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Thermal Therapy Approaches for Treatment of Brain Tumors in Animals and Humans

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Abstract

Primary brain tumors are often aggressive, with short survival from time of diagnosis even with standard of care therapies such as surgery, chemotherapy, and radiation therapy. Thermal therapies have been extensively investigated as both primary and adjuvant therapy. Although thermal therapies are not yet widely used clinically, there have been several promising approaches demonstrated in both animals and humans. This review presents thermal therapy approaches in animal and human studies, including both hyperthermia (temperatures ~42°C–45°C) and thermal ablation (temperatures > 50°C). Hyperthermia is primarily used as adjuvant to chemotherapy and radiotherapy, and is the most widely studied radiation sensitizer where enhanced efficacy has been shown in human patients with brain cancer. Hyperthermia has additional beneficial effects such as immunogenic effects, and opening of the blood-brain barrier to potentially enhance drug delivery, for example in combination with nanoparticle drug delivery systems. Thermal ablation uses high temperatures for direct local tumor destruction, and it found its way into clinical use as laser interstitial thermal therapy (LITT). This review presents various hyperthermia and ablation approaches, including a review of different devices and methods that have been used for thermal therapies, such as radiofrequency/microwaves, laser, high-intensity focused ultrasound, and magnetic nanoparticles. Current research efforts include the combination of advanced thermal therapy devices, such as focused ultrasound with radiation, as well as the use of thermal therapies to enhance chemotherapy delivery across the blood-brain barrier.

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I. INTRODUCTION

Brain tumors affect more than 360,000 people annually.¹ Primary brain tumors are often aggressive, with a rapid progression resulting in short survival time from the time of diagnosis, even with standard of care therapy. Therapy includes a combination of surgery, chemotherapy, and radiation therapy. Another potential therapy, which could increase survival time, is the addition of thermal therapy to standard of care treatment. Over the past few decades, research in both animals and humans has allowed for a dramatic increase in the cure rates of many cancers. As an example, pediatric medulloblastoma was once a nearly uniformly fatal disease; now, its long-term cure rate is 70% to 75%.² However, similar advances have not been made in high grade gliomas. This is, in part, due to poor animal models of the disease and, in part, due to the refractory and diffuse nature of these tumors. In order to develop novel therapeutic approaches such as thermal therapy to increase survival, appropriate brain tumor models are essential for understanding the molecular and anatomic impact of this approach. Once preclinical models are selected, engineering effective therapies for treating aggressive and currently incurable brain tumors will be possible.

Thermal therapies have been investigated extensively for treatment of brain cancer, both as primary treatment and as an adjuvant to chemotherapy and radiation therapy. Thermal therapies can be broadly classified into hyperthermia (~42°C –45°C) and ablation therapies (> 50°C). Hyperthermia is employed as an adjuvant in most studies, because it is one of the best-studied chemotherapy and radiation sensitizers.³ Hyperthermia can also enhance drug delivery by improving perfusion and enhancing the permeability of vessels (e.g., opening the blood-brain barrier) and cells.^{3–6} Thermal ablation uses higher temperatures, as 50°C for one to two minutes causes complete cell necrosis for normal and cancerous tissues. Although hyperthermia may be used as regional therapy, thermal ablation is typically highly localized and only the tumor visible under imaging is exposed to ablative temperatures. Thermal ablation induced by laser (termed laser interstitial thermal therapy [LITT]) is now used with increasing frequency for treatment of recurrent brain tumors in the clinical setting, as well as for epilepsy.^{7–9}

In this paper we will first review brain tumor surrogates (models), and then we will discuss the various thermal therapy approaches that have been investigated in animal models as well as in human patients.

II. BRAIN TUMOR SURROGATES

Brain tumor surrogates (models) are a challenge to develop, as the brain represents an uncommon environment, isolated from the general circulation by the blood-brain barrier. Additionally, the functions of the brain are anatomically varied and are often exquisitely sensitive to changes in pressure or neural communication that might be caused by tumor

growth and invasion. Current brain tumor surrogates frequently used in research include *in vitro* models, such as cell cultures and neurospheres, *in vivo* murine models, and large animal models.

A. *In Vitro* Surrogates

Cell cultures are useful for assessment of the molecular dynamics present in disease states as well as for verification that certain interventions are therapeutic against the tumor cells being studied. Cell cultures are often immortalized and are simple to maintain, as they can often be frozen between uses for the convenience of the investigator. They are likely the most affordable brain tumor surrogate. However, the immortalization process and multiple passages of cells in culture often leads to divergence in the cell characteristics (genetic drift), putting into question the translation of data from cell culture into human samples. In addition, cell cultures may not reproduce the heterogeneity present in tumors. On the other hand, cell cultures that are immortalized are reliable surrogates with consistent profiles of tumor markers.

Neurospheres are based on brain tumor stem cells that were allowed to grow into spherical cell clusters in culture solutions. These stem cells are maintained by allowing them to grow in the brain of an animal model (such as a mouse) so that there is no genetic drift from the original human tumor as occurs in cell cultures. These brain tumor stem cells are similar to normal neural stem cells with similar genotype, gene expression patterns, and *in vivo* biology.¹⁰ Cancer cells are extracted from such animals and frozen for future use; the cells can be passaged a handful of times before they lose their characteristic genetic and proteomic makeup to genetic drift. Cancer stem cell markers are tracked to determine the stem cell nature of these neurospheres and to monitor for genetic drift.¹¹

B. *In Vivo* Surrogates

Murine models are convenient in that mice and rats have short gestations and life spans and are frequently genetically modified for research purposes. This allows for specific research questions to be analyzed with reliable and reproducible results, when the scientific plan is rigorous. Because of their size, they are relatively affordable to generate and to maintain, requiring minimal overhead costs. However, murine brain tumor models are typically those of human tumor cells implanted into the brain of immunocompromised mice and rats. This eliminates the impact of the natural immune system in either the establishment of the tumor or the effect of the therapy.¹² Hence, research into immunotherapy in murine models can be quite a challenge, not to mention the difficulty inherent in the translation of information from an immunocompromised model into a relatively immunocompetent patient. However, there is a strategy for creation of immunocompetent tumor bearing murine models via lentiviral vectors that is occasionally used, though it uncommonly leads to cortical tumors (the expected location of most aggressive gliomas).¹³

Large animal brain tumor models are varied and can include any common large research animal (e.g., rabbit, cat, dog, pig, nonhuman primate). However, spontaneous brain tumors are most commonly diagnosed in companion cats and dogs. The canine brain tumor model has significant advantages over other species and is the most widely studied and clinically

treated. Specifically, immunocompetent companion dogs develop spontaneous brain tumors at a known rate (diagnosed via necropsy) of 2% to 4.5%. Their risk of primary brain tumor increases with increasing age and weight.¹⁴ Brachycephalic (“smushed face”) breeds, particularly Boxers and Boston Terriers, are overrepresented in glioma diagnoses, which account for a third or more of all primary intra-axial (inside the brain tissue) brain tumors in dogs.^{15–17} Dolicocephalic (long-nose) breeds, such as Golden Retrievers and German Shepherds, more often develop meningiomas, the most frequently reported primary extra-axial (outside the brain tissue) brain tumor in dogs.¹⁸ Together, gliomas and meningiomas account for about 80% to 90% of canine primary brain tumors,¹⁵ and these are two of the most common brain tumors to develop in humans as well.¹⁹ Similar to human patients, the prognosis for dogs with brain tumors is poor with a median survival of 65 days (4 studies, 138 dogs) with symptomatic therapy only, 312 days (6 studies, 108 dogs) with surgical treatment, and 351 (13 studies, 428 dogs) with radiation therapy.²⁰ Occasionally, long-term survivors live 2.5 to 5 years with multimodal therapy; although dogs with intra-axial tumors, which are predominantly gliomas, were not typically in this long-lived group.^{20,21}

A spontaneously occurring brain tumor in an animal with an intact immune system is an ideal model for investigation of therapeutic approaches for spontaneously occurring brain tumors in humans with intact immune systems. Not surprisingly, this is the most common situation for development of brain tumors in humans, though certain immunodeficiencies (such as lymphoma) and genetic predispositions (such as neurofibromatosis or von Hippel–Lindau disease) will also lead to brain tumors. The histological presentation and molecular variance in canine brain tumors is remarkably similar to that of common and severe brain tumors in humans. Specifically, canine high-grade gliomas express vimentin, glial fibrillary acidic protein (GFAP), vascular endothelial growth factor receptor-1 (VEGFR-1), epidermal growth factor receptor-1 (EGFR-1), and platelet-derived growth factor receptor- α (PDGFR- α) as is seen in human high-grade gliomas.^{16,22–25} Furthermore, dog Glioblastoma Multiforme (GBMs) demonstrate endothelial proliferation, which is common in human GBMs but not in murine models of GBM. In recent years, the dog model has become even more powerful, because the entire genome has been sequenced and more than 25% of the canine sequence aligns with the human genome.²⁶ In addition, multiple existing mouse reagents have been found to be useful for analyzing dog samples, such as anti-mouse Fox3P antibody that cross-reacts with canine Fox3P, allowing identification of T regulatory cells, and mouse monoclonal antibodies to GFAP and vimentin.^{16,27}

Granted, research in spontaneously occurring canine brain tumors requires that researchers have access to veterinary centers that treat these brain tumors in dogs. Fortunately, the Comparative Oncology Trials Consortium (COTC) of academic veterinary medical colleges was created by the National Cancer Institute in 2015 to do just that.¹⁹ Additional opportunities for treatment of companion dogs are also now widespread in veterinary multispecialty referral private practices that have veterinary neurologists, surgeons, and oncologists. In addition, clinical trials for companion dogs are frequently used to assess preclinical efficacy data for therapeutic approaches to brain tumors.^{28,29} Certain weaknesses to this approach exist, the most important of which is financial support for the trials, because pets are less likely to have health insurance than humans. The life span of dogs is about 1/10 that of humans, and while this is useful for gaining rapid information about the outcomes of

novel brain tumor treatments, it may also influence financial decisions of pet owners about treating canine brain tumors, which costs \$10,000 to \$20,000, and the survival period is likely to be less than one year. Another challenge for veterinary specialists diagnosing canine brain tumors is access to specialized equipment, such as stereotaxic equipment for image-guided biopsies, particularly for inoperable tumors located deep in the brain. It is the standard of care for a human to have a surgical biopsy, if not excision, at the time of diagnosis with a brain tumor. Surgical debulking and biopsy have become more commonplace in dogs in recent years, as CT and MRI imaging have become more accessible in veterinary medicine and compatible stereotaxic systems have been developed. Nevertheless, as of 2012, only one commercial canine stereotaxic system based on magnetic resonance imaging (MRI) had been reported.¹⁵ Therefore, histologic diagnosis for canine brain tumors is often delayed until necropsy, if they are diagnosed at all. A systematic literature review found 6 of 22 canine studies reporting histological diagnoses.²⁰ Presumptive diagnosis and treatment may rather be based on the appearance of the tumor on computed tomography (CT) or MRI images.^{17,18,30}

Current treatment options for companion dogs diagnosed with primary brain tumors include surgery, radiation, novel therapy, and palliative care. Minimal evidence currently exists in veterinary medicine for the benefit of chemotherapy, largely because published studies did not include histologic diagnosis, and local, targeted application is currently under refinement.^{15,27,31} Tumor-targeted chemotherapy, specifically convection-enhanced delivery of liposomes containing gadoteridol and irinotecan (CPT-11) into brain tumors, is offered at some academic veterinary centers.^{15,31} Gene therapy, small peptides, and immunotherapy have all been tried in recent years.^{15,16} Whole body hyperthermia (42°C) in combination with radiation therapy was compared in a clinical trial to radiation alone, but systemic complications of whole body hyperthermia and lack of benefit led to disappointing outcomes.³² Many canine brain tumor patients are offered palliative care, typically corticosteroids to treat swelling around the tumor and antiepileptic medications to control seizures, and, when these options are no longer effective, compassionate euthanasia is offered.

III. THERMAL THERAPIES IN BRAIN TUMORS

Thermal therapies are typically categorized as either hyperthermia (~42°C to 45°C) or ablation therapy (> 50°C). At higher temperatures employed for thermal ablation, thermally induced cell necrosis occurs, depending on temperature, within ~1 to 2 minutes at 50°C and within seconds at 60°C, both for normal and cancerous cells.^{33–35} The cell death mechanism is likely based on irreversible denaturation of structural membrane proteins.^{36,37} An advantage of thermal ablation over chemotherapy or radiation therapy is that cells do not develop resistance towards heat. In essence, retreatment without loss of efficacy is theoretically possible in cases of recurrence. Since thermal ablation is not selective for cancer cells, the ablation has to be accurately targeted to prevent damage to surrounding normal tissue.

Hyperthermia has a number of biological effects of potential therapeutic interest. While some studies evaluate the concept of whole-body hyperthermia, here we will focus on loco-

regional hyperthermia application, or hyperthermia applied to the brain. The observation that cancer cells are more sensitive than normal tissue to the range of temperatures used for hyperthermia has been known since the 1960s,³⁸ and it spawned considerable interest in hyperthermia as cancer therapy in the following decades. Hyperthermia affects numerous cellular mechanisms, including impairment of transmembrane transport and protein synthesis, apoptosis induction, protein denaturation, enhanced cell membrane permeability, induction of heat shock proteins, and inhibition of DNA damage repair pathways.^{3,39} Based on these cellular effects, recent studies suggest that hyperthermia could become an important strategy in targeting therapy-resistant cancer stem cells.⁴⁰ In addition, hyperthermia affects the tumor microenvironment in various ways. Among others, heat alters blood flow; although the effect varies between tumors, mild hyperthermia (< 42°C) has been shown to improve perfusion,⁴¹ and temperatures above 42°C are likely to reduce blood flow.^{42,43} In addition, hyperthermia increases vascular permeability, and this effect has been employed to enhance accumulation of drugs and nanoparticles.^{44–47} Similarly, hyperthermia opens barriers such as the blood-retinal barrier⁴⁸ and the blood-brain barrier (BBB) to allow delivery of molecules and nanoparticles,^{4,6,44,49–53} though some studies suggest that the temperature required for BBB opening is in the range of temperatures cytotoxic to normal tissue.^{5,54} Relevant to the use of hyperthermia as an adjuvant is the ability to sensitize cells for chemotherapy and/or radiation therapy.⁵⁵ When combined with radiation therapy, the timing of hyperthermia should ideally be close to radiation therapy, though it is not clear whether it is more advantageous to apply hyperthermia before or after (in clinical trials, both have been used).³ The enhancement effect when combining heat with chemotherapy depends highly on the temperature and on the specific agent. Some agents such as 5-Fluorouracil are not beneficial, but the effect of most drugs is enhanced by a factor between ~1.5 and 4 times.³ Finally, hyperthermia may also have important immunological effects of possible therapeutic benefit, which may lead to new types of cancer immunotherapy.^{56,57}

Next we will review various devices that have been employed for hyperthermia and thermal ablation in the brain.

A. Thermal Probes

Thermal probes are the most basic form of hyperthermia delivery used in the brain. These probes are generally very simple heat conductors (e.g., heated by circulating hot fluid) inserted into brain tissue and are ideal for animal studies, as they are inexpensive to create and maintain. They have been used since the 1970s to kill cancer cells and are typically used for localized hyperthermia.⁵⁸ Some initial clinical trials in the 1970s and 1980s used heated probes,⁵⁹ but they were replaced by more effective and advanced heating technologies in later trials.

B. High-Intensity Focused Ultrasound

High-intensity focused ultrasound (HIFU) employs ultrasound focused typically via transducer arrays deep into the tissue from outside the body (i.e., noninvasively). HIFU can be employed for both ablation and hyperthermia therapies. Advantages of HIFU over other heating modalities include its non-invasive nature and high targeting accuracy (~mm), though targeted heating through the intact skull is often still problematic depending on

location. Ultrasound induced hyperthermia is helpful in reducing tumor burden and to enhance drug delivery in glioma models. Historically, it has been a challenge to deliver into the human brain, but recently developed systems allow delivery of ultrasound through the skull.⁶⁰ In murine models, HIFU permeabilizes the blood-brain barrier noninvasively, demonstrated by delivery of gadolinium-based contrast (Magnevist) into the targeted brain tissue.⁶¹

Without craniotomy, delivery of HIFU into the brain is in its early phases. When used, HIFU is usually combined with MRI thermometry for accurate dosing of hyperthermia.⁵⁸ A recent case study reports a 63-year-old patient who received magnetic resonance-guided focused ultrasound surgery for a glioblastoma. Focused ultrasound sonication treatments lasted 10 to 25 seconds at 150 to 950 Watt acoustic power. Because of heating in the bone, cooling was required in several minute periods between sonications. The patient received 25 sonication treatments with increasing acoustic energy up to 19,950 J per sonication, and 17 out of 25 treatments had a peak temperature above 55°C, maximum temperature of 65°C, and 240 cumulative equivalent minutes at 43°C.⁶² A handful of other patients have been treated with HIFU to the brain (without craniotomy), although toxicity includes fatal cavitation-induced intracranial hemorrhage.⁶³

Access to the posterior fossa is a particular challenge for HIFU, given the large concentration of bone material in this area. However, a study done in rabbits demonstrated that increased (target) temperatures were attainable when combined with microbubbles (gaseous microspheres injected intra-venously).⁶⁴

C. Radiofrequency/Microwave Hyperthermia

Electromagnetic waves can be utilized to induce hyperthermia either by radiofrequency or by microwaves. Broadly, these electromagnetically based hyperthermia modalities can be classified as external or internal (i.e., interstitial). External heating by radiofrequency or microwave heating has been found to have limited applications for brain tumor therapy, mainly confined to preclinical studies,⁶⁵ due to the inability of precise control of heating location in deep tissue regions. In addition, most studies employ interstitial devices inserted into the brain or tumor. If necessary, multiple interstitial applicators can be placed in arrays to enlarge the hyperthermia region.

The use of microwave hyperthermia for brain tumor treatment has been investigated since the early 1980s,⁶⁶ and a first clinical trial in six patients with glioma demonstrated safety and feasibility.⁶⁷ Microwave antennas can be placed stereotactically into the brain, or capacitively coupled radiofrequency heating devices may allow for transcranial heating.⁵⁸ In 1994, a team at Dartmouth treated 23 patients with numerous microwave antennas to determine the number and placement of antennas needed to heat a specific volume.⁶⁸ Because the CSF volume is lower, pediatric brains may be more ideal for development of microwave beamforming for noninvasive localized hyperthermia than the adult brain. However, this technique is also still in the development stage.⁶⁹ Although there were several promising clinical trials conducted in the 1980s and 1990s that combined microwave or radiofrequency hyperthermia with radiotherapy,^{67,70–74} these techniques have so far not progressed into clinical practice.

D. LASER

Laser therapy has been investigated for treatment of brain tumors since the 1980s, and it is currently the only thermal therapy widely used clinically for brain tumor treatment (Fig. 1). Laser therapy works by causing thermal damage with laser light (typically infrared; i.e., light > 800 nm wavelength) photons that are absorbed by the molecules of the targeted tissues, causing excitation and release of thermal energy. This thermal energy is then redistributed via convection through the tissues and conduction in the blood flow.⁷⁵ Initially, the challenge with laser therapy was that the extremely high heat of the laser would vaporize the brain tumors,^{76,77} causing increased intracranial pressure. These technical issues have been addressed, in part by combining laser with real-time temperature monitoring by MRI thermometry. Laser interstitial thermal therapy (LITT) has been developed and engineered with cooling systems to allow for targeted necrosis (which occurs at temperatures above 43°C for longer than 10 to 60 minutes in human brain tissues).⁷⁵ LITT has been tested along with MRI thermometry in dog brains, given the previously mentioned relevance of dog brain studies for translation into human trials.⁷⁸ The compatibility of LITT with MRI allows also for planning and monitoring of the treatment in real time.⁵⁸ Now that LITT has been developed, it is safer than resection in certain areas of the brain, with a 10% absolute risk reduction, as it is associated with less long-term neurologic morbidity and an increased rate of gross total resection compared to surgical resection.⁷⁹

When used alone, LITT is demonstrated to completely kill glioma cells with adequate thermal dose and has been proven as safe therapy for brain tumors in adults and children.⁸⁰ However, in the margin of the thermal treatment zone, glioma cells often survive and are able to replicate.⁸¹ Hence, LITT has been combined with some nanoparticles to attain improved glioma tumor control. For example, gold-silica nanoshells have been used to facilitate more conformal heating of canine tumors when injected intravenously. However, this strategy does allow for a heterogeneous delivery of the nanoparticles into the tumor region, because once they are delivered intravenously, targeting of the nanoparticles is reduced. This could lead to an inconsistent increase of thermal damage delivered to tumor areas.⁸²

E. Magnetic Nanoparticles

When certain magnetic nanoparticles are exposed to alternating magnetic fields of high strength, magnetic losses in the particles generate heat. After local administration of such magnetic nanoparticles at the treatment site, they can be employed to induce local hyperthermia. These nanoparticles are generally made up of iron oxide (magnetite or maghemite) and are coated with cationic liposomes or other biocompatible materials.^{83,84} Once the nanoparticles are delivered, patients are exposed to alternating magnetic fields to cause excitation with resultant increase in temperature. Magnetic field strengths range from 11 to 30 kA/m with an oscillation frequency of around 100 kHz.⁸⁵ In the setting of hyperthermia, magnetic nanoparticles cause glioma cell death and tumor shrinkage in murine models.⁸⁶ Magnetic nanoparticles also cause mechanical destruction of malignant glioma cells when exposed to alternating field currents, regardless of the temperature effect of the nanoparticles, because excitation of the iron nanoparticles causes movement that

induces cell death.⁸⁷ In fact, some magnetic nanoparticles have antitumor effects in absence of any alternating magnetic field and act independently as chemotherapeutic agents.⁸⁸

Control of the area where these nanoparticles are delivered is achieved with a direct injection of the nanoparticles into the brain tumor (with water as carrier substance).⁸⁹ A limitation in the approach of injecting magnetic nanoparticles into the tumor is that the fluid injected does not evenly distribute throughout the tumor and is constrained in the infusion area. Hence, multiple injections of magnetic fluid are required to treat the entire tumor area. The lack of a noninvasive method for targeted nanoparticle delivery is currently a limitation of this approach. A postmortem report in patients with GBMs treated with magnetic nanoparticles reassuringly does not demonstrate harmful aggregates or reactions (including sarcomatous tumors, sterile abscesses, or foreign body giant cell reactions).⁹⁰

To date, magnetic nanoparticles have been combined with different therapies. *In vitro*, magnetic nanoparticles have been combined with microRNAs to increase the efficacy of hyperthermia cell kill. They have also been combined with radiotherapy in human trials, and a Phase II clinical trial has demonstrated prolonged survival in patients with recurrent GBM compared to historical controls.⁹¹

F. Combinations of Thermal and Drug Therapies

1. Hyperthermia and Free Drug—While there are extensive *in vitro* studies demonstrating synergy between hyperthermia and chemotherapy, there are comparably fewer *in vivo* studies on this combination. The combination of either cisplatin or carboplatin with hyperthermia was evaluated in rats, in glioma or GBM-like tumors implanted on the hind leg, and demonstrated enhanced efficacy. More recently, a combination of alternating magnetic field hyperthermia with magnetized nanoparticles and 1,3-bis(2-chlorethyl)-1-nitrosurea (BCNU) demonstrated an increase in efficacy when compared with the magnetic nanoparticles or BCNU alone (in the presence of the alternating magnetic field) in murine legs bearing implanted glioma tumors.⁹²

HIFU has been combined with systemic microbubbles to locally and transiently disrupt the blood-brain barrier in a targeted fashion. This approach has demonstrated enhanced delivery of BCNU, epirubicin, doxorubicin, gene-based therapies, and various immunotherapies, although these therapies are still in preclinical development.^{93,94}

In addition to investigating chemotherapy in combination with HIFU, sonosensitizers (such as porphyrins) have been demonstrated to increase glioma cell death when combined with sonication *in vitro*.⁹⁵

A combination of intra-arterial doxorubicin (Adriamycin) and radiofrequency induced hyperthermia was used to treat glioma in rats. This *in vivo* study demonstrated both increased doxorubicin delivery and longer survival for the rats after treatment with this combination of therapy.⁹⁶

2. Hyperthermia and Nanoparticles—Another combination therapy available against malignant brain tumors is the combination of hyperthermia and nanoparticles. For example,

a combination of hyperthermia and magnetite cationic liposomes carrying gadd 153, a stress-inducible *TNF- α* gene promoter, demonstrated arrest in tumor growth in glioma-bearing nude mice.⁹⁷ MicroRNA designed to modulate heat-shock proteins can also be packaged into nanoparticles and delivered in the setting of hyperthermia to kill glioma cells.⁹⁸ Alternatively, some nanoparticles are designed to be toxic in the setting of hyperthermia without additional components, such as silver nanoparticles that have also been shown to be active against glioma cells.⁹⁹

Chemotherapy can be packaged into nanoparticles for delivery in the setting of hyperthermia. For example, doxorubicin has been delivered into the brain in the setting of hyperthermia (produced by ultrasound) to treat a murine model of metastatic breast cancer (breast cancer cells were implanted into mice brains) with demonstration of doxorubicin delivery and efficacy against the implanted tumors.¹⁰⁰

In other recent studies, elastin-like polypeptides (ELP) have been investigated because of their thermoresponsive nature. One study recently developed ELP to target gliomas in rat brains. By combining these ELPs with cell-penetrating proteins and cMyc inhibitor peptide and hyperthermia, they delivered the cMyc peptide to the target tumor and reduced its progression.¹⁰¹ In a similar approach, Bernardi et al.¹⁰² targeted the Notch pathway by delivering a Notch inhibitory peptide by conjugating with an ELP and cell-penetrating protein.

Another group examined temperature sensitive liposomes that release the drug when heated to above ~40°C, filled with cisplatin for delivery to brain tumors in rats, and performed follow-up studies in a canine model.^{103,104} They demonstrated delivery of therapeutic doses of this chemotherapy to the tumors, resulting in survival benefit in the rat model. Also, doxorubicin encapsulated in temperature-sensitive liposomes has been demonstrated to improve drug delivery and survival in a rat glioma model.¹⁰⁵

3. Engineering New Thermal and Drug Combination Therapies—Both the heating technology and drug delivery system need to be carefully engineered to realize the full benefit of this combination therapy. The ideal heating technology would be noninvasive, allow accurate spatial control of the heated region, and expose the target region to a narrowly defined temperature range (to ensure therapeutic temperatures while preventing toxicity from overheating of normal brain tissue). None of the heating methods discussed previously completely fulfills all these requirements. HIFU combined with MRI thermometry comes close in many aspects, but it is still limited to heating primarily the central brain regions that are far from bony structures.

The drug delivery system has additional requirements to fulfill. First, it must be able to cross the blood-brain barrier (BBB) to reach the cancer cells. In tumor regions where the BBB is not compromised, additional means are necessary to open the BBB. Hyperthermia with carefully controlled temperature to prevent damage to normal brain tissue may be able to facilitate controlled penetration of the BBB. Otherwise, microbubble-mediated penetration of the BBB combined with focused ultra-sound is a highly promising method for transporting substances across the BBB. The drug delivery system must then allow delivery

to the targeted region while limiting uptake in nontargeted tissues. Such focused delivery could be facilitated by employing heat-responsive delivery systems (e.g., temperature-sensitive liposomes or polymers) to only release drug in the heated region, or by adding moieties specific to the targeted cells.

Finally, thermal therapies can be employed in another fashion: high temperatures ($> 50^{\circ}\text{C}$) allow direct and complete destruction of tumor regions without additional means (e.g., as used in LITT). In a potential combination approach, the central tumor regions could be treated by high temperatures. This is advantageous since these regions are often hypoxic and/or have limited perfusion, making chemotherapy and radiation therapy challenging. The same heating device could then be employed to facilitate hyperthermia and be combined with targeted drug delivery to the tumor margins and the region surrounding the visible tumor, possibly reducing recurrences.

G. Combinations of Hyperthermia and Radiation Therapy

Hyperthermia is the most widely studied radiation sensitizer, and the therapeutic combination of hyperthermia and radiotherapy has been studied since the 1970s. Also for brain tumors, this therapy combination has been widely investigated *in vitro*, *in vivo*, and in clinical trials.^{58,106,107} Hyperthermia sensitizes glioblastoma cells, which are resistant to radiation and/or chemotherapy, both *in vitro* and *in vivo*. One contributing factor is that hyperthermia inhibits several DNA repair mechanisms, thus enhancing the efficacy of DNA damaging radiation or chemotherapy agents.³⁹

The often limited efficacy of radiation therapy alone has been attributed to the heterogeneity of the tumors and the presence of stem-like cells,¹⁰⁸ which render the recurrent clonal tumors resistant to radiation.^{108,109} Several pathways have been implicated in this resistance.¹¹⁰ One study found that PI3K-AKT was involved in the development of resistance of these glioblastoma stem-like cells,¹¹⁰ and there has been increasing evidence that PI3K-AKT pathways play an important role in the repair of double-strand breaks caused by radiation.¹¹¹ It was found that hyperthermia sensitizes stem-like cells to radiation by inhibiting the AKT pathway.¹¹² Bao et al.¹¹⁴ showed that exposing glioblastoma cells to conventional radiation therapy led to the enrichment of CD133+ (Prominin-1, neural and hematopoietic cancer stem-like cell marker overexpressed in multiple cancers¹¹³) cells. In an *in vitro* study, Cha et al.¹¹⁵ exposed glioblastoma cells to electro-hyperthermia, causing a significant decrease in the population of CD133+ cells. Similarly, in a pancreatic tumor model, Garcia-Santos et al.¹¹⁶ used gemcitabine along with hyperthermia to decrease the CD133+ cells. The use of gemcitabine alone had little to no effect on the CD133+ cell population.

1. Clinical Trials of Hyperthermia with Radiation—Several clinical studies have demonstrated the benefit of adding hyperthermia to radiotherapy in a variety of tumors, including superficial tumors,¹¹⁷ melanoma,¹¹⁸ pelvic tumor,¹¹⁹ and breast cancer.¹²⁰ In the 1980s and 1990s, there were several clinical hyperthermia trials for brain tumors, which were summarized by Sneed et al.¹⁰⁷ In the late 1990s, Sneed et al.¹²¹ reported a Phase II/III clinical study for glioblastoma patients comparing radiation (brachytherapy) alone versus radiotherapy with hyperthermia. About 40 patients were enrolled into each of the two

treatment arms. The patients that received the combination therapy experienced significantly prolonged mean survival (85 versus 76 weeks).¹²¹ However, hyperthermia is currently not used clinically in combination with radiotherapy.

IV. FUTURE DIRECTIONS

There are several ongoing efforts in novel thermal therapy approaches. For example, there is currently one open clinical trial for hyperthermic opening of the blood-brain barrier in the setting of chemotherapy administration for treatment of brain tumors. In this study, MRI is used for guidance of LITT to induce hyperthermia. The chemotherapy administered includes doxorubicin and etoposide, and the tumors treated are multiple resistant pediatric brain tumors (clinical trial NCT02372409). There is currently great interest in various forms of immunotherapy, and hyperthermia also has several potentially beneficial immunotherapeutic effects that may be translated into clinical therapies.⁵⁶

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FIG. 1.

LITT therapy overview: A laser fiber is introduced under imaging guidance into the tumor, and the tumor is treated by heat while monitoring tumor and surrounding tissue temperature with MR thermometry. (Repinted with permission from John Wiley & Sons, Copyright 1998)⁷