Published in final edited form as:

Neurosurg Focus. 2012 January ; 32(1): E3. doi:10.3171/2011.10.FOCUS11252.

Focused ultrasound disruption of the blood brain barrier: a new frontier for therapeutic delivery in molecular neuro-oncology

Arnold B. Etame, MD^a, Roberto J. Diaz, MD^a, Christian A. Smith, PhD^a, Todd G. Mainprize, MD^a, Hynynen H. Kullervo, PhD^b, and James T. Rutka, MD, PhD^{a,*}

^aDivision of Neurosurgery, the Hospital for Sick Children, Department of Surgery, University of Toronto, Toronto, Canada

^bDepartment of Imaging Research at Sunnybrook Research Institute, University of Toronto, Toronto, Canada

Abstract

Recent advances in molecular neuro-oncology provide unique opportunities for targeted molecular-based therapies. However, the blood brain barrier (BBB) remains a major limitation to the delivery of tumor-specific therapies directed against aberrant signaling pathways in brain tumors. Given the dismal prognosis of patients with malignant brain tumors, novel strategies that overcome the intrinsic limitations of the BBB are therefore highly desirable. Focused ultrasound (FUS) BBB disruption is emerging as a novel strategy for enhanced delivery of therapeutics into the brain via focal, reversible and safe BBB disruption. This review examines the potential role and implications of FUS in molecular neuro-oncology.

Keywords

Brain tumors; blood brain barrier; focused ultrasound; chemotherapy

Background

Malignant glioma is the most common primary brain tumor in adults²⁵. These tumors demonstrate extensive invasion into the brain parenchyma thereby evading the current multimodal therapeutic paradigms of conventional chemotherapy, radiotherapy and surgery. Despite tremendous advances within this multimodal treatment scheme, the prognosis for patients with the disease remains dismal⁶⁹⁻⁷¹. The mean survival remains 12-16 months with over 70-80% of patients dead within 2 years⁷⁰. The highly invasive nature as well as the complex molecular underpinnings of the disease both account for its intransigence to current conventional therapies. Hence molecularly directed therapeutic targets are essential in order to clinically improve outcome. As a result, there is an impetus towards deciphering critical signaling pathways in hopes of identifying putative molecular targets.

*Corresponding Author: Prof. James T. Rutka, The Arthur and Sonia Labatt Brain Tumor Research Centre, The Hospital for Sick Children, Division of Neurosurgery, Suite 1503, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8
james.rutka@sickkids.ca.

However, a major challenge to the delivery of molecular pathway targeting therapeutics is the transport across the blood-brain barrier (BBB) which creates both structural and physiologic impediments to delivery⁵³. The BBB limits effectiveness of many conventional chemotherapeutic drugs, making systemic administration an ineffective option for the vast majority of chemotherapy agents¹⁷. For instance doxorubicin is a chemotherapy agent that does not appreciably cross the BBB, yet has been very effective against malignant gliomas in vitro⁸⁰. Similar delivery challenges plague molecular-based targeted glioma therapy given the various transport mechanisms that govern BBB delivery.

Generally, in order for therapeutics to cross the BBB, they must employ either passive or active transport mechanisms⁵¹. Small (<400 Da), non-polar lipophilic agents are easily transported passively⁵², while polar or water-based compounds generally require active transport mechanisms^{38,39}. Furthermore, the BBB expresses drug-efflux transporter proteins that physiologically exclude therapeutics from the brain. A well-known efflux transporter is the P-glycoprotein, which is a substrate for most chemotherapy agents^{2,33}. Another confounding factor that could significantly affect delivery of therapeutics into brain tumors is the potential variability in vascular permeability between tumors, and between tumors and normal brain^{3,5,6,16,34,46,47,55}. Potential solutions require either structural modifications of therapeutic agents or transient, safe and reversible modifications of the BBB in order to enable delivery into the brain. The latter strategy of BBB modification appears practically more appealing given the complexities of redesigning and modifying molecular therapeutic agents. Ideally, strategies that transiently increase BBB permeability should be focal, safe, reversible and noninvasive.

Focused ultrasound (FUS) disruption of the BBB is emerging as a very promising novel non-invasive technology that can circumvent some of the anatomical limitations of the BBB, thereby enhancing delivery of therapeutic agents into the brain^{22,42}. Low frequency ultrasound waves are delivered trans-cranially resulting in BBB disruptions in focal areas of the brain. Given the potential for focal and selective delivery in conjunction with the inherent advantage of enhanced therapeutic delivery, this review will examine the potential applications and implications of FUS in molecular neuro-oncology.

Conventional non-focal BBB disruption strategies

Since most therapeutic agents do not readily cross the BBB, several strategies to temporally disrupt the BBB have been embarked upon previously (See Table 1). Transient disruption of the BBB can be attained using an osmotic agent such as mannitol, which is delivered intra-arterially via carotid arteries¹. The feasibility of such an approach has been demonstrated in brain tumors^{45,57}. Furthermore alkylated alcohols such as alkyl-glycerol open the BBB in a similar fashion when delivered intra-arterially³². Besides osmotic agents, there are receptor-mediated mechanisms that can enhance permeability of the BBB. A notable example is the bradykinin system where analogues such as *RMP-7* have been employed for enhanced permeability of the BBB^{12,59}. Preclinical application of bradykinin analogues with intra-arterial carboplatin appeared promising with increased drug levels in the brain¹³. However, when a similar strategy was applied in Phase II trials for childhood brain tumors⁷⁷, and

recurrent gliomas⁵⁶, the results from clinical trials were not as promising. A major pitfall was failure to attain reasonable therapeutic concentrations within the brain.

Enhanced permeability of the BBB can have both beneficial and deleterious effects. Although the BBB limits delivery of therapeutics into the brain, it also protects the brain from systemic toxins. BBB disruption strategies that employ osmotic agents such as mannitol or bradykinin analogues can result in widespread BBB disruption with the potential for deleterious consequences. Therefore an obvious shortcoming of the above BBB disruption strategies is the lack of targeted delivery applications.

FUS disruption of BBB: Principles

A unique advantage of FUS disruption of the BBB over other conventional BBB disruption schemes, is the selective and regional permeability increases that result in enhanced local delivery within the brain – Table 2^{20-22,41,44}. The technique entails trans-cranial delivery of low-frequency ultrasound waves, which ultimately result in disruption of BBB (Figure 1)²³. Typically, ultrasonic exposure burst at 10-ms with pressure amplitudes less than 1 MPa are conventionally used for durations of 20–30 s repeated at the frequency of 1 Hz¹⁹. By employing low frequencies, the chances of permanent tissue damage are minimized. The technique can be employed in conjunction with MRI for both targeting purposes as well as documentation of focal BBB disruption, which is manifest by regional contrast extravasation (Figure 2). Incorporation of intravenously administered lipid-encased perfluorocarbon gas microbubbles (diameter ~1–5 μm) further lowers the frequency threshold for BBB disruptions thereby allowing for much lower and safer frequencies to be employed^{22,42,44}. The feasibility of microbubble-assisted FUS disruption of the BBB was first successfully demonstrated a decade ago²². The FUS BBB disruption effects are not as apparent in the absence of microbubbles since acoustic powers are two orders of magnitude lower²². As the microbubbles traverse capillaries, they can expand and collapse based on the ultrasonic input. It is hypothesized that FUS results in oscillation and concentration of microbubbles by the capillary walls, which in turn imparts mechanical forces that could result in BBB opening^{22,23,65}. Furthermore, the microbubbles emit acoustic signals that have been highly correlated with BBB disruption in the absence of vascular damage, thus suggesting that acoustic signals could serve as a surrogate for safety⁴⁰. The safety of FUS disruption of the BBB is well documented and the overall effects are transient and reversible with no overt neuronal injury^{21,22,44}. The ensuing BBB disruption lasts at most for approximately 4 hours⁶⁴.

FUS disruption of BBB: Design¹⁸

The overall schematic for preclinical FUS systems for BBB disruption is illustrated in Figure 3. The animal is anesthetized and positioned supine with the scalp submerged in a chamber containing degassed water. Low frequency ultrasound waves emitted from a focused transducer are transmitted through the degassed water into the cranium. Prior to BBB disruption, animals receive lipid micro-bubbles as well as the therapeutic agent of interest. The targeted area for sonication is selected, followed by burst low frequency ultrasound for BBB disruption. Magnetic resonance imaging (MRI) of the animal brain is

obtained prior to and after focused ultrasound BBB disruption. The region of BBB disruption is confirmed on T-1 contrast-enhanced MRI (Figure 2).

Preclinical applications of FUS (Table 3)

Delivery of antibodies into the brain

One of the most practical therapeutic attributes of FUS is the ability to deliver antibodies into the brain, which was demonstrated in a previous study³⁰. Here, Kinoshita et al. were able to demonstrate the BBB crossing of dopamine D(4) receptor-targeting antibody and subsequent antigen recognition within the brain via FUS mediation. Traditionally, a major hurdle in antibody therapeutics is the very limited ability of antibodies to cross the BBB in light of absent large water channels or active transport mechanisms for antibodies in cerebrovascular endothelium. Yet there are several neurological disorders for which antibody-mediated therapy would be advantageous. For instance, in Alzheimer's disease there is accumulation of A β plaques, which form the basis for toxicity and cognitive impairment⁶¹. Interestingly, direct intracranial administration of anti-A β antibodies in transgenic mice⁷⁴, as well as in normal animals⁷⁹ led to a substantial amount of plaque reduction. A downside of direct intracranial injections is the invasiveness. Most recently, using MRI-guided FUS, anti-A β antibodies in transgenic mice were intravenously delivered into transgenic mice and resulted in significant plaque reduction 4 days post treatment²⁶. The investigators also noted that with FUS, a much lower dose of antibodies was effective as well for plaque reduction.

Another potential area of interest pertaining to antibody therapy is cancer targeting. Anti-tumor monoclonal antibodies that have been successfully used to target systemic cancers could have potential applications for metastasis or primary cancers in the brain. For instance, Herceptin, which is an anti-HER2 monoclonal antibody and a therapeutic target of breast cancer, was successfully delivered across the BBB with MRI-guided FUS as a proof-of-concept²⁹. This proof-of-concept is very encouraging in light of potential extrapolations to other anti-tumor antibody candidates.

Delivery of conventional chemotherapy into the brain

Given that the BBB significantly compromises the bioavailability of conventional chemotherapy agents into the brain, several strategies have been previously employed with promising preclinical results, yet minimal benefits in clinical trials. One approach of augmenting brain concentration of chemotherapy agents has been the employment of biodegradable matrices^{27,78}. Matrices laden with chemotherapy agent are implanted within the tumor resection cavity. Other approaches have included convection-enhanced delivery systems^{35,54}, and intra-arterial chemotherapy⁶². However, even with these approaches, insufficient chemotherapy bioavailability renders their efficacy suboptimal.

As a result, preclinical studies have been undertaken with FUS in order to assess the feasibility of chemotherapy delivery into brain tumors. This strategy was first successfully applied for doxorubicin, which does not appreciably cross the BBB⁷⁵. The investigators successfully demonstrated a substantial increase in the concentration of liposome-encapsulated doxorubicin within the FUS treated hemispheres of normal rats when

compared to the non-treated hemisphere. On a subsequent follow-up study, the investigators demonstrated a substantial therapeutic benefit from FUS-mediated delivery of liposome-encapsulated doxorubicin in rats with intracranial gliomas⁷⁶.

The delivery of methotrexate into the brain using FUS disruption of the BBB has been investigated as well. Mei and colleagues assessed the FUS-assisted intracranial delivery of intravenous methotrexate into rabbit brains when compared to an intra-carotid injection⁴³. They noted targeted and enhanced delivery of intravenous methotrexate up to 10-fold with FUS. Furthermore, the FUS delivery strategy was noted to be more effective than intra-carotid delivery alone.

Most recently, Liu and colleagues investigated FUS-mediated delivery of BCNU chemotherapy in preclinical animals with brain tumours³⁶. They reported substantial increases in tumor BCNU bioavailability in animals treated with FUS compared to non-treated animals. Accordingly tumor progression was significantly compromised as evident by decreases in tumor size. They further demonstrated a significant survival benefit associated with FUS-mediated delivery of BCNU.

The preclinical data thus far for FUS-mediated delivery of chemotherapy agents into the brain appear promising. Preliminary results demonstrate both feasible and efficacy. Additional successful preclinical applications should pave the way for clinical studies.

Delivery of therapeutic nanoparticles into the brain

Nanotechnology-based delivery systems have generated a substantial amount of interest in light of demonstrated tumor-targeting applications²⁸. Through such unique features nano-delivery platforms could potentially circumvent some of the challenges associated with conventional chemotherapy for malignant brain tumors. However, the BBB remains a critical limitation for nano-platforms. For instance, gold nanoparticles (AuNP) have gained prominence in nano-mediated cancer targeting for systemic cancers^{7,10,50} yet AuNP are significantly limited in their biodistribution to the brain following systemic delivery^{11,67,73}. Therefore strategies such as FUS that can increase BBB permeability to nano-carriers could enhance applicability of nano-based targeting in malignant brain tumors.

Liu and colleagues recently assessed FUS-mediated delivery of an iron oxide magnetic nanoparticles (MNPs) conjugated to an antineoplastic agent epirubicin³⁷. They employed MNPs because of the favorable MRI characteristics, which could facilitate imaging. They demonstrated a substantial accumulation of MNPs as well as epirubicin up to 15-fold the therapeutic range in the brain when delivered with FUS. They further showed decreased tumor progression in animals with brain tumors that received MMP with epirubicin via FUS. Similar to intracranial magnetic nanoparticle targeted delivery, we have recently observed targeted enhanced parenchymal delivery of polyethylene glycol coated gold nanoparticles into normal brain and to the periphery of parenchymal brain tumors in a rat model (unpublished data).

The prospects of combining FUS with nano-delivery platforms create a whole new avenue for targeting opportunities. The nano-platforms are already very attractive for molecular

targeting since nanoparticles can be easily functionalized with small molecule inhibitors, proteins, nucleic acid, ligands and antibodies in any combination onto the surface of the nanoparticle.

Delivery of gene-based therapies into brain tumors

One of the major challenges with viral-based gene therapy for malignant brain tumors has been the inadequacy with respect to therapeutic delivery¹⁵. Delivery strategies employed have included intra-tumoral injection²⁴, convection-enhanced delivery systems^{8,58} as well as intraventricular delivery⁴⁹. However, all of the above modalities are invasive. In addition, there are toxicity-related issues especially with intraventricular delivery⁴⁹. On the other hand, intravascular delivery has been shown to be safe but it is plagued by BBB limitations, which can be alleviated with BBB disrupting agents^{4,48}. Since glioma gene therapy strategies are geared towards targeting invasive tumor cells that might have escaped local therapies of surgery and radiosurgery, focal and targeted delivery is very essential. Hence FUS could play a major role in this endeavor. The main preclinical assessment of FUS mediated delivery of viral vectors into the brain was performed using radio-labeled Herpes Simplex Virus(HSV) vector⁶³. Using a combination of autography and histological assessments, the investigators were able to demonstrate focal delivery of intravenously administered HSV within the hemisphere that was sonicated with FUS. While this study is encouraging, additional studies are warranted in order to assess functional efficiency of this mode of viral vector delivery into the brain. Transgene expression via FUS delivery of DNA constructs could serve as an alternative to viral intracellular delivery methods^{31,68,72}. FUS has been previously employed for intra-uterine gene delivery into fetal brain¹⁴. Furthermore, Shimamura and colleagues have demonstrated enhanced delivery and expression of the luciferase gene within rat central nervous system using the microbubble-enhanced ultrasound method⁶⁶. The use of FUS mediated targeted molecular therapy can be envisioned. For example, this technology could be used to deliver a gene that could complement a mutation in a tumor cell or by delivery of a silencing gene, such as microRNA or short hairpin RNA gene, to downregulate the expression of an aberrantly expressed protein. Reduction in the expression of Survivin, an inhibitor of apoptosis which is expressed in malignant glioma, has been achieved in a flank xenograft cervical cancer model using focused ultrasound delivery of a short hairpin RNA silencing vector targeting Survivin⁹. Further preclinical investigation to assess the potential of transcranial focused ultrasound for non-viral gene therapy in the central nervous system is warranted.

Future directions

Preclinical studies thus far suggest that FUS with microbubbles can safely facilitate the focal delivery of wide range therapeutic agents into the brain. In this regards, FUS affords selective and targeted delivery. In addition, the potential to successfully combine FUS with other specialized targeted delivery systems such as nanoparticles and viral vectors carries tremendous promise in molecular neuro-oncology. Furthermore, FUS applications on animal brain tumor models demonstrate correlations between enhanced delivery and decreased tumor progression with concomitant improved survival. Safety profiles have equally been established with FUS-mediated delivery strategies from a preclinical-standpoint. If these

preliminary preclinical results are sustainable, FUS mediated delivery will play a pivotal role in the molecular therapeutics of malignant brain tumors.

Acknowledgments

We acknowledge CIHR MOP-74610, NIH grant No. EB003268, Canada Research Chair program, Congress of Neurosurgeons Penfield Award, Canadian Cancer Society Research Institute, Brain Tumour Foundation of Canada, and brainchild Canada for financial support.

References

1. Abbott NJ, Revest PA. Control of brain endothelial permeability. *Cerebrovasc Brain Metab Rev*. 1991; 3:39–72. [PubMed: 2036300]
2. Alvarez M, Paull K, Monks A, Hose C, Lee JS, Weinstein J, et al. Generation of a drug resistance profile by quantitation of mdr-1/P-glycoprotein in the cell lines of the National Cancer Institute Anticancer Drug Screen. *J Clin Invest*. 1995; 95:2205–2214. [PubMed: 7738186]
3. Arosarena O, Guerin C, Brem H, Laterra J. Endothelial differentiation in intracerebral and subcutaneous experimental gliomas. *Brain Res*. 1994; 640:98–104. [PubMed: 8004469]
4. Barnett FH, Rainov NG, Ikeda K, Schuback DE, Elliott P, Kramm CM, et al. Selective delivery of herpes virus vectors to experimental brain tumors using RMP-7. *Cancer Gene Ther*. 1999; 6:14–20. [PubMed: 10078959]
5. Blasberg RG, Kobayashi T, Horowitz M, Rice JM, Groothuis D, Molnar P, et al. Regional blood flow in ethylnitrosourea-induced brain tumors. *Ann Neurol*. 1983; 14:189–201. [PubMed: 6625536]
6. Blasberg RG, Molnar P, Horowitz M, Kornblith P, Pleasants R, Fenstermacher J. Regional blood flow in RT-9 brain tumors. *J Neurosurg*. 1983; 58:863–873. [PubMed: 6854379]
7. Brown SD, Nativo P, Smith JA, Stirling D, Edwards PR, Venugopal B, et al. Gold nanoparticles for the improved anticancer drug delivery of the active component of oxaliplatin. *J Am Chem Soc*. 132:4678–4684. [PubMed: 20225865]
8. Chen MY, Hoffer A, Morrison PF, Hamilton JF, Hughes J, Schlageter KS, et al. Surface properties, more than size, limiting convective distribution of virus-sized particles and viruses in the central nervous system. *J Neurosurg*. 2005; 103:311–319. [PubMed: 16175862]
9. Chen ZY, Liang K, Qiu RX. Targeted gene delivery in tumor xenografts by the combination of ultrasound-targeted microbubble destruction and polyethylenimine to inhibit survivin gene expression and induce apoptosis. *J Exp Clin Cancer Res*. 29:152. [PubMed: 21092274]
10. Cheng Y, A CS, Meyers JD, Panagopoulos I, Fei B, Burda C. Highly efficient drug delivery with gold nanoparticle vectors for in vivo photodynamic therapy of cancer. *J Am Chem Soc*. 130:10643–10647. [PubMed: 18642918]
11. De Jong WH, Hagens WI, Krystek P, Burger MC, Sips AJ, Geertsma RE. Particle size-dependent organ distribution of gold nanoparticles after intravenous administration. *Biomaterials*. 2008; 29:1912–1919. [PubMed: 18242692]
12. Emerich DF, Dean RL, Osborn C, Bartus RT. The development of the bradykinin agonist labradimil as a means to increase the permeability of the blood-brain barrier: from concept to clinical evaluation. *Clin Pharmacokinet*. 2001; 40:105–123. [PubMed: 11286321]
13. Emerich DF, Snodgrass P, Dean R, Agostino M, Hasler B, Pink M, et al. Enhanced delivery of carboplatin into brain tumours with intravenous Cereport (RMP-7): dramatic differences and insight gained from dosing parameters. *Br J Cancer*. 1999; 80:964–970. [PubMed: 10362103]
14. Endoh M, Koibuchi N, Sato M, Morishita R, Kanzaki T, Murata Y, et al. Fetal gene transfer by intrauterine injection with microbubble-enhanced ultrasound. *Mol Ther*. 2002; 5:501–508. [PubMed: 11991740]
15. Fulci G, Chiocca EA. The status of gene therapy for brain tumors. *Expert Opin Biol Ther*. 2007; 7:197–208. [PubMed: 17250458]
16. Groothuis DR, Fischer JM, Lapin G, Bigner DD, Vick NA. Permeability of different experimental brain tumor models to horseradish peroxidase. *J Neuropathol Exp Neurol*. 1982; 41:164–185. [PubMed: 7062086]

17. Haroun RI, Brem H. Local drug delivery. *Curr Opin Oncol*. 2000; 12:187–193. [PubMed: 10841189]
18. Huang Y, Hynynen K. MR-guided focused ultrasound for brain ablation and blood-brain barrier disruption. *Methods Mol Biol*. 711:579–593. [PubMed: 21279624]
19. Hynynen K. Ultrasound for drug and gene delivery to the brain. *Adv Drug Deliv Rev*. 2008; 60:1209–1217. [PubMed: 18486271]
20. 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 sonications. *Neuroimage*. 2005; 24:12–20. [PubMed: 15588592]
21. Hynynen K, McDannold N, Vykhodtseva N, Jolesz FA. Non-invasive opening of BBB by focused ultrasound. *Acta Neurochir Suppl*. 2003; 86:555–558. [PubMed: 14753505]
22. Hynynen K, McDannold N, Vykhodtseva N, Jolesz FA. Noninvasive MR imaging-guided focal opening of the blood-brain barrier in rabbits. *Radiology*. 2001; 220:640–646. [PubMed: 11526261]
23. Hynynen K, McDannold N, Vykhodtseva N, Raymond S, Weissleder R, Jolesz FA, et al. Focal disruption of the blood-brain barrier due to 260-kHz ultrasound bursts: a method for molecular imaging and targeted drug delivery. *J Neurosurg*. 2006; 105:445–454. [PubMed: 16961141]
24. Immonen A, Vapalahti M, Tyynela K, Hurskainen H, Sandmair A, Vanninen R, et al. AdvHSV-tk gene therapy with intravenous ganciclovir improves survival in human malignant glioma: a randomised, controlled study. *Mol Ther*. 2004; 10:967–972. [PubMed: 15509514]
25. Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, et al. Cancer statistics, 2006. *CA Cancer J Clin*. 2006; 56:106–130. [PubMed: 16514137]
26. Jordao JF, Ayala-Grosso CA, Markham K, Huang Y, Chopra R, McLaurin J, et al. Antibodies targeted to the brain with image-guided focused ultrasound reduces amyloid-beta plaque load in the TgCRND8 mouse model of Alzheimer's disease. *PLoS One*. 5:e10549. [PubMed: 20485502]
27. Judy KD, Olivi A, Buahin KG, Domb A, Epstein JI, Colvin OM, et al. Effectiveness of controlled release of a cyclophosphamide derivative with polymers against rat gliomas. *J Neurosurg*. 1995; 82:481–486. [PubMed: 7861228]
28. Kim BY, Rutka JT, Chan WC. Nanomedicine. *N Engl J Med*. 363:2434–2443. [PubMed: 21158659]
29. 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*. 2006; 103:11719–11723. [PubMed: 16868082]
30. Kinoshita M, McDannold N, Jolesz FA, Hynynen K. Targeted delivery of antibodies through the blood-brain barrier by MRI-guided focused ultrasound. *Biochem Biophys Res Commun*. 2006; 340:1085–1090. [PubMed: 16403441]
31. Kuliszewski MA, Kobulnik J, Lindner JR, Stewart DJ, Leong-Poi H. Vascular gene transfer of SDF-1 promotes endothelial progenitor cell engraftment and enhances angiogenesis in ischemic muscle. *Mol Ther*. 19:895–902. [PubMed: 21364544]
32. Lee HJ, Zhang Y, Pardridge WM. Blood-brain barrier disruption following the internal carotid arterial perfusion of alkyl glycerols. *J Drug Target*. 2002; 10:463–467. [PubMed: 12575736]
33. Lee JS, Paull K, Alvarez M, Hose C, Monks A, Grever M, et al. Rhodamine efflux patterns predict P-glycoprotein substrates in the National Cancer Institute drug screen. *Mol Pharmacol*. 1994; 46:627–638. [PubMed: 7969041]
34. Lesniak MS, Brem H. Targeted therapy for brain tumours. *Nat Rev Drug Discov*. 2004; 3:499–508. [PubMed: 15173839]
35. Lidar Z, Mardor Y, Jonas T, Pfeffer R, Faibel M, Nass D, et al. Convection-enhanced delivery of paclitaxel for the treatment of recurrent malignant glioma: a phase I/II clinical study. *J Neurosurg*. 2004; 100:472–479. [PubMed: 15035283]
36. 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. [PubMed: 20413754]
37. Liu HL, Hua MY, Yang HW, Huang CY, Chu PC, Wu JS, et al. Magnetic resonance monitoring of focused ultrasound/magnetic nanoparticle targeting delivery of therapeutic agents to the brain. *Proc Natl Acad Sci U S A*.

38. Loscher W, Potschka H. Drug resistance in brain diseases and the role of drug efflux transporters. *Nat Rev Neurosci.* 2005; 6:591–602. [PubMed: 16025095]
39. Loscher W, Potschka H. Role of drug efflux transporters in the brain for drug disposition and treatment of brain diseases. *Prog Neurobiol.* 2005; 76:22–76. [PubMed: 16011870]
40. McDannold N, Vykhodtseva N, Hynynen K. Use of ultrasound pulses combined with Definity for targeted blood-brain barrier disruption: a feasibility study. *Ultrasound Med Biol.* 2007; 33:584–590. [PubMed: 17337109]
41. McDannold N, Vykhodtseva N, Raymond S, Jolesz FA, Hynynen K. MRI-guided targeted blood-brain barrier disruption with focused ultrasound: histological findings in rabbits. *Ultrasound Med Biol.* 2005; 31:1527–1537. [PubMed: 16286030]
42. McDannold NJ, Vykhodtseva NI, Hynynen K. Microbubble contrast agent with focused ultrasound to create brain lesions at low power levels: MR imaging and histologic study in rabbits. *Radiology.* 2006; 241:95–106. [PubMed: 16990673]
43. 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.* 2009; 28:871–880. [PubMed: 19546329]
44. Mesiwala AH, Farrell L, Wenzel HJ, Silbergeld DL, Crum LA, Winn HR, et al. High-intensity focused ultrasound selectively disrupts the blood-brain barrier in vivo. *Ultrasound Med Biol.* 2002; 28:389–400. [PubMed: 11978420]
45. Neuwelt EA, Barnett PA, Bigner DD, Frenkel EP. Effects of adrenal cortical steroids and osmotic blood-brain barrier opening on methotrexate delivery to gliomas in the rodent: the factor of the blood-brain barrier. *Proc Natl Acad Sci U S A.* 1982; 79:4420–4423. [PubMed: 6289301]
46. Neuwelt EA, Frenkel EP, D'Agostino AN, Carney DN, Minna JD, Barnett PA, et al. Growth of human lung tumor in the brain of the nude rat as a model to evaluate antitumor agent delivery across the blood-brain barrier. *Cancer Res.* 1985; 45:2827–2833. [PubMed: 2985254]
47. Neuwelt EA, Frenkel EP, Gumerlock MK, Brazier R, Dana B, Hill SA. Developments in the diagnosis and treatment of primary CNS lymphoma. A prospective series. *Cancer.* 1986; 58:1609–1620. [PubMed: 3756785]
48. Nilaver G, Muldoon LL, Kroll RA, Pagel MA, Breakefield XO, Davidson BL, et al. Delivery of herpesvirus and adenovirus to nude rat intracerebral tumors after osmotic blood-brain barrier disruption. *Proc Natl Acad Sci U S A.* 1995; 92:9829–9833. [PubMed: 7568227]
49. Oshiro EM, Viola JJ, Oldfield EH, Walbridge S, Bacher J, Frank JA, et al. Toxicity studies and distribution dynamics of retroviral vectors following intrathecal administration of retroviral vector-producer cells. *Cancer Gene Ther.* 1995; 2:87–95. [PubMed: 7621261]
50. Paciotti GF, Myer L, Weinreich D, Goia D, Pavel N, McLaughlin RE, et al. Colloidal gold: a novel nanoparticle vector for tumor directed drug delivery. *Drug Deliv.* 2004; 11:169–183. [PubMed: 15204636]
51. Pardridge WM. Blood-brain barrier biology and methodology. *J Neurovirol.* 1999; 5:556–569. [PubMed: 10602397]
52. Pardridge WM. Blood-brain barrier drug targeting: the future of brain drug development. *Mol Interv.* 2003; 3:90–105. 151. [PubMed: 14993430]
53. Pardridge WM. Drug and gene targeting to the brain with molecular Trojan horses. *Nat Rev Drug Discov.* 2002; 1:131–139. [PubMed: 12120094]
54. Parney IF, Kunwar S, McDermott M, Berger M, Prados M, Cha S, et al. Neuroradiographic changes following convection-enhanced delivery of the recombinant cytotoxin interleukin 13-PE38QQR for recurrent malignant glioma. *J Neurosurg.* 2005; 102:267–275. [PubMed: 15739554]
55. Pathak AP, Schmainda KM, Ward BD, Linderman JR, Rebro KJ, Greene AS. MR-derived cerebral blood volume maps: issues regarding histological validation and assessment of tumor angiogenesis. *Magn Reson Med.* 2001; 46:735–747. [PubMed: 11590650]
56. Prados MD, Schold SJS, Fine HA, Jaeckle K, Hochberg F, Mechtler L, et al. A randomized, double-blind, placebo-controlled, phase 2 study of RMP-7 in combination with carboplatin administered intravenously for the treatment of recurrent malignant glioma. *Neuro Oncol.* 2003; 5:96–103. [PubMed: 12672281]

57. Rapoport SI. Osmotic opening of the blood-brain barrier: principles, mechanism, and therapeutic applications. *Cell Mol Neurobiol.* 2000; 20:217–230. [PubMed: 10696511]
58. Saito R, Krauze MT, Noble CO, Tamas M, Drummond DC, Kirpotin DB, et al. Tissue affinity of the infusate affects the distribution volume during convection-enhanced delivery into rodent brains: implications for local drug delivery. *J Neurosci Methods.* 2006; 154:225–232. [PubMed: 16472868]
59. Sanovich E, Bartus RT, Friden PM, Dean RL, Le HQ, Brightman MW. Pathway across blood-brain barrier opened by the bradykinin agonist, RMP-7. *Brain Res.* 1995; 705:125–135. [PubMed: 8821743]
60. Schizas C, Tzinieris N, Tsiroidis E, Kosmopoulos V. Minimally invasive versus open transforaminal lumbar interbody fusion: evaluating initial experience. *Int Orthop.* 2008
61. Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, et al. Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med.* 2008; 14:837–842. [PubMed: 18568035]
62. Shapiro WR, Green SB, Burger PC, Selker RG, VanGilder JC, Robertson JT, et al. A randomized comparison of intra-arterial versus intravenous BCNU, with or without intravenous 5-fluorouracil, for newly diagnosed patients with malignant glioma. *J Neurosurg.* 1992; 76:772–781. [PubMed: 1564540]
63. Sheikov N, McDannold N, Jolesz F, Zhang YZ, Tam K, Hynynen K. Brain arterioles show more active vesicular transport of blood-borne tracer molecules than capillaries and venules after focused ultrasound-evoked opening of the blood-brain barrier. *Ultrasound Med Biol.* 2006; 32:1399–1409. [PubMed: 16965980]
64. Sheikov N, McDannold N, Sharma S, Hynynen K. Effect of focused ultrasound applied with an ultrasound contrast agent on the tight junctional integrity of the brain microvascular endothelium. *Ultrasound Med Biol.* 2008; 34:1093–1104. [PubMed: 18378064]
65. Sheikov N, McDannold N, Vykhodtseva N, Jolesz F, Hynynen K. Cellular mechanisms of the blood-brain barrier opening induced by ultrasound in presence of microbubbles. *Ultrasound Med Biol.* 2004; 30:979–989. [PubMed: 15313330]
66. Shimamura M, Sato N, Taniyama Y, Yamamoto S, Endoh M, Kurinami H, et al. Development of efficient plasmid DNA transfer into adult rat central nervous system using microbubble-enhanced ultrasound. *Gene Ther.* 2004; 11:1532–1539. [PubMed: 15269716]
67. Sonavane G, Tomoda K, Makino K. Biodistribution of colloidal gold nanoparticles after intravenous administration: effect of particle size. *Colloids Surf B Biointerfaces.* 2008; 66:274–280. [PubMed: 18722754]
68. Song S, Shen Z, Chen L, Brayman AA, Miao CH. Explorations of high-intensity therapeutic ultrasound and microbubble-mediated gene delivery in mouse liver. *Gene Ther.*
69. Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol.* 2009; 10:459–466. [PubMed: 19269895]
70. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med.* 2005; 352:987–996. [PubMed: 15758009]
71. Stupp R, Roila F. Malignant glioma: ESMO clinical recommendations for diagnosis, treatment and follow-up. *Ann Oncol* 20 Suppl. 2009; 4:126–128.
72. Taniyama Y, Tachibana K, Hiraoka K, Aoki M, Yamamoto S, Matsumoto K, et al. Development of safe and efficient novel nonviral gene transfer using ultrasound: enhancement of transfection efficiency of naked plasmid DNA in skeletal muscle. *Gene Ther.* 2002; 9:372–380. [PubMed: 11960313]
73. Terentyuk GS, Maslyakova GN, Suleymanova LV, Khlebtsov BN, Kogan BY, Akchurin GG, et al. Circulation and distribution of gold nanoparticles and induced alterations of tissue morphology at intravenous particle delivery. *J Biophotonics.* 2009; 2:292–302. [PubMed: 19434616]
74. Thakker DR, Weatherspoon MR, Harrison J, Keene TE, Lane DS, Kaemmerer WF, et al. Intracerebroventricular amyloid-beta antibodies reduce cerebral amyloid angiopathy and

- associated micro-hemorrhages in aged Tg2576 mice. *Proc Natl Acad Sci U S A*. 2009; 106:4501–4506. [PubMed: 19246392]
75. Treat LH, McDannold N, Vykhodtseva 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*. 2007; 121:901–907. [PubMed: 17437269]
76. Treat, L.H.; Z, Y.; McDannold, N.; Hynynen, K. Impact of focused ultrasound-enhanced drug delivery on survival in rats with glioma. In: ES, E., editor. *AIP Conference Proceedings*; 8th International Symposium on Therapeutic Ultrasound; Minneapolis, Minn. 2008. p. 443-446.
77. Warren K, Jakacki R, Widemann B, Aikin A, Libucha M, Packer R, et al. Phase II trial of intravenous lobaradimil and carboplatin in childhood brain tumors: a report from the Children's Oncology Group. *Cancer Chemother Pharmacol*. 2006; 58:343–347. [PubMed: 16408203]
78. Westphal M, Lamszus K, Hilt D. Intracavitary chemotherapy for glioblastoma: present status and future directions. *Acta Neurochir Suppl*. 2003; 88:61–67. [PubMed: 14531563]
79. Wilcock DM, DiCarlo G, Henderson D, Jackson J, Clarke K, Ugen KE, et al. Intracranially administered anti-Aβ antibodies reduce beta-amyloid deposition by mechanisms both independent of and associated with microglial activation. *J Neurosci*. 2003; 23:3745–3751. [PubMed: 12736345]
80. Wolff JE, Trilling T, Molenkamp G, Egeler RM, Jurgens H. Chemosensitivity of glioma cells in vitro: a meta analysis. *J Cancer Res Clin Oncol*. 1999; 125:481–486. [PubMed: 10480340]

Figure 1. Schematic of enhanced BBB delivery following FUS disruption of BBB

FUS delivers low frequency ultrasound waves which cause mechanical oscillations in microbubbles resulting in disruption of endothelial cells (EC) tight junctions leading to enhanced BBB permeability to agents.

Figure 2. MR-guided FUS disruption of BBB

Coronal gadolinium enhanced T1-weighted MRI of rat brain showing contrast enhancement (arrow) within the sonicated right hemispheric parenchymal. The non-sonicated left hemisphere lacks contrast enhancement.

Figure 3. Preclinical focused ultrasound blood-brain-barrier disruption system

The animal is positioned with skull partially submerged in a de-gassed water tank and microbubbles are intravenously administered. A focused transducer attached to a network power and computer system delivers low frequency ultrasound, which disrupts the BBB. MRI is incorporated for targeting as well as BBB disruption visualization.

Table 1

Enhanced BBB delivery strategies

•	Intra-arterial via carotids
•	Intraventricular
•	Osmotic agents such a mannitol
•	BBB permeation analogues such as bradykinin
•	Convection enhanced interstitial delivery

Table 2

Advantages of FUS-mediated therapeutic delivery

<ul style="list-style-type: none">• Focal and targeted delivery minimizes problems seen with widespread BBB disruption• Transient disruption of BBB, hence reversible• Noninvasive trans-cranial technique• Enhanced delivery of chemotherapy, gene therapy, monoclonal antibodies across BBB
--

Table 3**Preclinical animal studies of FUS delivery across BBB**

Reference	Study
Kenoshita et al., 2006{Kinoshita, 2006 #137}	Delivery of dopamine D(4) receptor-targeting antibody into the brain.
Sheikov et al., 2006{Sheikov, 2006 #172}.	Focal delivery of intravenously administered HSV-engineered vector across the BBB of normal rats.
Kenoshita et al., 2006{Kinoshita, 2006 #137}	Delivery of Herceptin (anti-HER2 monoclonal antibody) into the brain.
Treat et al., 2007{Treat, 2007 #47}	Successful enhanced delivery of doxorubicin across the BBB of normal rats.
Treat et al., 2008{Treat LH, 2008 #48}	Successful enhanced delivery of doxorubicin across the BBB of rat glioma models.
Mei et al., 2009 {Mei, 2009 #174}	Successful enhanced delivery of doxorubicin across the BBB of rabbit brain.
Liu et al., 2010{Liu, #52}.	Enhanced delivery of BCNU with FUS led to tumor remission and prolonged survival in rat glioma model.
Jordao et al., 2010{Jordao, #138}.	Delivery of anti- A β antibodies via FUS led to Alzheimer plaque reduction in transgenic mice model.
Liu et al., 2010{Liu, #49}.	FUS delivery of an iron oxide magnetic nanoparticles (MNPs) conjugated to epirubicin led to enhanced delivery, tumor remission and prolonged survival in rat glioma models.