Safety and Feasibility of Repeated and Transient Blood-Brain Barrier Disruption by Pulsed Ultrasound in Patients with Recurrent Glioblastoma

Ahmed Idbaih[1], Michael Canney[2], Lisa Belin[4], Carole Desseaux[2], Alexandre Vignot[2], Guillaume Bouchoux[2], Nicolas Asquier[2,6], Bruno Law-Ye[3], Delphine Leclercq[3], Anne Bissery[5], Yann De Rycke[5], Clementine Trosch[7], Laurent Capelle[7], Marc Sanson[1], Khe Hoang-Xuan[1], Caroline Dehais[1], Caroline Houillier[1], Florence Laigle-Donadey[1], Bertrand Mathon[7], Arthur André[7], Cyril Lafon[6], Jean-Yves Chapelon[6], Jean-Yves Delattre[1], Alexandre Carpentier*[7,8]

[1]Sorbonne Université, Inserm, CNRS, UMR S 1127, Institut du Cerveau et de la Moelle épinière, ICM, AP-HP, Hôpitaux Universitaires La Pitié Salpêtrière - Charles Foix, Service de Neurologie 2-Mazarin, F-75013, Paris, France

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

[3]AP-HP, Hôpitaux Universitaires La Pitié-Salpêtrière–Charles Foix, Service de Neuroradiologie, F-75013 Paris, France

[4]Sorbonne Université, AP-HP, Hôpital Pitié-Salpêtrière, Département Biostatistique Santé Publique et Information Médicale, INSERM, CIC-1421, Paris, France

[5]Clinical Research Unit, Assistance Publique–Hôpitaux de Paris (AP-HP), Hôpitaux Universitaires La Pitié-Salpêtrière, F-75013 Paris, France

[6]LabTAU, INSERM, Centre Léon Bérard, Université Lyon 1, Univ Lyon, F-69003, LYON, France

[7]Assistance Publique–Hôpitaux de Paris (AP-HP), Hôpitaux Universitaires La Pitié-Salpêtrière, Service de Neurochirurgie, F-75013 Paris, France

[8]Sorbonne Université, UPMC Univ Paris 06, F-75013 Paris, France

***Corresponding author**:

Alexandre Carpentier MD PhD (alexandre.carpentier@aphp.fr)

Chairman, Department of Neurosurgery
Pierre & Marie Curie Professor of Neurosurgery, Sorbonne University School of Medicine

Hôpital de la Pitié-Salpêtrière

Batiment Babinski, 47-83 Boulevard de l'Hopital

75013 Paris, FRANCE

Phone: +33 1 4216 3405

Fax: +33 1 4216 3495

Financial support: Assistance Publique–Hôpitaux de Paris (AP-HP) University Hospital La Pitié-Salpêtriere, CarThera, and Banque Publique d'Investissement (BPI).

Conflict of Interest: A.I. reports travel support from CarThera and personal fees from Bristol-Myers Squibb, Hoffmann-La Roche, and Cipla. M.S. received travel grants from Abbvie and personal fees from Abbvie and Genenta. A.C., M.C., C.L., and J.Y-C. have ownership in CarThera, a spin-off from Paris Sorbonne University and AP-HP. A.C., M.C., C.L. have submitted a patent application on the technology via Sorbonne Université, and AP-HP, which licensed it to CarThera. M.C. and A.C. are paid consultants to CarThera. C.L. and J.Y-C. received financial support from CarThera to support research on related topics. C.D., A.V., G.B., and N.A. are employees of CarThera.

Running Title: Blood-Brain Barrier Disruption by Ultrasound in GBM

Keywords: Glioblastoma, Brain Tumor, Blood-brain Barrier, Ultrasound, Carboplatin

Word Count: 3993

Number of Figures: 4

Number of Tables: 2

Translational Relevance

Numerous clinical trials with new drug therapies and/or combination treatments in glioblastoma (GBM) patients have failed over the past several decades. The last drug to significantly improve overall survival in a randomized trial was temozolomide, which was introduced 20 years ago. One of the major limitations of new GBM therapies is their penetration within the tumor and surrounding region due to the presence of the blood-brain barrier (BBB). This study was designed to evaluate a new technique for improving drug penetration into the tumor and infiltrative regions using pulsed ultrasound to first transiently disrupt the BBB. This work demonstrates that a new technique for treating GBM patients is safe and not burdensome in a cohort of 21 patients. The pulsed ultrasound add-on treatment presented herein can be extended and used with numerous other existing and novel drug therapies to enhance drug penetration in GBM patients.

ABSTRACT

Purpose: The blood-brain barrier (BBB) limits the efficacy of drug therapies for glioblastoma (GBM). Pre-clinical data indicate that low-intensity pulsed ultrasound (LIPU) can transiently disrupt the BBB and increase intracerebral drug concentrations.

Experimental Design: A first-in-man, single-arm, single-center trial (NCT02253212) was initiated to investigate the transient disruption of the BBB in patients with recurrent glioblastoma (rGBM). Patients were implanted with a 1 MHz, 11.5-mm diameter cranial ultrasound device (SonoCloud-1, CarThera, Paris, France). The device was activated monthly to transiently disrupt the BBB before intravenous (IV) carboplatin chemotherapy.

Results: Between 2014 and 2016, 21 patients were registered for the study and implanted with the SonoCloud-1; 19 patients received at least one sonication. In 65 US sessions, BBB disruption was visible on T1w MRI for 52 sonications. Treatment-related adverse events observed were transient and manageable: a transient edema at H1 and at D15. No carboplatin-related neurotoxicity was observed. Patients with no or poor BBB disruption (*n*=8) visible on MRI had a median progression-free survival (PFS) of 2.73 months, and a median overall survival (OS) of 8.64 months. Patients with clear BBB disruption (*n*=11) had a median PFS of 4.11 months, and a median OS of 12.94 months.

Conclusion: SonoCloud-1 treatments were well tolerated and may increase the effectiveness of systemic drug therapies, such as carboplatin, in the brain without inducing neurotoxicity.

INTRODUCTION

Glioblastoma (GBM) is the most aggressive diffuse glioma of astrocytic lineage with an annual incidence of three per 100,000 people. The standard of care—maximal safe surgical resection followed by radiotherapy with concomitant and maintenance temozolomide chemotherapy—was introduced in 2005 and has been shown to extend overall survival (OS) from 12 to 15 months (1). The introduction of the tumor-treating field device has recently been shown to further extend OS to 20.5 months in newly diagnosed patients not progressing after chemoradiation therapy (2).

At recurrence, a range of treatment options are available and include additional debulking surgery, if possible, with administration of chemotherapies such as nitrosoureas with/without antiangiogenic therapy using bevacizumab. These additional treatment strategies have not been standardized and results in randomized clinical trials have failed to show a significant survival benefit, leading many patients to participate in clinical trials of investigational therapies (3–5).

GBM is a diffuse and infiltrative tumor, making complete surgical resection impossible. At recurrence, a range of treatment options are available and include additional debulking surgery, if possible, with administration of chemotherapies such as nitrosoureas with/without antiangiogenic therapy using bevacizumab. In infiltrative regions, where the BBB remains intact, intravenously administered drugs do not consistently reach adequate therapeutic concentrations in the brain. Results of clinical trials of both new and existing drug therapies for recurrent GBM have failed to show a significant survival benefit, likely due to the BBB (3).

One method to enhance drug delivery to the brain is to disrupt the BBB, allowing for drug therapies to penetrate in increasing concentrations using low intensity pulsed ultrasound (LIPU) in combination with systemic administration of micron-sized bubbles (6). LIPU can be used to disrupt the BBB (7), increase the concentrations of systemically administered drug therapies in the brain parenchyma (8–14), and enhance survival in preclinical glioma models (15). LIPU has furthermore been shown to be safe in long-term studies after repeated BBB disruption in non-human primates (16–18).

In this work, results from the first safety and feasibility study using an implantable LIPU device, SonoCloud-1, are presented. The SonoCloud-1 device was used to repeatedly and temporarily disrupt the BBB in 21 patients with recurrent glioblastoma prior to carboplatin infusion. Safety and efficacy data after a 1-year follow-up period are reported.

MATERIALS AND METHODS

Study design and participants

This study is a prospective, open-label, single-center, single-arm, dose escalation, phase 1/2a clinical trial, enrolling GBM patients at any recurrence. This investigator-driven study was developed, conceived, and performed at the Assistance Publique–Hôpitaux de Paris (AP-HP) University Hospital La Pitié-Salpêtriere. All patients provided written informed consent and the study was conducted in accordance with Good Clinical Practices guidelines and the Declaration of Helsinki. Additionally, an Independent Safety Committee was formed to assure that patient safety was maintained and that current standards for clinical research were met. Approval was obtained from the French National Agency for Medicines and Health Products (ANSM) and Ile-de-France VI ethical committee (ref. CPP/38-14). The trial was registered as NCT02253212, EudraCT 2014-000393-19, and IDRCB: 2014-A00140-47. Preliminary data for the first 15 patients treated (41 sonications) in the first five cohorts up to the end of January 2016 was previously reported (19). Here, data from all 21 patients recruited into the trial (7 cohorts, 65 sonications) are reported, including follow-up of safety and efficacy.

The trial was designed as an ultrasound-escalation study, in which the US pressure was increased throughout the study, starting at 0.41 MPa and increasing to 1.15 MPa through seven different levels (0.41, 0.53, 0.66, 0.78, 0.90, 1.03, 1.15 MPa). A minimum of three patients were treated at each ultrasound pressure level.

Patients experiencing recurrence (first, second, or third) of a histologically proven *de novo* GBM, after at least the first-line standard of care (radiation with concurrent and adjuvant temozolomide) were recruited. Qualifying patients were required to have a growing contrast-enhanced tumor of less than 35 mm in diameter and be eligible for carboplatin-based chemotherapy.

Procedures

The procedures were previously described (19). The SonoCloud-1 device (**Figure 1**) was implanted within the skull bone overlying the tumor area (contrast-enhancing region or high-signal FLAIR region). If the patient was eligible for a debulking surgery under general anesthesia, the device was implanted during this surgical procedure within a burr hole after dura mater closing and before skin closure. If surgical resection was not indicated, the device was implanted during a dedicated surgical procedure in an ambulatory fashion under local anesthesia. This procedure consisted of a 3-cm skin opening, creation of a burr hole without dura mater opening, and, finally, implantation of the device and closure of the skin. In all cases, neuronavigation systems could be used to position the device in the desired location. As a result, the transducer was in contact with the external face of the dura mater with no residual bone in between to have no distortion and no attenuation of emitted US. The US output intensity was then known from a calibration of the implant performed during its production, so no MRI monitoring during sonications was required.

Patients received US for BBB disruption followed by IV carboplatin chemotherapy every four weeks. Carboplatin was started after BBB disruption and was intravenously infused for 60 to 90 min. Carboplatin dose was calculated on the basis of the area under the curve (AUC) with the Calvert formula taking the renal function into account (20). The starting dose was AUC5, further adapted (AUC4 or AUC6) on the basis of clinical and biological monitoring. Carboplatin is prescribed as a third-line chemotherapy for GBM with limited efficacy; however, the clinical literature provides significant evidence that carboplatin is effective against gliomas when sufficient brain concentrations are reached, and without major brain toxicity at high doses (21–27).

Patients were treated monthly until Dose Limiting Toxicity (DLT), serious adverse event (SAE), or evidence of disease progression. Intra-patient dose escalation was allowed. The first treatment was performed at the initial ultrasound dose level for the inclusion group, the second treatment was performed at the next highest ultrasound dose level, and escalation was continued in subsequent cycles in the absence of toxicity. Patients were treated monthly for up to a maximum of six treatments or beyond if clinical benefit was expected by investigators or until there was evidence of tumor progression.

Dose-limiting toxicity (DLT) was defined as the occurrence of an adverse effect directly related to the US emission during the first cycle of treatment, which would include the following: a neurological deficit starting within two days after the procedure and persisting at day 15, localized brain edema not pre-existing before the procedure, occurrence of cerebral midline shift not controlled by routine treatment or requiring a salvage surgical procedure, partial epilepsy induced or enhanced after the procedure and not controlled by routine therapy, irreversible focal encephalopathy in the area of the BBB disruption, bleeding or ischemia of more than 1 cm in diameter in the area of the BBB disruption occurring within two days of the procedure, and brain herniation requiring salvage surgery. Dose level cohort size was three patients, although the first three patients were included sequentially following an accelerated titration design (28). If none of the patients experienced DLT, the next dose level was opened. If one patient experienced DLT, three additional patients would have been enrolled at the same dose level. If no additional patients experienced DLT, the dose level was increased after approval by the independent scientific monitoring committee. The maximum tolerated dose is defined as the highest dose level at which six patients are started and fewer than two experience first-course DLT.

Blood biological parameters were assessed for all patients to monitor for potential carboplatin toxicity. Patients were treated with carboplatin only when platelet counts reached >100,000 cells/ml.

All patients were assessed monthly using MRI, blood sampling, and clinical neurological evaluation. Two days before each planned US treatment, tumor status was evaluated by MRI according to the Response Assessment in Neuro-Oncology (RANO) criteria (29). If tumor progression was observed, the patient exited the trial and received alternative therapies. In the absence of tumor progression and toxicity, the BBB disruption session

was scheduled before the carboplatin infusion. A subsequent MRI exam was performed starting within 10 min after US treatment to assess BBB disruption and safety of the US procedure.

A 3.0T GE Signa MRI (GE Medical Systems) was used for the imaging exams. At each exam, standard fluid-attenuated inversion recovery (FLAIR), T1-weighted gadolinium contrast-enhanced (0.2 ml/kg, Dotarem®), susceptibility-weighted angiography (SWAN), and diffusion sequences were obtained. T1-weighted MR images were analyzed to grade the magnitude of BBB disruption observed after sonications. Four different grading stages were defined as follows, as described previously (19,30): grade 0, no BBB opening; grade 1, contrast enhancement in sub-arachnoid space; grade 2, contrast enhancement in subarachnoid space and gray matter; and grade 3, contrast enhancement in subarachnoid space, gray matter, and white matter. Two neuro-radiologists (B.L.Y, D.L.) and a neurosurgeon (A.C.) independently reviewed the grading of BBB disruption. When discordant, consensus was performed by all three readers.

The SonoCloud-1 Device

The SonoCloud-1 implant (CarThera, Paris, France) consisted of a 10-mm-diameter US emitter with a resonance frequency of 1.05 MHz, encased in an 11.5-mm-diameter biocompatible housing, as shown in **Figure 1**. The emitter was operated with a burst length of 25,000 cycles (23.8 ms) at a pulse repetition frequency of either 0.5 or 1 Hz (1.2 or 2.4% duty cycle) for a total duration of 150-270 seconds. To activate the device, a transdermal needle connection device is connected to the implant and plugged into a proprietary external radiofrequency (RF) generator. The external generator is custom-designed with a graphical user interface that guides the user step-by-step through the treatment protocol. As part of efforts to refine the calibration procedure for the SonoCloud-1, new measurements were performed, which give a more accurate and reliable estimation of the absolute nominal treatment pressures previously reported (19). These values are reported in **Supplementary Table 1**.

The sonication was initiated at the beginning of a bolus injection of SonoVue microbubbles (Bracco, Milan, Italy). The initial clinical protocol calls for a dose of SonoVue corresponding to 0.1 ml/kg with a maximum dose of 4.8 ml (one vial of SonoVue). After 18 treatments in nine patients who showed limited BBB disruption, the Agence Nationale de Sécurité du Médicament et des produits de santé (ANSM) authorized an increase in SonoVue based on the weight of the patient to 0.1 ml/kg.

Outcomes

The primary objective of the study was to evaluate the safety and tolerance to sonication with the SonoCloud-1 device and to determine the Maximum Tolerated Dose of US. The secondary objectives are: (i) to evaluate the disruption of the BBB using the SonoCloud-1 system, (ii) to estimate the Progression-Free Survival and the Overall Survival of the patients treated with SonoCloud-1 device, (iii) to determine the biocompatibility of the device, and (iv) to document the practical feasibility for future clinical trials.

Statistical Analysis

Qualitative variables were described by frequencies and quantitative variables were described by their medians with range. Overall survival (OS) was defined as the time elapsed between the date of inclusion and the date of death for any cause. Progression-free survival (PFS) was defined as the time elapsed between the date of inclusion and the date of progression according to the RANO criteria or death. Patients still alive without progression were censored at their last known contact date. Survival functions were estimated using the Kaplan-Meier method, survival rates and 95% confidence interval were provided using Greenwood variance. To study the prognostic value of BBB disruption on PFS and OS, BBB disruption was used as time dependent variable in a Cox model to estimate the hazard ratio and its 95% confidence interval. Graphical representation of this result has been obtained using the Simon and Makuch estimate of survival curves (31).

RESULTS

Patient enrolment and treatment characteristics

Between July 2014, and September 2016, 21 patients were enrolled in the trial (**Table 1**). Two patients were judged ineligible after implantation due to radionecrosis (*n*=1) and detection of microhemorrhages on pre-sonication MRI (*n*=1). In the 19 remaining patients, the 11.5-mm SonoCloud-1 US device was implanted within the skull bone either during a planned debulking procedure (*n*=12) or in a dedicated procedure (*n*=9).

The procedure and US device were well-tolerated by patients. The entire BBB disruption procedure from the needle connection, preparation of microbubbles, and sonication procedure lasted a median duration of 9 minutes (range=4-16 minutes). The only minimal irritation was some pain reported during the transdermal needle connection process (median visual analog scale pain was 2 out of a range of 0-7). No patients complained of the device implantation (median visual analog scale pain was 3 out of a range of 0-5) and none of them asked for device removal after progression/end of treatment.

Safety of repeated BBB disruption

A total of 65 sonication procedures were performed in 19 patients. The median number of monthly sonications per patient was three, with a range of 1-10 sonications. No DLTs were reported during the study, even in the patients sonicated at a maximum ultrasound dose of 1.15 MPa. Neurological deficits (Common Terminology Criteria for Adverse Events, CTCAE grade 2) did appear within two days after US in one patient treated at 0.90 MPa and in another treated at 1.03 MPa, but these events resolved within 15 days. Thus, the maximum dose of US was not reached in the study.

Table 2 provides a full summary of adverse events (AEs) reported in sonicated patients. Sixty-seven percent (67%) of the AEs reported during the study were of CTCAE grade 1 or 2. The most frequently reported AEs include hematological disorders (32%) and general complaints, such as fatigue (23%). Nervous system disorders which represent 19% of overall events include headaches (26%), brain edema (11%) and faintness (11%). No severe neurological adverse events occurred during or after the sonications, even in patients sonicated in eloquent zones. A transient neurological deficit (transient facial palsy) occurred immediately after sonication at cycles 7-10 in Patient 15 at an acoustic pressure of 0.90 MPa but resolved within two hours under steroids. Patients with no history of seizures did not present any US-induced epileptic seizures and did not require any anti-epileptic drugs. No patients complained of the device implantation or the treatment process. A few patients complained of pain during the needle insertion. Nine patients were explanted at the end of 6 months as requested by the protocol, 12 patients asked to not remove the device even after treatments were finished. No AE had been documented during explantation. One patient had a device that had technical issues, and which was replaced during a dedicated surgical procedure (Patient 19). After device replacement, the patient resumed treatments.

Two occurrences of transient edema occurred (Grade 4) and were considered by the Data Safety Monitoring Board (DSMB) as related to the procedure. Patient 14 reported a local, transient, steroid-responding edema at 15 days after sonication (cycle 1, 0.78 MPa) and was hospitalized. It resolved within 48 hours after the event. Patient 19 had transient, steroid-responding edema at 1 hour after sonication (cycle 1, 1.03 MPa) that resolved within two hours. In both cases, the edema was resolved within several hours of the event and steroid levels returned to pre-sonication levels. Neither of these patients underwent additional surgical debulking/resection of the tumor during device implantation and both patients had a large residual tumor diameter of >30 mm. One death other than for disease progression occurred during the trial. Patient 10, who received four US treatments (from 0.66 to 0.90 MPa) followed by carboplatin at AUC5, died from systemic B-cell lymphoma one year after the last sonication.

Overall, the US treatment followed by carboplatin was well-tolerated. Carboplatin infusion was started at an average of 106 min after sonications (range 24-185 minutes). The dose of carboplatin (mg/mL/min) was AUC4 in 16 procedures and AUC5 in 48 procedures. One patient did not receive carboplatin after BBB disruption. (S)AEs tended to be related to tumor progression or known side effects of carboplatin chemotherapy. No US dose dependence or cumulative toxicity of repeated sonications was observed in events reported.

Efficacy

A representative image of a Grade 3 BBB disruption is shown in **Figure 2** for Patient 15. This patient received a total of 10 sonications over the course of 12 months at an acoustic pressure of 0.90 MPa. At each sonication, the BBB was repeatedly disrupted on MRI and was re-closed in appearance on each subsequent pre-sonication MRI.

Out of the 65 sonication procedures performed in 19 patients, 52 showed evidence of BBB disruption on post T1w contrast-enhanced imaging (grade 1-2-3) and 34 showed evidence of at least grade 2 BBB disruption. The degree of BBB disruption increased with acoustic pressure as 0% (0.41 MPa), 0% (0.53 MPa), 18% (0.66 MPa), 57% (0.78 MPa), 80% (0.90 MPa), 77% (1.03 MPa), and 66% (1.15 MPa) of sonications show at least a grade 2-3 of BBB disruption (**Supplementary Table 2**). No decrease in BBB disruption was observed after repeated monthly treatments (with up to 10 treatments for two patients).

Representative FLAIR and SWAN MR images for Patients 15 and 18 are shown in **Figure 2**. Patient 15 showed no changes in SWAN or FLAIR MR images after repeated disruption of the BBB at an acoustic pressure of 0.90 MPa. Patient 18 received three sonications—one at 1.03 MPa and two at the highest acoustic pressure of 1.15 MPa. No evidence of additional edema or micro-bleeding was observed on post-sonication MRIs in this patient or any others treated at the highest acoustic pressures.

Overall median PFS for all sonication patients (*n*=19) was 3.45 months while overall OS was 10 months (**Supplementary Table 3**). Sonicated patients who had clear BBB disruption extending beyond the subarachnoid space (Grade 2/3 BBB disruption after sonication on MRI) had a trend toward longer PFS and OS. As shown in **Figure 3**, Patients with Grade 2/3 disruption had a PFS of 4.11 months versus a PFS of 2.73 months in patients with a Grade 0/1 opening. Overall survival (OS) was increased to 12.94 months from 8.64 months in patients that had clear BBB disruption. The hazard ratio for PFS with clear BBB disruption after at least one sonication group was 0.39 (95% CI [0.11 ; 0.94], p = 0.03) and 0.49 (95% CI [0.16 ; 1.14], p = 0.09) for survival.

DISCUSSION

Historically, recurrent GBM patients receiving additional chemotherapy have had limited additional benefit (32). In studies of GBM patients treated by carboplatin monotherapy, PFS of 2-3 months and OS of 6-9 months were reported (21–26). The PFS and OS results for patients experiencing minimal or no BBB disruption (Grade 0/1, median PFS=2.73 months, median OS=8.64 months) align with this historical data. For patients with greater BBB disruption (Grade 2/3), median PFS and OS were extended to 4.11 months and 12.94 months, respectively. The results herein suggest a potentially greater efficacy of carboplatin when used in combination with US-induced disruption of the BBB, though results are limited by the small number of patients in this trial receiving treatment (*n*=19) and must be confirmed in a larger clinical trial.

To our knowledge, the study results herein are the only reported safety results showing large regions of repeated BBB disruption using this technique in GBM patients. In our previous publication (19), the interim safety results from 41 sonications in 15 patients were reported. Herein, we completed the recruitment of this study with 21 patients recruited and 65 sonications performed in 19 of these patients. These results further confirm the safety of BBB disruption using LIPU prior to carboplatin infusion and point towards the potential efficacy of this technique, as shown in **Figure 3**.

It has been estimated that 90% of recurrences for GBM are within 2 cm of the primary site (33). When progression was observed on MRI in FLAIR or contrast-enhanced T1w sequences, it tended to occur outside the zone of the acoustic field of the US implant, as shown in **Figure 2** and **Figure 4**. This sonication volume was generally not large enough to cover the entire tumor and infiltrative region. Nevertheless, as shown in **Figure 4**, repeated sonications in some patients showed tumor reduction in the field of the SonoCloud-1 implant. This effect was quantified in Patient 19, who received 10 monthly sonications prior to receiving carboplatin chemotherapy. Pre-ultrasound contrast-enhanced T1 hypersignal (+15% threshold) volume was measured inside a 25-mm diameter cylindrical zone around the implant axis (emitter ROI), and outside this zone. Compared to the first treatment, the hypersignal volume decreased progressively up to treatment 8 by 49%

inside the sonication ROI, and by only 2% outside this volume. This indicates that tumor progression may have been controlled more efficiently in the sonicated zone and its surroundings, which corresponds well to the zone with enhanced carboplatin concentration observed in primates treated with the SonoCloud device (34). In future studies, a device with a larger sonication area is being developed to improve the efficacy of this approach and to enhance drug delivery to a larger region of the brain.

An additional limitation of the current study was the time from sonication to start of carboplatin infusion (mean=106 min), which was longer than initially planned for in the clinical protocol (60 min max). Although the BBB has been shown to close in 6 to 24 hours (35), partial closing begins immediately after sonication, thus the ideal time to begin chemotherapy is immediately after sonications. In future clinical trials, chemotherapy will be started immediately after sonications as a post-sonication MRI will no longer be necessary to verify safety.

The safety data from our study in GBM patients has been used to initiate an additional study in Alzheimer's Disease (AD) patients (NCT03119961) at the optimal acoustic pressures reported here (0.90 MPa – 1.03 MPa). Although a lower proportion of Grade 3 BBB disruption was reported at the highest pressure levels (Supplementary Table 2), these findings were primarily due to a single patient that had a large residual tumor that obscured grading and the optimal acoustic pressure was determined to be 1.03 MPa. BBB disruption by US without additional drug administration has been shown to reduce beta-amyloid and tau pathology in animal models of AD. Recently, a team using a transcranial focused ultrasound approach has shown the safety of this technique in five patients with AD (36). Future studies will further evaluate the value of this technique in additional brain indications as well as in a larger group of patients, potentially using other drug therapies.

ACKNOWLEDGMENTS

We thank AP-HP for sponsoring P120905 and funding the clinical trial with the support of CarThera. We would like to thank the members of the data monitoring committee, which included the clinical research unit Groupe Hospitalier Pitié-Salpêtrière consisting of M. Bourgoin, N. Mansour, L. Gambotti,, A. Mallet, F. Tubach; members of the Direction de la Recherche Clinique consisting of C. Hoffart-Jourdain, M. Dabbech, A. Bergera, A. Souag, S. Dalibey and P.-A. Jolivot; and members of the Independent Scientific Committee consisting of S. Katsahian (methodologist and president), J.-S. Guillamo (neurologist and vice president), S. Pujet (neurosurgeon), J. Savatovsky (neuro-radiologist), C. Fernandez (pharmacist), and A. Grandvuillemin (pharmacist and pharmacovigilance).

AUTHORS' CONTRIBUTIONS

A.I. was principal investigator of the trial; A.C. invented the SonoCloud device; A.C., L.C., C.T., B.M., and A.A. performed the device implantations and assisted in BBB disruption treatments; C.T. assisted with sonications; A.C., M.C., C.D., L.B., and A.I. wrote the manuscript; M.C., A.V., G.B., A.C., C.L., and J-Y.C. developed the technology; D.L. and B.L-Y. assisted in MRI data acquisition, interpretation and analysis; N.A. and G.B. assisted in MR data processing; J-Y.D., M.S., A.I., K-H.X., C.D., C.H., F.L-D. assisted in patient recruitment; A.V. and C.D. prepared technical and regulatory documentation of the medical device; L.B., A.B., and Y.R. performed data analysis; A.I. served as principal investigator for the trial. All authors approved the final version of the paper.

REFERENCES

1. Stupp R, Mason WP, Van Den Bent MJ, Weller M, Fisher B, Taphoorn MJB, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. Mass Medical Soc; 2005;352:987–96.

2. Stupp R, Taillibert S, Kanner AA, Kesari S, Steinberg DM, Toms SA, et al. Maintenance therapy with tumor-treating fields plus temozolomide vs temozolomide alone for glioblastoma: a randomized clinical trial. JAMA. 2015;314:2535–43.

3. Vanderbeek AM, Rahman R, Fell G, Ventz S, Chen T, Redd R, et al. The clinical trials landscape for glioblastoma: Is it adequate to develop new treatments? Neuro Oncol. 2018;20:1034–43.

4. Brown CE, Badie B, Barish ME, Weng L, Ostberg JR, Chang WC, et al. Bioactivity and safety of IL13Rα2-redirected chimeric antigen receptor CD8+T cells in patients with recurrent glioblastoma. Clin Cancer Res. 2015;21:4062–72.

5. Wick W, Fricke H, Junge K, Kobyakov G, Martens T, Heese O, et al. A phase II, randomized, study of weekly APG101+reirradiation versus reirradiation in progressive glioblastoma. Clin Cancer Res. 2014;20:6304–13.

6. McDannold N, Vykhodtseva N, Hynynen K. Effects of acoustic parameters and ultrasound contrast agent dose on focused-ultrasound induced blood-brain barrier disruption. Ultrasound Med Biol. 2008;34:930–7.

7. Hynynen K, McDannold N, Vykhodtseva N, Jolesz FA. Noninvasive MR Imaging–guided Focal Opening of the Blood-Brain Barrier in Rabbits. Radiology. 2001;220:640–6.

8. Beccaria K, Canney M, Goldwirt L, Fernandez C, Piquet J, Perier MC, et al. US-Induced Opening of the Blood-Brain Barrier to Enhance Temozolomide and Irinotecan Delivery: A Preclinical Study in Rabbits. J Neurosurg. 2016;124:1602–10.

9. Wei K-C, Chu P-C, Wang H-YJ, Huang C-Y, Chen P-Y, Tsai H-C, et al. Focused ultrasound-induced blood-brain barrier opening to enhance temozolomide delivery for glioblastoma treatment: a preclinical study. PLoS One. 2013;8:e58995.

10. Newman WC, Amankulor NA. Focused Ultrasound Enhances Central Nervous System Delivery of Bevacizumab for Malignant Glioma Treatment. Neurosurgery. 2016;79:N12.

11. Park J, Zhang Y, Vykhodtseva N, Jolesz FA, McDannold NJ. The kinetics of blood brain barrier permeability and targeted doxorubicin delivery into brain induced by focused ultrasound. J Control Release. 2012;162:134–42.

12. Kovacs Z, Werner B, Rassi A, Sass J, Martin-Fiori E, Bernasconi M. Prolonged survival upon ultrasound-

enhanced doxorubicin delivery in two syngenic glioblastoma mouse models. J Control Release. 2014;187C:74–82.

13. 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. PNAS. 2006;103:11719–23.

14. Kobus T, Zervantonakis IK, Zhang Y, McDannold NJ. Growth inhibition in a brain metastasis model by antibody delivery using focused ultrasound-mediated blood-brain barrier disruption. J Control Release. 2016;238:281–8.

15. Treat LH, McDannold N, Zhang Y, Vykhodtseva 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. 2012;38:1716–25.

16. 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. 2017;126.

17. Downs ME, Buch A, Sierra C, Karakatsani ME, Chen S, Konofagou EE, 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. 2015;10:e0125911.

18. McDannold N, Arvanitis CD, Vykhodtseva 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. 2012;72:3652–63.

19. Carpentier A, Canney M, Vignot A, Reina V, Beccaria K, Horodyckid C, et al. Clinical trial of blood-brain barrier disruption by pulsed ultrasound. Sci Transl Med. 2016;8:343re2.

20. Calvert AH, Newell DR, Gumbrell LA, O'Reilly S, Burnell M, Boxall FE, et al. Carboplatin dosage: prospective evaluation of a simple formula based on renal function. J Clin Oncol. 1989;7:1748–56.

21. Yung WK, Mechtler L, Gleason MJ. Intravenous carboplatin for recurrent malignant glioma: a phase II study. J Clin Oncol. 1991;9:860–4.

22. Poisson M, Pereon Y, Chiras J, Delattre JY. Treatment of recurrent malignant supratentorial gliomas with carboplatin (CBDCA). J Neurooncol. 1991;10:139–44.

23. Prados MD, Warnick RE, Mack EE, Chandler KL, Rabbitt J, Page M, et al. Intravenous carboplatin for recurrent gliomas. A dose-escalating phase II trial. Am J Clin Oncol. 1996;19:609–12.

24. Lunardi P, Osman Farah J, Mastronardi L, Puzzilli F, Lo Bianco FM. Intravenous administration of high doses of carboplatin in multimodal treatment of high grade gliomas: a phase II study. Acta Neurochir. 1996;138:215–20.

25. Murray LJ, Bridgewater CH, Levy D. Carboplatin chemotherapy in patients with recurrent high-grade glioma. Clin Oncol (R Coll Radiol). 2011;23:55–61.

26. Roci E, Cakani B, Brace G, Bushati T, Rroji A, Petrela M, et al. Platinum-based chemotherapy in recurrent high-grade glioma patients: retrospective study. Med Arch. 2014;68:140–3.

27. White E, Bienemann A, Pugh J, Castrique E, Wyatt M, Taylor H, et al. An evaluation of the safety and feasibility of convection-enhanced delivery of carboplatin into the white matter as a potential treatment for high-grade glioma. J Neurooncol. 2012;108:77–88.

28. Simon R, Freidlin B, Rubinstein L, Arbuck SG, Collins J, Christian MC. Accelerated titration designs for phase I clinical trials in oncology. J Natl Cancer Inst. 1997;89:1138–47.

29. Wen PY, Macdonald DR, Reardon DA, Cloughesy TF, Sorensen AG, Galanis E, et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. J Clin Oncol. 2010;28:1963–72.

30. Asquier N, Bouchoux G, Canney M, Martin C, Law-Ye B, Leclercq D, et al. Blood-brain barrier disruption in humans using an implantable ultrasound device: quantification with MR images and correlation with local acoustic pressure. J Neurosurg. 2019;in press:DOI:10.3171/2018.9.JNS182001.

31. Simon R, Makuch RW. A non-parametric graphical representation of the relationship between survival and the occurrence of an event: Application to responder versus non-responder bias. Stat Med. 1984;3:35–44.

32. Stupp R, Wong ET, Kanner AA, Steinberg D, Engelhard H, Heidecke V, et al. NovoTTF-100A versus physician's choice chemotherapy in recurrent glioblastoma: a randomised phase III trial of a novel treatment modality. Eur J Cancer. 2012;48:2192–202.

33. Hochberg FH, Pruitt A. Assumptions in the radiotherapy of glioblastoma. Neurology. 1980;30:907–11.

34. Goldwirt L, Canney M, Horodyckid C, Poupon J, Mourah S, Vignot A, et al. Enhanced brain distribution of carboplatin in a primate model after blood-brain barrier disruption using an implantable ultrasound device. Cancer Chemother Pharmacol. 2016;77:211–6.

35. O'Reilly MA, Hough O, Hynynen K. Blood-Brain Barrier Closure Time After Controlled Ultrasound-Induced Opening Is Independent of Opening Volume. J Ultrasound Med. 2017;36:475–83.

36. Lipsman N, Meng Y, Bethune AJ, Huang Y, Lam B, Masellis M, et al. Blood–brain barrier opening in Alzheimer's disease using MR-guided focused ultrasound. Nat Commun. 2018;25:2336.

FIGURE LEGEND

Figure 1. (a) SonoCloud-1 device illustration. (b) The SonoCloud-1 implant, a 11.5 mm diameter biocompatible implant containing a 1 MHz planar ultrasound emitter, is implanted in the skull bone and connected to (c) an external RF generator using a transdermal needle. At each treatment session, the device was activated to send low intensity pulsed ultrasound for a duration of 150-270 seconds to disrupt the BBB prior to carboplatin chemotherapy.

Figure 2. MR imaging of BBB disruption and safety of repeat sonications. (top row) BBB disruption on T1w contrast-enhanced MRI at two days prior ("pre-sonication") and within 30 min after BBB disruption ("post-sonication") by pulsed ultrasound in Patient 15. This patient received a total of 10 monthly sonications to disrupt the BBB. The T1w images shown and enhancement are from the sixth sonication session. The pre-sonication T1w contrast-enhanced MRI prior to sonication 10 is also shown to illustrate the location of tumor recurrence (white arrows), which was outside the region of BBB disruption (shown in the white rectangle). (bottom row) MRI using SWAN (to show any signs of bleeding) and FLAIR (to show any signs of edema/inflammation) sequences in Patients 15 and 18 are shown. No signs of micro-hemorrhages were observed on SWAN images, including in Patient 15, who received 10 sonications. Patient 18 was treated at the highest acoustic pressure level (1.15 MPa) and did not show any signs of adverse effects.

Figure 3. Progression-free survival (PFS, left) and overall survival (OS, right) estimated by the Simon and Makuch method (31). Nineteen (19) patients were separated according to BBB disruption grade based on T1w contrast-enhanced MRI immediately after sonications, with 8 patients having Grade 0/1 ("No BBB disruption") and 11 patients having Grade 2/3 ("BBB disruption"). Overall median PFS for all patients was 3.45 months while overall median OS was 10 months. Patients with at least Grade 2/3 disruption on post-sonication MRI had a PFS of 4.11 months versus a PFS of 2.73 months in patients with a Grade 0/1 opening; OS was also increased in this group to 12.94 months from 8.64 months.

Figure 4. Tumor response to 10 repeated monthly treatments to disrupt the BBB prior to carboplatin administration (Patient 19). The monthly pre-sonication (2 days prior) T1w images are shown. Gadolinium-enhanced T1 hypersignal from the contrast-enhancing tumor (red contour) was used as a surrogate measurement of tumor volume, and decreased by up to 49% in the sonication zone (blue box), while the calculated tumor volume decreased by only 2% outside of the sonication zone.

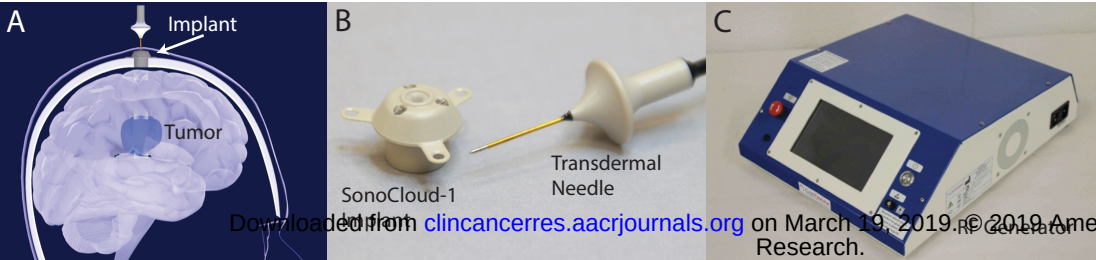
Table 1. Baseline patient characteristics, by BBB disruption grade. Data are median (range).

Characteristic	Grade 0/1 (*n*=8)	Grade 2/3 (*n*=11)	All patients (*n*=19)
Age (years)	73 (38-77)	58 (41-67)	59 (38-77)
Sex			
Men	4	9	13
Women	4	2	6
Time from initial diagnosis (months)	24.8 (14.1 – 74.9)	20.3 (8.3 – 54.2)	20.7 (8.3 – 74.9)
Recurrence #			
1	2	6	8
2	6	5	11
Karnofsky Index	80 (70-100)	90 (70-100)	90 (70-100)
Extent of surgery (at device implantation)			
None	4	4	8
Additional resection	4	7	11
Corticosteroid therapy at inclusion	2	4	6
Antiepileptic therapy at inclusion	6	6	12
Diameter of maximum enhancing tumor at inclusion (mm)	32.5 (20-35)	30 (25-35)	30 (20-35)
*IDH*1			
Wildtype	7	10	17
Mutated	1	0	1
Not done/unknown	0	1	1

IDH2			
Wildtype	8	10	18
Mutated	0	0	0
Not done/unknown	0	1	1

Table 2. Treatment-emergent adverse events which occurred during treatment or up to 30 days after the end of therapy. The occurrence of each adverse event is listed as well as the total number of patients affected as some patients might have experienced the same adverse event multiple times over the course of therapy.

	No. of Events by Grade			
***n* =19 patients**	Grade 1-2	Grade 3	Grade 4	No. of Patients
Haematological adverse events				
Thrombocytopenia	6	1	0	**5 (26%)**
Neutropenia	4			**3 (16%)**
Leucopenia	3	1		**2 (11%)**
Anemia	1			**1 (5%)**
Lymphopenia	1	1	0	**1 (5%)**
Central nervous system adverse events				
Headache	4	1		**5 (26%)**
Edema cerebral			2	**2 (11%)**
Syncope		2		**2 (11%)**
Dizziness	1			**1 (5%)**
Drowsiness	1			**1 (5%)**
Facial Palsy	3			**1 (5%)**
Ischaemic Stroke	1			**1 (5%)**
Left Sensorimotor Deficit	1			**1 (5%)**
Other adverse events				
Fatigue	8			**8 (42%)**
Nausea	3			**3 (16%)**
Urinary Urgency	2			**2 (11%)**
Vomiting	2			**2 (11%)**
Alteration of General Status			1	**1 (5%)**
Appendicitis			1	**1 (5%)**
Chemotherapy induced Phlebitis		1		**1 (5%)**
Cystic Evolution	1			**1 (5%)**
Device Failure		1		**1 (5%)**
Nausea and Vomiting	1			**1 (5%)**
Pain (transdermal needle)	1			**1 (5%)**
Papular Erythema	1			**1 (5%)**
Pulmonary Embolism			1	**1 (5%)**
Scar Tissue Pain	1			**1 (5%)**
Subdural Hygroma	1			**1 (5%)**
Tinnitus	1			**1 (5%)**



Author Manuscript Published OnlineFirst on March 19, 2019; DOI: 10.1158/1078-0432.CCR-18-3643
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

Figure 2

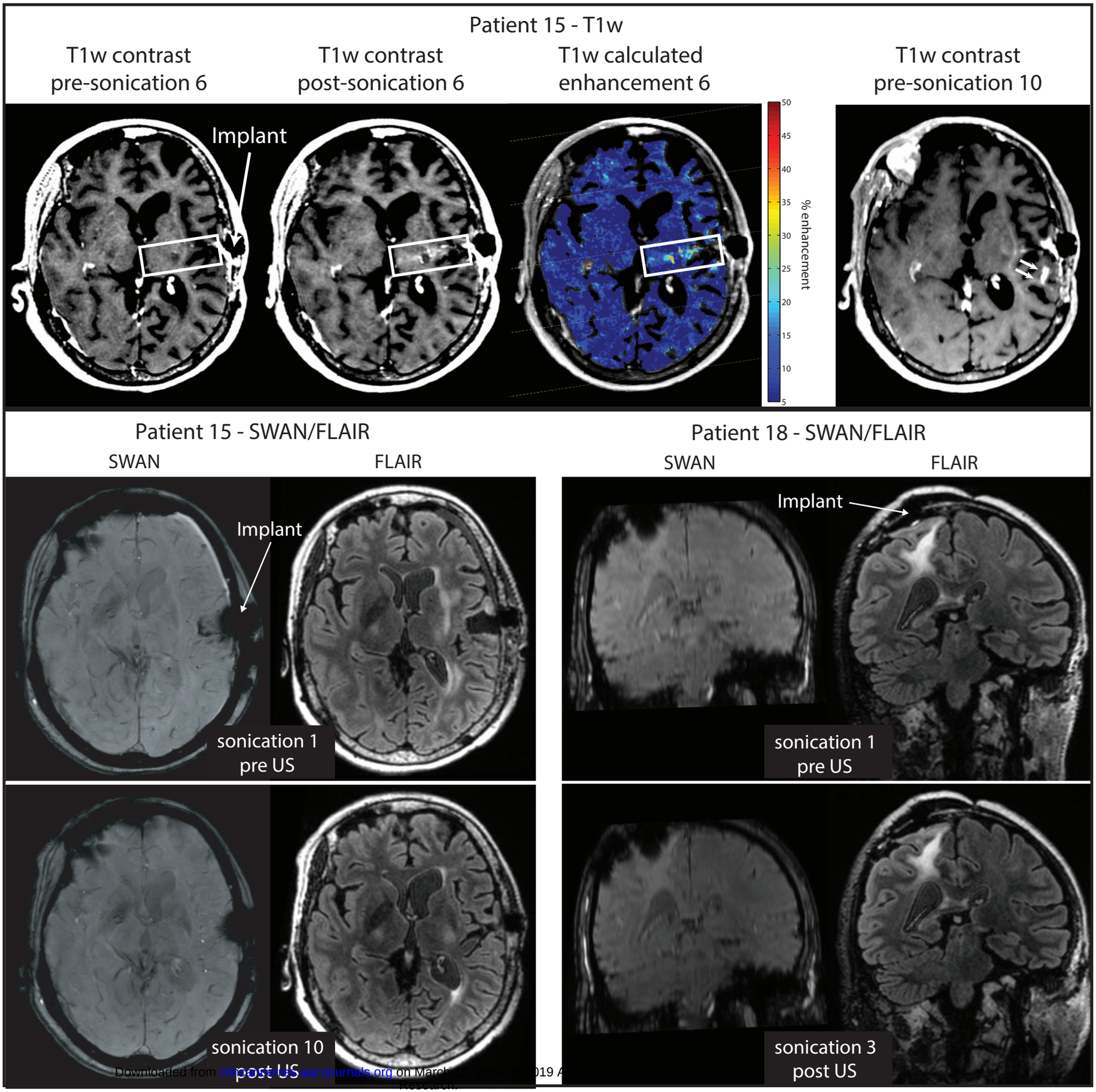


Figure 3

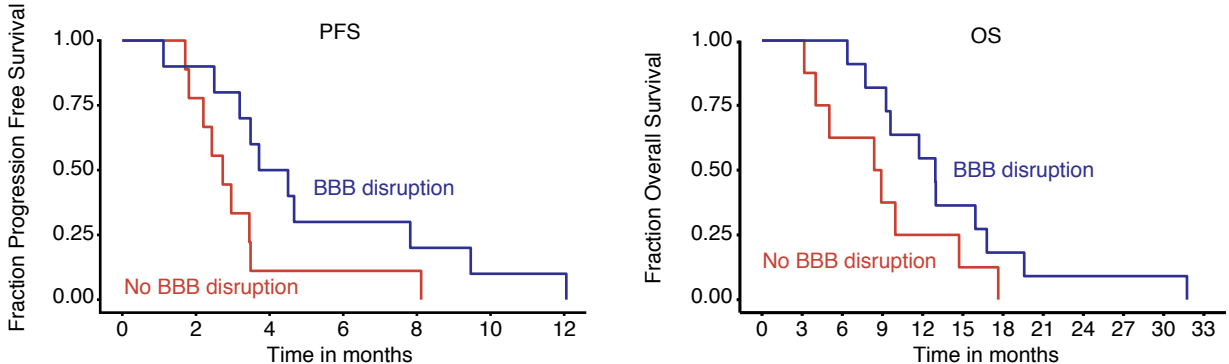
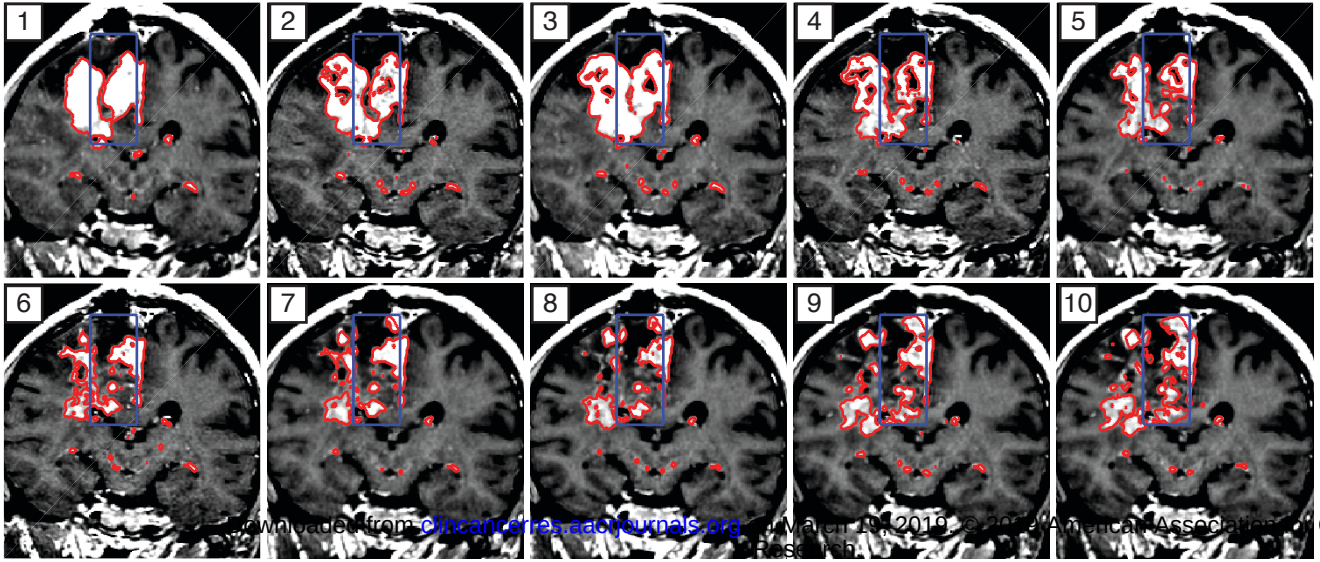


Figure 4



AACR American Association for Cancer Research

Clinical Cancer Research

Safety and Feasibility of Repeated and Transient Blood-Brain Barrier Disruption by Pulsed Ultrasound in Patients with Recurrent Glioblastoma

Ahmed Idbaih, Michael Canney, Lisa Belin, et al.

Clin Cancer Res Published OnlineFirst March 19, 2019.