Rational Nanomedicine Design Enhances Clinically Physical Treatment-Inspired or Combined Immunotherapy

Qiaoqiao Liu, Wei Zhang, Rong Jiao, Zheng Lv, Xia Lin, Yunping Xiao,* and Kun Zhang*

Independent of tumor type and non-invasive or minimally-invasive feature, current physical treatments including ultrasound therapy, microwave ablation (MWA), and radiofrequency ablation (RFA) are widely used as the local treatment methods in clinics for directly killing tumors and activating systematic immune responses. However, the activated immune responses are inadequate and incompetent for tumor recession, and the incomplete thermal ablation even aggravates the immunosuppressive tumor microenvironment (ITM), resulting in the intractable tumor recurrence and metastasis. Intriguingly, nanomedicine provides a powerful platform as they can elevate energy utilization efficiency and augment oncolytic effects for mitigating ITM and potentiating the systematic immune responses. Especially after combining with clinical immunotherapy, the anti-tumor killing effect by activating or enhancing the human anti-tumor immune system is reached, enabling the effective prevention against tumor recurrence and metastasis. This review systematically introduces the cutting-edge progress and direction of nanobiotechnologies and their corresponding nanomaterials. Moreover, the enhanced physical treatment efficiency against tumor progression, relapse, and metastasis via activating or potentiating the autologous immunity or combining with exogenous immunotherapeutic agents is exemplified, and their rationales are analyzed. This review offers general guidance or directions to enhance clinical physical treatment from the perspectives of immunity activation or magnification.

1. Introduction

Cancer is still one of the major human health burdens and greatly endangers human life.[1,2] Differing from conventional treatment methods[3–5] that suffer from a series of side effects,[6,7] physical treatments featuring high efficiency, high specificity, and low side effects are urgently expected and acquire significant breakthroughs in repressing tumor progression and reducing the recurrence and metastasis rates.[8] Typically, some clinically-common physical treatment approaches such as ultrasound (US) treatment including sonodynamic therapy (SDT) and high-intensity focused ultrasound (HIFU) ablation, microwave ablation (MWA), and radiofrequency ablation (RFA) featuring non-invasive and minimally-invasive penetration are receiving increasing attention. These means are anticipated to address the above side effects that conventional treatment methods encountered and realize the objective expectations because they, as the general method, can exert the potent anti-tumor actions without dependence on the type, site, and malignancy degree of cancers. Nevertheless, the local operation manner dictates the limited immune responses of these physical treatment methods. Regarding this, a surge of combined therapy with immunotherapy or nanomedicine to boost the immune responses, is accessible.[9–13] In particular, various nanomedicines and nanobiotechnologies such as photodynamic therapy (PDT),[14–17] photothermal ablation (PTA),[18,19] radiotherapy,[20] chemodynamic therapy (CDT) or nanocatalytic medicine,[21,22] have been documented to activate immune responses and potentiate immunotherapy after combining them (e.g., immune checkpoint blockade). Great efforts and significant advances have been made in these combined therapies to resist tumor recurrence or metastasis via directly elevating energy utilization efficiency, remodeling immunosuppressive tumor microenvironment (ITM) and increasing antigen exposure, and presentation and promotion of immune cell infiltration.[23–26] These inspiring cases provide experiences and basis to the combined therapies of clinically physical treatment methods with immunotherapy under the assistance of nanomedicine or nanobiotechnologies. Nanomedicine alone could induce some biophysical activities of these clinically physical treatment methods via inducing reactive oxygen species (ROS) production, and enhancing inertial cavitation and/or...
Figure 1. a) Nanosonosensitizers promoted the production of ROS. b) Acoustic cavitation effect enhanced by nanoparticle-based cavitation nuclei. c) Nanoparticles enhanced the efficiency of thermal ablation. d) Nanoparticles captured tumor-associated antigens (TAA) and presented them to antigen presenting cells (APCs). e) Cell-membrane-coated nanoparticles directly activated T cells.

elevating heat effect, which are harnessed to promote the birth, exposure, and presentation of antigens and activate the autologous immune responses to exert anti-tumor immunotherapy without adding exogenous immunotherapeutic agents,[27–29] as evidenced in Figure 1. More significantly, other imaging unit integration confers them with imaging-guided function, which is preferable for treatment precision elevation and treatment progress monitoring.[30–32] Previous reviews focused on the nanomedicine or nanobiotechnology-enhanced energy utilization of each physical treatment and discussed the rationales of nanomedicine, while few reviews systematically highlighted the autologously activated immune responses or combined with exogenous immunotherapeutic agents to enhance the anti-tumor outcomes of the above clinically physical treatment methods.

In this review, we briefly introduced some signal pathways related to tumor immunity, with emphasis on the progress
of nanobiotechnologies. The corresponding nanomaterials that could furnish reliable tools to enhance physical treatment efficiency against tumor progression, relapse, and metastasis via activating or potentiating the autologous immunity or combining with exogenous immunotherapeutic agents was underlined. Herein, we first offered an outlined glimpse at the category and principle of each physical treatment means, based on which the cutting-edge progress and direction associated with efficiency elevation and immune activation were emphasized. Subsequently, we expatiated the roles of rational nanomedicine design in the combined therapy of common physical treatments (ultrasound, MWA, RFA, and RFA) with immunotherapy with some typical paradigms and analyzed their rationales. More significantly, we highlighted the activation and magnification of systematic immune responses using the nanomaterials when referring to enhancing the local physical treatment efficiency. Eventually, we pointed out the unresolved concerns, provided the potential solution, and discussed the challenge and direction of physical treatment and future fundamental research so as to guide new development of nanomedicine and nanobiotechnology in clinical physical treatments.

2. Classic Immune Signal Pathway

The innate immune system can recognize pathogens and identify dead and abnormal cells in the body, thus initiating a protective response. This recognition relies on pattern recognition receptors (PRRs) that can sense pathogen-related molecular patterns (PAMP) and damage-related molecular patterns (DAMP). Among them, membrane-bound PRRs contain Toll-like receptors (TLRs) and retinoic acid-induced gene I (RIG-I) like receptors, while cytoplasmic PRRS contain NOD-like receptors (NLRs). NLR can be divided into NLRP and NLRC. In addition, cGAS-STING can recognize a wide range of double-stranded DNA (dsDNA) and participate in immune activation pathways.

2.1. TLRs

TLRs are one of the PRRs and are accepted to be the important in vivo anti-pathogen or endogenous release molecules, which are made up of extracellular leucine-rich repeat sequence (LRR) domains, while intracellular Toll/IL-1 receptors (TIR) domains constitute the type I transmembrane receptor family.21,22,35,36 Therein, LRR interacts with DAMP, which allows TIR domain to bind to cytoplasmic proteins, causing the activation of downstream networks, such as C-Jun N-terminal kinase, NF-kB, and p38 MAP kinase pathway.35,36 Agonists of TLR receptors have been discovered and applied to tumor immunotherapy. As immunomodulators, TLR agonists can stimulate natural killer cells (NK) and cytotoxic lymphocytes, induce dendritic cells (DCs) maturation to activate innate immunity, and cause long-term adaptive immunity, which can also enhance the sensitivity of tumor cells to other treatments such as chemotherapy, radiation therapy, and immunotherapy.37,38

2.2. RIG-I

The retinoic acid-inducible gene I (RIG-I)-like receptor (RLRs) is another pattern-recognition receptor that recognizes molecules of double-stranded RNA (dsRNAs) and ssRNA virus pathogens, constituting a defense line against RNA viruses. Short dsRNA with the 5’-triphosphate moiety is the specific ligand of RIG-I, and when binding with RNA, RIG-I activates interferon regulator factor 3 (IRF3), mitose-activated protein kinases, and NF-kB pathway through mitochondrial antiviral signaling proteins (MAVS). In addition to playing an important role in the induction of antiviral cytokines, RIG-I causes tumor cell death through IRF-1–independent and IFN-1–primed mechanisms.37,38

2.3. NLRP3 Inflammasome

NLRP3 inflammasome is an important component of the response system to microbial infection and cellular damages, and it is a multimeric cytoplasmic protein complex produced in response to pathogen-associated molecular patterns (PAMP) and damage-associated molecular patterns (DAMP).39,40 NLRP3 can activate cysteine protease caspase-1 to promote IL-1β and IL-18 secretions as well as inflammatory cell death. Tumor-associated inflammation is caused by inflammasome and by a variety of immune cells such as macrophages, dendritic cells, natural killer cells (NK), neutrophils, and T and B lymphocytes.41 In the course of fighting against tumors, the impact of NLRP3 inflammasome on tumor immunity has a double-edged role, which is decided by tumor type. In brief, it plays an inflammatory protective role in colorectal cancer, while it favors invasive growth of breast cancer, gastric cancer, lung cancer, and so on.42

2.4. cGAS-STING

Cyclic GMP-AMP synthase (cGAS)–interferon gene stimulator (STING) pathway is an important DNA sensing mechanism in innate immunity and virus defense, which can be activated by dsNDA. In this pathway, cGAS interacts with dsDNA to activate its catalytic activity, leading to the production of the second messenger molecule, that is, 2’-3’ cyclic GMP-AMP (cGAMP). Then, cGAMP activates STING in the endoplasmic reticulum to recruit TANK binding kinase 1 (TBK1) protein and phosphorylates, activates interference regulator factor 3 (IRF3), and induces type 1 interferon after they enter nuclei to activate innate immunity. cGAS can recognize a wide range of foreign and self-originated DNA, and its role has been extended to cancer.43,44

3. Clinical Physiotherapy-Activated or Combined Immunotherapy

Currently, the prevalent physical treatment methods include ultrasound treatment, MWA treatment, and RFA treatment. According to their treatment principles, different nanotechnology and nanomaterials were designed to elevate the energy transformation efficiency for acquiring high physical treatment outcomes. High physical treatment efficiency can activate systematic immune responses including more antigen exposures and presentations, ITM mitigation, and effector T infiltration, to favor autologous immunotherapy and combined immunotherapy against tumor progression, relapse, and metastasis. As a result,
nanotechnology or nanomaterials-enhanced physical treatment is the premise of autologously-activated or combined immunotherapy.

3.1. Ultrasound Treatment

Ultrasound is a mechanical wave, which can be used in ultrasonic diagnosis and ultrasound treatment.[45–47] In terms of treatment, two types of US treatment methods were classified; one is SDT and another is HIFU ablation, both of which are non-invasive methods with deep tissue penetration, high efficiency, low side effects, and low cost.[48] In terms of clinical application of SDT, a breast cancer-bearing patient aged 55 years received a combined SDT including Gc protein-derived macrophage-activating factor (GcMAF) and hormone therapy in a medical record report. After the treatment, the symptoms were improved significantly, for example, lesion disappearance and tumor marker decrease, with which slight side effects caused by hormones were accompanied.[49] In another pathological report, a patient aged 77 years with non-small cell lung cancer received GcMAF and oral colostrum MAF-based immunotherapy that were combined with SDT, TTF, and Ozone therapy. The level of tumor marker (i.e., neuron-specific enolase [NSE]) was decreased to within the normal range, and the tumor volume did not increase within 15 months.[50]

SDT depends on sonosensitizers, where low-intensity ultrasound irradiates sonosensitizers to stimulate immobilized electron transfer to adjacent free oxygen-contained species and produce ROS to kill tumor.[51–54] HIFU depends on the hyperpyrexia, where the mechanical energy of ultrasound waves is switched into heat to ablate tumor.[55] In the field of acoustics, ultrasound cavitation is usually accompanied, which is available for benefiting SDT and HIFU.[56,57] Therefore, cavitation is identified as the origin of ultrasound treatment as it often induces some biophysical effects to enable SDT or HIFU potentiation.[28,55] Generally, cavitation is classified into inertial cavitation and stable cavitation.[55,58] As cavitation proceeds, energy is released to give birth to shock waves, microjets, high temperature, high pressure, and so on.[59,60] and these biophysical effects could be used to enhance HIFU temperature and sonocatalytic ROS production for killing tumor cells.

3.1.1. Design Rationales of Nanobiotechnologies-Augmented SDT

With the comprehensive and deep understandings of SDT, SDT principle was progressively figured out, as outlined in Figure 2. In detail, several steps were generalized, sonoluminescence process,[18] sonosensitizers-mediated electron (e⁻)–hole (h⁺) pairs separation, electron transfer to adjacent free oxygen-contained molecules for producing ROS (e.g., 1O₂, ·OH, etc.), and the last irreversible damages to the tumor cells and induced tumor cell necrosis or apoptosis.[59,61,62] In SDT, sonosensitizers are indispensable to convert mechanical energy of ultrasound waves into ROS-represented chemical energy. Generally, sonosensitizers cover organic nanosonosensitizers and inorganic nanosonosensitizers.[18] In light of the fact that SDT are considered to derive from PDT,[56,63] most photosensitizers usually serve as sonosensitizers, which were loaded in nanocarriers to improve the bioavailability, pharmacokinetics, biosafety, and ultrasonic stability.[64] The level of sonocatalytic ROS production is identified as the source of SDT and also closely correlates with immunotherapy as ROS level dictates immunogenic cell death (ICD) and mitigates ITM to magnify autologous immunotherapy and combined immunotherapy.[28] It determines that developing sonosensitizers featuring high catalytic activity is highly desirable and is regarded as one direction to elevate ROS level. As materials science and nanotechnology proceed, various pathways referring to design sonocatalytic active sites-contained nanomaterials have been developed, wherein the electron (e⁻)–hole (h⁺) separation and recombination inhibition were first highlighted via various methods, for example, surface functionalization,[56,65] noble metal doping,[56] and 2D nanosheets supporting.[51,64,67] In addition, thermal effect was found to boost the sonocatalytic activity of SDT and benefit ROS production.[68] In particular, the recent advances in the field of SDT reveal that the piezoelectric materials and nonstoichiometric metal-based nanomaterials with rich inherent defects are sprouted and attract increasing attention as another appealing route for enhancing ROS production.[69–72] In detail, the rich defects in burgeoning nonstoichiometric metal-based sonosensitizers allow the free and unpaired electrons to activate adsorbed oxygen-contained species to generate ROS.[73,74] The piezoelectric materials will be deformed upon experiencing sound pressure and then the electron-hole pairs are separated to induce ROS birth.[72,75] Impressively, the acoustic cavitation dose correlates with ROS generation because cavitation can split H₂O and further stimulate the sonosensitizers to produce electron (e⁻)–hole (h⁺) pairs and then, ROS evolution.[52,76,77] Regarding this point, exogenous cavitation nuclei are introduced to augment ROS level, which are also used to combine with drug delivery to further elevate the anti-tumor outcome (Figure 3).[78]

Besides rationally developing new sonosensitizers, tumor microenvironment (TME) also decides ROS production. It is well-known that ROS production process requires oxygen.[79,80] Actually, solid tumor tissue is usually hypoxic due to the abnormal metabolism, which will greatly discount the production of ROS and then affect the therapeutic effect of SDT.[70,81] Therefore, hypoxia mitigation to supply adequate oxygen grasped the essence, and various O₂-release nanomaterials using microbubble, perfluorocarbon or hemoglobin-mediated O₂ delivery,[23,81] photo- or electron-catalytical O₂ production,[75] Fenton or Fenton-like O₂ release,[20] and so on as the design rationales, have been rationally constructed. The O₂ release could not only mitigate hypoxic TME but also liberate the hypoxia-induced resistance to ROS-based anti-tumor actions.[20,28] Besides hypoxia, redox balance is another TME component which can restrict ROS accumulation via depleting ROS with reducing agents (e.g., glutathione [GSH]).[16,51] Regarding this, GSH depletion and GSH synthesis blockade are two means to reduce the intratumoral GSH level or disrupt GSH metabolism,[16,51,82] break the redox balance, and favor abundant ROS accumulation in tumor.[83,84] Notably, nitrogen/oxygen balance disruption is also favorable for enhancing ROS-based anti-tumor outcome.[80,85] It is found that switching ROS into reactive nitrogen species (RNS) was undertaken to prolong the anti-tumor action time as RNS have longer lifetime than ROS and can circumvent ROS annihilation before they can
enter and destroy cell nuclei or mitochondria (Figure 4a). They constructed a nano-sized platform that could produce ROS and switch them into RNS, eventually receiving the most robust anti-tumor effects against MCF-7 cells in vitro (Figure 4b) and significantly inhibiting the growth of MCF-7 tumors in vivo (Figure 4c,d).

Genetic-engineering technology was also combined to modulate TME to enhance SDT. Pu et al. developed a metal–organic skeleton (MOF) integrated with acoustic-controllable and ROS-sensitive sonosensitizers using gene-editing technology. The nanoparticles could convert ultrasonic energy into $^{1}\text{O}_2$ and then induce the controllable release of CRISPR-Cas9 system to initiate genome editing. The ROS-sensitive MTH1 gene knockdown by the CRISPR-Cas9 system significantly enhanced the therapeutic effect of SDT via sensitizing tumor cells to SDT and hindered the self-healing function of tumor cells. In addition, Chen et al. connected the nanobubbles (NBs) with the sonosensitizer (indocyanine green [ICG]) and constituted an all-in-one nanoplatform carrying RAS plasmid (RSL3, ferroptosis promoter) (abbreviated into RSL3@O$_2$-ICG NBs) (Figure 4e). The experimental
group RSL3@O₂-ICG NBs caused obvious decreases and damages of mitochondrial membrane potential of HepG2 and Huh7 cells compared to other groups (Figure 4f), suggesting the occurrence of ferroptosis. In contrast, ferroptosis inhibitor (Fer-1) introduction resisted the GSH drop via blocking ferroptosis (Figure 4g,h). These experimental results show that RSL3@O₂-ICG NBs indeed realized the ferroptosis-enhanced SDT.⁸⁷ As the programmed cell death, apoptosis and ferroptosis are two dominant cell death pathways, and thus, the apoptosis or ferroptosis-enhanced SDT is expected to enable the antigen exposures and presentations, which will further activate the systematic immune responses to favor the subsequent autologous immunotherapy and combined immunotherapy against tumor progression and metastasis.

### 3.1.2. Nanobiotechnology-Augmented SDT for Activating Immune Responses

Besides directly oxidizing cell membrane and DNA, sonocatalytic ROS production in SDT could also potentiate ICD via releasing tumor-associated antigens (TAAs), facilitating TAAs engulfment and presentation by antigen-presenting cells (e.g., dendritic cells [DCs]), and activating T cells maturing and effector T cell infiltrations especially after combining with ultrasound-targeted nanobubble destruction (UTND) technology.⁸⁹ The nanobiotechnology or nanomedicine can boost ROS level through various pathways, wherein various nanoparticles that had been engineered into new nanosonosensitizers received increasing attentions.¹⁰¹ As a paradigm, Yin et al. designed continuous CO₂ bubbling nanoreactors based on the reversible CO₂ adsorption/desorption by amino groups, and the continuous CO₂ release enabled the continuous inertial cavitation, which continuously and tremendously produced ROS.²⁸ After directly killing tumor cells, the tumor cell fragments served as tumor antigens to induce the robust anti-tumor immune responses and brought about massive ICD (Figure 5). From the perspective of molecular level, the hallmarkers of ICD including adenosine triphosphate, high mobility group protein 1 (HMGB1), and calreticulin (CRT) were detected,¹⁰² and these indicators were highly expressed, promising the occurrence of ICD. The increases of DCs, T-lymphocytes (CT8⁺ T cells, CTLs), and NK cells were upregulated and the infiltration of CD8⁺ T cells were found to rise in metastatic tumor tissues. In addition, cytokines such as tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and interleukin-2 (IL-2) were also activated. More significantly, the protumorigenic M2-type macrophages were polarized into M2-type ones, mitigating the ITM, which further strengthened the anti-tumor immune actions.

The above strategies for elevating ROS from the perspective of rationally designing sonosensitizers were also believed to be utilized to potentiate the SDT-activated immune responses. Typically, robust ICD of cancer cells can be induced by GSH depletion-enhanced SDT especially after uniting with oxaliplatin (Oxa[IV]).⁹⁰ In detail, Shen et al. synthesized a new multifunctional nano-drug THPP Oxa [IV]-PEG, which can release oxaliplatin in response to GSH. In other words, it can deplete GSH. By detecting the expression levels of typical ICD markers (CRT, HMGB1), it is proved that GSH depletion-enhanced SDT as well as chemotherapy can effectively induce immunogenic cancer cell death. Concurrently, it was found that the numbers of mature DCs in inguinal lymph nodes and CD3⁺ CD8⁺ T cells and CD3⁺ CD4⁺ T cells in tumors were increased significantly, indicating that this treatment could activate the systematic anti-tumor immune responses. Impressively, the effective immune memory effect was activated to prevent the recurrence of the tumor.

### 3.1.3. Nanobiotechnology-Augmented SDT in Combination With Immunotherapy

Immunotherapy is different from traditional surgical resection, radiotherapy, and chemotherapy, and it relies on autoimmunity. Immunotherapy can kill tumor cells and tissues by activating or enhancing the human immune system, which thereby, plays a remarkable role in tumor prevention and can be regarded as the sole strategy for cancer eradication without relapse and metastasis.¹⁰³ Effective immunotherapy can also promote the formation of memory immunity objective to tumor cells and exert the positive preventive actions on distant metastasis and recurrence of tumor.¹⁰⁴ Inspiringly, the above advances of sonosensitizers driven by nanobiotechnologies can mitigate the ITM and activate and increase effector T cells infiltrations when high sonocatalytic activity is equipped to produce ROS in SDT process (Figure 6). This pre-treatment of nanobiotechnology-augmented SDT that alters TME and promotes the transformation from a “cold” tumor in the immunosuppressive state to a “hot” tumor in the immune activation one,¹⁰⁵ deserves to be exploited to contribute to the enhanced immunotherapy such as immune checkpoint blockade (ICB). To comprehensively understand it, the nanobiotechnology-augmented SDT for activating anti-tumor immune responses and combined therapy with immunotherapy is summarized in Table 1.

ICB immunotherapy has become the first-line treatment choice for multiple solid tumors (Figure 7a), but the clinical benefit rate is limited, ranging from 10% to 40% and only a small portion of patients respond to ICB, which severely limits their clinical application.¹⁰⁶,¹⁰⁷ In this regard, combined therapy with
Figure 4. a) The dynamic N/O balance disruption for enhancing ROS-based anti-tumor via switching ROS into RNS with longer life-time. b) Confocal laser scanning microscopy (CLSM) images of cell treated with different groups. c) Tumor volume changes in each treatment group during the experiment. d) Tumor weight at the end of the experiment. Reproduced under the terms of the Creative Commons CC-BY license. Copyright 2021, The Authors. Published by Wiley-VCH. e) Schematic of self-assembled RSL3@O2-ICG NBs for enhancing SDT and ferroptosis. f) CLSM images of JC-1 monomers (green channel) and aggregates (red channel) in mitochondria of HepG2 and Huh7 cells in different experimental groups. g,h) GSH levels in HepG2 (g) and Huh7 (h) cells treated with different experimental groups. Reproduced with permission. Copyright 2022, Dove Press Ltd.
Figure 5. Principle schematic on the continuous inertial cavitation for promoting ROS production and enhancing ICD.

Figure 6. Illustration on SDT in combination with immunotherapy for synergistically activating and enhancing antitumor immunity.

Various therapeutic methods are usual and have gained a large number of explorations. Especially, the combination of SDT with ICB is burgeoning, and Lin et al. studied SDT combined with immune checkpoint inhibitor aPD-L1 (TiO2-Ce6-CpG/SDT/aPD-L1) based on nanosonosensitizer (TiO2-Ce6-CpG). This combined therapy enabled the activation and maturation of DCs, the infiltration of CD8+ T cells in tumor tissues, and the immune escape target (PD-L1) blockade (Figure 7b). In vitro experiments revealed that TiO2-Ce6-CpG showed an efficient internalization into the cells (Figure 7c). After various experimental analysis, it was found that CD11c and CD86 as the representative markers of DCs maturation were up-regulated,
indicating that nanosonosensitizer TiO2-Ce6-CpG could significantly promote DCs maturation in vitro. In the in vivo experiment of female C57BL/6 mice inoculated with Hepa1-6, it was found that the treatment with such composite sonosensitizers (TiO2-Ce6-CpG) combination with PD-L1 inhibitor could not only effectively inhibit the primary tumor but also significantly delay the growth of distant tumors (Figure 7d). Moreover, the survival time of mice in the experimental group was significantly prolonged (Figure 7e). In such combined therapy of SDT with ICB, immunologic adjuvants play an important role associated with immunity promotor. Yue et al. synthesized a liposomes-supported SDT nanoplatform co-loading sonosensitizers and immunologic adjuvants, where the strong immune responses and immune memory effects were activated to repress primary and distant tumors and rechallenged ones. Further, genetics regulation was harnessed to assist the ultrasound-guided immunotherapy especially after integrating with nanobiotechnology. Typically, Li et al. designed ultrasound-guided immunotherapy nanocomplexes, wherein the nanocomplexes were obtained based on APCs-targeting microbubbles that were coated with 2’3’-cyclic guanosine monophosphate-adenosine monophosphate (cGAMP)-adsorbed biopolymers. Through binding APCs, the loaded cGAMP was readily delivered into cytosol via microbubble cavitation-enhanced membrane permeability to activate interferon-related cGAS-STING pathway and proinflammatory pathways and further stimulate antigen-specific T cells, which successfully connected innate and adaptive anti-tumor immunity to repress local and metastasized tumors especially after combining ICB immunotherapy.

In brief, SDT based on nanomaterials can effectively activate the systematic anti-tumor immune responses, which could not only delay the primary tumors and produce remarkable therapeutic effects against metastatic tumors but also actively prevent tumor recurrence. It is believed that SDT combination with immunotherapy will make large breakthroughs as nanobiotechnology moves forward.

### 3.1.4. Nanobiotechnology-Enhanced HIFU Ablation for Activating Immune Responses

High-intensity focused ultrasound (HIFU) as one of the prevalent tumor thermal ablation means is recognized all over the world to treat various cancers, such as liver cancer, kidney tumor, pancreatic cancer, and prostate cancer and also to relieve the pain of tumors. HIFU plays the thermal and mechanical roles in the tumor ablation. The thermal effect can make the local tissue temperature rise to 60 °C rapidly and then induce tissue coagulative necrosis, which is the main cause in HIFU ablation treatment. The treatment methods of HIFU include ultrasound-guided high-intensity focused ultrasound...
(USgHIFU) and magnetic resonance-guided high-intensity focused ultrasound (MRgHIFU). USgHIFU uses diagnostic ultrasound to locate the target area and observe the treatment response in real-time. With regards to MRgHIFU, Deng et al. constructed pluronic polymer and Gd-DTPA-modified MSN (Pluronic-Gd-MSN) nanoparticles, and the obtained nanoparticles could respond to HIFU, change local T1 (spin-lattice relaxation time), and enhance image contrast. Under MRI guidance, the focused ultrasound transducer could make their focus on tissue sites as small as 1.5 mm³ for treatment. MRgHIFU also provided a noninvasive real-time temperature measurement function, which accurately controlled temperature and heat dose for ablating deep bone metastasis.

Thermal ablation can effectively activate a human anti-tumor immune response. The activated immunity mechanism is summarized as follows: 1) Tumor fragments and related antigens after HIFU treatment served as tumor vaccines to enhance the anti-tumor immunity. 2) HIFU induced Th1 response and enhanced the activity of DCs and CTLs. 3) HIFU balanced the immunosuppressive TME. It has been reported that the average value of NK cells, CD3⁺ T cells, CD4⁺ T cells, and CD4⁺/CD8⁺ T cells in the peripheral blood of patients with advanced pancreatic cancer after HIFU treatment were all increased. After USgHIFU treatment was administered in 100 patients with pancreatic cancer (PaC) and in 30 patients with uterine myoma (UF), the early subclinical systemic inflammatory responses were triggered as serum LDH, leukocyte, C-reactive protein (CRP), and IL-6 were increased. The increased LDH indicated the protein release in cells, and the increase of leukocyte and CRP denoted the beginning of inflammatory responses in vivo. The increase of IL-6 may be caused by the enhancement of macrophage and T-lymphocyte activity in the acute stage. These results indicated that the early inflammatory environment of the whole body caused by HIFU treatment was the precondition for anti-tumor immunity. In a research hosted by Zhu et al., 25 of 48 breast cancer patients underwent a modified radical mastectomy and another 23 patients underwent HIFU radical resection. After HE and immunohistochemical analysis of axillary lymph nodes, HIFU was observed to stimulate the immune responses of the whole body and significantly increase the number of effector T cells, activated CTLs, and NK cells.

Although many benign tumors can be treated with HIFU, the coagulation necrosis caused by thermal effect is unsatisfactory in inducing immune responses. To address it, combined therapy with other treatment methods is highlighted to magnify ablation outcomes for potentiating the immune responses after HIFU treatment. Notably, chemotherapy was employed to combine with HIFU to enhance ablation consequence and activate the systematic immune responses to further augment immunotherapy. In the treatment of triple-negative breast cancer (TNBC), HIFU was combined with gemcitabine (GEM) to treat TNBC (4T1 tumor) in mice. Results showed that the combined therapy significantly inhibited the TNBC tumor, prolonged the overall survival time of mice, and proved that the
Another study proved that MRgHIFU could repolarize tumor-related macrophages (TAM) into M1 subtypes and enhance the infiltration of T cells in the TNBC model. As they were used in mouse neuroblastoma, immune escape blockade using aPD-L1 and mAb was implemented after HIFU ablation, and they cooperatively caused the potent anti-tumor immune responses of the whole body and reduced the recurrence and metastasis of advanced tumors.[120,125] Similar results were obtained in the mouse pancreatic cancer model.[126] PEI-PLGA-NaHCO₃-NPs developed by the Wang et al. showed an effective tumor-targeting effect in the 4T1 breast cancer model, which was much preferable for enhancing HIFU treatment.[129] Intriguingly, Shen et al. used neutrophils as carriers to entrap and encapsulate PEGylated liposome adriamycin (PLD) nanoparticles to establish the composite therapeutic drug (PLD@NES) (Figure 8a).[130] With Hepa1-6 cells and corresponding tumor-bearing mouse models, in vivo experiments showed that PLD@NES could effectively target and remove the residual tumor tissues after HIFU ablation with higher efficiency and lower side effects (Figure 8b,c).
consequently receiving the most robust anti-tumor consequences. Comprehensive immune characterizations revealed that the mechanical stress deriving from HIFU could promote ICD of human breast cancer cells through tumor necrosis-associated signal pathways, which significantly increased the damages-related molecular patterns (e.g., CRT, HSP70, and HMGB-1) and inflammatory cytokines (IFN-γ, IL-1α, IL-1β, and IL-18), and drove the secretion of chemokine (IL-8) relating to M1 macrophage activation.[131]

Besides combined therapy, enhanced energy utilization efficiency using various methods is also expected to make massive antigens exposure and activate immune responses for repressing tumor. In light of the fact that cavitation will elevate energy utilization efficiency and induce ICD,[79] exogenously artificial cavitation nuclei were also introduced to reinforce HIFU ablation-based anti-tumor outcomes.[132–135] DL-menthol (DLM)-based and various microbubbles-based cavitation nuclei have been constructed to realize it. Especially, DLM-based cavitation nuclei featured multiple enhancements under one injection,[134] which completely matched the pulsed and multi-point operations of HIFU ablation, determining that DLM-based cavitation nuclei outperformed micro- or nano-sized bubbles cavitation nuclei in boosting energy utilization efficiency and ablation outcomes.

Based on acoustic cavitation, histotripsy ablation was developed to destroy tissue (M-HIFU), which could produce an adaptive immune response to a tumor in spite of no thermal effects. A lot of evidence shows that histotripsy activation could stimulate the specific immune responses objective to tumor and further amplify the immunotherapy effect of the checkpoint inhibitor.[136,137] Under the help of nanotechnology and nanomaterials, Li et al. bridged a contrast agent and a chemotherapy drug to engineer smart nanodroplets and such nanodroplet exhibited the M-HIFU/GSH dual-responsive drug release property. Experimental results showed that the combined multiple effects activated DCs maturation, induced robust ICD, and enhanced the infiltrations of effector T cells to effectively inhibit tumor growth, providing an effective method for tumor treatment of nanoparticles combined with HIFU.[134] Liquid fluorocarbon-encapsulated nanobubbles or nanoemulsion as cavitation nuclei could further enrich the functions. Typically, perfluorooctane bromothane (PFOB)-based nanoemulsion could be engineered as vehicles to load MnO2 nanoparticles (MBP)[138] and simultaneously retained the dual-mode MRI/CT imaging functions as PFOB and MnO2 could serve as CT and MRI contrast agents, respectively. It is worth noting that MnO2-mediated Fenton-like reaction could consume GSH, which along with PFOB cavitation-enhanced HIFU and its activated immune responses, contributed to the significantly-increased ICD and eventually enhanced the inhibitory effects against tumor growth and lung metastasis.

3.2. MWA Treatment

MWA is still considered a local treatment for tumors. It emits a microwave oscillating electric field through a needle, which greatly induces temperature rise and coagulation necrosis of tumor tissues of interest, thus, reducing the tumor burden. Nowadays, it has become a reliable local treatment for various solid tumors, for example, liver cell carcinoma, renal cell carcinoma, lung cancer, and bone tumor.[139–141] Xu et al. reported a case with multiple lung metastases of endometrial cancer. After chemotherapy and endocrine therapy were ineffective, the focus underwent MWA. Results showed that the metastases gradually disappeared, and no new metastases were found during the following 3-year follow-up.[142] According to the medical record report by Wei et al., a 47-year-old and a 55-year-old female patient with non-small cell lung cancer received camrelizumab administering (200 mg every 2 weeks) one week after MWA. After four treatment cycles, the tumor on the 47-year-old patient treated with MWA reached complete ablation, and the metastatic tumor reached complete response. Similarly, the primary lesion of the 55-year-old patient was completely ablated, and other lesions showed partial response (PR).[143] Compared with other ablation methods such as RFA, MWA can generate higher temperature (more than 100 °C), larger ablation volume, and shorter ablation time.[144] In addition, MWA is also suitable for larger tumor treatment because it is almost not affected by the change of tissue impedance and can effectively pass through fibrous tissues. Compared with other thermal ablation techniques, MWA can rapidly heat tissue and is insusceptible to the heat sink effect.[145,146] However, akin to RFA, MWA is usually used as a single treatment, accompanied with the postoperative tumor recurrence rate remaining high, and the prognosis is unsatisfactory. Therefore, it is necessary to develop new solutions to overcome these concerns. In an attempt to address them, MWA sensitizers and combined therapy with other treatment methods for expanding MWA outcomes as much as possible were prevalent.

3.2.1. MWA-Activated Immune Responses

It is also reported that MWA treatment could inhibit the metastasis of primary breast cancer via activating the macrophage/IL-15/NK cell axis. In this study, two types of stage-IV breast cancer mice models were used to determine whether MWA could inhibit lung metastasis of breast cancer and improve the survival rate of mice. Robust evidence revealed that macrophages were activated and IL-15 was produced after MWA treatment, and NK cells were instigated to inhibit tumor metastasis.[144] Zhou et al. found that MWA could activate the ICOS pathway to induce acquired immunity in the treatment of breast cancer.[147] It was also found that the proinflammatory cytokines including IL-1β and IL-6 were increased in the circulatory system after MWA treatment, suggesting the occurrence of inflammatory responses.[148] Another team used the single-cell sequencing technology to analyze and compare the peripheral blood mononuclear cells (PBMCs) of six patients with early breast cancers before and after MWA treatment. Macroscopically, MWA treatment was found to induce systemic immune responses and confirmed that B cells played an important role in MWA-activated immune responses.[149]

The relationship between the specific immune response and T-cell response and tumor treatment outcome was analyzed in the study conducted by Katharina et al. who traced and assayed their levels in the peripheral blood of patients with liver cancer after MWA.[150] Several results were obtained, for example, 1) The TAAs for specifically activating T cell responses were enhanced after MWA. 2) After MWA, the activated T cells and B
cell subsets were increased in patients with long-term remission.
3) The antigen-specific T-cell responses after MWA gave rise to a long disease-free survival period. The phenomenon disclosed that MWA had a significant anti-tumor immune correlation effect in patients with liver cancer and provided reliable evidence for the combination of local MWA and immunotherapy. In addition, combining MWA with TIGIT blockers may be a potential clinical treatment strategy. In Chen et al.'s study, the expressions of anti-human t-globulin (ATG) and anti-tumor module domain (TIGIT) based on tyrosine were prominently upregulated after MWA. The combination of TIGIT blocker and MWA significantly promoted the increase of CD8+ TILs in tumor. These results showed that TIGIT blocker introduction in MWA could up-regulate the expressions of CXCL9 and CXCL10 in TAMs, boost CXCR3 expression in T cells, and promote CD8+ TILs activation and infiltration, which may inhibit tumor growth and enhance anti-tumor immunity.\[^{131}\] Furthermore, to explore the mechanism, some scholars used image-guided ablation (IGA) to analyze the plasma protein profile and shed light on the principle including molecular signaling pathway after small renal tumor treatment. One hundred sixty-four circulating proteins in the blood of patients with small renal tumors treated by ablation were analyzed to determine the immune effects on body system and to understand ablation effects on the tumor and immune system. This study provided fundamental information for the design of MWA in combination with immunotherapy.\[^{152}\]

3.2.2. Nanobiotechnology-Enabled MWA Sensitizers for Activating or Potentiating Immunity

MWA nanosensitizers based on nanobiotechnology are an important direction of activating immune responses via magnifying MWA outcomes with lowering MWA power or time. Usually, exogeneous cavitation nuclei are common for enhancing MWA via inertial cavitation-mediated energy transformation, akin to those in HIFU. As the nanotechnology proceeded, great efforts have been focused on metal-based nanoparticles, where the enriched ions’ friction with each other are activated and ion oscillation in the presence of MWA field is induced, both of which are responsible for the heat elevation.\[^{153–155}\]

To overcome the shortcomings of current exogeneous cavitation nuclei and metal-based nanoparticles for enhancing MWA, that is, uncontrollable and nonpersistent enhancement actions and inevitable ion disruption-enabled dyshomeostasis of normal tissues, respectively, biocompatible non-ionic MWA nanosensitizers were constructed.\[^{156}\] In this non-ionic MWA nanosensitizer, polar but non-ionic ethyl formate (EF) molecules as the liquid inner core were successfully loaded in liposomes along with doxorubicin (DOX). EF with high volatility could realize three enhancement actions (Figure 9a), that is, EF bubbles-mediated cavitation, polar oscillation-mediated electricity-heat energy conversion, and EF metabolism-enabled chemical ablation after degradation into ethanol. The three actions were sufficiently validated...
and leveraged to enhance MWA outcome, which further cooperated with released DOX to perform the best in repressing tumor progression and recurrence of multiple orthotopic nodules (Figure 9b,c). Mechanistic analysis revealed that the excellent anti-tumor outcome was attributed to the systematic immune activations and ITM mitigation induced by the three actions, including the increased infiltrations of CD8+ CTLs, NK cells, and memory CD1+ T cells; boosted anti-tumor cytokine secretions (TNF-α and IFN-γ); and decreased myeloid-derived suppressor cells (MDSCs) in tumor. Zhou et al. conducted an interesting work, and they developed mannose-derived carbon dots (MAN-CDs) nanoparticles on the basis of the fact that MWA can dissolve tumor cells and release cell fragments containing tumor-related antigens. Although the introduced nanoparticles failed to significantly enhance MWA, they could effectively capture many “danger signals” (DSs) after MWA and present these DSs to DCs so as to induce the body to produce strong anti-tumor immune responses. The treatment protocol could not only inhibit the growth of primary tumors and distant tumors but also brought about the long-term immune memory effects to eliminate the re-attacked tumor cells (Figure 9d). The two cases referring to activated or enhanced immune responses using their corresponding MWA immune promoters lay a solid foundation to subsequent combined therapy of MWA with immunotherapy.

3.2.3. Nanobiotechnology-Enabled MWA-Combined Immunotherapy

There is increasing evidence that MWA can not only physically eliminate tumors but also play a therapeutic role in the location of metastatic lesions especially after combining with immunotherapy. In a case report study, a 69 year old patient with metastatic squamous lung cancer was reported. Despite generating the immunotherapy resistance, the clinical treatment results showed that MWA produced the durable ablation consequences. This phenomenon found that local ablation not only eliminated the primary lesion but also inhibited the metastatic lesion by enhancing the systematic immune responses.

Shen et al. found that the immunosuppression enhancement mediated by bone marrow cells after incomplete microwave ablation (iMWA) was the main cause of high invasiveness that residual tumors were confronted with. In order to solve this issue, they used phosphatidylinositol 3-kinase (PI3Kγ) inhibitor (IP1549) to target bone marrow cells and concurrently, oxaliplatin (OX) was combined in hydrogels after MWA (Figure 9e). The injectable hydrogels usually served as the reservoir of drugs and cells, which can magnify local treatment outcomes of various lesions. Such OX&IP1549@Gel treatment significantly inhibited tumor growth after iMWA on colorectal cancer (CT26)-bearing mice model (Figure 9f,g). Intriguingly, MWA combined with the chemotherapy and immune activation promoter could provide long-term immune memory function, protect mice from the re-attack of tumor cells after eliminating the initial tumor, and also wake the strong anti-tumor immune responses to inhibit metastasis and proliferation (Figure 9h,i).

MWA combination with anti-PD-1 treatment-represented ICB can inhibit the growth of distant tumors, establish a systematic anti-tumor immune environment, and reduce recurrence. Studies have shown that the combined therapy of MWA with anti-PD1 (aPD-1)/CTLA-4 could prolong the survival time of mice and prevent tumor recurrence in the hepatocellular carcinoma mouse model. The main mechanism unraveled was that MWA activated T cell immune responses against hepatoma cells and enhanced the intratumoral infiltration of cytotoxic T lymphocytes (CTLs) induced by MWA. The combined treatment also enhanced the immune response of Th1 cells, reduced Th2 cytokines, led to Th1 cell polarization, and displayed the specific antitumor immunity. In the study conducted by Huang et al., a hepatocellular carcinoma model was established in mice and then treated with MWA combined with aPD-1. They showed that the survival period of the combined group was longer than that of MWA or aPD-1 alone, and the distant tumor growth was considerably delayed. In addition, the MWA&aPD-1 combined therapy improved the level of Th1 cytokines in peripheral blood and showed a stronger ability to induce a specific anti-tumor immune response than the treatment of aPD-1 or MWA alone. These results unveiled that MWA combined with immunotherapy could induce and enhance the anti-tumor immune responses, thus, reducing the recurrence rate and improving the survival rate of tumor patients.

3.3. RFA Treatment

Local ablation including RFA and MWA is the first choice for the treatment of early HCC, and RFA is another minimally-invasive and local treatment means. RFA is driven by a high-frequency electric pulse to produce radio frequency waves and then heat tumor. The electricity-heat conversion principle depends on the friction motion of ions under the action of high-frequency oscillation current, and the heat effect can make the temperature at the central region reach above 60 °C. The high temperature leads to the solidification necrosis of tumor tissues around the electrode, enabling RFA to become the main ablation method for early HCC with a diameter of less than 5 cm. Similar to other treatment methods, the eradication of tumor needs the participation of activated autoimmunity or combined therapy-potentiated immunity.

3.3.1. RFA-Activated Immune Responses

RFA can induce a large number of heat solidification necrosis by radiofrequency wave and produce corresponding antigens, which induce tumor-specific T cell response. At the same time, it can further induce the adaptive immune response to the tumor and treat the primary tumor and distant metastasis tumor. In detail, RFA can produce a large number of cell fragments during the apoptosis and necrosis of cancer cells. These damaged tumor cells may provide impetus to induce tumor immunity in vivo, which can produce immune responses by promoting the maturation of APCs. The current evidence shows that RFA not only has a positive effect on tumor immunity at a local level but also triggers systemic immune responses. In brief, RFA has a better therapeutic effect, good safety, and few complications, similar to the vaccines.

In the oligometastasized breast cancer, RFA therapy benefits oligometastases regression and in colorectal cancer lung...
metastasis, the invasive rate of RFA treatment was much lower than that of surgical resection.\cite{170} Despite this, according to clinical experiences, with RFA, it is very difficult to treat large tumors (>5 cm) because the adequately-high temperature field is difficult to cover the whole tumor and results in incomplete RFA. In addition, cancer patients with multiple site metastasis (n > 3) are also found difficult to appropriate for RFA.\cite{164} Moreover, the low energy conversion efficiency in part contributes to the incomplete RFA.\cite{79,171} Therefore, RFA as a single treatment alone fails to induce sufficient immune responses to eliminate residual cancer cells and avoid tumor recurrence. More significantly, the incomplete RFA has been validated to inevitably pose local recurrence and ITM potentiation and heighten the tumor resistance to immunotherapy, consequently resulting in the failure of combined immunotherapy and accelerating tumor metastasis.\cite{172} Inspiringly, the marriage of RFA and immunosuppressants can significantly evoke the T cell response toward tumor antigens and inhibit the growth of distant tumors, which is expected to lower the recurrence and metastasis rates of incomplete RFA.\cite{168}

3.3.2. Nanobiotechnology-Enabled RFA Enhancers for Activating or Potentiating Immunity

Akin to MWA, EFA enhancer that could elevate the energy utilization efficiency and improve RFA outcome for activating and magnifying immunity also deserves expectation to resist incomplete RFA and repress tumor progression, relapse, and recurrence via activating immune responses. In the design of RFA enhancers, magnetic nanoparticles (MNPs) are the most prevalent one because they could mediate the magnetothermal conversion under the alternating electromagnetic field during RFA to improve the energy utilization efficiency. Depending on the high energy conversion property, cavitation effect was introduced and qualified to serve as the foundation of RFA enhancer design. To realize the continuous inertial cavitation, Zhang et al. developed a radiofrequency solidoid vaporation (RSV) strategy based on DLM, and they utilized polyacrylic acid glycolic acid (PLGA) carriers to encapsulate DLM.\cite{175} The obtained DLM@PLGA nanocapsules could respond to RFA-unlocked heat and trigger the continuous solid–liquid–gas tri-phase transition of DLM, eventually harvesting the largest RFA volume with descended power and time. Furthermore, chemotherapeutic drugs were co-loaded in PLGA carriers along with DLM and then active targeting ligands were chelated,\cite{174} which realized the targeting enhancement of RFA and combined therapy with chemotherapy for resisting tumor progression.

To further elevate the energy utilization efficiency, the magnetic Fe\textsubscript{3}O\textsubscript{4} nanoparticles-mediated magnetothermal conversion and DLM-enabled continuous cavitation were integrated (Figure 10a).\cite{175} Mesoporous Fe\textsubscript{3}O\textsubscript{4} (m-Fe\textsubscript{3}O\textsubscript{4}) that played the roles of magnetothermal conversion agents and containers to load DLM were used to engineer the composite DLM@m-Fe\textsubscript{3}O\textsubscript{4}. Thanks to the combined actions, DLM@m-Fe\textsubscript{3}O\textsubscript{4} outperformed m-Fe\textsubscript{3}O\textsubscript{4} alone and DLM alone in augmenting RFA volume (Figure 10b). More significantly, the DLM tri-phase transition made m-Fe\textsubscript{3}O\textsubscript{4} exposed away from DLM and altered the DLM-arisen hydrophobic microenvironment of m-Fe\textsubscript{3}O\textsubscript{4} into hydrophilic one, which successfully varied the longitudinal relaxations and MRI signal and eventually enabled the RFA monitoring and outcome assessment.

3.3.3. Nanomedicine-Enhanced RFA-Combined Immunotherapy

Immunotherapy in combination with local treatment such as RFA is believed to be a direction to control the progress of early liver cancer.\cite{176} Compared with the combination therapy, the single ICB shows limited clinical benefits.\cite{177} The combination of immunotherapy and other therapies elongated the overall survival (OS) and progression-free survival (PFS). A clinical study showed that 59 patients with stage I or II liver cancer received adjuvant autologous cytokine-induced killer (CIK) cell immunotherapy after radical resection or RFA, and the recurrence-free survival (RFS) was significantly prolonged.\cite{178} In recent years, significant breakthroughs have been made in the combined therapy of RFA and immunotherapy. It is extensively accepted that RFA can destroy tumor cells and enhance anti-tumor immunity by releasing tumor antigens from the killed tumor cells, and immunosuppressants (ICIs) can enhance the antitumor immune response induced by local therapy.\cite{179} Thereby, the two actions synergize to activate and potentiate anti-tumor immunity in different dimensions such as time and space.\cite{180} There is lots of evidence that the combination of locoregional treatment (LRT) and ICB can produce synergistic effects on tumor immune response.\cite{165,181} Tremelimumab is a human anti-CTLA-4 monoclonal antibody and it can improve its efficacy by combining RFA. In the treatment of local advanced pancreatic cancer, immunotherapy combined with RFA can increase the blocking activity of the checkpoint and the presentation of antigens to the immune system.\cite{182} Huang et al. treated hepatoma mice with aPD-1 immunosuppressant and RFA in a preclinical model study. The results showed that the tumor decreased significantly in the combined treatment group, and the tumor-infiltrating lymphocytes increased significantly, validating that this combination therapy exerted a significant antitumor effect.\cite{183} Animal experiments confirmed that the immune response mediated by T cells increased significantly after RFA.\cite{184} RFA could destroy primary liver cancer, stimulate the release of tumor antigens, and increase immune responses, which indicated that RFA in combination with immunotherapy was a promising new treatment method. Some studies used anti-PD-1 and anti-CTLA4 antibodies to treat residual tumor after RFA-mediated tumor size reduction. This dual-targets blockade strategy restored the antitumor immunity of HCC by increasing antitumor T cell responses so as to prevent tumor recurrence and progression.\cite{186}

To some extent, nanomedicine can overcome the limitations of current tumor treatment and provide more distinctive insights into tumor treatment.\cite{185} On the one hand, nanoparticles can maximize the delivery of genes, imaging units, or chemotherapy drugs; and on the other hand, the modified nanoparticles can spontaneously aggregate into tumor tissue and release killer substances so as to achieve the purpose of targeted tumor cell killing.\cite{31,32,45,184} In recent years, researchers have proposed a new anti-cancer treatment method which applies external physical stimulations and nanoparticles at the same time. The synergistic effect succeeds in inducing the apoptosis of cancer cells
Yang et al. encapsulated the tumor-killing HLCaP nanoreactor (NRs) prepared by lipoxygenase and heme in PLGA copolymer (Figure 10c). After RFA, the obtained nanomaterials inhibited the residual tumor by triggering ferroptosis and improved the efficacy of RFA. As an immunogenic nano-drug, it could induce robust anti-tumor immune responses and produce strong anti-tumor effect (Figure 10d,e) so as to inhibit the further development of primary and distant tumors (Figure 10f,g). Taking all above together, RFA enhancers that can promote the ablation outcomes are designed to favor more antigen exposures and systematic immune response activation and to strengthen immunotherapy, which will arouse more interest in the near future.

4. Conclusions and Challenges

Compared with traditional tumor treatment protocols, SDT, HIFU, RFA, MNP, and MWA combined immunotherapy that served as a new treatment scheme which can release tumor-specific antigens as they lyse tumor tissues. The released antigens further activate and enhance the body’s anti-tumor immune responses, change the tumor microenvironment, promote the transformation of TME from “cold” to “hot”, and eventually inhibit tumor recurrence and metastasis. In this process, nanomedicine shows great potential for adjuvant therapy as it could potentiate the physical treatment-activated immunity. In addition, other functions such as nanomaterial-assisted imaging could be added to achieve highly precise treatment. Different surface modifications of nanomaterials can not only significantly enhance the outcomes of corresponding treatment modes (e.g., SDT) but also realize the immune escape blockade and targeted delivery of drugs in vivo, and even achieve the controllable release of drugs in an on-demand manner. In a word, the development of nanomedicine built a bridge for the combined treatment of different medical means. It is believed that the progressive development of different treatment methods such as ultrasound therapy, immunotherapy, and ablation therapy in the near future, will also provide concerned points to propel nanomedicine development. Despite acquiring massive achievements, there have many concerns that are yet to be investigated. First, the synergistic mechanism of nanomaterials, clinical physical treatment, and immunotherapy remains to be clear. The early detection
using immune checkpoint inhibitor biomarkers to predict clinical benefits needs to be explored. Personalized treatment, multidisciplinary treatment, and optimal treatment strategy determination according to the immune specificity of each patient is still challenging but desirable. Besides the rational design of nanomedicine in terms of enhancing physical treatment outcomes and boosting immune responses, biocompatibility is another concern that should be taken into serious account.

Acknowledgements
This work was supported by the National Outstanding Youth Science Fund Project of the National Natural Science Foundation of China (Grant Number: 82022033), the Shanghai Talent Development Fund (Grant No. 2019QA1406800), the Fundamental Research Funds for the Central Universities (Grant Number: 82022033), the Shanghai Rising-Star Program (Grant No. 19QA1406800), and the program for Shanghai Young Top-Notch Talent.

Conflict of Interest
The authors declare no conflict of interest.

Author Contributions
QL. and W.Z. contributed equally to this work. Conceived this theme, organized the structure, and proposed the skeleton: K.Z. Wrote the original manuscript: Q.L., W.Z., and R.J. Applied for copyright transfer: Q.L., X.L., and Z.L. Selected and organized the figures: Q.L. and K.Z. Revised the manuscript: K.Z. All authors commented on this manuscript.

Keywords
clinical physical treatment, immunity activation, nanomedicine, thermal ablation, ultrasound treatment

Received: July 7, 2022
Revised: August 3, 2022
Published online: August 24, 2022

[1] M. Latifi, A. Hay, J. Carroll, N. Dervisis, L. Arnold, S. L. Coutermarsh-Ott, K. R. Kierski, S. Klahn, J. C. Allen, E. Vlaisavljevich, J. Tuohy, Vet. Comp. Oncol. 2021, 19, 411.
[2] R. L. Siegel, K. D. Miller, H. E. Fuchs, A. Jemal, CA-Cancer J. Clin. 2022, 72, 7.
[3] R. Baskar, K. A. Lee, R. Yeo, K. W. Yeoh, Int. J. Med. Sci. 2012, 9, 193.
[4] J. E. Krook, C. G. Moertel, L. L. Gunderson, H. S. Wieand, R. T. Collins, R. W. Beart, T. P. Kubista, M. A. Poon, W. C. Meyers, J. A. Mailliard, D. I. Twito, R. F. Morton, M. H. Veeder, T. E. Witzig, S. Cha, S. C. Vidyarthi, N. Engl. J. Med. 1991, 324, 709.
[5] C. T. Wolf, N. Engl. J. Med. 1991, 324, 1685.
[6] J. J. Shi, P. W. Kantoff, R. Wooster, O. C. Farokhzad, J. Clin. Oncol. 2017, 17, 20.
[7] C. L. Shapiro, A. Recht, N. Engl. J. Med. 2001, 344, 1997.
[8] Y. Cao, C. Chen, Y. Tao, W. Lin, P. Wang, Pharmaceutics 2021, 13, 03.
[9] Z. F. Hu, Q. L. Wei, H. M. Zhang, W. N. Tang, Y. K. Kou, Y. Q. Sun, Z. C. Dai, X. W. Zheng, J. Mater. Chem. B 2022, 10, 339.
[10] Y. Ding, Y. Wang, Q. Hu, Exploration 2022, 1, 20210106.
Qiaoqiao Liu received his Bachelor’s degree in Medicine from the Xi’an Jiaotong University in 2020. He is currently doing his Master’s degree at the Guangxi Medical University. His current research focuses on the biological targeted therapy of hepatocellular carcinoma.

Wei Zhang is MD, Associate Professor, Director at the Department of Radiology, Liuzhou People’s Hospital Affiliated to the Guangxi Medical University. He received his doctor’s degree from the Guangxi Medical University in July, 2019. His current research focuses on the radiology molecular imaging, diagnostic, and clinical research in disease imaging, and nanomedicine.
Yunping Xiao is Professor, Vice President at the Liuzhou People’s Hospital affiliated to the Guangxi Medical University. He received his Master’s degree from the Xiangya Medical College, Central South University in June, 2007. His current research focuses on radiology molecular imaging and nanomedicine.

Kun Zhang is Ph.D., Professor, Deputy director, Central Laboratory at the Shanghai Tenth People’s Hospital, Tongji University School of Medicine, National Science Fund for Outstanding Young Scholars, Shanghai Top-notch Youth Talent Support Program and Chair Professor for Bayu Scholar Program, etc. His research fields include nanomedicine, ultrasound theranostics, immunotherapy, gene editing, tumor microenvironment modulation and monitoring, epigenetic modulation, regenerative medicine, and other interdisciplinarity research.