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MR-guided focused ultrasound–induced blood-brain barrier opening for brain metastasis: a review

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Although the therapeutic armamentarium for brain metastases (BMs) has been expanded from innovative surgical techniques and radiotherapy to include targeted therapies and immunotherapy, the prognosis of BMs remains poor. Despite the proven efficacy of numerous compounds in preclinical studies, the limited penetration of promising therapeutic agents across the blood-brain barrier (BBB) remains an unaddressed issue. Recently, low-intensity magnetic resonance–guided focused ultrasound (MRgFUS) in combination with microbubbles has been shown to overcome vascular and cellular transport barriers in the brain and tumor microenvironment, resulting in increased drug diffusion and preliminary effective results. Preclinical studies have investigated the increased penetration of many therapeutic agents including doxorubicin, trastuzumab, and ipilimumab into the CNS with promising results. Furthermore, anticancer drugs combined with MRgFUS-induced BBB opening have been demonstrated to improve animal survival and slow tumor progression. Accordingly, the first clinical trial has recently been launched and hopefully the results will provide evidence for the safety and efficacy of drug delivery enhanced by MRgFUS-induced BBB opening in BMs. This review aims to provide an overview of transcranial low-intensity MRgFUS application for BBB disruption and a comprehensive overview of the most relevant evidence in the treatment of BMs.

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Brain metastases (BMs) are the most common CNS tumors, causing significant morbidity and mortality in cancer-affected patients. BMs are diagnosed in approximately 10%–30% of adults, with 97,800–170,000 estimated new cases per year.[1] Due to the histopathological variability of BMs, the incidence as well as survival rate differ based on the specific histology. Lung cancer, breast cancer, and melanoma account for 67%–80% of all BMs.[2,3]

Currently, MRI is often performed as part of tumor staging,[4] resulting in many patients with subclinical BM identified at the time of presentation. While the therapeutic value of resection of single BMs in patients with controlled systemic disease remains indisputable, surgery should be also considered for large BMs (> 3 cm in diameter) resulting in neurological impairment,[5] for BMs in posterior fossa locations, and in cystic or necrotic BMs.[5]

Stereotactic radiosurgery is performed in patients with lesions 3–3.5 cm in maximum diameter, for lesions in surgically inaccessible locations, in advanced systemic cancer, or in patients with serious comorbidities.[5] Whole-brain radiotherapy, usually 20–30 Gy in 5–10 fractions, has been used either as an established treatment after local therapy or as the primary treatment modality for patients with multiple BMs.[5] Chemotherapy effectiveness remains largely uncertain due to the variable penetration of chemotherapeutic agents into the CNS across the blood-brain barrier (BBB).[6] It is well known that the anatomical and functional constitution of the BBB plays an essential role in maintaining brain homeostasis, but at the same time, can complicate the treatment of neurological diseases by hindering the diffusion of therapeutic agents into the CNS. Overcoming the BBB for therapeutic purposes has been attempted without success and with questionable safety.

ABBREVIATIONS BBB = blood-brain barrier; BM = brain metastasis; DOX = doxorubicin; FUS = focused ultrasound; LeDOX = liposome-encapsulated DOX; LIFU = low-intensity focused ultrasound; MRgFUS = magnetic resonance–guided FUS; NSCLC = non–small cell lung cancer; ROS = reactive oxygen species; SDT = sonodynamic therapy; tcMRgFUS = transcranial MRgFUS; tFUS = transcranial FUS; TMZ = temozolomide.

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In this scenario, advancement in the transcranial focused ultrasound (tFUS) technique has opened new avenues in manipulating the BBB for therapeutic purposes. Consistent with this line of research, a number of preclinical studies and a clinical trial have begun; hopefully, the future results of these studies will transform the treatment of several neurological diseases. This review aims to provide the state of the art on tFUS application for BBB disruption and a comprehensive overview of the most relevant evidence in the treatment of BMs.

Transcranial Focused Ultrasound

Transcranial FUS is a noninvasive technique that uses ultrasound transmitted through the skull to reach specific areas of the brain. Currently, the most common clinical and experimental tFUS setups use an FUS system coupled with a 1.5- or 3-T MRI machine (MR-guided FUS [MRgFUS]). The use of an MRI scanner allows real-time monitoring and feedback during sonication by MR thermometry,[7] acoustic monitoring, and intraoperative imaging,[8–11] which together with real-time patient feedback results in a groundbreaking closed-loop technology. Specifically, MR thermometry can measure absolute temperature or temperature changes, allowing for noninvasive monitoring of the thermal distribution and heat deposition in real time during MR-guided procedures.[12] MR thermometry may rely on detecting various temperature-sensitive MR parameters; the most extensively adopted parameter to guide thermal ablation techniques such as tFUS is the proton resonance frequency thermometry method, considered the current clinical gold standard.[13] When focused into soft tissues, the ultrasound energy can induce biological effects also related to nonthermal (mechanical) mechanisms.[14] Mechanical effects are predominately associated with acoustic cavitation, which is a stable or inertial oscillation of microbubbles in the ultrasound field. A cavitation bubble can be produced when a strong ultrasound wave is applied to a liquid and its molecular structure undergoes alternating expansion and compression cycles. During the expansion phase, the sudden pressure drop can create bubbles of gaseous substances in the liquid. These bubbles grow under the subsequent ultrasound expansion cycles until they reach an unstable size and then violently collapse. This process is the so-called inertial cavitation that produces locally high pressures and temperatures, and if it occurs close to cells, can lead to cellular damage or hemorrhage in biological tissues. There are therapeutic ultrasound modalities that exploit physical and thermal effects of cavitation,[14] and to guarantee a safe treatment, an accurate knowledge of the location of the cavitation inception point is needed. Therefore, cavitation detection and monitoring are important strategies to improve the safety of the tFUS procedure.

There are two main types of tFUS, i.e., low and high intensity. Low-intensity focused ultrasound (LIFU) uses a lower-power ultrasound wave primarily for neuromodulation applications, and when combined with intravenous injection of the ultrasound contrast agent (microbubbles), can provide transient opening/disruption of the BBB in targeted regions of the CNS by increasing the likelihood of inertial cavitation in the targeted area. High-intensity tFUS systems are also known as transcranial MRgFUS (tcMRgFUS) and use a higher-power ultrasound beam to create thermal lesions in specific brain regions. These systems are used for ablative purposes, such as treating movement disorders like essential tremor or Parkinson's disease, neuropathic pain, or psychiatric illnesses such as obsessive-compulsive disorder.

Sonodynamic Therapy

Sonodynamic therapy (SDT) is a noninvasive treatment, often used for tumor irradiation, that utilizes LIFU for sonosensitizer agent activation.[15] Both in vitro and in vivo studies have shown that low-intensity ultrasound can increase the permeability of the plasma membrane without causing complete cell destruction.[16–18] One of the attractive features of SDT relies on the ability to focus ultrasound energy on deep-seated malignancies and locally activate a preloaded sonosensitizer.[19] High efficacy of drug uptake will result in lower doses of sonosensitizer being required to significantly damage malignant growths. Starting with the first observation that hematoporphyrin, a well-known photosensitizer, exerted high cytotoxicity for cancer cells under ultrasound irradiation,[20,21] interest in these chemotherapeutic agents as a potential treatment for brain tumors has risen in recent years.

The mechanisms through which SDT exerts its cytotoxic effects on brain tumors are still unclear; some theories include cavitation effects, reactive oxygen species (ROS) generation, apoptosis induction in cancer cells, antitumor immunity improvement, restraining angiogenesis, and hyperthermia induction.[22] To understand SDT-associated mechanisms and improve SDT therapeutic effects, researchers have focused on ROS generation by sonosensitizers.[23] Although the mechanisms linking ultrasound stimulation and ROS production by sonosensitizers are still under investigation, it has been suggested that pyrolysis and sonoluminescence play an important role in the ultrasonic cavitation effect.[23] The generated ROS leads to irreversible cell damage and the collapse of cavitation bubbles produces heat and strong shear forces, triggering tumor cell death.[24]

The choice of the ideal sonosensitizer depends on its ROS generation efficiency under ultrasound irradiation, tumor targeting capacity, water solubility, and biocompatibility.[25,26] Overall, several sonosensitizers have been investigated in experimental contexts. Among these, 5-ALA and fluorescein are routinely used in current practice for targeting malignant brain tumor resection due to their selective accumulation in glial cells. Recently, some studies have addressed the feasible role of SDT for the treatment of metastases;[27,28] overall, chemo-sonodynamic therapy not only inhibited tumor growth and metastasis with reduced metastatic protein expression, but also caused an immune response due to the release of tumor-associated antigens.[27]

BBB Opening

The BBB consists of tight junctions and membrane transporters, receptors, and channels that strictly regulate

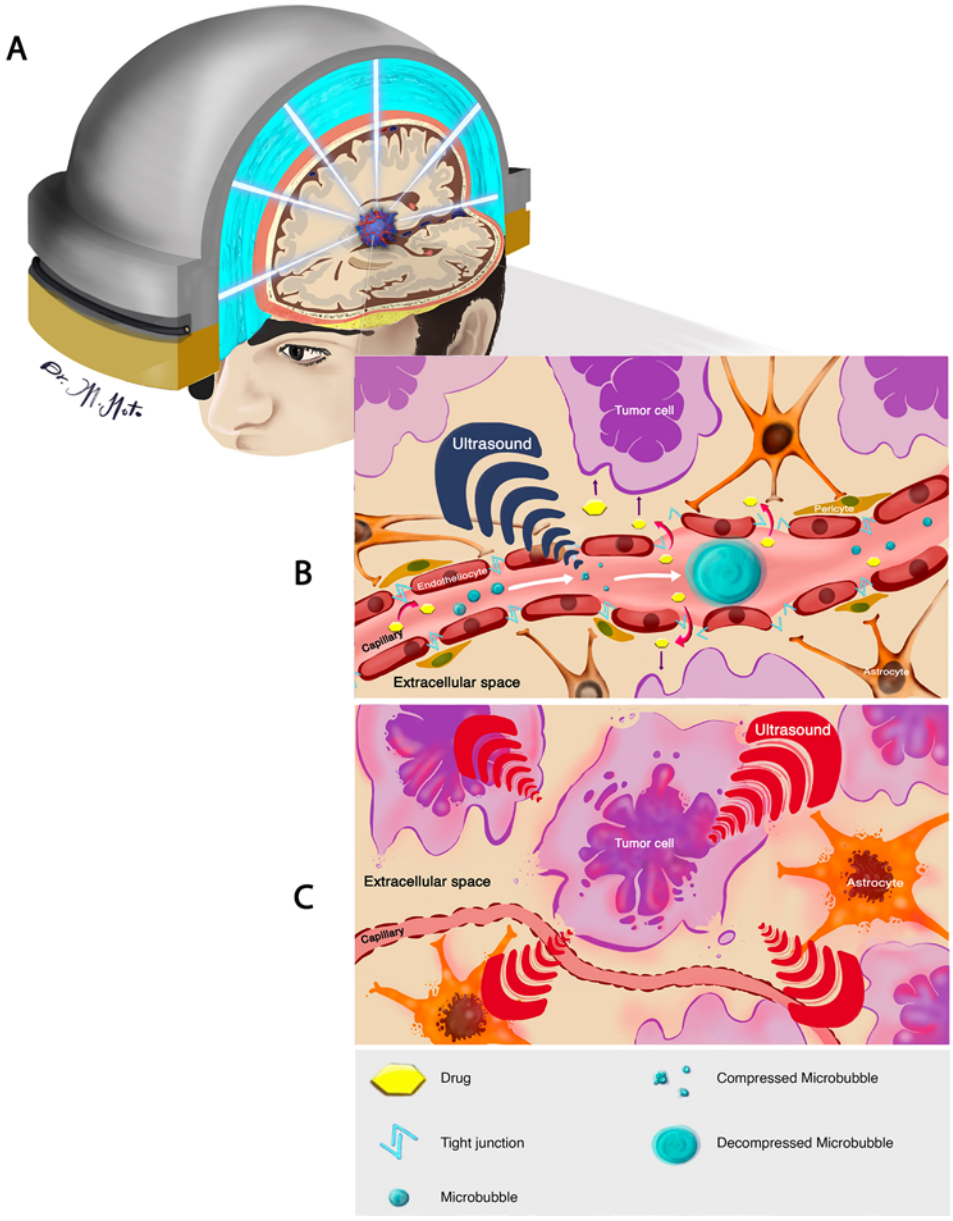

FIG. 1. Illustration showing the MRgFUS procedure. **A:** During the procedure, the base of the device is attached to the patient's head inside the MRI suite. **B:** Schematic of the low-intensity MRgFUS mode causing transient disruption of the BBB through collapse and inertial expansion of systemically administered microbubbles, allowing penetration of drugs into the CNS. **C:** Schematic of the high-intensity MRgFUS mode, creating irreversible thermal lesions for ablative purposes. © Manfredi Noto, published with permission.

the circulation of substances between systemic blood and brain parenchyma.[29] The BBB exerts an important role in CNS homeostasis by regulating interstitial fluid composition, peripheral and central cellular communication, and immunity.[30,31] Its anatomical and functional nature, however, represents an obstacle for diffusion of anticancer drugs into the CNS.[32,33]

Many attempts to bypass the BBB have been made. Briefly, hypertonic solutions,[34] receptor-modifying nanoparticles, intranasal injections, and chemo-agent wafers[35–37] have been used with questionable results. In the last few years, the potential to modulate BBB permeability by using pulsatile LIFU has been investigated. Pulsatile LIFU can interfere with BBB permeability without causing permanent lesions. Numerous preclinical studies have shown that LIFU, combined with microbubble administration, can be an effective technique for BBB opening.[38,39] The FUS makes microbubbles alternate between expansion and compression cycles, thus creating penetrable gaps in the BBB's tight endothelial junctions (Fig. 1). The best agent for opening the BBB should easily reach stable cavitation to induce structural and functional disruption of the tight junctions, decrease expression of P-glycoprotein, and increase the formation of caveolae.[40] Microbubbles are

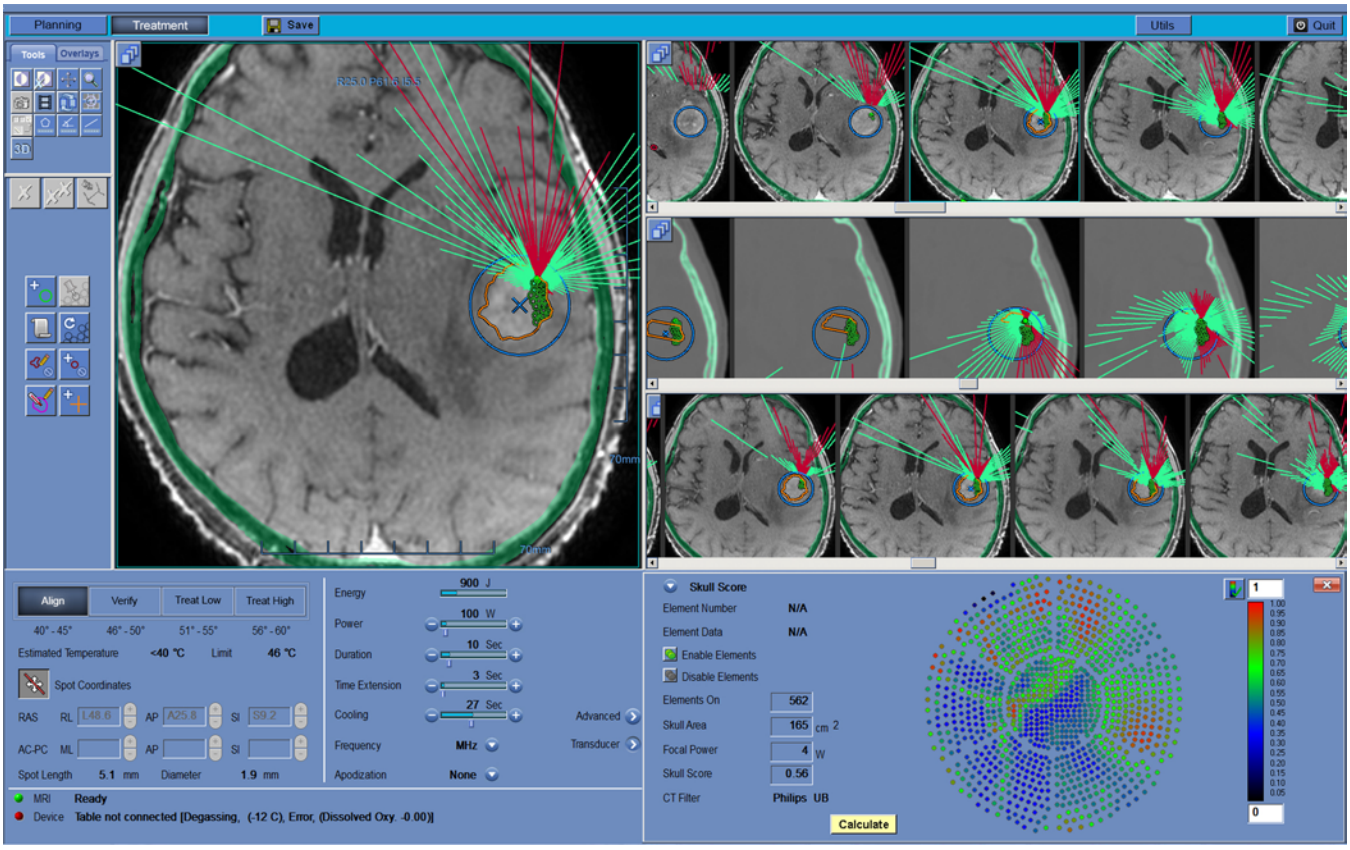

FIG. 2. Treatment planning simulation of a low-intensity MRgFUS procedure in a patient with metastatic brain disease. The region of treatment was manually delineated and split into multiple subvolumes, each of which will be targeted during a different sonication cycle, coupled with intravenous microbubble injection during real-time monitoring including acoustic monitoring, MR thermometry, and patient feedback.

administered through continuous infusion, even though more data are needed to define the optimal administration protocol between continuous infusion and intravenous bolus injection.[41,42] One of the advantages of this technique is that the temperature rise is negligible, and the power used to obtain opening the BBB is typically three times lower than that required for thermoablation.[43] Although there are no well-defined optimal sonication parameters for BBB opening, it can be assumed that the best parameters are those that will allow the maximum drug delivery with minimal tissue damage. Hence, the best FUS settings for inducing BBB opening can vary based on the size of the molecule being delivered and the type of microbubble being used.[44]

Before MRgFUS treatment, it is important to verify the increased permeability of the BBB to the target area before the administration of systemic chemotherapy. The most common and safest method to confirm BBB opening is T1-weighted contrast-enhanced MRI, although better imaging modalities to quantify or confirm the increased concentration of the chemotherapeutic drug into the target area should be introduced.[45,46] Figure 2 depicts the treatment planning simulation of a low-intensity MRgFUS procedure in a patient with metastatic brain disease.

To date, the safety and feasibility of FUS-induced BBB opening has been established with three different clinical devices: Insightec Exablate,[47] Carthera SonoCloud,[48] and NaviFUS.[49] The feasibility and preliminary efficacy of FUS-assisted targeted delivery of cancer therapeutics have been demonstrated in various animal models. Additionally, there are preliminary data regarding the safety and feasibility of FUS BBB opening with coadministration of carboplatin in patients with gliomas.[50] Notwithstanding the potential advantages of MRgFUS-induced BBB opening for therapeutic delivery in patients with BM, potential side effects such as hemorrhage, ischemia, and brain swelling should be considered. Future clinical trials will provide answers to a number of crucial issues.

Preclinical and Clinical Evidence

Many preclinical studies have explored the efficacy of BBB opening following MRgFUS for enhanced delivery of chemotherapeutic agents and targeted therapy to the CNS, but the safety and efficacy of this therapeutic approach in the clinical setting still needs to be addressed. Recently, the first results of enhanced brain penetration of trastuzumab with MRgFUS in patients with HER2-positive breast cancer and brain metastases have been reported (ClinicalTrials.gov; registration no. NCT03714243).[51] In this study, 4 patients affected by progressive intracranial metastasis and stable systemic disease were enrolled in a

single-arm open-label study. Twenty treatments combining tcMRgFUS with concomitant standard-of-care intravenous trastuzumab-based therapies were administered. The treatment was safe and increased drug delivery into MRgFUS-targeted lesions compared with nontargeted lesions, demonstrating the promise of this technology for a broad range of CNS diseases. Below, we report the evidence from the literature and discuss futures perspectives.

Chemotherapeutic Agents

Doxorubicin (DOX) has been shown to be an effective treatment for solid tumors through its action on topoisomerase II and inhibition of DNA and RNA synthesis.[52] Due to its low BBB permeability, however, systemic DOX has limited clinical use in BMs. In contrast, intratumoral administration of DOX using the Ommaya reservoir was associated with a durable tumor response and a low rate of side effects in patients with recurrent high-grade gliomas.[53]

Animal studies indicated that MRgFUS could increase the penetration of DOX across the BBB and improve tumor control and survival.[54] Lin et al.[55] investigated the in vivo extracellular kinetics of DOX using microdialysis in glioblastoma-bearing mice after MRgFUS-induced BBB disruption. Drug administration with sonication raised the DOX ratio of tumor to normal brain of the target tumors by approximately 2.35-fold, and the mean peak concentration of DOX dialysate was 10 times greater than without sonication.[55]

To overcome systemic and neuronal toxicity of this chemotherapeutic agent, the liposome-encapsulated DOX (LeDOX) associated with MRgFUS-induced BBB opening has been investigated in an animal model and showed that DOX could achieve target therapeutic concentration, with nontargeted MRgFUS brain regions showing significantly lower DOX concentration.[56] Subsequently, LeDOX plus MRgFUS has been studied in animal brain tumor models, showing a significantly increased retention and survival compared with controls receiving LeDOX alone.[56–59] Consequently, LeDOX plus MRgFUS has been studied in animal brain tumor models showing a significantly increased drug retention and survival compared with controls receiving LeDOX alone.[57–59] In the clinical setting, a single phase I, single-arm, open-label clinical trial evaluated the safety and efficacy of low-intensity MRgFUS with the Exablate Neuro Type 2 system (Insightec) with microbubble administration (Definity, Lantheus Medical Imaging) for BBB opening associated with systemic chemotherapy (liposomal DOX n = 1, or temozolomide [TMZ] n = 4) 1 day before resection.[60] The procedure was well tolerated, with radiographic evidence of consistent BBB opening. Biochemical analysis of sonicated versus unsonicated tissue suggested a greater concentration of LeDOX and TMZ in FUS-targeted regions compared with control areas.[60]

In this context, several chemotherapies associated with MRgFUS-induced BBB opening have been investigated in brain tumor therapy, including liposomal paclitaxel,[61] TMZ,[60] methotrexate,[62] and bevacizumab.[63] Although most of these chemotherapeutic agents have been administered in high-grade glioma models, with subsequent data about safety and pharmacokinetics, the promising results from these studies have the potential to extend its clinical investigation in BMs.

Targeted Therapies

The increasing knowledge of crucial driving pathways of various tumors, such as non–small cell lung cancer (NSCLC), breast cancer, and melanoma, has led to the identification of effective therapeutic agents. Tailored molecules or antibodies, able to selectively inhibit abnormally activated signaling pathways, demonstrated significant improvements in the survival of some subgroups of patients with NSCLC (epidermal growth factor receptor mutations or ALK translocations), breast cancer (HER2-positive) and melanoma (*BRAF* V600E–negative mutant).

A substantial scientific impetus has been provided for developing innovative targeted agents with satisfactory BBB penetration.[64] Although the results from preclinical studies have increased optimism for a number of novel agents, the main limitation remains the paucity of available phase III randomized trial data for patients with BMs.[1]

Trastuzumab, a monoclonal antibody targeting HER2-positive breast cancer, has an extremely low permeability through the BBB due to its size (approximately 150 kD). In an animal study, the amount of trastuzumab delivered to the brain tissue was correlated with the extent of the MRI-monitored BBB opening, allowing investigators to indirectly estimate the amount of monoclonal antibody delivered.[65] Specifically, the brain concentration of trastuzumab in unsonicated tissues was below the threshold in 8 of 9 cases, while following sonication it increased significantly up to 3.257 ng/g. Furthermore, Park et al.[66] investigated whether BBB permeability in the tumor area and surrounding brain tissue induced by MRgFUS and microbubbles could slow tumor growth and improve survival in a breast cancer BM model in rats receiving trastuzumab. The resulting mean tumor volume of the interventional group was significantly smaller than that of the control group. Moreover, trastuzumab significantly increased the median survival of the trastuzumab plus MRgFUS group. Collectively, data from these and other similar studies[67] suggests the need for clinical investigations of targeted agents plus MRgFUS-induced BBB opening to establish the efficacy of this therapeutic approach.

Immunotherapy

Immune checkpoint inhibitors have changed the therapeutic approach to the metastatic stage of melanoma, kidney, and bladder cancer, showing promising outcomes particularly in lung cancer and melanoma.[1] The first clinical trial demonstrating the efficacy of ipilimumab, an anticytotoxic T lymphocyte antigen 4, in metastatic brain melanoma was published by Margolin et al. in 2012.[68] Using ipilimumab, the authors showed substantial disease control in patients with melanoma and small and asymptomatic BMs. Hence, this drug did not provide unexpected toxic effects in the cohort of patients treated. Moreover, the administration of ipilimumab plus nivolumab, an anti–PD-1 antibody, resulted in notable intracranial response

rates of 45%–60% in patients with brain-metastatic melanoma.[69]

Despite these achievements, passive brain tumor immunotherapy faces the same challenges of limited blood-brain permeability. The introduction of MRgFUS represents an attractive strategy for successful treatment of brain malignancy by using immunotherapeutic agents. In this regard, successful animal studies have shown preliminary evidence for the safety and efficacy of immunological enhancers following MRgFUS-induced BBB opening.[70,71] The clinical translation has not taken long: a prospective, multicenter, randomized, two-arm, controlled phase III clinical trial to evaluate the safety and efficacy of MRgFUS-induced BBB opening is ongoing (ClinicalTrials.gov; registration no. NCT05317858), using the Exablate Model 4000 Type 2 for the treatment of NSCLC brain metastases in patients undergoing pembrolizumab monotherapy for their primary disease. This study aims to demonstrate the superiority of Exablate BBB disruption targeted to BMs over the standard of care (without Exablate BBB disruption) with respect to the percentage of patients achieving an objective response rate by 6-month follow-up. The results of this study will hopefully provide evidence for the safety and efficacy of immunotherapy plus MRgFUS-induced BBB opening in BMs.

Future Perspectives

With advancements in imaging, radiation therapy, targeted agents, immunotherapy, and genomics, our understanding of and treatment modalities for BMs have greatly improved over the past decade. Improvements in surgical,[72–74] radiotherapeutic, and chemotherapeutic treatments have favorably changed outcomes, shortened treatment time, and alleviated adverse effects. The introduction of new possible therapeutic approaches, such as that of MRgFUS, has broadened the horizon of care and makes the era of individualized treatment a much closer reality.[75]

Current MRgFUS systems are built to treat neurofunctional brain disorders in which the target is deeply located in the brain. In a neurooncological scenario, the need to address more eccentric and superficial targets, often in proximity to the skull bone, remains a challenge. Furthermore, the speed of current systems in terms of volume of brain parenchyma treated per minute does not allow them to cover a large area of treatment (for example, in high-grade glioma or large metastases). Although staged treatments are possible, the current setups of the most common MRgFUS equipment require a complete shave of the head and fixation of the stereotactic frame. The introduction of faster, frameless, and shaveless systems, along with MR-navigated, rather than MR-guided, systems able to perform periodically repeated BBB-opening/disruption could overcome many drawbacks of such a technology and provide a consistent solution for effective BM treatment.

Conclusions

Low-intensity MRgFUS in combination with microbubbles is a blossoming technology that holds promise to overcome vascular and cellular transport barriers into the brain and tumor microenvironment. These features make this fascinating technology a potential tool able to increase the diffusion of chemotherapeutic agents into the neoplasms with effective results, as already shown by preliminary studies. Future clinical studies hopefully will provide evidence for the safety and efficacy of drug delivery enhanced by MRgFUS-induced BBB opening in BMs, thus opening new avenues for individualized treatments.

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Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: Grasso, Torregrossa, Bartolotta, Gagliardo. Acquisition of data: Torregrossa, Buscemi, Bartolotta, Gagliardo. Analysis and interpretation of data: Torregrossa, Noto, Bruno. Drafting the article: Grasso, Torregrossa, Bruno, Buscemi, Gagliardo. Critically revising the article: Bruno, Feraco, Bartolotta, Gagliardo. Reviewed submitted version of manuscript: Feraco, Bartolotta, Gagliardo. Approved the final version of the manuscript on behalf of all authors: Grasso. Administrative/technical/material support: Torregrossa, Noto, Buscemi. Study supervision: Grasso, Bartolotta.

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