vector delivery with BBBD to the brain of rats after intravenous injection of the vector [61]. This resulted in long-term and efficient transduction of the gene marker, GFP, in neurons located in the targeted parenchyma. Local inflammation was observed during the first day after the sonication, but no astrocytosis or microgliosis were detected in either sonicated or nonsonicated brain at 2 weeks, 2 months, or 6 months after sonication plus AAV delivery. It is unclear how long the BBB-opening ultrasound triggers microglia activation. Specifically, Iba1-positive microglia were found to be activated in both TgCRND8 mice (model of Alzheimer's disease) and non Tg mice from 4 h to 4 days after FUS BBB opening. In the same study, GFAP-positive astrocytes were activated in both the TgCRND8 mice and non Tg mice at 4 days after sonication. By 15 days, activation of GFAP-positive astrocytes remained only in the TgCRND8 mice, and activation of microglia had subsided in both TgCRND8 mice and non Tg mice. In another study, a mild inflammation with macrophage infiltration and activation of microglia was initially observed but was reduced at 4 weeks [62], indicating that the kinetics of the ultrasound with specific types of immune modulation will need to be considered.

US-induced BBB disruption and innate immune response

Accumulation of macrophages has been observed and associated with scattered hemosiderin deposits after single or repeated BBBD sessions [25, 28, 62]. The macrophage infiltration of the brain after BBBD has been specifically studied using MRI and super-paramagnetic iron oxides (SPIO) [63]. No significant inflammatory responses were observed with optimal US parameters. However, with higher US parameters that can induce intracerebral hemorrhage, a significant cellular reaction occurred, corresponding to activity of SPIO-laden monocytes and monocyte-derived macrophages in the hemorrhagic brain from 4 to 24 h after sonication. In another study, migration of systemic fluorescently-labeled superparamagnetic iron oxide nanoparticles (FISPION)-labeled CD68+ macrophages into the sonicated parenchyma was observed 6 days after sonication, with optimal and safe ultrasound parameters being observed [57] (without parenchymal damage or microhemorrhages).These results suggest that (1) macrophage infiltration may be associated with distinct parameters that are used during the ultrasound and the extent of erythrocyte extravasation and (2) that recruitment of macrophages originating from the blood circulation can occur after US-induced BBBD.

US-induced BBB disruption and cellular immune response

Chen et al. did not observe significant changes in the T-cell population in normal rat brains after exposure to ultrasound, aside from a slight but nonsignificant increase in Th cells [55]. Specifically, there were no changes in the numbers of CTLs or Tregs, either with an intact BBB opening (0.36 MPa) or BBB opening with red blood cell extravasation (0.7 MPa) [55]. In contrast, in tumor-bearing rats, exposure to ultrasound significantly increased CD3+ CD8+ lymphocyte infiltration in the tumor (C6 glioma, about twofold relative to control). Tumor infiltration by CD3+ CD8+ , CD3+ CD4+ , and CD4+ CD25+ lymphocytes was significantly enhanced after IP injection of IL-12 in association with BBBD, compared with controls, IP injection of IL-12 monotherapy, or BBBD alone. In addition, both BBBD and IL-12 alone resulted in an increased CTL/Treg ratio relative to the control group in glioma tissue. This ratio was more significantly increased when BBBD and IL-12 injection were used in combination. The immunological response was targeted to the brain, as no changes in the lymphocyte population percentages were observed systemically, either in the spleen or in mesenteric lymph nodes. However, these results should be considered with some caution because the C6 model is known to be able to induce vigorous immune reactions that are not reflective of human gliomas [64]. Nonetheless, sonication may allow for infiltration of immune cells through the open BBB along with local exposure of the tumor to the systemic immune system. BBB opening ultrasound may also allow the release of antigenic tumor particles into the peripheral blood circulation as previously described for U87 mice [65].

Conclusions and perspectives

US-induced opening of the BBB is a promising technique that could facilitate the entry of a wide range of substances into the brain and increase their concentration within it, from drugs or antibodies, to cells. In the context of immunotherapy, this strategy could be used to increase the concentrations of antibodies whose target resides in the CNS. Such targets could either be tumor antigens or immune modulatory antibodies in which the target cell population, such as microglia, resides in the CNS. Notably, there may not be additional therapeutic value of using US-induced opening of the BBB if the immune modulatory effect is primarily mediated at the periphery and if the immune effector cell gains adequate access to the tumor. A second potential area for future investigation is to ascertain whether this strategy, by increasing the number of effector cells in the tumor microenvironment, can enhance other immune therapeutic strategies. US-induced BBBD may allow for delivery of immune cells into the tumor microenvironment, thereby converting an immunologically "cold" tumor into a "hot" one, which may enable responses to immune checkpoint blockade strategies. A hindrance to several types of adoptive immunotherapies, such as NK cells, adoptive T cells, and chimeric antigen receptor T cells, for brain tumors has been the lack of adequate CNS delivery, and as such, US-induced BBBD may allow delivery of multiple dosing to focal areas of the brain. Another strategy to consider is the use of US-induced BBBD to specifically deposit either benign cells or nanoparticles elaborating immune chemokines or immune modulatory cytokines to modulate the local tumor immune responses. Modulation of antigen-presenting cells could also be considered, either by depositing these cells (dendritic cells) directly into the tumor, which would allow for T-cell stimulation in the local microenvironment, or by liberating tumor antigens into the blood circulation through the disrupted BBB to activate circulating antigen-presenting cells. Clearly, the emerging technology of BBB-opening ultrasound offers multiple therapeutic opportunities to enhance immunotherapy for glioma patients.

Acknowledgements The authors acknowledge graphic designer Quentin Beccaria for his help in creating Fig. 1. Special thanks go to David M. Wildrick, Ph.D. and Audria Patrick for editorial and administrative support.

Author contributions Manuscript writing: KB, ABH. Manuscript editing: KB, MC, AC, JG, ABH, AS.

Compliance with ethical standards

Conflict of interest M. Canney is an employee of CarThera. A. Carpentier is a paid consultant to CarThera. K. Beccaria was previously an employee of CarThera. A. Carpentier, K. Beccaria, and M. Canney are inventors on intellectual property related to the SonoCloud device that has been licensed to CarThera. A. Carpentier and M. Canney have ownership interested in CarThera.

References

1. Medawar PB (1948) Immunity to homologous grafted skin. III. The fate of skin homografts transplanted to the brain, to subcutaneous tissue, and to the anterior chamber of the eye. Br J Exp Pathol 29:58–69
2. Aloisi F, Ria F, Adorini L (2000) Regulation of T-cell responses by CNS antigen-presenting cells: different roles for microglia and astrocytes. Immunol Today 21:141–147
3. Hickey WF, Hsu BL, Kimura H (1991) T-lymphocyte entry into the central nervous system. J Neurosci Res 28:254–260. https://doi.org/10.1002/jnr.490280213
4. Ransohoff RM, Kivisakk P, Kidd G (2003) Three or more routes for leukocyte migration into the central nervous system. Nat Rev Immunol 3:569–581. https://doi.org/10.1038/nri1130
5. Cserr HF, Harling-Berg CJ, Knopf PM (1992) Drainage of brain extracellular fluid into blood and deep cervical lymph and its immunological significance. Brain Pathol 2:269–276. https://doi.org/10.1111/j.1750-3639.1992.tb00703.x
6. Cserr HF, Knopf PM (1992) Cervical lymphatics, the blood-brain barrier and the immunoreactivity of the brain: a new view. Immunol Today 13:507–512. https://doi.org/10.1016/0167-5699(92)90027-5
7. Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, Derecki NC, Castle D, Mandell JW, Lee KS, Harris TH, Kipnis J (2015) Structural and functional features of central nervous system lymphatic vessels. Nature 523:337–341. https://doi.org/10.1038/nature14432
8. Ledford H (2011) Engineered antibodies cross blood-brain barrier. Nat News. https://doi.org/10.1038/news.2011.319
9. Wang Q, Hu B, Hu X, Kim H, Squatrito M, Scarpace L, deCarvalho AC, Lyu S, Li P, Li Y, Barthel F, Cho HJ, Lin YH, Satani N, Martinez-Ledesma E, Zheng S, Chang E, Gabriel Sauve CE, Olar A, Lan ZD, Finocchiaro G, Phillips JJ, Berger MS, Gabrusiewicz KR, Wang G, Eskilsson E, Hu J, Mikkelsen T, DePinho RA, Muller F, Heimberger AB, Sulman EP, Nam DH, Verhaak RGW (2018) Tumor evolution of glioma-intrinsic gene expression subtypes associates with immunological changes in the microenvironment. Cancer Cell 33:152. https://doi.org/10.1016/j.ccell.2017.12.012
10. Wei J, Marisetty A, Schrand B, Gabrusiewicz K, Hashimoto Y, Ott M, Grami Z, Kong L-Y, Ling X, Caruso H, Zhou S, Wang YA, Fuller GN, Huse J, Gilboa E, Kang N, Huang X, Verhaak R, Li S, Heimberger AB (2019) Osteopontin mediates glioblastoma-associated macrophage infiltration and is a potential therapeutic target. J Clin Invest 129:137–149
11. Hussain SF, Yang D, Suki D, Aldape K, Grimm E, Heimberger AB (2006) The role of human glioma-infiltrating microglia/macrophages in mediating antitumor immune responses. Neuro-oncology 8:261–279
12. Chongsathidkiet P, Jackson C, Koyama S, Loebel F, Cui X, Farber SH, Woroniecka K, Elsamadicy AA, Dechant CA, Kemeny HR, Sanchez-Perez L, Cheema TA, Souders NC, Herndon JE, Coumans JV, Everitt JI, Nahed BV, Sampson JH, Gunn MD, Martuza RL, Dranoff G, Curry WT, Fecci PE (2018) Sequestration of T cells in bone marrow in the setting of glioblastoma and other intracranial tumors. Nat Med 24:1459–1468. https://doi.org/10.1038/s41591-018-0135-2
13. Lidar Z, Mardor Y, Jonas T, Pfeffer R, Faibel M, Nass D, Hadani M, Ram Z (2004) Convection-enhanced delivery of paclitaxel for the treatment of recurrent malignant glioma: a phase I/II clinical study. J Neurosurg 100:472–479. https://doi.org/10.3171/jns.2004.100.3.0472
14. Chen W, Wu Q, Mo L, Nassi M (2013) Intra-arterial chemotherapy is not superior to intravenous chemotherapy for malignant gliomas: a systematic review and meta-analysis. Eur Neurol 70:124–132. https://doi.org/10.1159/000346580
15. Ashby LS, Smith KA, Stea B (2016) Gliadel wafer implantation combined with standard radiotherapy and concurrent followed by adjuvant temozolomide for treatment of newly diagnosed high-grade glioma: a systematic literature review. World J Surg Oncol 14:225. https://doi.org/10.1186/s12957-016-0975-5
16. Farra R, Sheppard NF Jr, McCabe L, Neer RM, Anderson JM, Santini JT Jr, Cima MJ, Langer R (2012) First-in-human testing of a wirelessly controlled drug delivery microchip. Sci Transl Med 4:121–122. https://doi.org/10.1126/scitranslmed.3003276
17. Rapoport SI, Hori M, Klatzo I (1972) Testing of a hypothesis for osmotic opening of the blood-brain barrier. Am J Physiol 223:323–331. https://doi.org/10.1152/ajplegacy.1972.223.2.323
18. Black KL, Cloughesy T, Huang SC, Gobin YP, Zhou Y, Grous J, Nelson G, Farahani K, Hoh CK, Phelps M (1997) Intracarotid infusion of RMP-7, a bradykinin analog, and transport of gallium-68 ethylenediamine tetraacetic acid into human gliomas. J Neurosurg 86:603–609. https://doi.org/10.3171/jns.1997.86.4.0603
19. Pardridge WM (1995) Transport of small molecules through the blood-brain barrier: biology and methodology. Adv Drug Deliv Rev 15:5–36
20. Ozduman K, Wollmann G, Piepmeier JM, van den Pol AN (2008) Systemic vesicular stomatitis virus selectively destroys multifocal glioma and metastatic carcinoma in brain. J Neurosci 28:1882–1893. https://doi.org/10.1523/JNEUROSCI.4905-07.2008
21. Hynynen K, McDannold N, Vykhodtseva N, Jolesz FA (2001) Noninvasive MR imaging-guided focal opening of the blood-brain barrier in rabbits. Radiology 220:640–646. https://doi.org/10.1148/radiol.2202001804
22. Idbaih A, Canney M, Belin L, Desseaux C, Vignot A, Bouchoux G, Asquier N, Law-Ye B, Leclercq D, Bissery A, De Rycke Y, Trosch C, Capelle L, Sanson M, Hoang-Xuan K, Dehais C, Houillier C, Laigle-Donadey F, Mathon B, Andre A, Lafon C, Chapelon JY, Delattre JY, Carpentier A (2019) Safety and feasibility of repeated and transient blood-brain barrier disruption by pulsed ultrasound in patients with recurrent glioblastoma. Clin Cancer Res 25:3793–3801. https://doi.org/10.1158/1078-0432.CCR-18-3643
23. Sheikov N, McDannold N, Vykhodtseva N, Jolesz F, Hynynen K (2004) Cellular mechanisms of the blood-brain barrier opening induced by ultrasound in presence of microbubbles. Ultrasound Med Biol 30:979–989. https://doi.org/10.1016/j.ultrasmedbio.2004.04.010
24. Hynynen K, McDannold N, Sheikov NA, Jolesz FA, Vykhodtseva N (2005) Local and reversible blood-brain barrier disruption by

noninvasive focused ultrasound at frequencies suitable for transskull sonications. Neuroimage 24:12–20. https://doi.org/10.1016/j.neuroimage.2004.06.046
25. Kobus T, Vykhodtseva N, Pilatou M, Zhang Y, McDannold N (2016) Safety validation of repeated blood-brain barrier disruption using focused ultrasound. Ultrasound Med Biol 42:481–492. https://doi.org/10.1016/j.ultrasmedbio.2015.10.009
26. Horodyckid C, Canney M, Vignot A, Boisgard R, Drier A, Huberfeld G, Francois C, Prigent A, Santin MD, Adam C, Willer JC, Lafon C, Chapelon JY, Carpentier A (2017) Safe long-term repeated disruption of the blood-brain barrier using an implantable ultrasound device: a multiparametric study in a primate model. J Neurosurg 126:1351–1361. https://doi.org/10.3171/2016.3.JNS151635
27. Downs ME, Buch A, Sierra C, Karakatsani ME, Teichert T, Chen S, Konofagou EE, Ferrera VP (2015) Long-term safety of repeated blood-brain barrier opening via focused ultrasound with microbubbles in non-human primates performing a cognitive task. PLoS ONE 10:e0125911. https://doi.org/10.1371/journal.pone.0125911
28. McDannold N, Arvanitis CD, Vykhodtseva N, Livingstone MS (2012) Temporary disruption of the blood-brain barrier by use of ultrasound and microbubbles: safety and efficacy evaluation in rhesus macaques. Cancer Res 72:3652–3663. https://doi.org/10.1158/0008-5472.CAN-12-0128
29. Beccaria K, Canney M, Goldwirt L, Fernandez C, Piquet J, Perier MC, Lafon C, Chapelon JY, Carpentier A (2016) Ultrasound-induced opening of the blood-brain barrier to enhance temozolomide and irinotecan delivery: an experimental study in rabbits. J Neurosurg 124:1602–1610. https://doi.org/10.3171/2015.4.JNS142893
30. Kinoshita M, McDannold N, Jolesz FA, Hynynen K (2006) Targeted delivery of antibodies through the blood-brain barrier by MRI-guided focused ultrasound. Biochem Biophys Res Commun 340:1085–1090. https://doi.org/10.1016/j.bbrc.2005.12.112
31. Hsu YH, Liu RS, Lin WL, Yuh YS, Lin SP, Wong TT (2017) Transcranial pulsed ultrasound facilitates brain uptake of laronidase in enzyme replacement therapy for Mucopolysaccharidosis type I disease. Orphanet J Rare Dis 12:109. https://doi.org/10.1186/s13023-017-0649-6
32. Wang F, Shi Y, Lu L, Liu L, Cai Y, Zheng H, Liu X, Yan F, Zou C, Sun C, Shi J, Lu S, Chen Y (2012) Targeted delivery of GDNF through the blood-brain barrier by MRI-guided focused ultrasound. PLoS ONE 7:e52925. https://doi.org/10.1371/journal.pone.0052925
33. Huang Q, Deng J, Wang F, Chen S, Liu Y, Wang Z, Wang Z, Cheng Y (2012) Targeted gene delivery to the mouse brain by MRI-guided focused ultrasound-induced blood-brain barrier disruption. Exp Neurol 233:350–356. https://doi.org/10.1016/j.expneurol.2011.10.027
34. Alkins R, Burgess A, Ganguly M, Francia G, Kerbel R, Wels WS, Hynynen K (2013) Focused ultrasound delivers targeted immune cells to metastatic brain tumors. Cancer Res 73:1892–1899. https://doi.org/10.1158/0008-5472.CAN-12-2609
35. Ting CY, Fan CH, Liu HL, Huang CY, Hsieh HY, Yen TC, Wei KC, Yeh CK (2012) Concurrent blood-brain barrier opening and local drug delivery using drug-carrying microbubbles and focused ultrasound for brain glioma treatment. Biomaterials 33:704–712. https://doi.org/10.1016/j.biomaterials.2011.09.096
36. Treat LH, McDannold N, Zhang Y, Vykhodtseva N, Hynynen K (2012) Improved anti-tumor effect of liposomal doxorubicin after targeted blood-brain barrier disruption by MRI-guided focused ultrasound in rat glioma. Ultrasound Med Biol 38:1716–1725. https://doi.org/10.1016/j.ultrasmedbio.2012.04.015
37. Drean A, Lemaire N, Bouchoux G, Goldwirt L, Canney M, Goli L, Bouzidi A, Schmitt C, Guehennec J, Verreault M, Sanson M, Delattre JY, Mokhtari K, Sottilini F, Carpentier A, Idbaih A (2019) Temporary blood-brain barrier disruption by low intensity pulsed ultrasound increases carboplatin delivery and efficacy in preclinical models of glioblastoma. J Neurooncol 144:33–41. https://doi.org/10.1007/s11060-019-03204-0
38. Leinenga G, Gotz J (2015) Scanning ultrasound removes amyloid-beta and restores memory in an Alzheimer's disease mouse model. Sci Transl Med 7:233–278. https://doi.org/10.1126/scitranslmed.aaa2512
39. Clement GT, White J, Hynynen K (2000) Investigation of a large-area phased array for focused ultrasound surgery through the skull. Phys Med Biol 45:1071–1083. https://doi.org/10.1088/0031-9155/45/4/319
40. Hynynen K, Jolesz FA (1998) Demonstration of potential noninvasive ultrasound brain therapy through an intact skull. Ultrasound Med Biol 24:275–283
41. McDannold N, Clement GT, Black P, Jolesz F, Hynynen K (2010) Transcranial magnetic resonance imaging- guided focused ultrasound surgery of brain tumors: initial findings in 3 patients. Neurosurgery 66:323–332. https://doi.org/10.1227/01.NEU.0000360379.95800.2F (**discussion 332**)
42. Crake C, Brinker ST, Coviello CM, Livingstone MS, McDannold NJ (2018) A dual-mode hemispherical sparse array for 3D passive acoustic mapping and skull localization within a clinical MRI guided focused ultrasound device. Phys Med Biol 63:065008. https://doi.org/10.1088/1361-6560/aab0aa
43. Mainprize T, Lipsman N, Huang Y, Meng Y, Bethune A, Ironside S, Heyn C, Alkins R, Trudeau M, Sahgal A, Perry J, Hynynen K (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. https://doi.org/10.1038/s41598-018-36340-0
44. Beccaria K, Canney M, Goldwirt L, Fernandez C, Adam C, Piquet J, Autret G, Clement O, Lafon C, Chapelon JY, Carpentier A (2013) Opening of the blood-brain barrier with an unfocused ultrasound device in rabbits. J Neurosurg 119:887–898. https://doi.org/10.3171/2013.5.JNS122374
45. Goldwirt L, Canney M, Horodyckid C, Poupon J, Mourah S, Vignot A, Chapelon JY, Carpentier A (2016) Enhanced brain distribution of carboplatin in a primate model after blood-brain barrier disruption using an implantable ultrasound device. Cancer Chemother Pharmacol 77:211–216. https://doi.org/10.1007/s00280-015-2930-5
46. Raymond SB, Treat LH, Dewey JD, McDannold NJ, Hynynen K, Bacskai BJ (2008) Ultrasound enhanced delivery of molecular imaging and therapeutic agents in Alzheimer's disease mouse models. PLoS ONE 3:e2175. https://doi.org/10.1371/journal.pone.0002175
47. Jordao JF, Ayala-Grosso CA, Markham K, Huang Y, Chopra R, McLaurin J, Hynynen K, Aubert I (2010) Antibodies targeted to the brain with image-guided focused ultrasound reduces amyloid-beta plaque load in the TgCRND8 mouse model of Alzheimer's disease. PLoS ONE 5:e10549. https://doi.org/10.1371/journal.pone.0010549
48. Jordao JF, Thevenot E, Markham-Coultes K, Scarcelli T, Weng YQ, Xhima K, O'Reilly M, Huang Y, McLaurin J, Hynynen K, Aubert I (2013) Amyloid-beta plaque reduction, endogenous antibody delivery and glial activation by brain-targeted, transcranial focused ultrasound. Exp Neurol 248:16–29. https://doi.org/10.1016/j.expneurol.2013.05.008
49. Kinoshita M, McDannold N, Jolesz FA, Hynynen K (2006) Noninvasive localized delivery of Herceptin to the mouse brain by MRI-guided focused ultrasound-induced blood-brain barrier disruption. Proc Natl Acad Sci USA 103:11719–11723. https://doi.org/10.1073/pnas.0604318103
50. Nisbet RM, Van der Jeugd A, Leinenga G, Evans HT, Janowicz PW, Gotz J (2017) Combined effects of scanning ultrasound and

a tau-specific single chain antibody in a tau transgenic mouse model. Brain 140:1220–1230. https://doi.org/10.1093/brain/awx052
51. Janowicz PW, Leinenga G, Gotz J, Nisbet RM (2019) Ultrasound-mediated blood-brain barrier opening enhances delivery of therapeutically relevant formats of a tau-specific antibody. Sci Rep 9:9255. https://doi.org/10.1038/s41598-019-45577-2
52. Park EJ, Zhang YZ, Vykhodtseva N, McDannold N (2012) Ultrasound-mediated blood-brain/blood-tumor barrier disruption improves outcomes with trastuzumab in a breast cancer brain metastasis model. J Control Release 163:277–284. https://doi.org/10.1016/j.jconrel.2012.09.007
53. Kobus T, Zervantonakis IK, Zhang Y, McDannold NJ (2016) Growth inhibition in a brain metastasis model by antibody delivery using focused ultrasound-mediated blood-brain barrier disruption. J Control Release 238:281–288. https://doi.org/10.1016/j.jconrel.2016.08.001
54. Alkins R, Burgess A, Kerbel R, Wels WS, Hynynen K (2016) Early treatment of HER2-amplified brain tumors with targeted NK-92 cells and focused ultrasound improves survival. Neuro-oncology 18:974–981. https://doi.org/10.1093/neuonc/nov318
55. Chen PY, Hsieh HY, Huang CY, Lin CY, Wei KC, Liu HL (2015) Focused ultrasound-induced blood-brain barrier opening to enhance interleukin-12 delivery for brain tumor immunotherapy: a preclinical feasibility study. J Transl Med 13:93. https://doi.org/10.1186/s12967-015-0451-y
56. Liu HL, Hsu PH, Lin CY, Huang CW, Chai WY, Chu PC, Huang CY, Chen PY, Yang LY, Kuo JS, Wei KC (2016) Focused ultrasound enhances central nervous system delivery of bevacizumab for malignant glioma treatment. Radiology 281:99–108. https://doi.org/10.1148/radiol.2016152444
57. Kovacs ZI, Kim S, Jikaria N, Qureshi F, Milo B, Lewis BK, Bresler M, Burks SR, Frank JA (2017) Disrupting the blood-brain barrier by focused ultrasound induces sterile inflammation. Proc Natl Acad Sci USA 114:E75–E84. https://doi.org/10.1073/pnas.1614777114
58. Kovacs ZI, Burks SR, Frank JA (2018) Focused ultrasound with microbubbles induces sterile inflammatory response proportional to the blood brain barrier opening: attention to experimental conditions. Theranostics 8:2245–2248. https://doi.org/10.7150/thno.24181
59. McMahon D, 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. https://doi.org/10.7150/thno.21630
60. McMahon D, Hynynen K (2018) Reply to Kovacs et al.: concerning acute inflammatory response following focused ultrasound and microbubbles in the brain. Theranostics 8:2249–2250. https://doi.org/10.7150/thno.25468
61. Stavarache MA, Petersen N, Jurgens EM, Milstein ER, Rosenfeld ZB, Ballon DJ, Kaplitt MG (2018) Safe and stable noninvasive focal gene delivery to the mammalian brain following focused ultrasound. J Neurosurg 130:989–998. https://doi.org/10.3171/2017.8.JNS17790
62. McDannold N, Vykhodtseva N, Raymond S, Jolesz FA, Hynynen K (2005) MRI-guided targeted blood-brain barrier disruption with focused ultrasound: histological findings in rabbits. Ultrasound Med Biol 31:1527–1537. https://doi.org/10.1016/j.ultrasmedbio.2005.07.010
63. Liu HL, Wai YY, Hsu PH, Lyu LA, Wu JS, Shen CR, Chen JC, Yen TC, Wang JJ (2010) In vivo assessment of macrophage CNS infiltration during disruption of the blood-brain barrier with focused ultrasound: a magnetic resonance imaging study. J Cereb Blood Flow Metab 30:177–186. https://doi.org/10.1038/jcbfm.2009.179
64. Parsa AT, Chakrabarti I, Hurley PT, Chi JH, Hall JS, Kaiser MG, Bruce JN (2000) Limitations of the C6/Wistar rat intracerebral glioma model: implications for evaluating immunotherapy. Neurosurgery 47:993–999 (**discussion 999–1000**)
65. Zhu L, Cheng G, Ye D, Nazeri A, Yue Y, Liu W, Wang X, Dunn GP, Petti AA, Leuthardt EC, Chen H (2018) Focused ultrasound-enabled brain tumor liquid biopsy. Sci Rep 8:6553. https://doi.org/10.1038/s41598-018-24516-7