CNS tumors represent the most common solid tumor and the first cause of cancer death in childhood, adolescence, and young adulthood. Current treatments are far from optimal in most of these tumors and the prognosis remains dismal for many of them. One of the main causes of the failure of current medical treatments is in part due to the existence of the blood-brain barrier (BBB), which limits drug delivery to tumors. Opening of the BBB with low-intensity pulsed ultrasound (LIPU) has emerged during the last 2 decades as a promising technique for enhancing drug delivery to the brain. In preclinical models, enhanced delivery of a wide range of therapeutic agents, from low-molecular-weight drugs, to antibodies and immune cells, has been observed as well as tumor control and increased survival. This technique has recently entered clinical trials with extracranial and intracranial devices. The safety and feasibility of this technique has furthermore been shown in patients treated monthly for recurrent glioblastoma receiving carboplatin chemotherapy. In this review, the characteristics of the BBB in the most common pediatric brain tumors are reviewed. Then, principles and mechanisms of BBB disruption with ultrasound (US) are summarized and described at the histological and biological levels. Lastly, preclinical studies that have used US-induced BBB opening in tumor models, recent clinical trials, and the potential use of this technology in pediatrics are provided.
drugs. Intraarterial chemotherapy injection consists of local-regional delivery of the drug via the cerebral arteries; a systematic review and meta-analysis has shown that this invasive approach is not superior to intravenous chemotherapy in terms of efficacy and OS. Osmotic disruption of the BBB is performed using hypertonic agents, such as mannitol, injected into a cerebral artery. Although it can increase delivery of drugs into the brain compared to systemic injection alone, the delivery is largely reversed within 10 minutes and not targeted to the tumor, and this technique may induce seizures and increase intracranial pressure. Moreover, osmotic disruption of the BBB in association with carboplatin was inactive in pediatric high-grade and brainstem gliomas. The functional activity of the BBB can also be targeted by inhibition of the drug efflux transporter system. Inhibition of P-glycoprotein with cyclosporin A was evaluated in patients with DIPG in association with etoposide, vincristine, and radiation therapy, but the regimen proved excessively toxic. Coregional delivery of the drug via the cerebral arteries; a systematic review and meta-analysis has shown that this technique.29

In this review, the characteristics of the BBB in the most common pediatric brain tumors are reviewed. Then, we focus on ultrasound (US)—induced opening of the BBB to review the principles, mechanisms, and histological and biological effects of BBBD with US; we summarize preclinical studies that have used US-induced BBB opening on tumor models; and we report recent clinical trials that have been initiated.

**Methods**

A comprehensive review of the literature was performed using a PubMed search and the ClinicalTrials.gov website (https://clinicaltrials.gov) with the following keywords: (blood-brain-barrier, blood-tumor barrier) / (ependymoma, medulloblastoma, glioma, diffuse intrinsic pontine glioma, brain tumor) / (pediatric, children) and (blood-brain barrier, blood-tumor barrier) / ultrasound / (disruption, opening). Relevant articles published in English were selected based on individual merit and included basic science research, human subjects research, clinical trials, and reviews. The reference lists of included articles were searched for additional studies.

**The BBB in Pediatric Brain Tumors**

Although few studies have focused on specific features of the BBB in pediatric brain tumors, this certainly plays a major role in drug resistance and may explain the discrepancies between some encouraging preclinical in vitro results and failure of treatments once translated into clinical practice. Diffuse gliomas are very invasive tumors characterized by their capacity to infiltrate the brain parenchyma. In both DIPG and supratentorial malignant gliomas, tumor cells can be mixed with normal brain parenchyma, distant from the primary tumor mass. These tumors generally show little or no contrast enhancement on MRI, indicating an intact BBB. As many as 82.4% and 17.7% of WHO grade III and IV pediatric high-grade gliomas (pHGGs), respectively, do not exhibit enhancement. Contrast enhancement only involves 0%—25% of the tumor volume on average in DIPG. The extent of tumor infiltration compared to the small amount or absence of contrast enhancement implies that tumor cells can infiltrate brain areas protected by an intact BBB that prevents efficient delivery of systemically administered drugs to tumor cells and the microenvironment. Based on a theoretical model, it was estimated that only 15% of drugs currently administered to patients with DIPG may be likely to spontaneously cross the BBB and reach therapeutic concentration through an intact BBB. Different factors may affect BBB permeability in pHGG, and the microenvironment likely plays an important role. The heterogeneity of BBB permeability was confirmed in a genetic mouse model of pHGG, where it was shown that BBB permeability is 67% higher in cortical pHGG compared to brainstem pHGG. Permeability was not significantly affected by H3.3-K27M mutations, but was significantly correlated with tumor volume. Clinicians hypothesize that the low permeability observed in brainstem gliomas may explain the poor prognosis of these tumors. A similar observation was inferred from a clinical series of DIPGs in which contrast enhancement differed from tumor to tumor, and was more often associated with H3.1-K27M tumors, which have a better prognosis compared to H3.3-K27M tumors. ABC drug efflux transporters are expressed in both brain endothelial cells and glioma cells and transport their substrates from these cells back into the blood circulation, leading to a reduced delivery of many drugs in gliomas. All three major ABC efflux transporters—P-glycoprotein (ABCBI), breast cancer resistance protein (BCRP; ABCG2), and multidrug resistance-associated proteins (ABCC1)—are present in the microvasculature of pHGG, including DIPG. It has been suggested that both P-glycoprotein and BCRP limit the efficacy of dasatinib in a genetic brainstem glioma mouse model.

The permeability of the BBB is very variable as well in medulloblastomas. Signal enhancement after contrast injection varies from 85% to 100% of patients and can be subtle and heterogeneous (see review in Dangouloff-Ros et al.). Group 4 medulloblastomas generally lack or have minimal enhancement and have a poor prognosis; it was also observed that non-Wnt/non–sonic hedgehog tumors with extensive gadolinium enhancement had a worse prognosis in comparison with tumors with no or weak enhancement. However, gadolinium is a small molecule that can pass easily through a damaged BBB. Thus, contrast enhancement may overestimate BBB permeability and give a false appreciation of the ability for drugs to be delivered to a tissue. It has been recently described that Wnt-medulloblastomas, curable tumors even when metastatic, present BBB features that could explain their better prognosis. These tumors have significant hemorrhagic
Concept and Mechanisms of US-Induced BBBD

BBBD using LIPU in combination with injection of US resonators (preformed gas microbubbles) has been in preclinical development for more than 20 years. \(^{29}\) BBBD magnitude varies depending on acoustic parameters (acoustic pressure, frequency, burst length)\(^{11,45}\) and microbubble size and concentration.\(^{14,47}\) BBBD is transient and the integrity of the BBB has been shown to be rapidly restored after sonication; it begins to close immediately after disruption and is fully closed in 6–24 hours.\(^{29,57}\)

When US stimulates systemically administered microbubbles (1–10 \(\mu\)m size), the bubbles expand and contract, resulting in mechanical stretching of the vessel walls in capillaries. This leads to endothelial cell modification with increased transcytosis activity, formation of transendothelial fenestration, and opening of the tight junctions, particularly occludin and claudin-5 proteins.\(^{35,58}\) Beyond modification of the physical barrier, US and microbubbles may also modify the functional aspect of the BBB. P-glycoprotein expression is inhibited up to 48 hours after sonication, confirming an effect of US on drug efflux mechanisms,\(^{3}\) without the functional impairment of endothelial cells.\(^{13}\) These different mechanisms appear progressively in time, and BBBD occurs in two different phases with early/fast leakage, and late/slow leakage.\(^{55}\) Figure 1 represents mechanisms underlying US-induced BBBD with LIPU.

Preclinical Evaluation of US-Induced BBBD Safety

The safety of BBBD has been assessed through preclinical studies in both small and large animal models. The impact of US and microbubbles in the brain parenchyma depends on US parameters. BBBD has been obtained with acoustic pressures up to 0.5 MPa with few extravasated erythrocytes and very scarce ischemic neurons or apoptotic cells in the sonicated area with a frequency of 690 kHz suitable for transcranial sonication.\(^{28}\) Few erythrocyte extravasations without ischemic lesions were also observed after sonication in rabbits with acoustic pressures up to 0.6 MPa with a skull-implantable nonfocused 1-MHz US device.\(^{3}\) A sterile inflammatory response mediated by the NF-\(\kappa\)B pathway has been described up to 24 hours after sonication in the rat brain;\(^{36}\) however, this response is dependent on microbubble dose. Safe sonifications without induction of the NF-\(\kappa\)B signaling pathway have been performed in the same animal at lower microbubble dosages.\(^{45}\) Moreover, no microgliosis or astrocytosis has been observed up to 6 months after US-induced BBBD and adeno-associated virus delivery.\(^{60}\) Repeated BBBDs appear to be as safe as single sessions,\(^{34}\) and multiple studies have confirmed the feasibility and safety of the technique in nonhuman primates (NHPs) with different US devices. Feasibility of transcranial, cavitation-guided disruption of the BBB in NHPs was first described in the visual cortex with a 500-kHz focused single-element transducer.\(^{41,67}\) MCDAnold et al. used a noninvasive multi-array 1024-element device and demonstrated that multiple transcranial BBBDs are safe in deep and superficial targets. Animals repeatedly sonicated in the visual cortex recovered from each session without behavioral deficit or loss in visual acuity, and no signs of brain damage were observed in histological and MRI studies.\(^{43}\) Finally, a multiparametric study assessing behavioral, neurophysiological, imaging, and histological parameters proved that safe repeated BBBDs are possible with an implantable US device placed into the skull of NHPs in front of eloquent brain areas.\(^{27}\) BBBD can be observed on MRI as a pressure-dependent contrast enhancement after gadolinium injection in T1-weighted sequences.\(^{29}\)

US-Induced BBBD and Preclinical Tumor Models

BBBD with LIPU has been shown to enhance the delivery of a wide variety of agents into the brain in preclinical models. Chemotherapeutic drugs used in current neurocological protocols such as doxorubicin,\(^{65}\) temozolomide,\(^{6}\) irinotecan,\(^{6}\) carboplatin,\(^{69}\) BCNU,\(^{39}\) cytarabine,\(^{72}\) or methotrexate\(^{48}\) have been delivered in significant amounts after US-induced BBBD. Larger molecular weight molecules, such as monoclonal antibodies (trastuzumab\(^{37}\)), as well as cells (neuronal stem cells,\(^{8}\) natural killer [NK] cells\(^{8}\)) have also been significantly delivered to rodent healthy brain parenchyma and metastases in the brain after BBBD permeabilization. Different strategies have been developed to improve local drug/cell delivery and efficacy after US-induced BBBD. These strategies include the use of liposomes,\(^{73}\) nanoparticles,\(^{63}\) drug-loaded microbubbles,\(^{21}\) or magnetic attraction of cells.\(^{59}\)

BBBD with LIPU was evaluated in preclinical models of metastasis in the brain. Although results were not uniform, a complete disappearance of tumors has been observed in rats treated by trastuzumab associated with US and microbubbles in a breast cancer brain metastasis model (BT-474 HER2-positive human breast ductal carcinoma cells).\(^{52}\) Growth control was also obtained in an MDA-MB-361 HER2-positive model after weekly treatments of rats with trastuzumab and pertuzumab associated with BBBD. Results were also heterogeneous, and no complete response was observed in this study.\(^{55}\) The heterogeneity observed in these studies may be due to variations in tumor vasculature or in the US distribution. In the
same MDA-MB-361 HER-2 positive model, a reduction in tumor volume and an increase in survival time after applying multiple sonications were observed when HER2-specific NK cells were injected intravenously in association with US.  

A large panel of rodent glioma models has also been studied. Tumor control and improved survival was obtained in C6 glioma models treated with BCNU and BCNU-loaded microbubbles, in 9L gliosarcoma and glioblastoma (GBM) 8401 models treated with liposomal doxorubicin, in a 9L glioma model treated with temozolomide, and in a U87 model treated with bevacizumab. All of these later studies were performed with focused US devices. Recently, significantly increased survival with a trend for tumor growth control was observed in U87 and patient-derived GBM cell-line models in mice treated with carboplatin and BBBD with an unfocused US device. 

Clinical Translation of US-Induced BBBD

This emerging technique has recently been translated to the clinic, with either extracranial noninvasive devices or minimally invasive implantable devices (Table 1). In both cases, the skull represents the main obstacle for the application of US in the field of neurooncology, because bone induces distortion and attenuation of the US and causes rapid heating inside the skull. Three external US systems and 1 implantable US system are currently in clinical trials, as reviewed below (Fig. 2). Presently, no children have been included in clinical trials assessing US-induced BBBD. The ExAblate system, developed by InSightec, was first designed for thermal ablation applications and then extended for use in BBBD. Planning and monitoring of BBBD with this device can be performed using the dual-mode hemispheric array. Several ongoing clinical trials are evaluating the safety and feasibility of BBBD with the ExAblate system in adult patients with high-grade gliomas (www.clinicaltrials.gov nos. NCT03551249, NCT03616860, NCT03712293, NCT02343991), and breast cancer brain metastases (no. NCT03714243). Recently, 5 patients with malignant brain tumors were treated in a phase I, single-arm study (study no. NCT02343991). BBBD was observed at tumor margins, in volumes ranging from 972 to 2430 mm$^3$. The BBB integrity was confirmed to be restored 20 hours later. The procedure was well tolerated with no new or worsening symptoms during the 24 hours following the sonication, and no significant intracerebral hemorrhage or edema on control MRI. Two patients were previously treated with either oral temozolomide or intravenous doxorubicin, and increased concentrations of temozolomide and, to a lesser extent, doxorubicin were measured in sonicated tissue relative to unsonicated tissue (3.47 x 10$^{-4}$ ng/mg vs 0.45 x 10$^{-4}$ ng/mg for temozolomide and 0.22 ng/mg vs 0.15 ng/mg for doxorubicin, respectively). NaviFUS, a Taiwanese biotech company, has designed an external, multichannel hemispheric phased-array US system, the NaviFUS System. The system has been recently assessed in a single-arm dose escalation study in patients with recurrent GBM (study no. NCT03626896). Results have not yet been published. Finally, a single-element, transcranial, focused US system has recently been approved by the FDA for a pilot clinical trial for Alzheimer’s disease (Columbia University). The treatment procedure is guided and controlled by neuronavigation and a passive cavitation detection device. To improve the safety of BBBD with extracranial devices, real-time monitoring...
### TABLE 1. Clinical trials on BBBD with LIPU indexed in https://clinicaltrials.gov in the field of neurooncology

| Study Title                                                                 | PI Location          | Status                        | Condition                                    | US Device        | Drug                        | NCT No.       |
|-----------------------------------------------------------------------------|----------------------|-------------------------------|----------------------------------------------|------------------|-----------------------------|---------------|
| The use of focused ultrasound and microbubble infusion for altering brain perfusion and the blood brain barrier | Santa Monica, CA, USA | Not yet recruiting           | Low-grade glioma of brain                    | —                | —                           | NCT04063514  |
| Safety and efficacy of transient opening of the blood-brain barrier (BBB) with the SonoCloud-9 | Paris, France        | Recruiting                    | Glioblastoma                                 | SonoCloud-9      | Carboplatin                 | NCT03744026  |
| Blood-brain barrier disruption using transcranial MRI-guided focused ultrasound | Toronto, ON, Canada  | Active, not recruiting        | Brain tumors                                 | ExAblate         | Doxorubicin                 | NCT02343991  |
| Assessment of safety and feasibility of ExAblate blood-brain barrier (BBB) disruption | College Park, MD, USA | Recruiting                    | High-grade glioma                            | ExAblate         | Temozolomide                | NCT03551249  |
| Safety of BBB opening with the SonoCloud                                   | Paris, France        | Completed                     | Glioblastoma                                 | SonoCloud        | Carboplatin                 | NCT02253212  |
| Assessment of safety and feasibility of ExAblate blood-brain barrier (BBB) disruption for treatment of glioma | Toronto, ON, Canada  | Recruiting                    | Glioblastoma                                 | ExAblate         | Temozolomide                | NCT03616860  |
| Safety and efficacy of SonoCloud device combined with Nivolumab in brain metastases from patients with melanoma | Paris, France        | Not yet recruiting            | Malignant melanoma brain metastasis          | Sonocloud        | Nivolumab alone or w/ ipilimumab | NCT04021420  |
| Safety of BBB disruption using NaviFUS system in recurrent glioblastoma multiforme (GBM) patients | Taoyuan City, Taiwan | Completed                     | Glioblastoma                                 | NaviFUS          | —                           | NCT03626896  |
| Blood brain barrier disruption (BBBD) using MRFUS in the treatment of Her2-positive breast cancer brain metastases | Toronto, ON, Canada  | Not yet recruiting            | HER2-positive breast cancer brain metastases | ExAblate         | —                           | NCT03714243  |
| ExAblate blood-brain barrier disruption for glioblastoma in patients undergoing standard chemotherapy | Seoul, Republic of Korea | Recruiting                   | Glioblastoma                                 | ExAblate         | Temozolomide                | NCT03712293  |

NCT = National Clinical Trial; PI = principal investigator.

**FIG. 2.** Schematic representation of US devices developed for clinical application of BBBD with LIPU. 
A: Extracranial hemispheric focused US arrays (ExAblate, NaviFUS). 
B: Extracranial mono-element focused device. 
C: Implantable, unfocused single-emitter US device (SonoCloud-1). 
D: Implantable, unfocused 9-emitter US device (SonoCloud-9).
of acoustic activity (microbubble cavitation) has been developed. This system allows for a stepwise increase of acoustic pressure during the procedure, based on the spectral information received in real-time by an associated hydrophone, thus reducing variability of BBBD for transcranial US devices where the in situ acoustic pressure is unknown.

Another strategy to overcome the bone interface consists of inserting a US device into the skull. The SonoCloud-1 device, developed by CarThera, is an implantable unfocused US device that can be placed in a burr hole and activated using a transcutaneous connection. The first clinical trial using the SonoCloud-1 technology has been performed in adults with recurrent GBM treated with intravenous carboplatin (study no. NCT02253212). Intra- venous carboplatin injection was started on average 106 minutes after sonication. The BBBD procedures were well tolerated, without severe adverse events, including when sonicating eloquent brain regions. Both median progression-free survival (PFS) and OS were increased relative to historical data (4.11 vs 2–3 months for PFS and 12.94 vs 6–9 months for OS, respectively), and better tumor control in the sonication field was observed. Another clinical trial is underway to evaluate the safety and feasibility of BBBD using the SonoCloud-1 device in patients with melanoma brain metastases (study no. NCT04021420). The SonoCloud-9 device has been designed to sonicate the tumor and surrounding infiltrative region for patients with GBM. The device, with 9 cm–diameter transducers arranged on an implantable grid, is currently being assessed in an international multicenter clinical trial in patients with recurrent GBM (study no. NCT03744026). The SONOKID trial is planned to start in 2020 in Paris (France), and will assess the safety and feasibility of BBBD using the SonoCloud-1 device in association with intravenous carboplatin chemotheraphy in recurrent supratentorial malignant primitive tumors (any histological type) in the pediatric population. It will be the first clinical trial on US-induced BBBD in the pediatric population.

Both extracranial and implantable devices have advantages and drawbacks. Extracranial devices are noninvasive, and can focus on deep and variable targets in the brain, but they imply shaving of patients, and long and immobile procedures (2–4 hours); sonication is limited to small brain volumes (1–4 cm³), with difficulties in targeting superficial lesions. Implantable devices allow for fast procedures (4–15 minutes) and BBBD in larger volumes (4–140 cm³), but they imply the device has to be implanted during a tumor debulking or biopsy surgical procedure and the targeted volume is fixed in a 1 device/volume manner. Thus, clinical use of these devices in the future may be complementary, depending on the particular indication to be treated. Large, superficial, or infiltrative lesions such as extensive high-grade glioma or DIPG may be good targets for implantable devices, while smaller and deep-seated lesions, such as hypothalamic or basal ganglia lesions, may be ideally treated with extracranial devices.

Conclusions and Perspectives in Pediatric Neurooncology

Although there have been significant advances in understanding the biology of pediatric brain tumors, the treatment of these rare neoplasms is still challenging for neurooncologists and neurosurgeons, in part due to limited drug delivery through the BBB. BBBD with LIPI may be a method to overcome this limitation. This technique has many advantages compared to other strategies: 1) non- or minimal invasiveness; 2) local and targeted disruption; 3) possible targeting of both superficial and deep lesions; 4) transient disruption; 5) possible delivery of large molecules or immune cells; and 6) proven safety in preclinical and clinical studies. BBBD with LIPI has recently entered clinical trials in adults, with encouraging results, and clinical trials assessing the feasibility and safety of the technique in the pediatric population are planned to begin in the coming year. It will be the first step toward treatment of pediatric brain tumors with this technique in association with standard drugs, and emerging therapies such as targeted or immune therapies.

Different treatment protocols have been described in preclinical studies, with variable results, and both delivery before and after sonication have been assessed in clinical trials. The optimal treatment schedule is likely therapy dependent. This implies taking into account the type of treatment (cell, molecule), the pharmacokinetics of the drug (if any) and its route of delivery (oral, intravenous), and the formulation of the treatment (loaded-microbubble, liposome). Thus, each treatment protocol will have to be adapted to the agent delivered.

Moreover, some obstacles specific to the pediatric population will have to be overcome. Skull bone is expected to be similar or have less attenuation to US in children compared with adults, therefore the same transcranial US systems can likely be used. The feasibility of transcranial US ablation of centrally located tumors in pediatric patients performed with the Insightec ExAblate 4000 system is being evaluated (study no. NCT03028246). The thinner skull bone could be compensated for by adjusting the geometry of implantable devices and placing silicon spacers between the bone and the transducer. Anatomical considerations for deep-seated and posterior fossa lesions have to be taken into consideration with implantable devices. The US emitter shape and frequency can be optimized to efficiently cover large and deep tumor areas, and design of the transducer and the connection system will need adjustment for implantation in the posterior fossa due to the orientation of the occipital bone and the cervical muscle insertions. Neuronavigation systems may be needed to accurately insert the devices, especially for those targeting the brainstem. The lengthy procedure needed with extracranial systems can be a limitation in young children. Although constraining, general anesthesia is feasible under certain conditions without interfering with US-mediated disruption, as shown in preclinical studies. The overall transcranial treatment duration can also be reduced with advanced real-time monitoring and rapid electronic beam steering techniques or by designing a single-element US emitter with a relatively large focal size associated with neuronavigation.

Acknowledgments

We acknowledge graphic designer Quentin Beccaria for his help in creating Figures 1 and 2.
References
1. Alkins R, Burgess A, Ganguly M, Francia G, Kerbel R, Wels WS, et al: Focused ultrasound delivers targeted immune cells to metastatic brain tumors. Cancer Res 73:1892–1899, 2013
2. Alkins R, Burgess A, Kerbel R, Wels WS, Hynynen K: Early treatment of H3R2-amplified brain tumors with targeted NK-92 cells and focused ultrasound improves survival. Neuro Oncol 18:974–981, 2016
3. Aryal M, Fischer K, Gentile C, Gitto S, Zhang YZ, McDaniel N: Effects on P-glycoprotein expression after blood-brain barrier disruption using focused ultrasound and microbubbles. PLoS One 12:e0166061, 2017
4. Bartels U, Hawkins C, Vezina G, Kun L, Souweidane M, Bouffet E: Proceedings of the diffuse intrinsic pontine glioma (DIPG) Toronto Think Tank: advancing basic and translational research and cooperation in DIPG. J Neurooncol 105:19–125, 2012
5. Beccaria K, Canney M, Goldwirt L, Fernandez C, Adam P, Ciquet J, et al: Opening of the blood-brain barrier with an unfocused ultrasound device in rabbits. J Neurosurg 119:887–898, 2013
6. Beccaria K, Canney M, Goldwirt L, Fernandez C, Piquet J, Perier MC, et al: Ultrasound-induced opening of the blood-brain barrier to enhance temozolomide and intrathecal delivery: an experimental study in rabbits. J Neurosurg 124:1602–1610, 2016
7. Bode U, Zimmermann M, Moser O, Rutkowski S, Warmuth-Metz M, Pietsch T, et al: Treatment of recurrent primitive neuroectodermal tumors (PNET) in children and adolescents with high-dose chemotherapy (HDC) and stem cell support: results of the HITREZ 97 multicentre trial. J Neurooncol 120:635–642, 2014
8. Burgess A, Ayala-Grosso CA, Ganguly M, Jordao JF, Aubert T, Scherrmann JM: Role of ABC transporters in the chemoresistance of human gliomas. Cancer Res 79:433–445, 2009
9. Crake C, Brinker ST, Coviello CM, Livingstone MS, McDannold NJ: 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, 2018
10. Dangouloff-Ros V, Varlet P, Levy R, Beccaria K, Puget S, Dufour C, et al: Imaging features of medulloblastoma: conventional imaging, diffusion-weighted imaging, perfusion-weighted imaging, and spectroscopy: from general features to subtypes and characteristics. Neurochirurgie [epub ahead of print], 2018
11. Decléves X, Amiel A, Delattre JY, Scherrmann JM: Role of ABC transporters in the chemoresistance of human gliomas.Curr Cancer Drug Targets 6:433–445, 2006
12. Downs ME, Buch A, Sierra C, Karakatsani ME, Teichert T, Chen S, et al: 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, 2015 (Erratum in PLoS One 10:e0130860, 2015)
13. Dréan A, Lemaire N, Bouchoux G, Goldwirt L, Canney M, Goli L, et al: 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, 2019
14. El-Khouly FE, van Vuurden DG, Stroink T, Hulleman E, Kaspers GJL, Hendrikse NH, et al: Effective drug delivery in diffuse intrinsic pontine glioma: a theoretical model to identify potential candidates. Front Oncol 7:254, 2017
15. Fan CH, Ting CY, Chang YC, Wei KC, Liu HL, Yeh CK: Drug-loaded bubbles with matched focused ultrasound excitation for concurrent blood-brain barrier opening and brain-tumor drug delivery. Acta Biomater 15:89–101, 2015
16. Ginguéucé C, Champier J, Maalem S, Strazielle N, Jouve A, Fèvre-Montanges M, et al: P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2) localize in the microvesels forming the blood-tumor barrier in ependymomas. Brain Pathol 20:926–935, 2010
17. Greenberg ML, Fisher PG, Freeman C, Korones DN, Bernstein M, Friedman H, et al: Etoposide, vincristine, and cyclosporin A with standard-dose radiation therapy in newly diagnosed diffuse intrinsic brainstem gliomas: a pediatric oncology group phase I study. Pediatr Blood Cancer 45:644–648, 2005
18. Hargrave D: Pediatric diffuse intrinsic pontine glioma: can optimism replace pessimism? CNS Oncol 1:137–148, 2012
19. Hargrave D, Bartels U, Bouffet E: Diffuse brainstem glioma in children: critical review of clinical trials. Lancet Oncol 7:241–248, 2006
20. Heiss JD, Jamshidi A, Shah S, Martin S, Wolters PL, Argeringer DP, et al: Phase I trial of convection-enhanced delivery of IL13-Pseudomonas toxin in children with diffuse intrinsic pontine glioma. J Neurosurg Pediatr 23:333–342, 2018
21. Horodyckid C, Canney M, Vignot A, Boisgard R, Drier A, Huberfeld G, et al: 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, 2017
22. Hynynen K, McDannold N, Sheikov NA, Jolesz FA, Vykhodtseva N: Local and reversible blood-brain barrier disruption by noninvasive focused ultrasound at frequencies suitable for trans-skull sonication. Neuroimage 24:12–20, 2005
23. Hynynen K, McDannold N, Vykhodtseva N, Jolesz FA: Noninvasive MR imaging-guided focal opening of the blood-brain barrier in rabbits. Radiology 220:640–646, 2001
24. Idbaih A, Canney M, Belin L, Desseaux C, Vignot A, Bouchoux G, et al: 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, 2019
25. Johnson KJ, Cullen J, Barnholtz-Sloan JS, Ostrom QT, Langer CE, Turner MC, et al: Childhood brain tumor epidemiology: a brain tumor epidemiology consortium review. Cancer Epidemiol Biomarkers Prev 23:2716–2736, 2014
26. Jones RM, Deng L, Leung K, McMahon D, O’Reilly MA, Hynynen K: Three-dimensional transcranial microbubble imaging for guiding volumetric ultrasound-mediated blood-brain barrier opening. Theranostics 8:2909–2926, 2018
33. Kinoshita M, McDannold N, Jolesz FA, Hynynen K: Noninvasive localized delivery of Herceptin to the mouse brain by MRI-guided focused ultrasound-induced blood-brain barrier disruption. *Proc Natl Acad Sci U S A* 103:11719–11723, 2006

34. Kobus T, Vykhotdeva N, Pilatou M, Zhang Y, McDannold N: Safety validation of repeated blood-brain barrier disruption using focused ultrasound. *Ultrasound Med Biol* 42:481–492, 2016

35. Kovacs ZI, Kim S, Jitkaria N, Qureshi F, Milo B, Lewis BK, et al: Disrupting the blood-brain barrier by focused ultrasound induces sterile inflammation. *Proc Natl Acad Sci U S A* 114:E75–E84, 2017

36. Lastowska M, Jurkiewicz E, Trubicka J, Daszkiewicz P, Kobus T, Zervantonakis IK, Zhang Y, McDannold N: Blood-brain barrier disruption using focused ultrasound. *J Control Release* 255:415–425, 2010

37. Mainprize T, Lipsman N, Huang Y, Meng Y, Bethune A, Meng Y, et al: Contrast enhancement pattern predicts poor survival for patients with non-WNT/SHH medulloblastoma tumors. *J Neurooncol* 123:65–73, 2015

38. Liu HL, Hsu PH, Lin CY, Huang CW, Chai WY, Chu PC, et al: Focused ultrasound enhances central nervous system delivery of bevacizumab for malignant glioma treatment. *Radiology* 281:99–108, 2016

39. Liu HL, Hua MY, Chen PY, Chu PC, Pan CH, Yang HW, et al: Blood-brain barrier disruption with focused ultrasound enhances delivery of chemotherapeutic drugs for glioblastoma treatment. *Radiology* 255:415–425, 2010

40. Mainprize T, Lipsman N, Huang Y, Meng Y, Bethune A, Ironside J, et al: Blood-brain barrier opening in primary brain tumors with non-invasive MR-guided focused ultrasound: a clinical safety and feasibility study. *Sci Rep* 9:321–327, 2019

41. Marquet F, Tung YS, Teichert T, Ferrera VP, Konofagou EE: Noninvasive, transient and selective blood-brain barrier opening in non-human primates in vivo. *PLoS One* 6:e22598, 2011

42. Mata-Mbemba D, Zapotocky M, Laughlin S, Taylor MD, Ramaswamy V, Raybould C: MRI characteristics of primary tumors and metastatic lesions in molecular subgroups of pediatric medulloblastoma: a single-center study. *AJNR Am J Neuroradiol* 39:949–955, 2018

43. McDannold N, Arvanitis CD, Vykhotdeva N, Livingstone MS: 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, 2012

44. McDannold N, Clement GT, Black P, Jolesz F, Hynynen K: Transcranial magnetic resonance imaging-guided focused ultrasound surgery of brain tumors: initial findings in 3 patients. *Neurosurgery* 66:323–332, 2010

45. McDannold N, Vykhotdeva N, Hynynen K: Effects of acoustic parameters and ultrasound contrast agent dose on focused-ultrasound induced blood-brain barrier disruption. *Ultrasound Med Biol* 34:930–937, 2008

46. McDannold N, Zhang Y, Vykhotdeva N: Blood-brain barrier disruption and vascular damage induced by ultrasound bursts combined with microbubbles can be influenced by choice of anesthesis protocol. *Ultrasound Med Biol* 37:1259–1270, 2011

47. McMahon D, Hynynen K: Acute inflammatory response following increased blood-brain barrier permeability induced by focused ultrasound is dependent on microbubble dose. *Theranostics* 7:3989–4000, 2017

48. Mei J, Cheng Y, Song Y, Yang Y, Wang F, Liu Y, et al: Experimental study on targeted methotrexate delivery to the rabbit brain via magnetic resonance imaging-guided focused ultrasound. *J Ultrasound Med* 28:871–880, 2009

49. Mittappali RK, Chung AH, Parrish KE, Crabtree D, Halvorsen KG, Hu G, et al: ABCG2 and ABCB1 limit the efficacy of dasatinib in a PDGF-B-driven brainstem glioma model. *Mol Cancer Ther* 15:819–829, 2016

50. O’Reilly MA, Hynynen K: Blood-brain barrier: real-time feedback-controlled focused ultrasound disruption by using an acoustic emissions-based controller. *Radiology* 263:96–106, 2012

51. Partridge WM: Blood-brain barrier delivery. *Drug Discov Today* 12:54–61, 2007

52. Park EJ, Zhang YZ, Vykhotdeva N, McDannold N: 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, 2012

53. Phoenix TN, Patmore DM, Boop S, Boulous N, Jacus MO, Patel YT, et al: Medulloblastoma genotype dictates blood brain barrier phenotype. *Cancer Cell* 29:508–522, 2016

54. Ramaswamy V, Remke M, Bouffet E, Bailey S, Clifford SC, Doz F, et al: Risk stratification of childhood medulloblastoma in the molecular era: the current consensus. *Acta Neuro-pathol* 131:821–831, 2016

55. Raymond SB, Skoch J, Hynynen K, Bacskai BJ: Multiphoton imaging of ultrasound/Option mediated cerebrovascular effects in vivo. *J Cereb Blood Flow Metab* 27:393–403, 2007

56. Sabin ND, Merchant TE, Li X, Li Y, Klimo P Jr, Boop FA, et al: Quantitative imaging analysis of posterior fossa ependymoma location in children. *Childs Nerv Syst* 32:1441–1447, 2016

57. Sheikov N, McDannold N, Sharma S, Hynynen K: Effect of focused ultrasound applied with an ultrasound contrast agent on the right junctional integrity of the brain microvascular endothelium. *Ultrasound Med Biol* 33:1093–1104, 2008

58. Sheikov N, McDannold N, Vykhotdeva N, Jolesz F, Hynynen K: Cellular mechanisms of the blood-brain barrier opening induced by ultrasound in presence of microbubbles. *Ultrasound Med Biol* 30:979–989, 2004

59. Shen WB, Anastasiadis P, Nguyen B, Yarnell D, Yarowsky PJ, Frenkel V, et al: Magnetic enhancement of stem cell-targeted delivery into the brain following MR-guided focused ultrasound for opening the blood-brain barrier. *Cell Transplant* 26:1235–1246, 2017

60. Stevarache MA, Petersen N, Jurgens EM, Milstein ER, Rosenfeld ZB, Ballon DJ, et al: Safe and stable noninvasive focal gene delivery to the mammalian brain following focused ultrasound. *J Neurosurg* 130:989–998, 2018

61. Subashi E, Cordero FJ, Halvorsen KG, Qi Y, Nolus JC, Becher OJ, et al: Tumor location, but not H3.3K27M, significantly influences the blood-brain-barrier permeability in a genetic mouse model of pediatric high-grade glioma. *J Neurooncol* 126:243–251, 2016

62. Tanter M, Pernot M, Aubry JF, Montaldo G, Marquet F, Mink J: Compensating for bone interfaces and respiratory motion in high-intensity focused ultrasound. *Int J Hyperthermia* 23:141–151, 2007

63. Timbie KF, Afzal U, Date A, Zhang C, Song J, Wilson Miller PJ, Frenkel V, et al: Magnetic enhancement of stem cell-targeted delivery to the mammalian brain following focused ultrasound. *Ultrasound Med Biol* 33:1210–1236, 2007

64. Ting CY, Fan CH, Liu HL, Huang CY, Hsieh HY, Yen TC, et al: Concurrent blood-brain barrier opening and local drug delivery using drug-carrying microbubbles and focused ultrasound for brain glioma treatment. *Biomaterials* 33:704–712, 2012

65. Treat LH, McDannold N, Vykhotdeva N, Zhang Y, Tam K, Hynynen K: Targeted delivery of doxorubicin to the rat brain at therapeutic levels using MRI-guided focused ultrasound. *Int J Cancer* 121:901–907, 2007

66. Treat LH, McDannold N, Zhang Y, Vykhotdeva N, Hynynen K: 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, 2012

67. Tung YS, Marquet F, Teichert T, Ferrera V, Konofagou EE: Feasibility of noninvasive cavitation-guided blood-brain barrier opening using focused ultrasound and microbubbles in nonhuman primates. Appl Phys Lett 98:163704, 2011

68. Varlet P, Le Teuff G, Le Deley MC, Giangaspero F, Haberler C, Jacques TS, et al: WHO grade has no prognostic value in the pediatric high-grade glioma included in the HERBY trial. Neuro Oncol [epub ahead of print], 2019

69. Veringa SJE, Biesmans D, van Vuurden DG, Jansen MHA, Wedekind LE, Horsman I, et al: In vitro drug response and efflux transporters associated with drug resistance in pediatric high grade glioma and diffuse intrinsic pontine glioma. PLoS One 8:e61512, 2013

70. Warren K, Jakacki R, Widemann B, Aikin A, Libucha M, Packer R, et al: Phase II trial of intravenous lobradimil and carboplatin in childhood brain tumors: a report from the Children’s Oncology Group. Cancer Chemother Pharmacol 58:343–347, 2006

71. Wei KC, Chu PC, Wang HYJ, Huang CY, Chen PY, Tsai HC, et al: Focused ultrasound-induced blood-brain barrier opening to enhance temozolomide delivery for glioblastoma treatment: a preclinical study. PLoS One 8:e58995, 2013

72. Wu SY, Aurup C, Sanchez CS, Grondin J, Zheng W, Kimmura H, et al: Efficient blood-brain barrier opening in primates with neuronavigation-guided ultrasound and real-time acoustic mapping. Sci Rep 8:7978, 2018

73. Yang FY, Horng SC: Chemotherapy of glioblastoma by targeted liposomal platinum compounds with focused ultrasound. Conf Proc IEEE Eng Med Biol Soc 2013:6289–6292, 2013

74. Yang FY, Wong TT, Teng MC, Liu RS, Lu M, Liang HF, et al: Focused ultrasound and interleukin-4 receptor-targeted liposomal doxorubicin for enhanced targeted drug delivery and antitumor effect in glioblastoma multiforme. J Control Release 160:652–658, 2012

75. Zeng HQ, Lü L, Wang F, Luo Y, Lou SF: Focused ultrasound-induced blood-brain barrier disruption enhances the delivery of cytarabine to the rat brain. J Chemother 24:358–363, 2012

Disclosures
M. Canney and G. Bouchoux are employees 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 of intellectual property related to the SonoCloud device that has been licensed to CarThera. A. Carpentier and M. Canney have ownership interest in CarThera.

Author Contributions
Conception and design: Beccaria. Acquisition of data: Beccaria. Analysis and interpretation of data: Beccaria, Canney. Drafting the article: Beccaria. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Beccaria. Study supervision: Beccaria.

Correspondence
Kévin Beccaria: Necker-Enfants Malades Hospital, Paris, France. kevbeccaria@gmail.com.