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REVIEW

FOCUSED ULTRASOUND THERAPY IN CANCER CARE

Gaetano **Romano** [1, *], Elisa **Ventura** [1, 2], Kailey Shanti **Abraham** [1], Francesca **Cersosimo** [1, 2], Antonio **Giordano** [1, 2]

[1] College of Science and Technology, Temple University, Philadelphia, PA, USA
[2] Sbarro Health Research Organization, Philadelphia, PA, USA
[*] Correspondence to: gromano@temple.edu, https://orcid.org/0000-0002-6340-5879.

ABSTRACT: Focused ultrasound (FUS) holds great therapeutic potential in cancer care. Both clinical and preclinical studies indicate that FUS may eventually become a safe and efficient approach for the treatment of oncological patients. FUS-induced anticancer responses comprise tumor growth inhibition, enhanced T lymphocytes infiltration within the tumor mass and increased permeability of the vascular system, including the temporary opening of the blood-brain barrier. On these grounds, FUS can be utilized to target the tumor microenvironment, increase the efficiency of therapeutic agents and, possibly, elicit local and/or systemic host immune responses against malignant tissues and/or cells.
Clinical and preclinical studies are currently in progress to optimize anticancer FUS applications in establishing long-term systemic host immune responses, which may ultimately lead to abscopal effects in patients.

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Impact statement: This article discusses the latest developments in the field of FUS in cancer care, in terms of technology and therapeutic effects that were reported among various oncological patients. In addition, the article focuses on clinical and preclinical studies that aim at characterizing the effects of FUS on the tumor microenvironment, the impact on the vascular system and modulation of host immune responses against malignant cells.

Key words: *Focused Ultrasound Therapy; abscopal effects; radiation therapy; cancer immunotherapy; CTLA4; PD1; PDL1; PDL2; immunomodulators; immunogenic cell death.*

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INTRODUCTION

Ultrasound techniques for therapeutic applications must utilize higher energy than ultrasound for diagnosis and/or imaging, otherwise the biological effects will be obviously negligible (1). Focused ultrasound (FUS) has been designed to propagate mechanical waves within a restricted volume to produce high energy density, which is then absorbed by the tissues of the site of the treatment (1, 2). The mechanical waves are generated by a focused transducer (**figure 1**). The biological effects induced by FUS depend on the type and location of the tissue or organ that is irradiated and a variety of ultrasound parameters (3-8). In addition, gas-filled therapeutic microbubbles or nanodroplets may be added to increase the efficiency of the ultrasound-based intervention (9-11).
The main FUS parameters that can be varied to optimize the biological effects in the treated tissue are frequency, treatment time, pressure, and duty cycle (12, 13): frequency is directly proportional to the energy that is imparted into the irradiated tissue and represents the number of sound wave cycles transmitted over time. Frequency is measured in hertz (cycles per second).
Treatment time constitutes the total period that is required to scan the entire area for the intervention.
Ultrasound pressure is measured in Pascals and depends on the perpendicular force that is exerted on a determined surface area. The ultrasound pressure equals the sound wave amplitude. High pressure is utilized for ablative treatments, whereas non-ablative treatments necessitate low ultrasound pressure (7).
Duty cycle is defined as the time percentage of the on-phase ultrasound application, during the total on-and-off pattern. Thermal-based treatments require long pulses because ultrasound waves are

quickly absorbed by the irradiated tissues in the form of heat. Conversely, non-thermal treatments are conducted with short and rapid pulses to prevent the release of heat (8).

The four ultrasound parameters may be utilized in several combinations to induce different types of thermal and/or mechanical stress in treated tissues or organs.

There are four modalities for FUS-based treatments, which can be classified as follows: I) thermal ablation for the induction of coagulative tissue necrosis; II) hyperthermia and thermal stress to generate a mild cell heating without inducing coagulation; III) mechanical stimulation without the production of thermal effects; IV) histotripsy for the mechanical destruction of the irradiated tissue. Interestingly, FUS allows for the immunomodulation of the tumor microenvironment (2-5) and enhances the vascular system permeability (14, 15). These two effects may result in local and/or systemic host immune responses against the tumor and enhance the efficiency of the administration of various therapeutic agents (16, 17). On these grounds, FUS is emerging as a promising technology for the treatment of patient with cancer.

FUS-based cancer therapy is a rather recent area of investigation, which is still addressing several challenges to define the conditions for an optimal antitumor response in patients (1-11) (**table I**). FUS has a wide variety of parameters that must be adapted for each tumor type and treatment site (3-8). Furthermore, each of the previously mentioned FUS modalities for cancer therapy are associated with a particular combination of immunological and vascular effects in tumors (2-5, 14, 15). In addition to an increase in the vascular permeability, FUS-induced responses against a malignancy include enhanced T cell infiltration in malignant tissues and inhibition of tumor growth (18-23).

Studies are currently underway for the characterization of FUS-induced effects on the tumor microenvironment, which are the surroundings that support the malignant mass (18-23). The tumor microenvironment is constituted by the stroma, extracellular matrix, signaling molecules, blood and lymphatic vessels, cells of the immune system and fibroblasts (24, 25). Immunologically speaking, the tumor microenvironment can be classified as either permissive or not permissive to the infiltration of cytotoxic T lymphocytes (19, 20). In a tumor microenvironment that is permissive to the infiltration of cytotoxic T lymphocytes, the tumor-associated antigens are easily available to the host immune system. Therefore, the exposure to the host immune system confers a good degree of immunogenicity to the tumor microenvironment. Conversely, in the absence of cytotoxic T lymphocytes, the tumor microenvironment is scantily immunogenic, despite the presence

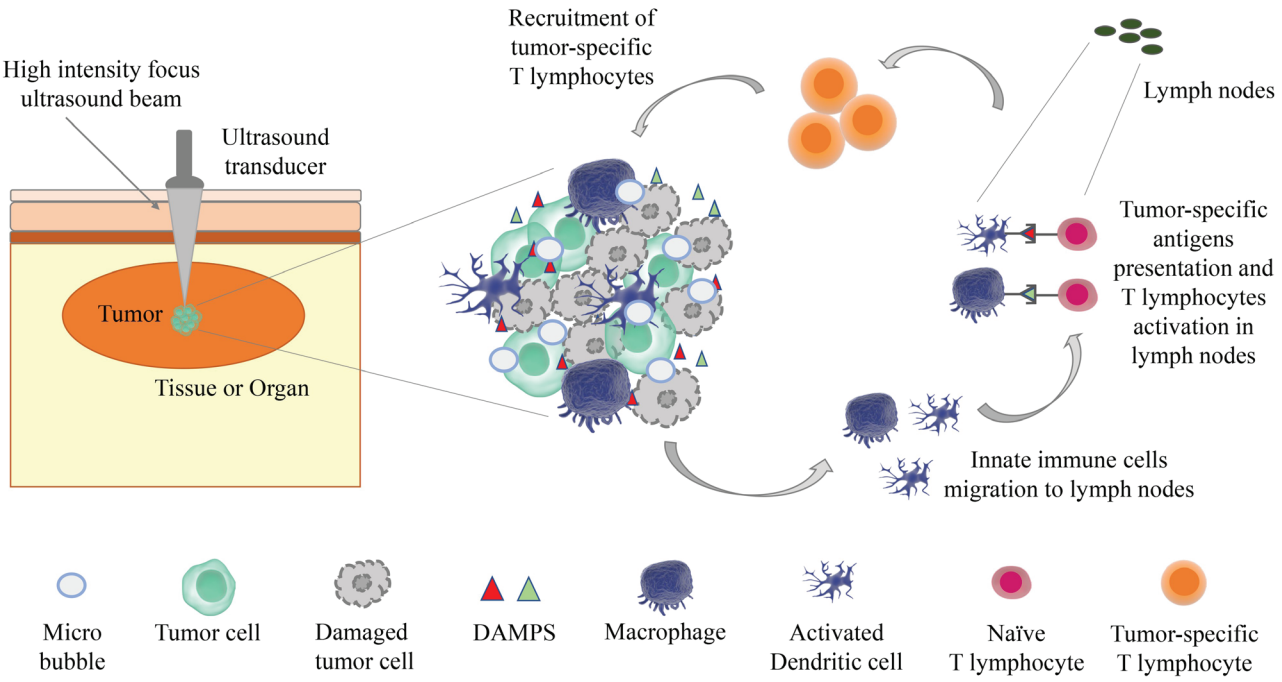

Figure 1. *Effects of an ultrasound transducer on the tumor mass. The transducer conveys the ultrasonic waves to a restricted volume of the tumoral tissue. The ultrasound acoustic waves, possibly combined with microbubbles, induce the death of malignant cells, which may elicit host immune responses against tumor associated antigens. These immunological effects are explained in greater detail in figure 3.*

Table I. *Summary of FUS modalities for the treatment of cancer.*

FUS MODALITY	BIOLOGICAL EFFECT(S)	COMBINATION OF FUS PARAMETERS TO OPTIMIZE THE FUS MODALITY
Thermal ablation. High Intensity Focused Ultrasound (HIFU).	Induction of coagulative tissue necrosis; removal of malignant tissues, without affecting healthy tissues and organs.	High pressure of sound waves transmission combined with intense duty cycle. The HIFU biological effects can be enhanced with microbubbles, or nanodroplets.
Hyperthermia and thermal stress.	Mild cell heating without inducing coagulation.	Hyperthermia and thermal stress temperature vary from 40 °C to 45 °C inside the treated area. Hyperthermia requires a treatment period of 30 to 90 minutes. Thermal stress is conducted in a shorter timeframe (from seconds to a few minutes).
Mechanical stimulation without thermal effects. (Mechanical perturbation). (LOFU).	Lack of thermal effects	Low to moderate pressure combined with high-duty cycle. Microbubbles, or cavitation may enhance the performance of mechanical stimulation.
Histotripsy for the mechanical destruction of the irradiated tissue. (M-HIFU, or cavitation cloud histotripsy). Boiling histotripsy.	Mechanical destruction and liquefaction of the irradiated tissue.	Very high pressure in combination with short-pulse ultrasound waves, which are in the range of microseconds. Boiling histotripsy requires longer pulses, which are still in the range of microseconds and produces boiling bubbles, and lower peak pressures than cavitation cloud histotripsy.

of other types of cells of the immune system. The absence of cytotoxic T lymphocytes in the tumor microenvironment may be related to a combination of factors, such as the constitution of the vasculature of the tumor and/or deficiency of priming and/or recruitment of CD8+ T cells (16, 17, 19-25). Recruiting CD8+ T cells may result from the activation of immune checkpoints pathways based on CTLA4, PD-1, PD-L1 and PD-L2 (16, 17), along with the release of other soluble factors like adenosine, which has immunosuppressive properties and is overexpressed in the tumor microenvironment (26-29).

FUS-BASED THERAPEUTIC APPROACHES IN CANCER CARE

FUS holds great therapeutic potential for the treatment of cancer because it is based on non-invasive practices that only require outpatient interventions, unlike most surgical interventions (30). Furthermore, the FUS treatments are very precise and do not involve the use of ionizing radiations (31, 32). Thus, FUS avoids the risk of infections, which are usually associated with surgical procedures, and it does not cause the adverse effects of ionizing radiations (31, 32).

FUS-based treatments are currently subdivided into four branches: thermal ablation, hyperthermia and thermal stress, mechanical stimulation without thermal effects and histotripsy. Each type of treatment has specific biological effects in malignant cells (1-11) and they can be produced by varying the four main parameters of FUS: frequency, treatment time, pressure and duty cycle (**table I**).

In addition, the use of gas-filled microbubbles, or nanodroplets can enhance the FUS-derived mechanical and/or thermal effects in targeted tumoral tissue (33-35). The increased efficiency of the treatment may allow for the reduction of acoustic waves intensity, along with a more precise intervention in the affected area (33-35). Gas-filles microbubbles or nanodroplets are administered via intravenous injection. The protocol for the production and purification of microbubbles and/or nanodroplets can be found at https://www.jove.com/it/t/62203/production-membrane-filtered-phase-shift-decafluorobutane. These gas-filled particles respond to the stimulation of acoustic waves with non-linear and fast oscillations, during which the acoustic radiation force (ARF) moves the oscillating microbubbles along the vascular walls. This phenomenon is termed cavitation (36, 37) and consists of an intricate series of events that derive from a variety of physical effects (38-40). Nevertheless, cavitation is simply summarized either as stable, or inertial phase (38-40). The FUS-induced gradual and constant oscillations give rise to a stable cavitation (38), whereas inertial cavitation consists of a fast expansion and violent collapse of the microbubbles in response to

FUS. Inertial cavitation produces various effects, such as: intense heat and pressure in the volume of the bubble core, presence of reactive oxygen molecules and local mini-streams that harm tissues and cells in the area of the intervention (39, 41, 42).

THERMAL ABLATION

The most common and best characterized ultrasound-based therapeutic approach is thermal ablation, which is also termed High Intensity Focused Ultrasound (HIFU) (43, 44). Thermal ablation is a noninvasive modality for the extirpation of malignant tissues that does not damage nearby healthy tissues and/or organs (44). The high pressure of sound waves transmission, in conjunction with the intense duty cycle, result in an amount of energy that is absorbed by the irradiated tissue and transformed into heat, with local temperatures ranging from 60 ˚C to 85 ˚C (44). The elevated local temperature within the core of the affected malignant tissue induces coagulation and necrotic cell death (**figure 2**), whereas a milder thermal stress affects the surrounding tissue that borders the focal spot and results in apoptotic cell death (**figure 2**) (5). The presence of microbubbles, nanodroplets, or cavitation of small bubbles in fluids may enhance the FUS-induced local heating and cause mechanically derived injuries within treated malignant tissues (45-47). Thermal ablation can be conducted with high precision, since FUS-induced lesions size can be narrowed down to the range of a millimeter (48). The precision of the treatment can be further increased either with sonography, or magnetic resonance imaging (MRI) to guide and monitor the heating effects induced by the thermal ablation (49). The Food and Drug Administration (FDA) has approved thermal ablation for the treatment of uterine fibroids (50), prostate cancer (51), management of bone metastases-related pain (52) and essential tremor care for patients with Parkinson's disease, or fragile X-associated tremor/ataxia syndrome (53).

Thermal ablation affects the tumor microenvironment by inhibiting cell proliferation, degradation and/or denaturation of a variety of cellular factors and causing the swelling of endothelial cells, which results in reduced blood flow inside the tumor mass (54). Thermal ablation-induced occlusion of feeder vessels causes the loss of vascular elasticity, destruction of the capillary endothelium, cavitation of peritubular cells, incomplete plasma membrane and sever damage of the tumor capillary ultrastructure (34). The ensuing traumatic cancer cell death produces debris, along with the discharge of damage associated molecular patterns (DAMPs) and

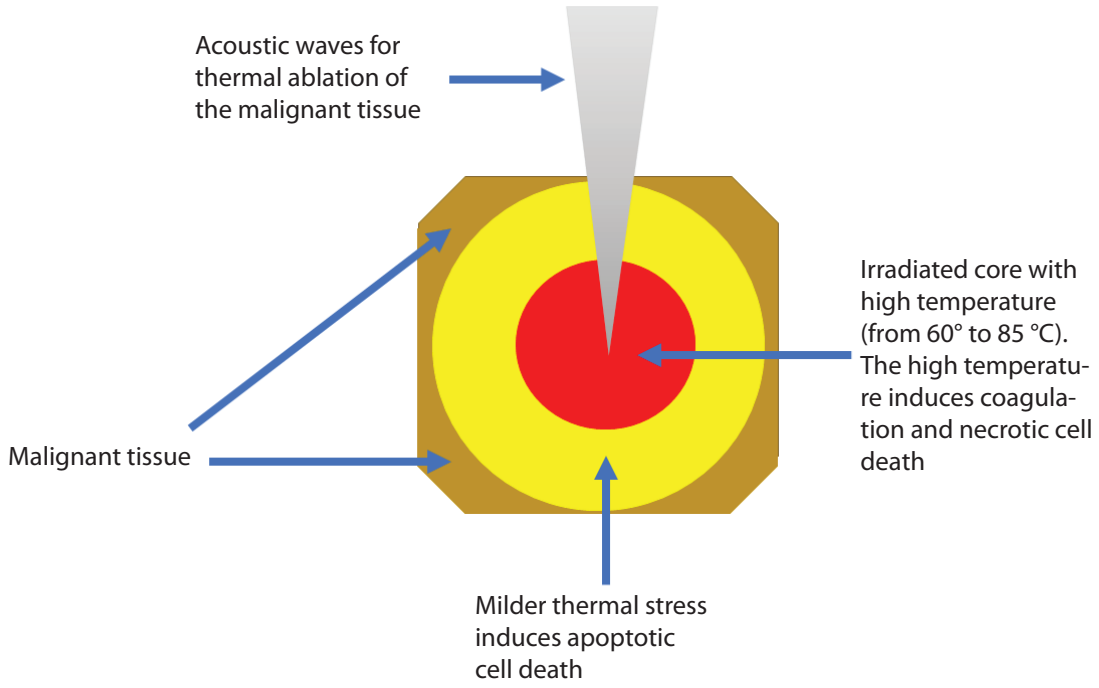

Figure 2. *Schematic representation of acoustic wave-induced thermal ablation of a tumor mass. The acoustic waves are denoted by the gray cone. The inner irradiated core with high temperature is reported in red, whereas the milder thermal stress is shown in yellow. The normal malignant tissue is represented in brown.*

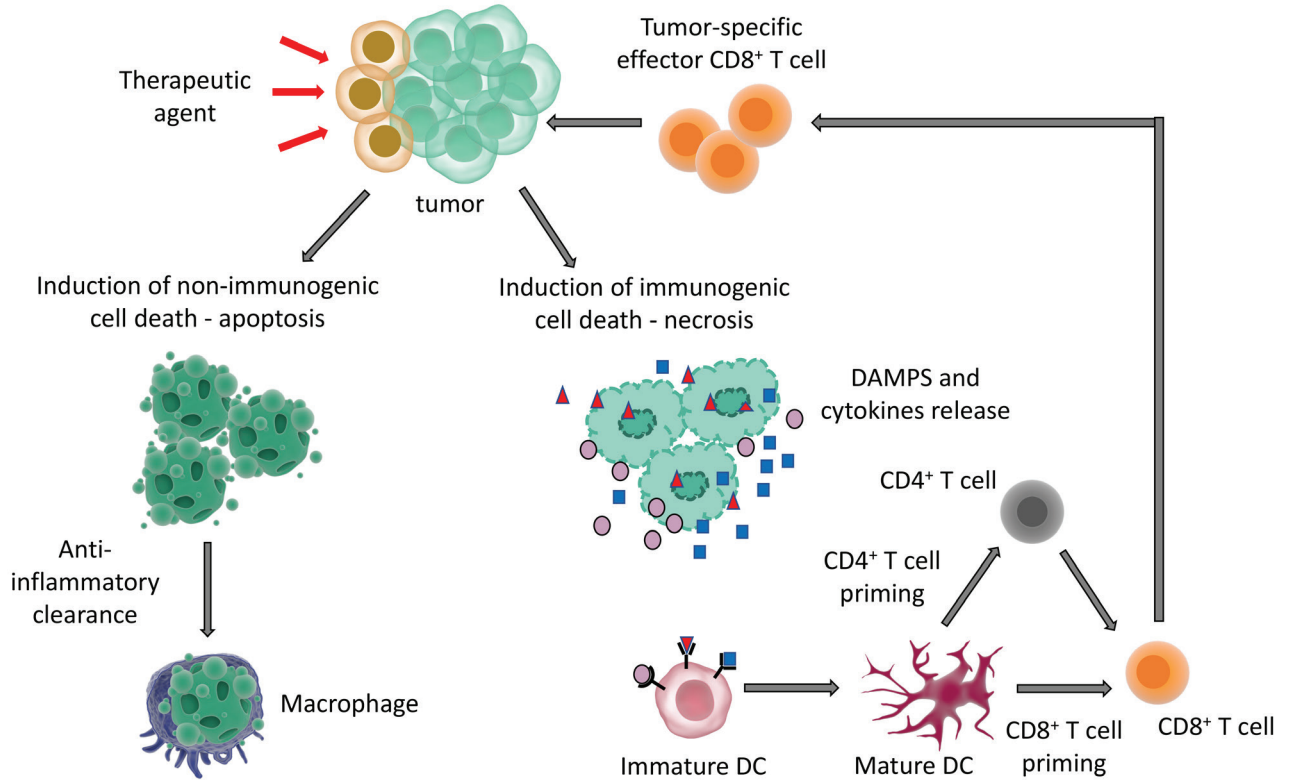

Figure 3. *Immunogenic and non-immunogenic cell death. A therapeutic agent destroys part of the malignant cells of the tumor mass. Some cells may undergo apoptosis, which does not elicit inflammatory reactions in the affected tissues, or organs. The cellular debris are subsequently removed by macrophages. Conversely, the necrotic or necro-apoptotic cell death releases cellular debris and other factors, which stimulate inflammatory reactions through DAMPs. The inflammation may ultimately lead to the maturation of dendritic cells, which then program tumor-specific cytotoxic CD8-positive T cells to attack the remaining cancer cells in the tumor mass and, possibly, in other regions of the organism (image taken from: Ventura E, Costa A, Dominguez RB, Romano G. Abscopal effects induced by localized interventions in oncological patients. Ann Res Oncol. 2021;1(4):272-84. doi: 10.48286/aro.2021.29).*

heat shock signals, which result in immunogenic cell death (16, 17) (**figure 3**). All the released inflammatory factors can be detected by surrounding antigen presenting cells, with consequent activation of host immune responses against tumor associated antigens (5, 16, 17, 55-60).

HYPERTHERMIA AND THERMAL STRESS

Both FUS-induced hyperthermia and thermal stress heat malignant tissues to milder temperatures compared to thermal ablation (61). Typically, areas treated with hyperthermia and thermal stress reach temperatures from 40 °C to 45 °C. The difference between hyperthermia and thermal stress depends on the time of the treatment. Hyperthermia requires a treatment period of 30 to 90 minutes, whereas thermal stress only requires a timeframe that ranges from seconds to a few minutes (61).

Hyperthermia and thermal stress have been applied in cancer care as adjuvants in order to elicit immune responses against the tumor, both in radiation therapy and chemotherapy and thermally activated drug delivery (61, 62).

Hyperthermia interferes with the cell cycle progression by targeting proteins and inducing DNA damage, which leads to traumatic cell death and/or apoptosis (63-65). Besides FUS, various techniques can be utilized to induce hyperthermia, such as: microwaves, radiofrequency and old-fashion physical methods based on warm water and heated air (66). In addition to inducing hyperthermia, FUS has the advantage of provoking mechanical damage in treated malignant tissues (67). At a cellular and molecular level, hyperthermia causes a short-term decrease in RNA synthesis and a longer-term decline in DNA synthesis in the S and M cell cycle phases, which result in a reversible cell proliferation arrest while some malignant cells undergo apoptosis (68). Moreover, hyperthermia sensitizes cancer cells to

radiation therapy and/or chemotherapy through the inhibition of the DNA repair pathways (69).

Hyperthermia induces vasodilation, which enhances blood flow and consequently causes a decline in hypoxia, interstitial tumor pressure and acidosis (67, 70). In summary, hyperthermia has a dual effect: it induces the death of malignant cells that releases DAMPs and increases the blood flow in the treated area, which signals cells of the immune system to enter the tumor mass. The combination of these two effects may activate the host immune system against cancer cells (16, 17).

Among the various systems for ultrasound-induced hyperthermia for clinical applications, the FDA has approved two devices named Sonotherm 1000 and Sonalleve (71, 72). Sonalleve is a commercially available magnetic resonance imaging system that guides hyperthermia-based treatments. FUS-induced hyperthermia has been utilized in clinical trials in combination with radiation therapy and/or chemotherapy for the treatment of a variety of malignancies, such as: ovarian cancer, metastatic pelvic tumors, prostate cancer, head and neck tumors, rectal cancer, glioma, hepatocellular carcinoma, and gastric cancer (73-81).

MECHANICAL STIMULATION WITHOUT THERMAL EFFECTS

The application of FUS to induce mechanical stimulation without thermal effects in irradiated tissues is also termed mechanical perturbation (82-87). The avoidance of thermal effects while imparting mechanical stimulation into cells and/or tissues can be achieved with low to moderate pressure combined with high-duty cycle (82-87). Sonoporation allows for transient apertures in cellular and/or tissues structures, such as cellular membranes and/or the three types of cellular junctions (86, 87). The focused ultrasound parameters for mechanical perturbation are adjusted to produce sonoporation-induced vasodilation, temporary opening of the blood-brain barrier and sonoporation of malignant tissues (88-92).

The effects of mechanical perturbation can be enhanced with microbubbles, by means of either acoustic pressure-induced fast expansion and contraction cycles, or cavitation (93). The rapid expansion and contraction of the microbubble is likely to cause a sudden rupture that may induce traumatic cell death by impairing the cellular membranes and affecting cellular metabolism (94, 95). In comparison, the biological effects of cavitation derive from the stable oscillation of the microbubbles, which creates a modest shear stress (93). The entity of the cellular membrane impairment may result either in apoptotic or necrotic cell death, and/or cell lysis (16, 17, 94, 95). Injured cells can repair minor cellular membrane damages and survive, or alternatively undergo apoptosis (94, 95). Conversely, extensive damage to the cellular membrane that cannot be repaired will only cause necrotic cell death (94, 95).

In preclinical studies, the increase of the vascular and cellular permeability induced by the combination of LOFU with microbubbles can be utilized to optimize the efficiency of nanoparticle carriers for drug and gene delivery (96). Following the interaction between the acoustic waves with the irradiated tissues in the presence of microbubbles, the acoustic radiation force (ARF) moves the oscillating microbubbles along the vascular system walls, enhancing the extent of the mechanical stress (97, 98).

MRI-guided sonoporation in combination with circulating microbubbles was applied in a phase 0 clinical trial for the temporary opening of the blood-brain barrier in patients with infiltrating gliomas (99). The transcranial administration of microbubble-enhanced sonoporation was safe and effective in causing the temporary opening of the blood-brain barrier (99). This finding is very promising for the development of novel and noninvasive interventions to treat patients with gliomas. For instance, the transient opening of the blood-brain barrier might optimize the efficiency of therapeutic agents in patients with brain tumors. Other studies have addressed the possibility of utilizing MRI-guided sonoporation for the transient opening of the blood-brain barrier (100, 101), which consequently may allow for the release of brain tumor markers into the bloodstream, such as circulating tumor DNA (100), or eventually, circulating tumor cells (101).

HISTOTRIPSY

Histotripsy is the most recent ultrasound-based technique for the modulation of host immune responses in cancer therapy (102, 103).

Histotripsy, also termed M-HIFU, or cavitation cloud histotripsy, requires very high pressure in combination with short-pulse ultrasound waves, which reduces the treated malignant tissue into an emulsion that is subsequently absorbed by the organism (102, 103). The pulse of these ultrasound waves is in the range of microseconds and pro-

duces condensate cavitating bubble clouds that undergo quick expansion and contraction (104), which, in turn, generates a mechanical force that smashes the affected tissue, with negligible thermal impairment (105).

A second type of histotripsy is termed boiling histotripsy. It requires longer pulses, which are still in the range of microseconds and produce boiling bubbles (106). The mechanical liquefaction of the treated soft tissue derives from the interaction between the boiling bubbles and the incident shockwaves (106). Boiling histotripsy fractionates the treated tissue into subcellular fragments without thermal impairment (107). By means of longer pulses, boiling histotripsy can also impart thermal impairment, in addition to the mechanical damage caused to the treated tissue (107).

Boiling histotripsy requires lower peak pressures than cavitation cloud histotripsy (106-108). For this reason, boiling histotripsy protocols can be more easily adapted to the needs of HIFU-based clinical applications (108). The consistency of the malignant tissue also plays a role in choosing the type of histotripsy for the treatment (109). For instance, tumor masses that have a high content of fibrotic tissues tend to be more resistant to histotripsy-based intervention (110). In these cases, cavitation cloud histotripsy might be more appropriate than boiling histotripsy for the treatment of the tumor.

Histotripsy-induced emulsification of tumor deposits is essentially mechanical, which may lead to the release of tumor-associated antigens that are not denatured by thermal effects and can stimulate an immune response (56, 110). Differences in eliciting host immune responses against a tumor between boiling histotripsy and thermal ablation based FUS were reported in a mouse EL4 thymoma model, using MRI and histopathology analyses (111). Boiling histotripsy-induced lesions exhibited forms of microhemorrhages on the border between the injured and unaffected tumoral tissue. The injured tumor mass contained a dense concentration of necrotic and apoptotic malignant cells and infiltration by macrophages and granulocytes was detected 4 days post-treatment (111). Instead, the thermal ablation-based FUS on the tumor exhibited the following effects: no sign of hemorrhage in the lesion produced by the treatment, heat-fixed malignant cells were observed in the central area of the treated tumor mass and macrophages and granulocytes were only present on the edges of the lesion (111). Preclinical studies are currently underway for the characterization of the effects of histotripsy on the vascular and immune systems in the context of different types of tumors (112-114).

Histotripsy was utilized for the first time in a clinical trial conducted in Barcelona, Spain for the treatment of 8 patients with either primary or multifocal liver metastases, derived from various kinds of primary tumors, such as colorectal cancer, hepatocellular carcinoma, breast cancer and gallbladder carcinoma (this information is available from: https://www.fusfoundation.org/the-technology/timeline-of-focused-ultrasound/first-histotripsy-clinical-trial/).

Histotripsy will be utilized in a clinical trial that is currently recruiting patients with primary or metastatic liver cancer (this information is available from: https://clinicaltrials.gov/ct2/show/NCT04572633). A similar approach will be adopted in another clinical trial for the treatment of patients with renal cancer (https://clinicaltrials.gov/ct2/show/NCT05432232).

CONCLUSIONS

FUS-based anticancer therapies are certainly safer than radiation therapy because acoustic waves do not induce the adverse effects that usually result from the use of ionizing radiations. Another main advantage is that FUS interventions do not require surgical procedures that necessitate hospitalization and, in turn, reduce the risk of infection.

Effective anticancer therapeutic approaches require long-term effects, in order to minimize the onset of metastases and/or the relapse of the disease in patients. In this regard, the FUS performance must be optimized for the treatment of oncological patients (1-15). To this end, preclinical studies are combining the effects of immune checkpoint inhibitors with various types of FUS-based treatments in attempt to produce long-term host immune responses against the tumor and potentially increase the occurrence of abscopal effects among oncological patients (16, 17, 115).

Another critical issue with FUS interventions is related to the lack of an appropriate characterization of the FUS-induced biological effects in oncological studies. For example, an interesting property of FUS applications in cancer therapy is associated with increased vascular permeability, which, on one hand, may optimize the delivery and efficacy of various types of anticancer agents, but, on the other hand, an increased vascular permeability might facilitate the dissemination of metastases in patients (116).

Lastly, additional studies must determine whether malignant cells may eventually become resistant to FUS-based treatments.

In summary, more efficient anticancer therapies may be achieved through a better understanding of FUS-associated biological effects in cancer therapy, along with the full characterization of the various combinations of the paraments that regulate and optimize the production of acoustic waves in FUS therapeutic applications in the field of oncology.

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Conflict of interests

The Authors have declared no conflict of interests.

Availability of data and materials

All the data supporting the findings of this study are available within the article.

Authors' contributions

All the Authors equally contributed to conception, data collection, analysis and writing of this paper.

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N/A.

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