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Kévin Beccaria, Michael Canney, Guillaume Bouchoux, Carole Desseaux, Jacques Grill, Amy B. Heimberger, Alexandre Carpentier

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Abstract

The treatment of primary brain tumors, especially malignant gliomas, remains challenging. The failure of most treatments for this disease is partially explained by the blood-brain barrier (BBB), which prevents circulating molecules from entering the brain parenchyma. Ultrasound-induced BBB disruption (US-BBBD) has recently emerged as a promising strategy to improve the delivery of therapeutic agents to brain tumors. A large body of preclinical studies has demonstrated that the association of low-intensity pulsed ultrasound with intravenous microbubbles can transiently open the BBB in a localized manner. The safety of this technique has been assessed in numerous preclinical studies in both small and large animal models. A large panel of therapeutic agents have been delivered to the brain in preclinical models, demonstrating both tumor control and increased survival. This technique has recently entered clinical trials with encouraging preliminary data. In this review, we describe the mechanisms and histological effects of US-BBBD and summarize the preclinical studies published to date. We furthermore provide an overview of the current clinical development and future potential of this promising technology.

Ultrasound-induced blood-brain barrier disruption for the treatment of gliomas and other primary CNS tumors

Kévin Beccaria, M.D.[1], Michael Canney, Ph.D.[2], Guillaume Bouchoux, Ph.D.[2], Carole Desseaux, Ph.D.[2], Jacques Grill, M.D., Ph.D.[3,4], Amy B. Heimberger, M.D.[5], Alexandre Carpentier, M.D., Ph.D.[6]

1. Department of Pediatric Neurosurgery, Necker Enfants Malades Hospital, APHP, Paris 5 University, Paris, France

2. CarThera, Institut du Cerveau et de la Moelle épinière (ICM), Paris F-75013, France

3. Department of Pediatric Oncology, Gustave-Roussy, Université Paris-Sud, Université Paris-Saclay, Villejuif, France

4. UMR8203 "Vectorologie et Thérapeutiques Anticancéreuses," CNRS, Gustave Roussy, Université Paris-Sud, Université Paris-Saclay, Villejuif, France

5. Department of Neurosurgery, The University of Texas MD Anderson Cancer Center, Houston, USA

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

Corresponding Author:

Dr Kévin Beccaria

Hôpital Necker – Enfants Malades

149 Rue de Sèvres

75015 Paris, France

kevin.beccaria@aphp.fr

Abstract

The treatment of primary brain tumors, especially malignant gliomas, remains challenging. The failure of most treatments for this disease is partially explained by the blood-brain barrier (BBB), which prevents circulating molecules from entering the brain parenchyma. Ultrasound-induced BBB disruption (US-BBBD) has recently emerged as a promising strategy to improve the delivery of therapeutic agents to brain tumors. A large body of preclinical studies has demonstrated that the association of low-intensity pulsed ultrasound with intravenous microbubbles can transiently open the BBB in a localized manner. The safety of this technique has been assessed in numerous preclinical studies in both small and large animal models. A large panel of therapeutic agents have been delivered to the brain in preclinical models, demonstrating both tumor control and increased survival. This technique has recently entered clinical trials with encouraging preliminary data. In this review, we describe the mechanisms and histological effects of US-BBBD and summarize the preclinical studies published to date. We furthermore provide an overview of the current clinical development and future potential of this promising technology.

Keywords:

brain tumors; blood-brain barrier; ultrasound; glioblastoma; low-intensity pulsed ultrasound

Abbreviations:

BBB: blood-brain barrier, BBBD: blood-brain barrier disruption, CNS: central nervous system, CTL: cytotoxic T lymphocyte, GBM: glioblastoma, IV: intravenous, LIPU: low-intensity pulsed ultrasound, MRI: magnetic resonance imaging, NHP: non-human primate, NK: natural killer ,

OS: overall survival, PFS: progression-free survival, P-gp: p-glycoprotein, Treg: regulatory T cell, US: ultrasound, US-BBBD: ultrasound-induced blood-brain barrier disruption

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Conflict of Interest

M. Canney and Guillaume Bouchoux are employees of CarThera. A. Carpentier is a paid consultant to CarThera. K. Beccaria has previously been employed by CarThera. A. Carpentier, K. Beccaria, and M. Canney, and G. Bouchoux are inventors on intellectual property related to the SonoCloud® device that has been licensed to CarThera. A. Carpentier and M. Canney have ownership interest in CarThera.

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1. Introduction

Primary brain tumors are the primary cause of solid cancer in the pediatric population, the third cause in young adults and the eighth one in adults older than 40 years old [1]. Mortality rates were estimated at 0.72 per 100,000, 0.96 per 100,000 and 9.01 per 100,000 people in these age groups, respectively, between 2011 and 2015 in the United States [1]. The most common of all malignant brain and other central nervous system (CNS) tumors are high-grade gliomas. These tumors have a particularly dismal prognosis in both children and adults, with 5-year survival rates of less than 20% in children [2] and around 5% in adults [1]. Although important advances have been made in our understanding of these tumors over the last decade, current treatment options remain limited and largely ineffective. Standard treatment of high-grade gliomas is based on maximal surgical resection, followed by adjuvant radiotherapy and chemotherapy. However, the diffuse and infiltrative nature of these tumors limits the efficacy of these treatments. Furthermore, total resection of these tumors is nearly impossible due to the infiltration of cancerous cells into the surrounding healthy tissues and the existence of the blood-brain barrier (BBB), which prevents the majority of systemic drug therapies from reaching the brain.

The BBB is a physiological barrier that protects the brain from potential toxins circulating in the systemic circulation. It is comprised of a system of tight junctions and transport proteins that prevent approximately 98% of small-molecule drugs (< 0.5 kD) and 100% of large-molecule drugs from crossing the intact BBB [3]. Thus, most existing and novel therapeutics for brain diseases cannot cross the intact BBB and be delivered to the brain [4]. Although hyperintense on T1w contrast-enhanced magnetic resonance imaging (MRI), showing a partially disrupted BBB, the primary tumor mass (which is typically resected) is surrounded by infiltrative tumor cells, surrounded by an intact BBB [5,6]. These surrounding regions of infiltrative cells lead to

recurrence of the tumor in nearly all GBM patients. In other brain tumors, such as medulloblastomas, it has been shown that the permeability of the BBB varied by patient and may have an impact on the response to therapy [7].

Several different methods to disrupt or bypass the BBB have been attempted to improve the delivery of drugs to patients with primary brain tumors. Methods previously tested clinically to increase the permeability of the BBB include mannitol administration to osmotically disrupt the BBB [8] and the use of the bradykinin agonist RMP-7 [9]. Direct injection of drugs into the brain has also been attempted using Rickham/Ommaya reservoirs placed in the ventricle [10] and convection-enhanced delivery devices [11,12]. Despite encouraging results in clinical trials, these methods have not gained widespread clinical use due to the difficulties associated with their routine implementation in the clinic.

An alternative method to enhance the concentrations of drugs in the brain for primary CNS tumors is to use low-intensity pulsed ultrasound (LIPU) in combination with intravenous (IV) injection of microbubbles. This technique, named ultrasound-induced blood-brain barrier disruption (US-BBBD), has been in pre-clinical development for over 20 years [13,14]. This technique has been demonstrated to be safe in a number of small and large animal model systems and preclinical glioma models. Furthermore, US-BBBD has shown efficacy in pre-clinical studies with a range of drug therapies that normally do not cross the BBB and numerous clinical trials have now been initiated within the past five years.

This review focuses on the significant progress made over the past 20 years as the technique of US-BBBD has advanced from a pre-clinical phase to initiation of multiple clinical trials that are now in progress using several different approaches.

2. Overview of BBB disruption using low intensity pulsed ultrasound

2.1. Mechanisms

When ultrasound stimulates systemically-administered microbubbles (1-10 microns in diameter), the bubbles expand and contract (a phenomenon called cavitation), inducing mechanical stresses on the capillary walls [15], stimulation of endothelial cells, and temporary BBB disruption (BBBD). Although the exact mechanisms are not fully understood, both mechanical and functional modifications of the BBB may be involved (Figure 1). Sonication allows for passive diffusion through extracellular pathways by inducing opening of the tight junctions [16], and transcellular transport by increasing transcytosis and creating transendothelial fenestrations [17]. Ultrasound and microbubbles may also modify the functional aspects of the BBB as P-glycoprotein (P-gp) expression is suppressed for up to 48 hours after sonication, reducing drug efflux mechanisms [18]. These different mechanisms appear progressively with time, and US-BBBD occurs in two different phases, with early/fast leakage, and late/slow leakage [19,20], which also affects the size of molecules that can pass at different time points [20]. After disruption, the integrity of the BBB is rapidly restored; it begins to close immediately after disruption and is fully closed in 6 to 24 h [20,21].

US-BBBD is not a binary effect, and the magnitude of BBBD depends on a range of parameters including acoustic pressure [22], ultrasound frequency [23], pulse duration [24] and burst pulse repetition frequency [25]. The opening of the BBB increases with the acoustic pressure and the threshold necessary to open the BBB has been shown to be linked to the mechanical index (peak negative pressure in vivo divided by the square root of the frequency) [26]. A wide range of

ultrasound frequencies can be used for US-BBBD, ranging from 200 kHz to 10 MHz [26]. Injection of systemic microbubbles is essential and the effect is correlated with both size [27] and concentration of microbubbles [28]. At a fixed acoustic pressure, the BBB opening intensity increases with total microbubbles gas volume administrated [29]. The microbubble contrast agents that have been tested in preclinical work have typically already been approved for diagnostic ultrasound imaging applications and include Definity® (Lantheus) [30], SonoVue®/Luminity® (Bracco) [31] and Optison® (GE Healthcare) [32], though specially formulated bubbles have also been used. **Table 1** presents the principle characteristics of these contrast agents [33].

2.2. Safety in small animal studies

BBB disruption using LIPU has been shown to be transient and safe in a wide range of animal models in recent clinical trials. US-BBBD is primarily due to mechanical effects, since thermal effects at the low ultrasound output intensities used are negligible [34]. These effects have largely been assessed in preclinical studies in small and large animals. The main side effects observed after US-BBBD are extravasation of red blood cells. The amount of extravasation is correlated with the acoustic pressure and may vary from none to large areas of hemorrhage depending on the acoustic parameters and microbubble concentrations used [35]. By choosing optimal acoustic pulsing parameters and microbubble dosage, it is possible to induce BBBD with no safety concerns with clinical significance [35,36]. Microvacuolation and damaged neurons (dark neurons) have been observed in sonicated brains of mice, and were correlated with the intensity of BBBD and the number of extravasated red blood cells in the same regions. At low acoustic pressures, their presence was limited to only a few areas [37]. US-BBBD can also induce vasospasm in microvessels [19]; however, very few cells showing evidence of apoptosis

or ischemia have been observed in sonicated areas up to four weeks after sonication in rabbits [38]. It was subsequently shown that no additional lesions were observed after repeated sonications compared to a single ultrasound session [19]. Finally, it has been observed that opening of the BBB was more intense in grey matter compared to white matter; this may be explained by the fact that grey matter is more densely vascularized than white matter [36,39].

2.3.Immunomodulatory aspects

Sonications may allow immune cells to transit through the transiently opened BBB, as shown with macrophages and T cells. Accumulation of macrophages originating from the blood circulation has been observed and associated with scattered hemosiderin deposits after single or repeated BBBD sessions [38–40]. This infiltration was not significant with ultrasound pressures optimal for BBBD, but was observed 4 to 24 hours after sonications at higher pressures, at which hemorrhages occurred [41]. On the contrary, Kovacs et al. described such an infiltration with ultrasound parameters compatible with US-BBBD without parenchymal damage or microhemorrhages [42]. Migration of systemic macrophages into the sonicated parenchyma was observed six days after sonication. In this study, the authors pointed out a sterile inflammatory reaction induced by US-BBBD from 5 minutes to 24 hours after sonication. BBBD was associated with increased expression of damage-associated molecular patterns and an NF-κB pathways-mediated sterile inflammatory reaction. This reaction included local production of chemotactic factors, heat-shock protein 70, and proinflammatory cytokines, and was characterized by microglial and astrocyte activation, and the macrophage migration described above. It was subsequently demonstrated that a lower microbubble concentration and lower acoustic pressures could reduce the magnitude of this acute inflammatory response [43]. Local inflammation has also been observed within days of sonication in combination with adeno-

associated virus 1/2 vector delivery [44]; although the sonication resulted in a long-term and efficient transduction of the gene marker in sonicated neurons, no astrocytosis or microgliosis was detected in either sonicated or nonsonicated brain for up to six months after sonication. It is still unclear as to how long the US-BBBD triggers microglia activation; this may vary from a few days to several weeks [38,45]. Finally, one study focused on T-cell population modulation by US-BBBD. Chen et al. did not observe significant changes in the T-cell population in normal rat brains after sonication, aside from a slight but nonsignificant increase in T helper cells [46]. In contrast, sonication of C6 glioma-bearing rats significantly increased CD3+CD8+ lymphocyte infiltration in the tumor, and tumor infiltration by CD3+ CD8+, CD3+ CD4+, and CD4+ CD25+ lymphocytes was significantly enhanced after intraperitoneal injection of IL-12 in association with BBBD. The CTL/Treg ratio was also significantly increased when BBBD and IL-12 injection were used in combination. The immunological response was limited to the brain, as no changes in the lymphocyte population percentages were observed systemically. However, the effects observed in the C6 model, which is not truly syngenic, may not be truly representative of human gliomas, which are known to be more immunosuppressive.

3. Safety of US-BBBD in non-human primates

The long-term safety of US-BBBD has been demonstrated in healthy non-human primates (NHP) by several independent teams, with multiple ultrasound approaches and devices.

Marquet et al. and Tung et al. [47,48] disrupted the BBB in male macaque monkeys with a single-element focused ultrasound transducer operating at 500 kHz. By using two types of microbubble agents (Definity® and in-house made microbubbles), they found that MRI contrast

enhancement and cavitation response were dependent on the targeted region and/or microbubble size. These preliminary results were confirmed with multiple sonications in the thalamus and basal ganglia of alert macaque monkeys while performing a behavioral task [49,50]. The procedures were well-tolerated, with no physiological effects. The application of focused ultrasound did not interrupt the behavioral tasks, but in fact, slightly improved performance. Mild transient edema without microhemorrhages was visible on T2-weighted MRI sequences, but was smaller than in procedures performed under general anesthesia. Finally, the maximum volume of US-BBBD observed was 534.2 ± 261.2 mm^3, 567.8 ± 251.6 mm^3, and 697.7 ± 181.8 mm^3 in the caudate, putamen and thalamus regions, respectively. This was larger than the volumes obtained in sleeping monkeys, likely due to the effect of oxygen used for anesthesia on cavitation activity.

McDannold et al. 2012 performed multiple transcranial BBBD in deep and superficial targets over several months in NHPs using an external hemispherical 1024-element phased array transducer operating at 220 kHz [39]. Animals sonicated repeatedly in the visual cortex recovered from each session without behavioral deficits or loss of visual acuity. The ultrasound pressure threshold for BBB disruption evaluated with gadolinium-enhanced T1-weighted MRI was lower than the threshold for microhemorrhages detected on T2* images. This confirmed the existence of an optimal acoustic pressure range between these two thresholds for safe and efficient BBB disruption. Histological analysis did not show significant neuronal damage; some red blood cell extravasations, correlated with higher acoustic pressures, were visible in the targeted tissue. No side effects were visible in histology or imaging out of the targeted area. Sonications of individual points corresponded to millimetric areas of US-BBBD; when multiple locations were targeted, the final volume of US-BBBD was up to 1 cm^3.

Horodyckid et al. 2017 performed seven sonications every two weeks for BBBD in three NHPs using a 1 MHz-implantable ultrasound device positioned in front of the motor area [51] of the brain. Animal behavior and motor function remained normal during the entire experiment. No modification of glucose metabolism was observed as assessed by positron emission tomography. No abnormal activity was registered on either EEG or SSEP recordings. Histological analysis of the sonicated brains only showed limited extravasation of a few red blood cells. MRI imaging after sonications did not show any anomalies except for a few cases of transient subarachnoid FLAIR hypersignal in front of the ultrasound emitter, with no signs of associated hemorrhage on T2*-weighted sequences. This work showed the safety of repeatedly disrupting the BBB over the course of several months in healthy NHPs.

MRI imaging has been the primary tool for planning, guidance, and evaluation of US-BBBD for extracranial devices [34]. T1w imaging using gadolinium contrast agents can be used to monitor BBBD after sonication procedures (Figure 2). Neuronavigation systems have also been developed for guiding procedures outside of an MRI [52,53]. Real-time monitoring and detection of microbubble activity has also been developed to safely guide extracranial devices, where the in situ acoustic pressure is unknown due to the presence of the skull bone [54]. The acoustic pressure applied to the target tissue is adapted to the measured cavitation activity, thus reducing the variability of BBBD for transcranial ultrasound devices and limiting potential side effects. Feasibility of 3D transcranial microbubble imaging with a hemispherical transmit/receive ultrasound phased array has been demonstrated in rabbits, and allows for calibration of acoustic exposure levels during sonications [55]. For implantable ultrasound devices, the in situ acoustic pressure is known and the treatment can be performed outside of an MRI. In clinical trials, post-

sonication MRI was performed to assess the safety and the extent of BBB disruption, but may be unnecessary in future routine clinical use.

4. US-BBBD has been assessed with a large panel of therapeutic agents in different preclinical tumor models

US-BBBD can significantly increase the concentrations of a wide range of systemically administered drugs in healthy brain (hemispheres and brainstem) and brain tumors. Preclinical studies have been performed using low-molecular-weight molecules [56–73] and larger molecules such as therapeutic antibodies [74–77] (**Table 2**). Cell therapies such as natural killer (NK) cells have also been delivered to the brain after US-BBBD [78–80].

After US-BBBD, the increased bioavailability of systemically administered molecules is not specific to a particular drug or class of drugs but depends on both the physicochemical characteristics of the molecule and ultrasound parameters. With higher magnitudes of BBBD, higher concentrations of drug can be reached in sonicated tissues. For example, doxorubicin concentrations were shown to correlate with both microbubble concentration [81] and acoustic pressure [59] in healthy rat brains; above a particular threshold of acoustic pressure, doxorubicin concentration in the brain reached a plateau [59]. For small-molecule drugs that already pass the BBB, there is a modest enhancement in brain drug concentration, relative to drugs that do not spontaneously cross the BBB. In a study in healthy rabbits, it was shown that temozolomide spontaneously crossed the BBB in a larger amount than irinotecan, and temozolomide concentrations in brain were increased to a lesser extent than irinotecan concentrations after US-BBBD [56]. MRI can be used as potential surrogate to monitor drug concentration. For example,

doxorubicin concentrations in brain tissue were correlated with gadolinium signal enhancement intensity [21,81].

Several techniques have been assessed to further optimize US-BBBD efficiency in association with systemic chemotherapy injection. This includes the use of drug-loaded microbubbles that not only transport and liberate the drug into the sonicated vessels but also protect drugs and prolong their circulatory half-life [82]. Moreover, drug-loaded microbubbles excited by focused ultrasound at a frequency of 10 MHz result in predominantly stable cavitation and significantly reduce the occurrence of potential hazards of exposure to biological tissues during the sonication [83]. Microbubbles have also been conjugated to superparamagnetic iron oxide (SPIO) particles to increase drug delivery with magnetic targeting [83]. Drug-loaded liposomes have been used in association with US-BBBD [68,81,84,85] as these long-circulating pegylated particles may allow drug to accumulate in the tumor core via the “enhanced permeability and retention” effect [86]. Finally, several types of nanoparticles have been used to further increase drug delivery after US-BBBD [87–90].

US-BBBD with LIPU in association with systemic chemotherapy has been studied in a large panel of rodent glioma models (**Table 2**): C6 glioma models treated with BCNU [59], microbubbles loaded with BCNU [82] or IL-12 [46]; a 9L gliosarcoma and a GBM 8401 models treated with liposomal doxorubicin [91,92]; a 9L glioma model treated with temozolomide [57]; a U87 model treated with bevacizumab [75] and carboplatin [64]; a F98 rat glioma cell line and a patient-derived glioblastoma (GBM) cell line treated with carboplatin [64,65]. Encouraging efficacy, showing increased survival and tumor control were reported in these studies [57,59,75,82]. One study demonstrated the feasibility of mRNA liquid biopsies in the peripheral

blood in two different murine glioma models (U87, GL261) [93]. In this study, acoustic pressures applied were significantly higher than acoustic pressures usually applied to safely open the BBB. US-BBBD was also evaluated in preclinical models of brain metastases. Complete disappearance of breast cancer brain metastases was observed in rats treated by monoclonal antibodies (trastuzumab, pertuzumab) in association with US-BBBD [76,77]. Cell delivery with HER2-specific NK cells was assessed in a breast cancer brain metastasis model [94]. A reduction in tumor volume and an increase in survival time were observed after multiple sonications.

5. Clinical trials of US-BBBD

5.1. Overview of clinical devices

The skull represents the principle obstacle for the application of ultrasound in the field of neuro-oncology. The thick human skull bone distorts and attenuates ultrasound at frequencies that are used for US-BBBD [95]. Three extracranial ultrasound systems and one implantable ultrasound system are currently in clinical development (Figure 3).

The ExAblate® system, developed by InSightec (InSightec, Tirat Carmel, Israel), is an extracranial device that has been approved for thermal ablation in the brain for patients with essential tremor. The device has been adapted for BBBD and is being used in clinical trials [96]. The latest version of the ExAblate® system consists of a hemispherical ultrasound helmet containing1024 transducers operating at a center frequency of 220 kHz and coupled with a 3T MR scanner (Signa MR750, GE Healthcare, Milwaukee, WI, USA) [97]. The device uses intraoperative MR imaging and real-time acoustic feed-back to guide treatments. During the procedure, the patient's head is shaved and fitted with a stereotactic frame. Pre-sonication MR sequences are first acquired for treatment planning. After IV injection of the microbubbles,

multiple sonications are performed, of around 50 seconds each, at an optimal power determined by a step-wise increase of the acoustic pressure in the protocol. A gadolinium injection is performed at the end of the procedure in order to confirm BBBD [97]. Several ongoing clinical trials are evaluating the safety and feasibility of BBBD with the ExAblate® system in adult patients with high-grade gliomas (NCT03551249, NCT03616860, NCT03712293, NCT02343991, NCT03322813), and HER2-positive breast cancer brain metastases (NCT03714243). The device is also being used for BBBD in clinical trials for Alzheimer's disease (NCT02986932, NCT03671889, NCT03739905), amyotrophic lateral sclerosis (NCT03321487), and Parkinson's disease (NCT03608553).

Another external, multichannel hemispherical phased-array ultrasound system, the NaviFUS® System, has been designed by a Taiwanese biotech company (NaviFUS). The system has been recently assessed in a single-armed dose-escalation study in patients with recurrent GBM (NCT03626896).

Finally, a single-element, extracranial, focused-ultrasound system is being developed at Columbia University [50] and has recently been approved by the FDA for a pilot clinical trial in Alzheimer's disease (NCT04118764). The ultrasound device consists of a single-element, 0.5 MHz FUS transducer coupled to an acoustic feedback monitoring. A neuronavigation system is used for brain targeting during sonications [53].

An alternative approach to extracranial, focused ultrasound systems is to implant the ultrasound emitters. The SonoCloud-1® is an implantable ultrasound device developed by CarThera (Paris, France) [36]. The implant portion is an 11.5 mm-diameter ultrasound transducer operating at a frequency of 1.05 MHz that can be placed in a burr hole during a surgical procedure for a biopsy or a tumor resection [98]. The device is totally covered by the skin and can be repeatedly

activated using a transcutaneous needle connection system prior to chemotherapy administration. After IV injection of the microbubbles, the device is activated for up to 270 seconds. A new generation device, the SonoCloud-9® device, consists of nine 1-cm diameter ultrasound transducers arranged on an implantable grid that increases the sonication volume by a factor of nine compared to the SonoCloud-1®. The SonoCloud-1® device has recently been assessed in a completed clinical trial in recurrent glioblastoma (rGBM) patients, with activation prior to carboplatin chemotherapy (NCT02253212). Additional clinical trials are underway to evaluate the safety and feasibility of US-BBBD using the SonoCloud-1® device in patients with melanoma brain metastases (NCT04021420) and in Alzheimer's disease (NCT03119961). The SonoCloud-9® device is currently being assessed in an ongoing international multicenter clinical trial in patients with rGBM (NCT03744026).

Both extracranial and implantable devices have advantages and drawbacks, listed in **Table 3**. Thus, routine clinical use of these devices in the future may be complementary and depend on the particular indication and tumor location to be treated. Large, superficial and infiltrative lesions such as extensive high-grade gliomas or diffuse intrinsic pontine gliomas may be good targets for implantable devices, whereas smaller, deep-seated and multiple lesions, such as hypothalamic or basal ganglia lesions or multiple metastases may be suited for treatment with extracranial devices.

5.2. Safety of US-BBBD observed in clinical trials of brain tumor patients

As shown in **Table 4**, several clinical trials are in progress using US-BBBD in brain tumor patients. All of the trials currently ongoing or completed are for either GBM or low-grade gliomas. These trials are designed as pilot trials to investigate the safety of a single US-BBBD

without drug, single US-BBBD with drug to evaluate drug concentrations, or multiple sessions of disruption to follow a typical course of chemotherapy that a brain tumor patient would receive. Two of these studies have been completed, as summarized below.

Mainprize et al. 2019 [97] (NCT02343991) performed US-BBBD in five patients with newly-diagnosed malignant glioma using the Exablate® transcranial focused-ultrasound system in association with Definity® microbubbles. All of the patients received either IV liposomal doxorubicin (*n*=1) or oral temozolomide (*n*=4) before the sonication at one day prior to surgical resection. BBBD was visualized using T1-weighted contrast-enhanced MRI. The procedure was safe and well-tolerated, with no new or worsening symptoms after the sonication, and no intracerebral haemorrhage or edema shown on MRI. Targeted volumes ranged from 972 to 2430 mm^3. BBBD was reversible, and BBB integrity was restored 20 hours later. Drug concentration in the sonicated tissue, compared with that in nonsonicated tissues were reported for two patients: increased concentrations of temozolomide and, to a lesser extent, doxorubicin, were measured in sonicated tissue relative to unsonicated tissue (3.47×10^{-4} ng/mg versus 0.45×10^{-4} ng/mg for temozolomide and 0.22 ng/mg versus 0.15 ng/mg for doxorubicin, respectively).

Idbaih et al. 2019 [99] (NCT02253212) reported the results of a clinical trial using the SonoCloud-1® in association with SonoVue® microbubbles. The device was implanted in patients with rGBM during tumor debulking under general anesthesia or a dedicated surgery under local anesthesia. The device was activated monthly in association with IV carboplatin chemotherapy at a dose of AUC5. Nineteen patients were treated, undergoing 65 monthly sonications. The median number of sonications per patient was three (range, 1-10 sonications). The trial was designed as an ultrasound-dose escalation study, in which the ultrasound pressure was increased progressively, from 0.41 MPa to 1.15 MPa; no dose-limiting toxicities were

observed in this acoustic pressure range. The BBBD procedures were well tolerated, without severe adverse events, including when sonicating eloquent brain regions. Both the median progression-free survival (PFS) and overall survival (OS) times were increased relative to historical data (4.11 months and 2-3 months for PFS and 12.94 months and 6-9 months for OS, respectively), and a trend for a better tumor control in the sonication field was observed.

Until now, no children have been included in clinical trials assessing US-BBBD. The ExAblate® system is currently being assessed for the thermal ablation (and not for BBBD) of benign intracranial tumors in children and young patients (NCT03028246). A clinical trial to assess the safety and feasibility of BBBD using the SonoCloud device in association with IV carboplatin chemotherapy in recurrent supratentorial malignant primitive tumors in the pediatric population is planned to begin in Paris, France in 2020.

6. Obstacles to overcome for wide clinical adoption of US-BBBD

Many preclinical studies have shown that US-BBBD may allow tumor control and increased survival in different murine models of brain tumors. However, tumor responses were varied [76,77]. The vascularization of the tumor, pharmacochemical characteristics of the drug used, ultrasound parameters, and other factors all influence the efficacy of this approach. Although a trend for tumor control and a better OS and PFS has been observed in the SonoCloud-1 clinical trial [99], no significant increases in survival have been demonstrated to date in humans. Thus, the next step in US-BBBD clinical development will be to confirm the benefits of this technique in a larger cohort of patients. Many preclinical studies have shown that opening of the BBB can be performed with minimal side effects, including in the case of repeated treatments. Although inflammatory reactions can be limited with optimal ultrasound parameters [43], many studies

have demonstrated that immune reactions occur in sonicated tissues [42,44]. The chronic effects of such reactions will have to be studied and monitored in future clinical trials, especially in long-surviving patients.

One challenge for neuro-oncologists will be to determine effective drugs to use in association with BBBD. For any drug choice, the potential neurotoxicity will need to be carefully examined. Indeed, the optimal treatment schedule is likely therapy-dependent and will have to be adapted to the agent delivered, taking into account the type of therapeutic agent (cell, small molecule, antibody) and its route of delivery (oral, IV), the pharmacokinetic characteristics of the drug (if any) and the formulation of the treatment (loaded-microbubble, liposome, nanoparticle). Moreover, immunotherapies will have to be assessed in combination with US-BBBD because different studies have shown that US-BBBD may modify local innate and cellular immunities, as well as the potential of the technique to deliver immunotherapies (antibodies, cells) to the brain.

Limited data exists on the systemic effects of repeated US-BBBD. One study has shown that systemic cellular immunity was not modified after repeated intraperitoneal IL-12 injections [46], while another has demonstrated that US-BBBD may allow the release of tumor DNA or antigens into the peripheral blood circulation [93]. More preclinical and clinical studies will be necessary in order to confirm these initial results. Release of particles from the brain through the disrupted BBB may be beneficial in order to induce an immune reaction against the tumor or to monitor tumor response from blood samples, but release of cells could also promote the development of peripheral metastasis and induce paradoxical local or systemic inflammatory reactions.

Although technological developments have drastically improved the devices available for US-BBBD during the last several decades, some limitations still exist. Implantable devices allow for

large volume of BBBD, especially with the new generation SonoCloud-9 device developed by CarThera (Paris, France), but the ultrasound field is limited in terms of target volume conformality as the ultrasound emitter's shape and frequency are fixed. The lengthy procedure (installation of stereotactic frame, access to MRI, head shaving, etc) required for sonications using multielement extracranial systems may be a limit for repeated use for some indications. Although constraining, general anesthesia is feasible under certain conditions without interfering with ultrasound-mediated disruption, as shown in preclinical studies [100]. The overall transcranial treatment duration could be reduced with advanced real-time monitoring and rapid electronic beam steering techniques [55]. Existing single-element ultrasound emitters with a relatively large focal size in combination with neuronavigation could also be used to decrease treatment times when using extracranial devices [52].

7. Conclusions

US-BBBD with LIPU has been investigated in numerous preclinical studies and has recently entered clinical trials with encouraging results. The technique allows for safe, repeatable, and targeted opening of the BBB and increased uptake of therapeutic agents into the brain parenchyma and brain tumors. The range of drugs used in preclinical studies shows the potential of this approach. These preliminary results will have to be confirmed in larger clinical trials for this technique to gain further acceptance and regulatory approvals.

Figure legends

Figure 1. A. Mechanical and functional endothelial cell modifications involved in ultrasound-induced blood-brain barrier disruption (US-BBBD). Circulating microbubbles (1) in the ultrasound beam (2) expand and contract (cavitation). This induces modification of the endothelial cells and opening of the blood-brain barrier (BBB). Tight junctions are transiently opened, creating paracellular routes (3). Efflux transporters are transiently inhibited, preventing drugs from being transported back into the blood (4). Formation and movement of vesicles from luminal to abluminal surfaces of endothelial cells induces transcellular transport (5). These vesicles can also merge and form large fenestrations and transendothelial channels (6). **B. US-BBBD assessed with evans blue penetration in brain parenchyma in mice after sonication with an unfocused ultrasound device.** On the left, cranial and caudal faces of whole brains; on the right, coronal slices. Right hemisphere has been sonicated (frequency 1.05 MHz, pulses length 25,000 cycles/23.8 ms, pulse repetition frequency 1 Hz and acoustic pressure 0.3 MPa) after intravenous microbubble injection (SonoVue®, 200 μL). Blue coloration corresponds to BBB opening regions, and is limited to the ultrasound beam**.**

Figure 2. MRI monitoring of BBB disruption in human with an implantable unfocused device. T1-weighted coronal images of brain parenchyma after gadolinium injection before sonication (left) and 30 minutes after sonication (right). Opening of the BBB appears as a contrast enhancement in the ultrasound beam (white arrows).

Figure 3. Schematic representation of ultrasound devices developed for clinical application of ultrasound-induced blood-brain barrier disruption (US-BBBD). A. Implantable, unfocused single emitter ultrasound device (SonoCloud-1®). **B.** Implantable, unfocused nine-

emitter ultrasound device (SonoCloud-9®). **C.** Extracranial hemispherical focused ultrasound arrays (ExAblate®, NaviFUS®). **D.** Extracranial monoelement focused device.

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Agent Name	**Luminity® / Definity®**	**Optison®**	**SonoVue® / Lumison®**
Manufacturer	Lantheus Medical Imaging	GE Healthcare	Bracco Diagnostics
Gas enclosed	Perflutren (C_3F_8)	Perflutren (C_3F_8)	Sulfur hexafluoride (SF_6)
Shell	Phospholipid	Albumin	Phospholipid
Mean bubble diameter (mm)	1.1 - 3.3	2.0 - 4.5	2.5
Bubble concentration (bubbles/ml)	1.2×10^{10}	$5\text{-}8 \times 10^{8}$	$1\text{-}5 \times 10^{8}$
Half-life (min)	1.9	1.3	2.0
Regulatory Approval	FDA approval 2001 EMEA approval 2006	FDA approval 1997 EMEA approval 1998	EMEA approval 2001 FDA approval 2014

Table 1. Comparison of the three main microbubble contrast agents used in preclinical studies for ultrasound-induced blood-brain barrier disruption. EMEA: European Medicines Evaluation Agency, FDA: Food and Drug Administration. Adapted from Miller et al. 2004 [33].

	Drug	Animal Model	Tumor model	Optimization strategies	Maximum concentration uptake		Tumor control	Increased survival	References
					Healthy brain	Tumor			
Platinum containing cytotoxics	Carboplatin	NHP			5,2				[58]
		Mouse	U87, PDCL		4,2		x	x	[64]
		Rat	F98		2,9	7,3	x	x	[65]
	Cisplatin	Rat	9L gliosarcoma, F98	Brain-penetrating nanoparticles			x	x	[87]
		Mouse	U251	Gold nanoparticles	2 – 3,5		x		[88]
	Liposomal cisplatin	Mouse	GBM8401	IL-4R-targeted liposomes			x		[84]
Alkylating agents	Temozolomide	Rat	9L glioma		1,5*		x	x	[57]
		Mouse	U87		2,7	1,9	x	x	[67]
		Rabbit				1,2			[31]
	BCNU	Rat	C6 glioma		3,4	2	x	x	[59]
		Rat	C6 glioma	MB-loaded		4	x	x	[82]
		Rat	C6 glioma	MB-loaded	5,8		x	x	[83]
		Rat	C6 glioma	MNP + MT	9,9 - 26		x	x	[89]
Topoisomerase inhibitors	Irinotecan	Rabbit			2,9				[31]
Cytotoxic antibiotics	Doxorubicin	Rat			2,7				[21]
		Rat	C6 glioma	MB-loaded		1,6			[66]
		Rat	C6 glioma	MB-loaded, SD-MB, MT		17 – 36			[71]
		Mouse	GL261, SMA-560 glioma		16,6	8,1	x	x	[69]
		Rat	9L glioma		9,1	2,7			[72]
		Mouse			56,8				[73]
	Liposomal doxorubicin	Rat		Liposomes	3,5				[81]
		Rat	9L gliosarcoma	Liposomes			x	x	[91]
		Rat	9L gliosarcoma	Liposomes		2,7			[68]
		Rat	9L glioma	Liposomes			x	x	[85]
		Mouse	GBM8401	IL-4R-targeted liposomes		~2-4	x	x	[92]
Microtubules inhibitors	Paclitaxel	Mouse	U87	Nanoparticles			x	x	[90]
	Liposomal paclitaxel	Mouse	U87	Liposomes		2	x	x	[63]
Antimetabolites	Methotrexate	Rabbit			13,6				[62]
	Cytarabine	Rat			4,4				[60]
		Rat			1,8				[61]
Monoclonal antibodies	Bevacizumab	Mouse	U87		6,7		x	x	[75]
	Trastuzumab	Rat	BT474**				x	x	[77]
		Mouse			1,9				[74]
	Pertuzumab + Trastuzumab	Rat	MDA-MB-361**				x	x	[76]
Interleukin	IL-12	Rat	C6 glioma			2,9	x	x	[46]

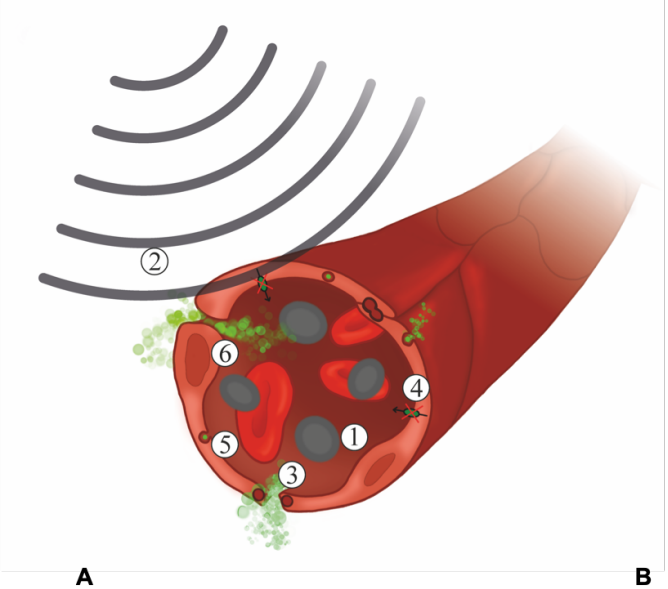
Table 2. Pre-clinical studies investigating various drugs using US-BBBD for primary CNS tumors. Maximum concentration uptakes are given when available; they correspond to maximum ratio of mean drug concentration in sonicated tissue (healthy brain or tumor) by mean concentration ratio in nonsonicated healthy brain. IL-4R: IL-4 receptor, MB: microbubbles, MNP: magnetic nanoparticles, MT: magnetic targeting, PDCL: patients-derived cell lines, SD-MB: SPIO (superparamagnetic iron oxide)-doxorubicin conjugated microbubbles. * concentrations in cerebrospinal fluid, ** HER2-positive human breast cancer cells.

Extracranial Devices		Implantable devices	
Advantages	**Drawbacks**	**Advantages**	**Drawbacks**
Non-invasive Appropriate for deep lesions Multiple targets possible Real-time MRI control / Neuronavigation control	Small volumes of BBBD (~1-4 cm^3) Lengthy procedure times (hours) MRI may be required General anesthesia may be needed during US procedure (pediatrics) Not appropriate for superficial lesions Head shaving required	Minimally-invasive Short procedures (minutes) Target BBBD volumes up to 45 cm^3 No head shaving required	Implantation during biopsy or resection procedure Target volume limited to the US beam (one device - one target) Not appropriate for deep lesions

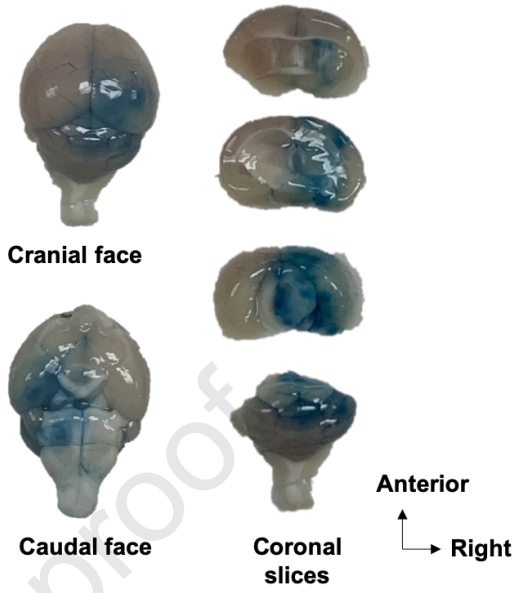
Table 3. Comparison of main advantages and drawbacks of extracranial and implantable devices. BBBD: blood-brain barrier disruption, MRI: magnetic resonance imaging, US: ultrasound.

Clinical Trial *Status*	Indication	Device	Drug	Reference
NCT03551249 *Recruiting*	nGBM	Exablate Neuro Model 4000 Type 2	Temozolomide	n/a
NCT03616860 *Recruiting*	nGBM	Exablate Neuro Model 4000 Type 2	Temozolomide	n/a
NCT03712293 *Recruiting*	nGBM	Exablate Neuro Model 4000 Type 2	Temozolomide	n/a
NCT03322813 *Recruiting*	nGBM	Exablate Neuro Model 4000 Type 2	No drug	n/a
NCT03744026 *Recruiting*	rGBM	SonoCloud-9	Carboplatin	n/a
NCT02253212 *Completed*	rGBM	SonoCloud-1, SonoCloud-3	Carboplatin	[95], [96]
NCT03626896 *Completed*	rGBM	NaviFUS	No drug	n/a
NCT02343991 *Completed*	Malignant glioma	Exablate Neuro	Temozolomide, Liposomal Doxorubicin	[94]
NCT040635514 *Not yet recruiting*	Low grade glioma	n/a	No drug	n/a

Table 4. Current clinical trials investigating ultrasound-induced blood-brain barrier disruption for primary central nervous system tumors. n/a: non available, nGBM: newly diagnosed GBM, rGBM: recurrent GBM



A



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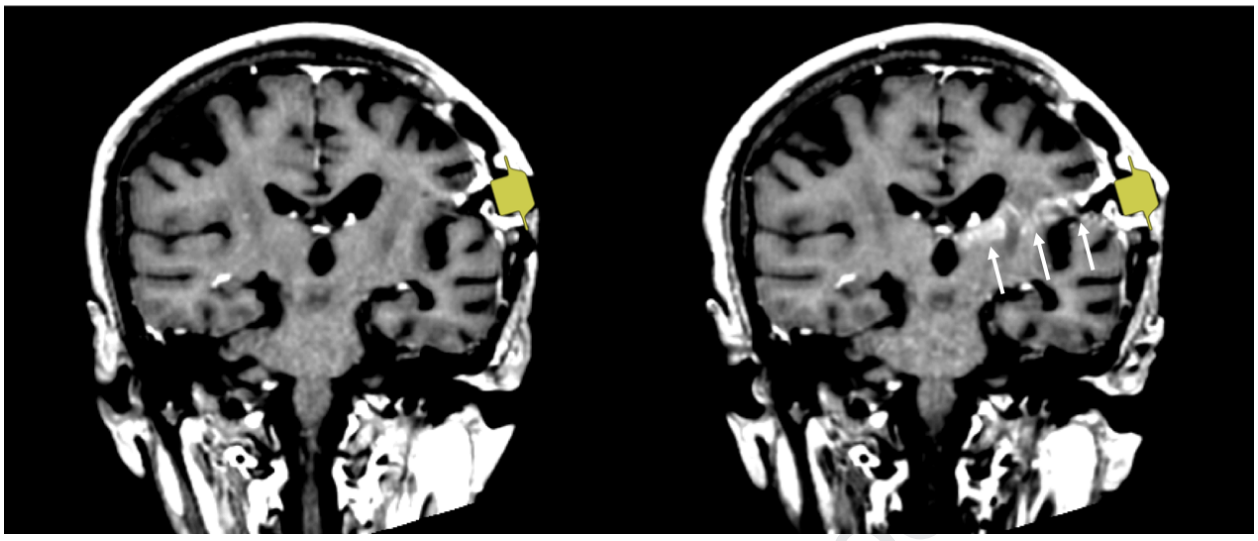

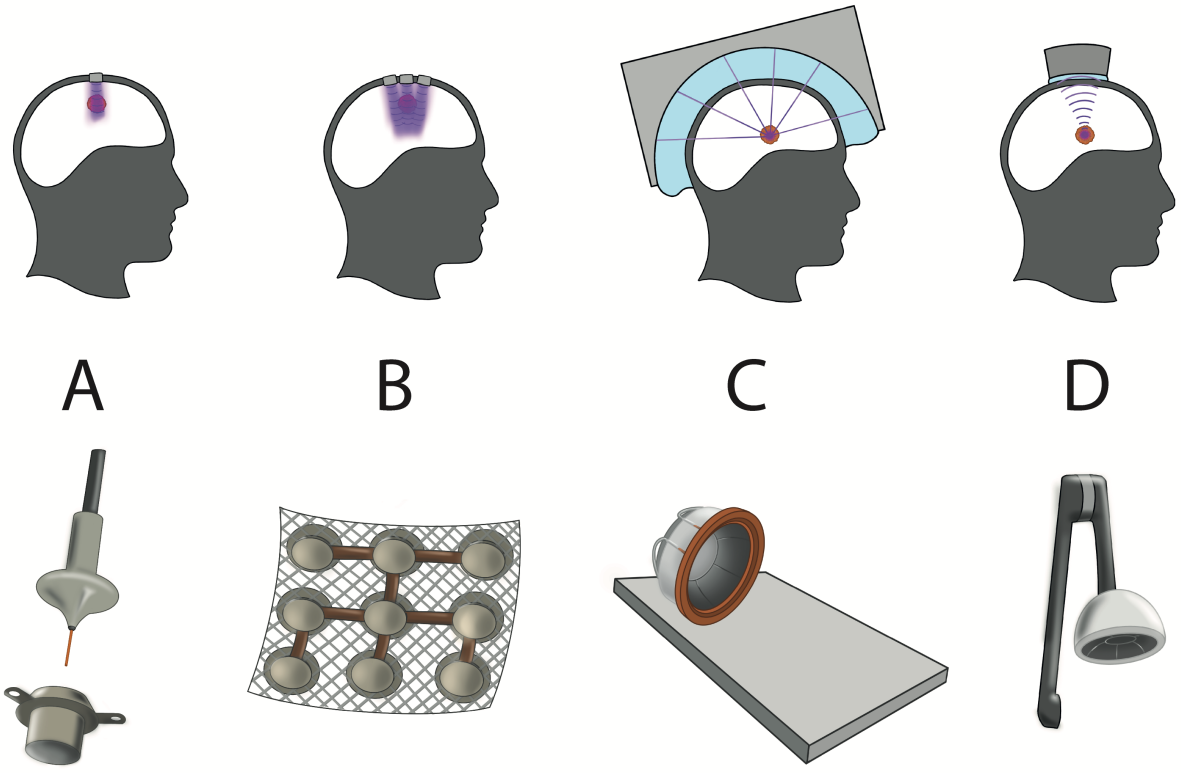

Highlights

- The blood-brain barrier prevents most drug therapies from reaching brain tumors
- Low-intensity pulsed ultrasound with systemic microbubbles can open the blood-brain barrier
- Opening of the blood-brain barrier with ultrasound is safe, localized and reversible
- A large panel of therapeutic agents can be delivered to the brain with ultrasound
- Extracranial and implantable ultrasound devices are currently in clinical trials

Conflict of Interest

M. Canney and Guillaume Bouchoux are employees of CarThera. A. Carpentier is a paid consultant to CarThera. K. Beccaria has previously been an employee of CarThera. A. Carpentier, K. Beccaria, and M. Canney, and G. Bouchoux 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.