





Review

Focused Ultrasound as Targeted Therapy for Colorectal Cancer: A Comprehensive Review

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Abstract: Traditional cancer treatments have not significantly improved the survival rates for individuals with colorectal cancer. As a result, there is a dire need to explore novel treatment modalities that can target cancer-specific niches, transform cold colorectal tumors into hot ones, and disrupt the tumor niche. Therapeutic focused ultrasound, recognized for its capacity to induce thermal and mechanical impacts on tissue, can potentially eliminate cancer cells and elicit the body's anti-cancer reaction by disrupting the tumor microenvironment. This article provides an overview of recent developments in employing therapeutic focused ultrasound (TFUS) to enhance the body's natural defenses against colorectal cancers. It also discusses studies examining the utility of TFUS in treating colorectal cancer patients and recent research indicating its potential to stimulate the body's anticancer response in various in vitro and in vivo colorectal cancer models. Furthermore, it explores the therapeutic effects of TFUS on the immune system in colorectal cancers. This article also highlights the safety and effectiveness of TFUS in managing colorectal cancer, providing relief from pain, and potentially improving survival rates. Given the indications that TFUS may bolster the body's immune response and augment the impacts of TFUS therapy in clinical and preclinical colorectal cancer models, it has the potential to emerge as a pivotal tool in clinical settings.

Keywords: therapeutic focused ultrasound; high-intensity focused ultrasound; tumor microenvironment; colorectal cancer



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1. Introduction

Colorectal cancer is the third most diagnosed cancer and the third most common death-causing cancer in the United States. It poses a significant global health challenge [1]. There are multiple risk factors associated with colorectal cancer. Obesity, influenced by various factors such as diet, lifestyle, and genetics, requires thorough weight management programs that includes dietary modifications, increased physical activity, behavioral therapy, and in some instances, pharmacotherapy or bariatric surgery to avoid the risk of colorectal cancer [2]. Meanwhile, managing diabetes requires proper diabetes management programs providing comprehensive care and continuous monitoring access by healthcare professionals [3]. Genetic predisposition to colorectal cancer mandates genetic testing for individuals with a family history of cancer, personalized risk assessment, and screening for those at high risk [4]. Other risk factors for colorectal cancer are dyslipidemia and non-alcoholic fatty liver disease (NAFLD), which entails integrating lipid screening into routine health assessments [5]. Approaches to prevent NAFLD include lifestyle changes promoting

weight loss and managing associated conditions like diabetes and dyslipidemia [6]. Dealing with issues surrounding nicotine dependence and quitting smoking requires the adoption of extensive tobacco control strategies and public awareness campaigns emphasizing the contradictory effects of smoking and its associated risk factors linked with colorectal cancer, as depicted in Figure 1 [7].

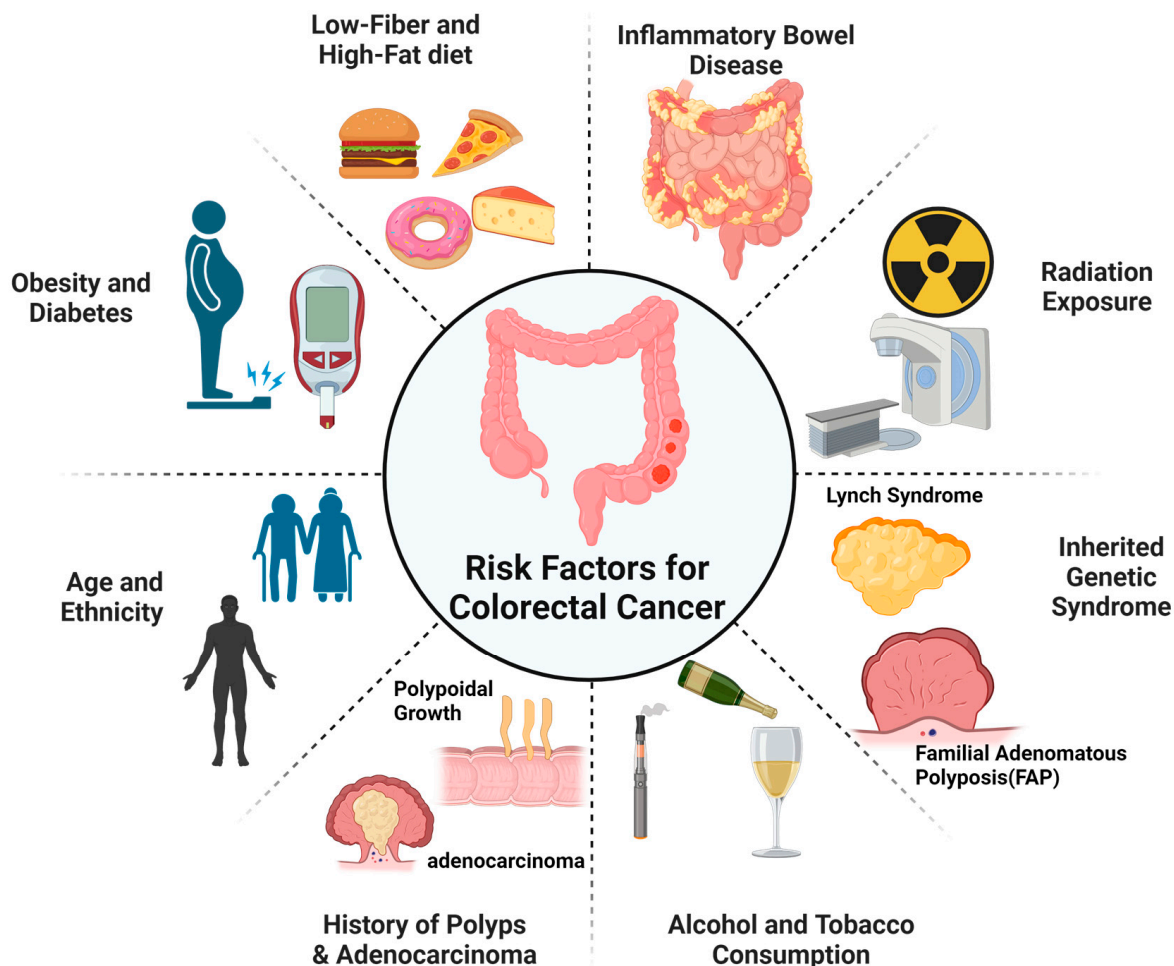


Figure 1. Association of risk factors linked with colorectal cancer.

Colorectal cancers exhibit phenotypic and genotypic diversity and can metastasize, invade, seed, and develop resistance to standard anticancer drugs [8]. Despite notable progress in conventional anticancer treatment options such as tumor resection, chemotherapy, monoclonal antibodies, and radiation, the outlook for individuals with colorectal cancer remains less than optimal, which underscores the need to transition towards more innovative and precisely targeted therapies [9]. Hence, there is an immediate imperative for novel strategies to effectively address this formidable disease. One particularly promising approach involves using focused ultrasound (FUS) as a non-invasive modality for disrupting the tumor microenvironment and targeting drug delivery in treating colorectal cancer [10]. Focused ultrasound (FUS) is an ultrasound with a curved transducer that focuses the ultrasound beam on the target tissue. There are two types of FUS: high-intensity focused ultrasound (HIFU) and low-intensity focused ultrasound (LIFU) [11]. HIFU works through a concave transducer that has a fixed focal length and aperture. According to the International Electrochemical Commission, in HIFU, a fundamental frequency of 0.8 to 2 MHz, a temporal-average intensity of 400 to 10,000 W cm⁻², and a pressure amplitude of 10 MPa give desirable therapeutic effects [12]. In HIFU, the ultrasound waves travel through the tissue and are converted into thermal heat, focusing on a single focal point and eventually causing necrosis at that focal point. Two types of effects are produced through

HIFU: mechanical and thermal effects. The mechanical effects are caused by the increased pressure effect of ultrasound waves, causing acoustic cavitation. Acoustic cavitation is the process of microbubble formation in the tissue due to pressure field differences produced by ultrasound waves in the targeted tissue [13]. This pressure difference causes oscillations and the collapse of those microbubbles, causing cell death. Thermal effects are caused by the ultrasound waves that cause physical heating effects in the tissue. This process of heating deposits energy doses in the targeted tissue [14]. At a low-dose energy deposition ($<55^{\circ}\text{C}$), hyperthermia increases cellular permeability, enabling drug delivery using nanoparticles [15]. At a high-dose energy deposition ($>55^{\circ}\text{C}$), hyperthermia indices cause coagulative necrosis in cells, leading to cell death [16]. Thus, HIFU is used in contrast to LIFU, which uses a low-intensity pulsed ultrasound beam and causes a minimal rise in target tissue temperature with no harm to surrounding healthy tissues if parameters are in control. In LIFU, a fundamental frequency of 500 kHz, a pulse repetition frequency of 1 kHz, and a peak temporal-average intensity of 23.87 W/cm^2 show desirable results in targeting the primary somatosensory cortex without damaging neighboring healthy tissues [17].

Utilizing FUS to precisely target tumor sites emerges as a promising non-invasive strategy in cancer therapy. Notably, it boasts minimal impact on adjacent healthy tissues, distinguishing it as advantageous over other non-invasive methods [18]. Its localized thermal and mechanical effects can potentially compromise the tumor microenvironment integrity, stimulating the local immune players and improving local drug delivery to tumors, thereby augmenting the effectiveness of chemotherapy and immunotherapy [19]. Furthermore, FUS shows promise in sensitizing tumors to radiation therapy and stimulating anticancer responses [20]. Both preclinical and clinical studies have emphasized the safety and effectiveness of FUS in treating colorectal cancer compared to traditional invasive and non-invasive methods of cancer treatment. Recent research highlights FUS's capacity to improve the delivery of chemotherapy drugs to colorectal cancers, such as 5-fluorouracil oxaliplatin and doxorubicin, leading to enhanced tumor shrinkage and extended life expectancy in animal models [21]. Clinical trials have corroborated its usefulness when combined with standard chemotherapy, demonstrating an increased tumor response rate to anticancer drugs and decreased adverse effects in colorectal cancer patients [22]. The advent of FUS as a non-invasive and targeted drug delivery method marks a significant stride in colorectal cancer therapy [23]. By harnessing ultrasound technology, researchers and clinicians hold the potential to redefine cancer treatment paradigms, offering renewed optimism to individuals grappling with this challenging disease [24]. Despite substantial advancements in molecular biology and immunotherapy over the past three decades, the prognosis for colorectal cancer patients has seen limited improvement. The typical progression of colorectal tumors starts with colorectal intraepithelial neoplasias (ColINs) and evolves into malignancies due to successive mutations [25]. These mutations often involve oncogenic KRAS mutations, inactivating mutations of the tumor suppressor TP53, the dysregulation of the cell cycle induced by Cyclin-dependent Kinase Inhibitor 2A mutations, and the inactivation of the tumor suppressor deletion in colorectal cancer. Mutations in these genes are present in colorectal tumors at different frequencies. Adenocarcinomas in the colon or rectum are the most common primary colorectal tumors, often leading to metastasis before detection due to late-stage diagnosis or screening. Consequently, only approximately 20% of patients qualify for curative surgery, with considerable recurrence rates even among those eligible [26]. Despite efforts with surgical excision, radiotherapy, chemotherapy, and immunotherapies, the five-year prognosis for colorectal cancer patients remains poor compared to other common malignancies. However, the evolution of chemotherapeutic strategies from 5-fluorouracil- to oxaliplatin-based regimens and later to combination therapies such as FOLFOX and FOLFIRI have improved survival rates [27]. Still, the low immunogenicity of colorectal tumors, dense fibrotic stroma, and resistance to treatments pose significant challenges [28]. Therapeutic attempts to clear the tumor microenvironment have failed, necessitating more resilient targeted approaches. Recent

research has demonstrated the potential of combining hyaluronan inhibitors with conventional treatments, underscoring the significance of targeted therapeutic strategies. While immunotherapy, notably immune checkpoint inhibitors (ICIs), has shown effectiveness across different cancers, its utility is hampered by toxicity and resistance mechanisms [29]. As a result, there is increasing interest in investigating new treatment approaches to make immunologically “cold” tumors more responsive to immunotherapy. Cold tumors are defined as tumors that have low inflammatory signatures, low immunoscores, and absent intra-tumoral CD8+ T cells [30]. FUS has emerged as a promising adjuvant tool for treating colorectal cancers. By non-invasively inducing local thermal and mechanical effects, FUS offers an appealing option for sensitizing tumors to treatments and modulating the tumor microenvironment [31]. FUS recently received approval from the US Food and Drug Administration (FDA) for various clinical and biomedical applications, affirming its safety and efficacy. In the context of colorectal cancer treatment, FUS holds significant promise in augmenting the effectiveness of current therapies through improved drug delivery, heightened immune responses, and the precise targeting of tumor cells [32]. Ongoing research endeavors are delving into the comprehensive benefits of FUS in colorectal cancer treatment, laying the groundwork for its incorporation into standard therapeutic protocols and ultimately enhancing patient prognosis. Despite being a promising technology for treating colorectal cancer, the use of FUS for treating colorectal cancer could come with some complications [33]. After a 3-month postoperative period, the patient may have episodes of pain, wound healing problems, deep vein thrombosis, UTI, and bowel dysfunction [34]. However, some significant factors that minimize FUS’s accuracy against colorectal cancers are obesity, bowel gases, and an empty bladder [35].

2. Immune Modulation Using Therapeutic Focused Ultrasound in Colorectal Cancer

FUS is increasingly recognized as a promising approach for colorectal cancer treatment thanks to its precision and non-invasive characteristics [36]. One key factor contributing to its efficacy is its ability to stimulate immune responses within the tumor niche, significantly impacting anticancer outcomes [37]. The immune effects triggered by FUS in colorectal cancer are attributed to several mechanisms, with cavitation and hyperthermia being particularly prominent [38]. Cavitation, characterized by the oscillation synthesis and collapse of gas bubbles within tissues exposed to ultrasound waves, generates mechanical forces that disrupt the tumor microenvironment, cancer cells, and tumor vasculature. Such disruption results in both direct cancer cell death and the heightened permeability of blood vessels, thereby promoting the infiltration of immune cells into the tumor microenvironment [39]. In contrast, hyperthermia involves raising tissue temperature to levels that induce cellular stress and death. FUS can precisely deliver thermal energy to targeted areas within the colorectal tumor, resulting in localized hyperthermia [23]. The thermal effects of FUS trigger diverse immunogenic responses, which include the liberation of damage-associated molecular patterns (DAMPs) from tumor cells undergoing apoptosis. These DAMPs act as messengers, alerting the immune system to the presence of cancerous cells, thereby prompting the mobilization and activation of immune effector cells such as dendritic cells (DCs), natural killer (NK) cells, and T lymphocytes [40]. Furthermore, hyperthermia induced by FUS enhances the immunogenicity of colorectal cancer cells by increasing the levels of heat shock proteins (HSPs) on their surface. HSPs are crucial in presenting tumor antigens to immune cells, stimulating adaptive immune responses against the tumor mass [41]. Moreover, hyperthermia can stimulate the secretion of pro-inflammatory cytokines and chemokines within the tumor microenvironment, fostering an immune-activating milieu that supports antitumor responses. In summary, the immune effects of FUS on colorectal cancer stem from a blend of mechanical and thermal mechanisms, such as cavitation and hyperthermia [42]. These effects collectively permute the tumor immune microenvironment, enhancing anticancer immune responses and improving treatment outcomes. Figure 2 explains the immunologically activated impacts of using FUS in colorectal cancer cells [40].

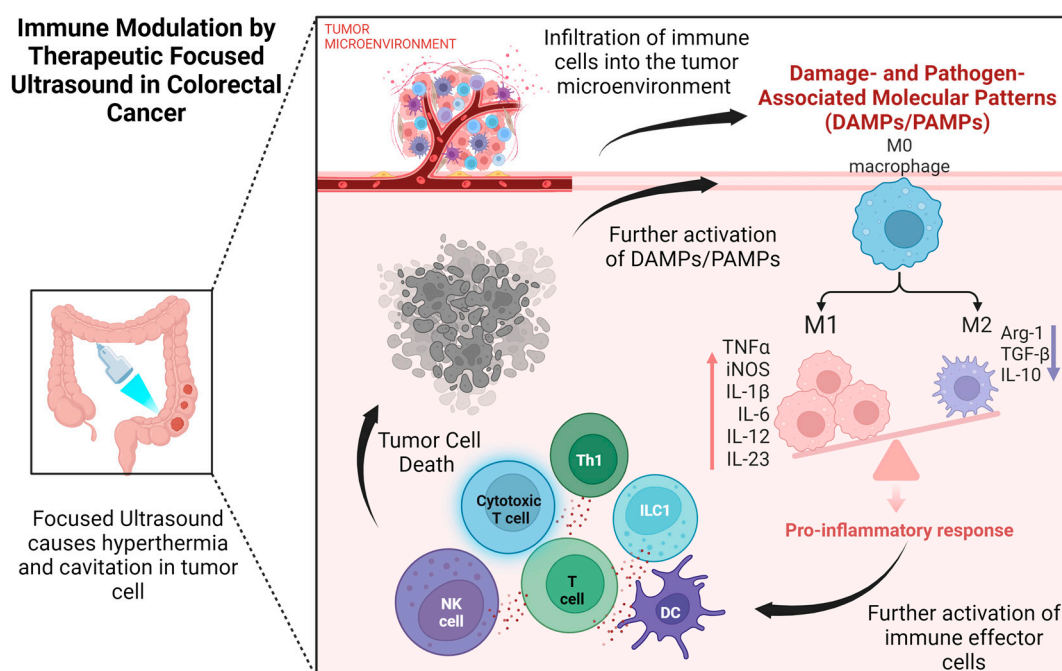


Figure 2. Immune modulation by therapeutic focused ultrasound in colorectal cancer. The high-intensity beam from HIFU targets tumor cells of colorectal cancer, disrupting the tumor microenvironment and triggering a cascade of events. As cavitation and hyperthermia cause damage to tissue cells, it attracts damage- and pathogen-associated molecular patterns (DAMPs/PAMPs). DAMPs/PAMPs activate the undifferentiated macrophages (M0) to characterize into pro-inflammatory macrophages (M1) and anti-inflammatory macrophages (M2). Because of DAMPs/PAMPs, M1 plays a significant and dominant role compared to M2. This pro-inflammatory response of M1 catalyzes the transport of cytotoxic T cells, Th1, ILC1, NK cells, and DC to the targeted tumor tissue causing necrosis and cell death. These dead cells again send signals in the form of DAMPs/PAMPs, and the pro-inflammatory response continues until the tumor and dead cells are wiped out. Black arrows show the event step by step, purple arrow: Decreased expression of Arg-1, TGF- β and IL-10, Orange arrow: Increased expression of TNF α , iNOS, IL-1 β , IL-6, IL-12, IL-23. Arg-1: Arginase-1; DAMPs: damage-associated molecular patterns; DC: dendritic cell IL: Interleukin; ILC1: innate lymphoid cell; iNOS: inducible nitric oxide synthesis; M0: non-activated macrophage; M1: pro-inflammatory macrophage; M2: anti-inflammatory macrophage; NK cell: natural killer cell; PAMPs: pathogen-associated molecular patterns; TGF- β : Tumor Growth Factor- β ; Th1: type 1 T-helper; TNF- α : tumor necrosis factor-alpha.

FUS presents a promising avenue for improving immunotherapy effectiveness in colorectal cancer by eradicating cancer cells, releasing tumor-specific antigens and DAMPs, and inducing immunogenic cell death (ICD) [43]. These mechanisms attract tumor-infiltrating lymphocytes (TILs), activate antigen-presenting cells (APCs) such as DCs, and enhance the permeability of the colorectal tumor stroma. This improves the accessibility of immune-based therapies [44]. The initial clinical investigation into the immunological impacts of HIFU in colorectal cancer revealed elevated natural killer (NK) cell activity and an increased abundance of TILs after thermal HIFU treatment [45]. The study involved 15 patients, averaging 62 years of age, with an average tumor size of 5.6 cm, who underwent ablative thermal HIFU using the FEP-BY01 system. Immune assessments conducted on peripheral blood samples from 10 patients revealed a statistically significant 25% increase in NK cells post treatment [46]. No statistically significant differences were observed in CD3+ and CD4+ cell counts compared to pre-HIFU treatment levels, with parameters ranging from 0.5 to 1.6 kW and beamed power from 1000 to 1400 kW. Recent evidence additionally confirms the immunomodulatory and anticancer effects of thermal HIFU in colorectal cancer [47]. A retrospective clinical study involving 100 patients with locally

advanced, inoperable colorectal tumors showcased these effects. The development of clinical systems assisted by ultrasound and magnetic resonance imaging (MRI), which can thermally ablate tissue, has prompted a comprehensive investigation into the immunogenic effects of “thermal” FUS in clinical and preclinical testing. In laboratory experiments, subjecting colorectal cancer cells to ablative and sub-ablative thermal iso-effective doses (TIDs) has demonstrated immunogenic cell death (ICD) with the release of DAMPs [48]. These comprise the release of calreticulin adenosine triphosphate (ATP) and heat shock protein 70 (HSP70), the exposure of HSP70 and HSP90 on the plasma membrane of cancerous cells, and the downregulation of CD47 [49]. In animal studies, utilizing thermal ablative-FUS on murine colorectal cancer tumors with a 2 cm diameter transducer led to a doubling in the cytotoxic activity of T lymphocytes [50]. Additionally, this method increased the release of interferons (IFNs) and tumor necrosis factor-alpha (TNF- α), along with a statistically significant maturation of DCs and a successive activation of cytotoxic T lymphocytes towards the tumor [35]. Treating a localized region within a larger B16 melanoma tumor using a 16-element MRI-guided HIFU annular array in combination with the Toll-like receptor (TLR) agonist CpG has led to the polarization of macrophages and DCs towards a pro-immune phenotype [51]. Furthermore, this strategy augmented intra-tumoral and draining lymph node antigen cross-presentation and amplified the production of type-I interferons (IFNs) compared to treatment with CpG alone. These findings highlight how thermal FUS promotes a tumor microenvironment conducive to immune activation by releasing DAMPs, stimulating T lymphocyte responses against tumor cells, and improving antigen presentation [52]. One of the earliest clinical investigations into HIFU involved applying the ultrasound-guided Model JC HIFU system (HAIFU Technology Co., Ltd., Chongqing, China) to treat colorectal cancer patients [53]. In these studies, a solitary treatment with the HIFU system was delivered to target primary tumors. After treatment, there was a noted rise in local T cells, T cytotoxic cells, and CD4+ T lymphocytes relative to their levels pre-treatment [15]. Later investigations employing the same HIFU system in patients with colorectal cancer revealed that the combination of HIFU with surgery resulted in an increased infiltration of diverse immune cells within the tumor [54]. These included DCs, macrophages, B lymphocytes, T lymphocytes, helper T lymphocytes, and cytotoxic T cells, compared to cases where only tumor resection was performed. Furthermore, the group receiving both HIFU and surgical excision exhibited a higher count of granzyme B and perforin-positive tumor-infiltrating lymphocytes (TILs) within the tumors compared to the group undergoing surgical resection alone, suggesting their activation [55]. These findings illustrate that the immunostimulatory impact of thermal ablation seen in preclinical studies can be reproduced in clinical settings, thereby influencing the immune microenvironments of patients [6]. While thermal tissue ablation can lead to cell destruction and the release of cancer-associated antigens, there is speculation that after HIFU treatment, some of these antigens may become thermally stable due to the drastic thermal escalation (>56 °C) induced within the tissue environment [7]. As a result, it is proposed that mechanical FUS proves to be more efficient than thermal methods in releasing antigens from immune activation. The innate immune response is activated when synergistic mechanical and thermal FUS effects with immunotherapy, anti-PD-1 antibodies, and CpG are employed [9]. HIFU treatment triggered the maturation of DCs, the concentration of tumor-specific IFN-secreting cells, and augmented effects of cytotoxic T lymphocytes as proposed by initial experimentation [11]. Recent investigations talk about the biological and immunological impacts of mechanical FUS, specifically relating to tumors. One research study found the emergence of an antitumor immune response when murine colorectal cancer and 4T1 breast tumors were subjected to HIFUS under ultrasound guidance [56]. This antitumor immune response was identified by decreased TGF- β , IL-12p40, IL-10, and IL-12p70 levels, associated with boosted ICAM, IL-17, CXCL10, and RANTES [47]. Additionally, both tumor types exhibited diminished populations of myeloid-derived suppressor cells (MDSCs), M1, regulatory T lymphocytes, and M2 macrophages, particularly noticeable in 4T1 breast cancer cells [57]. Reactive oxygen species (ROS) created by DNA damage from

ultrasound-induced cytosolic Ca^{+2} transients are responsible for the decreased cell population of MDSCs. A follow-up study observed the heightened regulatory T lymphocytes, Th1 cells, cytotoxic T lymphocytes, B lymphocytes, NK cells, and macrophages in colorectal cancer and 4T1 tumors treated with the specified HIFUS factors [58]. The key findings of these observations are the challenges associated with anticancer responses and changes occurring in the immune response to sharpen these anticancer effects. To address the need for robust immune responses, it has been proposed that releasing cancer-associated antigens and activating the cancer immunity cycle can be successful if histotripsy is used. Working with this hypothesis, the administration of histotripsy treatment with a specific focus on activating neoantigen-mimicking peptide GP33 to colorectal cancer resulted in the activation of cytotoxic T lymphocytes and an enhanced antitumor immune response. An increase in intracellular IFN expression demonstrated this activation. One study with 2 MHz HIFU transducers utilized the histotripsy treatment of breast cancer MDA-MB-231 cells, leading to the necrotic death of cancer cells and DAMPs such as HMGB1 and HSP70 [53]. This treatment also induced immunogenic cell death (ICD) and initiated the discharge of pro-inflammatory cytokines and chemokines. However, inside the living organism, these studies show the systematic inflammation induced by histotripsy. Preclinical results also indicates that large immune-refractory neuroblastoma tumors showing enhanced tumor-infiltrating lymphocytes (TILs) and the abscopal effect following HIFU treatment. Likewise, using ultrasound-guided boiling histotripsy for renal cell carcinoma leads to increased infiltration of cytotoxic T lymphocyte [59]. Figure 3 visually delineates the schematic representation of the progression of colorectal cancer tumors and the pathogenesis of colorectal cancer [60].

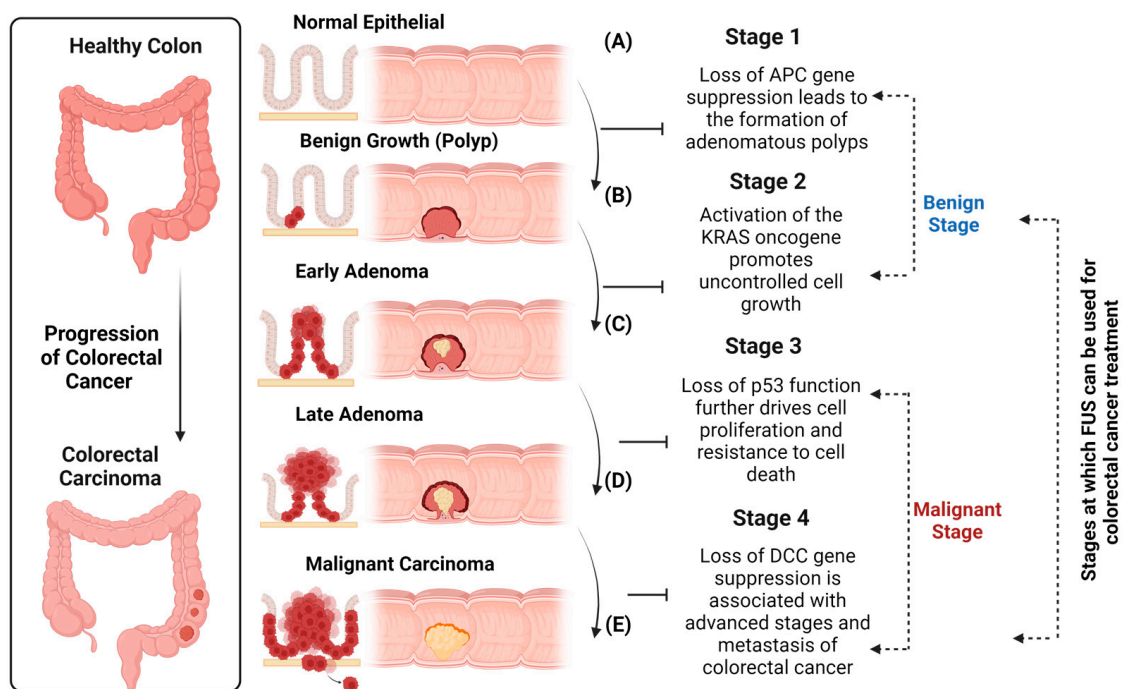


Figure 3. Schematic representation of progression of colorectal tumors. (A): APC genes of normal healthy intestinal cells are suppressed that lead to initiation of events for the formation of adenomatous polyps. (B): Adenomatous polyps started growing in the intestinal line but these polyps are still benign (C): Polyps becoming malignant, KRAS oncogene of these adenomatous polyps leads to the uncontrolled cell growth, forming early adenoma (D) Polyps are now in malignant stage, p53 genes are lost that accelerates cell proliferation and resist cell death, forming late adenoma (E): DCC genes are lost in the advanced stages and the polyp is now metastasized. Colorectal carcinoma is now malignant. Once a tumor, whether benign or malignant, is diagnosed, FUS could be used for the treatment of colorectal cancer.

These discoveries advocate for leveraging histotripsy, a method capable of tumor destruction, to initiate the cancer immunity cycle [54]. Initially, the clinical adoption of histotripsy was hindered by the requirement for higher peak negative pressures compared to thermal treatments. However, a phase I trial conducted with 25 patients diagnosed with benign prostate hyperplasia affirmed the safety of histotripsy utilizing the Vortx Rx system [9]. This system incorporates a 700-kHz, 36-element transducer equipped with cavitation detection through ultrasound imaging [15]. LIFU has also demonstrated immune effects, although the underlying biological mechanisms are not fully understood. As a result, fine-tuning ultrasound exposure parameters is essential to achieve the desired immune response [6]. Research has documented inflammatory responses linked to the NF-κB pathway and macrophage infiltration in the murine colon following LIFU treatment, particularly in scenarios necessitating colorectal cancer opening without notable immune reactions [11]. In colorectal cancer, the implementation of pulsed LIFU treatment through an ultrasound-guided FUS exposure system elicited prolonged elevations in tumor cytotoxic T lymphocytes and the regulatory T lymphocytes ratio relative to control groups [56].

Additionally, it led to the reversal of T lymphocyte tolerance and energy. LIFU interventions also enhanced cytokine production, decreased the expression of anergy-related genes, and promoted the maturation and activation of DCs, indicating a pro-immunogenic effect [59]. In summary, both thermal and mechanical FUS exposures have the potential to induce immune responses. These responses vary based on the type and stage of the tumor, with physical mechanisms causing tissue disruption and triggering tumor-specific biological effects [61]. Comprehending these mechanisms can guide the development of treatments to elicit potent and long-lasting anticancer immune responses with enhanced effectiveness and minimized side effects [62]. Crucial parameters such as frequency, intensity, duration, and other relevant factors govern FUS-mediated drug delivery tailored to colorectal cancer, as outlined in Table 1.

Table 1. Summary of preclinical and clinical trials and critical parameters using ultrasound-mediated therapeutics for colorectal cancer.

Research Topic	Focused Ultrasound Setup	Model	Treatment Parameters	Results	Reference
Improving cetuximab delivery to colon cancer xenografts in mice through pulsed HIFU therapy	Therapy Imaging Probe System, Philips Research, Briarcliff Manor, NY, USA	Pre-clinical mice model	Acoustic intensity: 191 W cm ⁻² Ultrasound frequency during sonication: 1.3 MHz Pulse repetition frequency: Set at 1 Hz Duty cycle: 5%, with 50 ms on and 950 ms off periods	HIFU therapy amplifies the antitumor efficacy of cetuximab against human colon cancer xenograft models in mice.	[63]
Using FUS and microbubbles to treat liver metastases in colorectal cancer patients undergoing chemotherapy	Ultrasound scanner, Vivid E9 (VE96975, GE Vingmed Ultrasound AS, Horten, Norway)	Clinical model	FUS was applied at a frequency of 1.67 MHz, with a mechanical index of 0.5, a pulse repetition frequency of 0.33 Hz, 33 oscillations, and a duty cycle ranging from 0.2% to 0.4%. Microbubbles (MBs) of SonoVue were administered for 35 min.	Combining FUS and MBs is a safe and feasible approach to enhance chemotherapy effectiveness in cancer patients.	[29]

Table 1. Cont.

Research Topic	Focused Ultrasound Setup	Model	Treatment Parameters	Results	Reference
Investigating the antitumor immune response triggered by HIFU in a murine cancer model	HIFU transducer (Model H-102, Sonic Concepts, Seattle, WA, USA)	Pre-clinical Mice model bearing MC-38 colon adenocarcinoma tumors	The focal length of 63 mm, operating at 3.3 MHz (3rd Harmonic) with sinusoidal signals, using a portable ultrasound imaging system (Terason 2000, Terason, Inc., Burlington, MA, USA) with a 5/10 MHz probe for tumor alignment	Thermal and mechanical HIFU resulted in a 3.1-fold rise in CD11c+ cells, while a 4-fold increase was observed with mechanical HIFU. In comparison, DC injection alone resulted in a 1.5-fold increase.	[64]
FUS application in colon cancer tumor suppression	FUS transducer (V3330) supplied by Olympus (Houston, TX, USA)	A pre-clinical orthotopic colorectal cancer (CC) mouse model was established by injecting CT26-Luc cells into BALB/c mice	Irradiation intensity: 9.3 MHz Power: 4.5 W Treatment technique: FUS treatment is initiated from the tumor center, expanding gradually to edges with 1.0 mm point spacing. Exposure time: 10 s per treatment point	FUS treatment alleviated intestinal tissue injury in CC mice, as indicated by morphological changes.	[65]
Investigation of thermal dose-induced cell death pathways in colon cancer cells	T-type thermocouples (catalog number 6212164, RS Instruments, Corby, UK)	Pre-clinical in vitro models using human HCT116 and HT29 colon cancer cell lines	The thermocouples were configured to measure temperatures within a range of -50°C to $+200^{\circ}\text{C}$, featuring a wire thickness of 0.2 mm, a probe diameter of 0.57 mm, and an accuracy of $\pm 0.5^{\circ}\text{C}$ up to 125°C	Treatment with ablative thermal doses resulted in the reduced viability of colon cancer cells.	[66]
Characterization of colorectal cancer	High-Frequency Endoscopic Ultrasound (EUS) System	Pre-clinical rabbit model of colorectal cancer	Cut-off frequency of 10 MHz in high pass and 63 MHz in low pass	High-frequency EUS system suitable for routine colonoscopy and potential functional tools.	[67]
Using high-intensity focused ultrasound to treat colorectal cancer liver metastases	JC FUS Tumor Treatment System Developed by Chongqing Haifu (HIFU) Technology Co., Ltd., Chongqing, China	Clinical model of patients with liver metastases from colorectal cancer after undergoing colectomy	Frequency: 0.8 MHz Focal length: 135 mm Physical focus region: $2\text{ mm} \times 2\text{ mm} \times 8\text{ mm}$ Treatment duration: 3800–12,000 s	HIFU demonstrates safety, effectiveness, and non-invasiveness in treating hepatic metastases from colorectal cancer.	[68]
Examining the biological impact of HIFUS on HT-29 colon cancer cell lines	HIFU (H-102 model) transducer from Sonic Concepts	Pre-clinical in vitro model using HT-29 human colorectal adenocarcinoma cell line	Fundamental frequency of H-102: 1.10 MHz Focal point: 62.6 mm from the transducer face HIFU was applied to the focused well for 30 s	A notable decrease in viability rates was observed with increasing thermal doses.	[69]

Table 1. Cont.

Research Topic	Focused Ultrasound Setup	Model	Treatment Parameters	Results	Reference
Enhancing the efficacy of systemic therapy for unresectable liver metastasis from colorectal cancer through HIFU ablation	HIFU (model-2001, Shanghai Jiao Tong University's Xindi Industrial Company, Shanghai, China)	Clinical model involving colorectal cancer liver metastasis patients	Ultrasonic frequency: 50 Hz Output power: 1 kW Effective treatment Depth: 30–150 mm Effect focus: 6 mm × 6 mm × 10 mm Focal Volume: 3 mm × 3 mm × 8 mm	HIFU ablation with systemic therapy controls the progression of colorectal cancer liver metastases.	[70]
Efficacy and safety of HIFU ablation for managing liver metastases from colorectal cancer	JC HIFU-200 system by Chongqing Haifu Medical Technology Co., Ltd., Chongqing, China	Clinical model (colorectal cancer liver metastasis patients)	The transducer, operating at 1.0 MHz, generates ultrasound energy with a focal region of 3 mm × 3 mm × 8 mm. It can smoothly move in six directions (X, Y, and Z axes) via computer control	HIFU ablation emerges as a safe and efficient treatment choice for patients with colorectal cancer liver metastases.	[71]
Inhibiting tumor angiogenesis in colon cancer through combined application of endostatin microbubbles and FUS	FUS (Sea Eagle Electronic Medical System Co. Ltd., Changzhou, China)	Pre-clinical in vivo model using BALB/c nude mice	Frequency: 238 kHz Output voltage: 400 Mv Focus incentive sound pressure: 1.4 MPa, irradiation sessions: 10 Time: 10 s	Targeted microbubbles, combined with FUS, effectively disrupt tumor microvasculature in subcutaneous colon cancer xenografts.	[72]
HIFU for hepatobiliopancreatic and digestive system	HIFU devices JC Model by Chongqing	Clinical model involving patients with colon, liver, and pancreas cancer	Acoustic power of 300 W, focal peak intensity of 20 KW/cm ² , applied for 30 min	HIFU consistently alleviates cancer-related pain.	[73]
HIFU ablation for colorectal liver metastases during open surgery	HIFU device with 7.5 MHz ultrasound imaging probe (Vernon, Tours, France)	Pre-clinical model using pigs with colorectal liver metastases	Ultrasound fields generated by eight emitters operating at 3 MHz, distributed in a toroidal geometry with a 70 mm diameter	HIFU enables treatment with real-time ultrasound guidance.	[74]
Clinical experience with HIFU therapy development at Oxford	HIFU device	Clinical study involving patients with colon, liver, kidney, prostate, and pancreas cancer	N/A	HIFU targets both benign and malignant tissues.	[75]
Enhanced therapeutic index of PD-L1 targeting immune-microbubble complex in murine colon cancer models	Utilized FUS system (VIFU 2000®, Alpinion Medical Systems, Seoul, Republic of Korea)	Pre-clinical colorectal liver metastasis mice model (BALB/c nude mice)	Applied 1.1 MHz frequency, 100 Watts power, 100 Hz pulse repetition frequency, 5% duty cycle, 5 s ultrasound exposure per spot, with 2 mm spot distance	Demonstrated improvement in the therapeutic index for PD-L1 antibodies.	[76]

Table 1. Cont.

Research Topic	Focused Ultrasound Setup	Model	Treatment Parameters	Results	Reference
Utilizing FUS for chemo-immunotherapy of colon cancer	Utilized integrated ultrasound-HIFUS Alpinion platform with specific specifications	Pre-clinical on Balb/c mice as an in vivo model of colon cancer	The parameters for HIFU treatment included a 35% duty cycle, a pulse repetition frequency of 5 Hz, and a power of 6 W, aiming to reach a mean target temperature of 40–42.5 °C within the heated focus for 60 s	Combinatorial therapy has the potential for the treatment of colorectal cancer.	[77]
Exploring the effects of HIFU on anti-tumor immune response in a murine tumor model	HIFU transducer (H-102, Sonic Concepts, Seattle, WA, USA) Thermocouple (Custom designed IT-23, Physitemp Inc., Clifton, NJ, USA)	Pre-clinical in vitro model (mice bearing MC-38 colon adenocarcinoma tumors)	Transducer focal length: 63 mm; frequency: 3.3 MHz	FUS treatment can trigger a systemic antitumor immune response.	[78]
Enhancing anticancer immune response with low-pressure pulsed FUS and microbubbles	The sonogram-guided MB-FUS exposure system (Sonic Concepts, Seattle, WA, USA)	Pre-clinical in vitro model (tumor-bearing BALB/mice)	FUS exposure was administered at power levels of 5 and 30 W, corresponding to acoustic negative-peak pressures measured at 0.6 and 1.4 MPa, respectively	Exposure to low-pressure FUS stimulates an anticancer immune response.	[68]
HIFU combined therapy with S-1 and oxaliplatin in metastatic colorectal cancer patients	HIFU developed by Jingyuande Medical Equipment Co., Ltd., Xuzhou, China. Treatment System (FEP-BY02 type)	Clinical model (patient with colorectal cancer)	D Input electric power level Control range 300–500 W Element emission time (t1) 1–2 s, the number of times per point (T) 5–10 time	The combination therapy involving HIFUS and SOX proves to be efficacious and well tolerated in late-stage colorectal cancer patients with pelvic masses.	[79]

3. Utilizing Therapeutic Focused Ultrasound for Colorectal Cancer Treatment

Considerable attention has been directed toward preclinical and clinical studies exploring the application of therapeutic FUS in managing colorectal cancer [80]. Over the past twenty years, more than 3500 patients diagnosed with colorectal cancer have undergone palliative therapy utilizing thermally ablative HIFU [81]. These interventions have shown feasibility, safety, and effectiveness, offering pain relief and, in some instances, tumor growth control [21]. Studies have noted enhancements in survival rates and positive clinical responses among recipients of such treatment. Moreover, preclinical investigations are delving into the potential benefits of mechanical FUS and LIFU in colorectal cancer therapy. Initial reports from China presented promising outcomes in late-stage colorectal carcinoma patients with HIFU. These individuals experienced pain alleviation and enhanced appetite, sleep quality, and mental well-being, with some showing weight gain [22]. Notably, tumors exhibited regression in several instances without severe complications like pancreatitis or damage to neighboring organs. Subsequent studies corroborated these findings, emphasizing chronic pain relief, tumor reduction, and minimal local complications in patients with unresectable colorectal tumors, underscoring the safety and efficacy of HIFU in colorectal cancer management [24].

Moreover, survival outcomes have shown notable improvement post HIFU treatment, with studies reporting median overall survival rates ranging from 6 to 10 months for individuals with advanced colorectal cancer [25]. Additionally, one-year survival rates of up to 30% have been documented, indicating a meaningful extension of life expectancy for treated patients. Remarkably, the utilization of repeated HIFU sessions has demonstrated even more significant survival benefits compared to single treatments, highlighting the potential of HIFU as a valuable adjunct to conventional therapies in managing colorectal cancer [26]. In Europe, studies from Italy and Germany have also demonstrated the safety and effectiveness of HIFU in treating patients with pancreatic cancer [27]. In Italy, patients diagnosed with pancreatic tumors underwent HIFU ablation with minimal complications, resulting in a noteworthy 1-year survival rate of approximately 43% [28].

On the contrary, a prospective study in Germany showed significant pain relief and tumor volume reduction among patients treated with ultrasound-guided HIFU. Moreover, investigating HIFU treatment alongside traditional oncological approaches like chemotherapy and radiotherapy has resulted in enhanced outcomes and minimal side effects for pancreatic cancer patients [29]. Clinical case reports have further underscored the efficacy of HIFU therapy in pancreatic cancer patients. Documented outcomes include successful tumor shrinkage, pain alleviation, and disease stability maintenance following HIFU treatment. Although some complications, such as transient fever, abdominal discomfort, and skin burns, have been noted, they were generally minor and manageable [30].

Moreover, preclinical studies investigating drug delivery and sonodynamic therapy have yielded promising results, suggesting enhanced anticancer effects in pancreatic tumors when combined with HIFU [31]. In summary, the evolving field of HIFU therapy holds great promise for managing colorectal cancer. Ongoing research and technological advancements indicate that HIFU may play a pivotal role in multimodal treatment approaches, potentially resulting in improved outcomes and enhanced quality of life for patients [32]. For more detailed insights, Table 1 provides a comprehensive overview of key findings from clinical trials investigating ultrasound-based treatments for colorectal cancer. This includes information on trial phases, interventions utilized, patient characteristics, and primary outcomes [33].

4. Investigating the Therapeutic Effects of Focused Ultrasound in Colorectal Cancer

Clinical trials exploring different combinations of antibodies and chemotherapy have shown limited advantages in individuals diagnosed with locally advanced or metastatic colorectal cancer [82]. Hence, it is imperative to investigate alternative therapeutic approaches to augment immunotherapy [44]. Patients received treatment using ultrasound-guided thermal HIFU administered via the JC TTS system manufactured by Haifu Medical Technology in Chongqing, China [56]. The system consisted of a treatment table equipped with a high-power focused ultrasound therapy transducer, which had a diameter of 20 cm and a focal length of 15 cm and operated at a frequency of 0.8 MHz [23]. Substantial decreases in tumor volumes were noted in patients between 6 weeks and 12 months post thermal ablation [24]. Compared to baseline levels, acute inflammation was also apparent, as indicated by elevated leukocyte counts at 2, 5, and 20 h after thermal ablation. Moreover, although not reaching statistical significance, increases in IL-6 levels compared to baseline were observed 20 h after ablation [25]. The treatment parameters were as follows: treatment time = 124 ± 46 min; sonication time = 932 ± 374 s; energy = 343.9 ± 156.2 kJ; power = 366 ± 91 W; energy/volume = 12.8 ± 4.5 kJ/mL. In a case study of a 48-year-old patient with unresectable metastatic colorectal cancer, thermal HIFU treatment using the JC HIFU system was implemented as palliative therapy [27]. This decision followed inadequate responses to gemcitabine/erlotinib and subsequent FOLFOX (folinic acid, oxaliplatin, and 5-FU) treatments. Following the HIFU procedure, the patient persisted with FOLFOX treatment, leading to reductions in tumor and metastatic lesion sizes noted at the 12-month mark post HIFU [63]. This observation suggests the potential induction of the abscopal effect. Key parameters during the procedure included a power of 103 W, a sonication

duration of 752 s, and detecting the first hyperechoic signal at 150 s. In a separate clinical scenario, a 74-year-old patient was diagnosed with anaplastic colorectal carcinoma [28]. Initially, a 3 cm lesion was identified in the colorectal body. Subsequently, after chemotherapy, the lesion progressed to 4.6 cm in size. Moreover, numerous pathological lymph nodes were identified in the retroperitoneal region. As a result, the patient underwent thermal HIFU ablation on the colorectal lesion using the JC HIFU system [83]. This procedure, performed as a locoregional palliative intervention, featured a frequency of 0.8 MHz, a focal length of 15 cm, and a transducer diameter of 20 mm [2]. Power Doppler was utilized to evaluate the ablation, with lesions vertically spaced by 5 mm. Imaging procedures were carried out using the MyLab70 imaging device manufactured by Esaote, based in Genova, Italy, which employed a 1.0 to 8.0 MHz imaging probe. A computed tomography (CT) scan conducted the day after the treatment revealed a 5.6 cm colorectal lesion and paraaortic lymphadenopathies [1]. Subsequently, the patient underwent five additional cycles of chemotherapy following the HIFU procedure. The patient became asymptomatic post HIFU, and a follow-up restaging CT scan conducted four months later indicated approximately a 30% reduction in the size of the colorectal head lesion and a 75% reduction in the size of the left paraaortic lymph node lesions. These findings suggest the induction of an abscopal effect following the HIFUS treatment [57]. Preclinical investigations have demonstrated that administering histotripsy treatment to approximately 60–75% of subcutaneous murine colorectal tumors using an ultrasound-guided transducer led to the formation of cavitation clouds, as confirmed by ultrasound imaging. This treatment resulted in tumor destruction and a reduction in tumor size. No discernible alterations were noted in immune cell populations within the tumor microenvironment at 24 h and 7 days following histotripsy treatment [10]. Nevertheless, a subsequent decline in macrophage levels (as a percentage of CD45+ cells) and regulatory T cells (as a percentage of total cells) and an elevation in dendritic cell numbers (as a percentage of CD45+ cells) were noted 14 days following histotripsy treatments. Furthermore, the release of damage-associated molecular patterns (DAMPs), such as HMGB1, was observed following the treatment of colorectal cells in vitro using identical histotripsy parameters [51]. These parameters included a frequency of 1 MHz, eight elements, a f-number of 0.68, a focal point at $0.98 \pm 0.93 \pm 3.9$ mm, pulses fewer than two cycles, and a pulse repetition frequency (PRF) of 250 Hz. Additionally, preclinical models of colorectal cancer have demonstrated the pro-immune effects of sonodynamic therapy. In a study involving animals with syngeneic bilateral KPC tumors, administering hypoxia-alleviating polymethacrylate-coated CaO₂ nanoparticles led to sonodynamic therapy with an SP100 Sonidel sonoprotector (Sonidel et al.) [48]. It increased T cytotoxic cells and decreased T regulatory cells in both target and off-target tumors, ultimately inducing the abscopal effect in off-target tumors. The parameters used included a frequency of 1 MHz, ISATP = 3 W cm^{-2} , d.c. = 30%, and a pulse repetition frequency (PRF) of 100 Hz [42]. Furthermore, microbubble-mediated sonodynamic therapy effectively managed tumor growth and triggered an abscopal effect in a bilateral syngeneic KPC tumor mouse model [38]. During the treatment, one tumor received ultrasound exposure while the systemic administration of a microbubble–Rose Bengal conjugate (MB-RB) and an injection of anti-PD-L1 were carried out. Notably, animals treated with sonodynamic therapy and anti-PD-L1 exhibited a significant decrease in target and off-target tumor volumes compared to sham-exposed animals [37]. Moreover, treated animals displayed an infiltration of CD4+ T lymphocytes and cytotoxic T lymphocytes in the off-target tumor. Studies and clinical trials have explored the utility of focused ultrasound for drug delivery in colorectal cancer tumors, aiming to enhance treatment efficacy and patient outcomes [84]. This innovative approach presents various potential applications, including targeting primary tumors, addressing metastatic lesions, and improving the effectiveness of chemotherapy. A notable study explored the potential of FUS for enhancing drug delivery in colorectal cancer and the thermal ablation of the tumors using heat deposition at the focal spot [84]. Researchers utilized ultrasound to facilitate the direct delivery of chemotherapy drugs to tumor sites, aiming to maximize therapeutic efficacy while minimizing systemic side ef-

fects. The results demonstrated promising outcomes regarding tumor response and patient tolerance to treatment [29]. Furthermore, clinical trials have been conducted to assess the feasibility and safety of FUS in treating colorectal cancer [46]. These trials often combine ultrasound with conventional chemotherapy or immunotherapy to enhance their effects and synergistically improve overall treatment outcomes, as illustrated in Figure 4 [47].

Mechanism of Focused Ultrasound for Colorectal Carcinoma Treatment

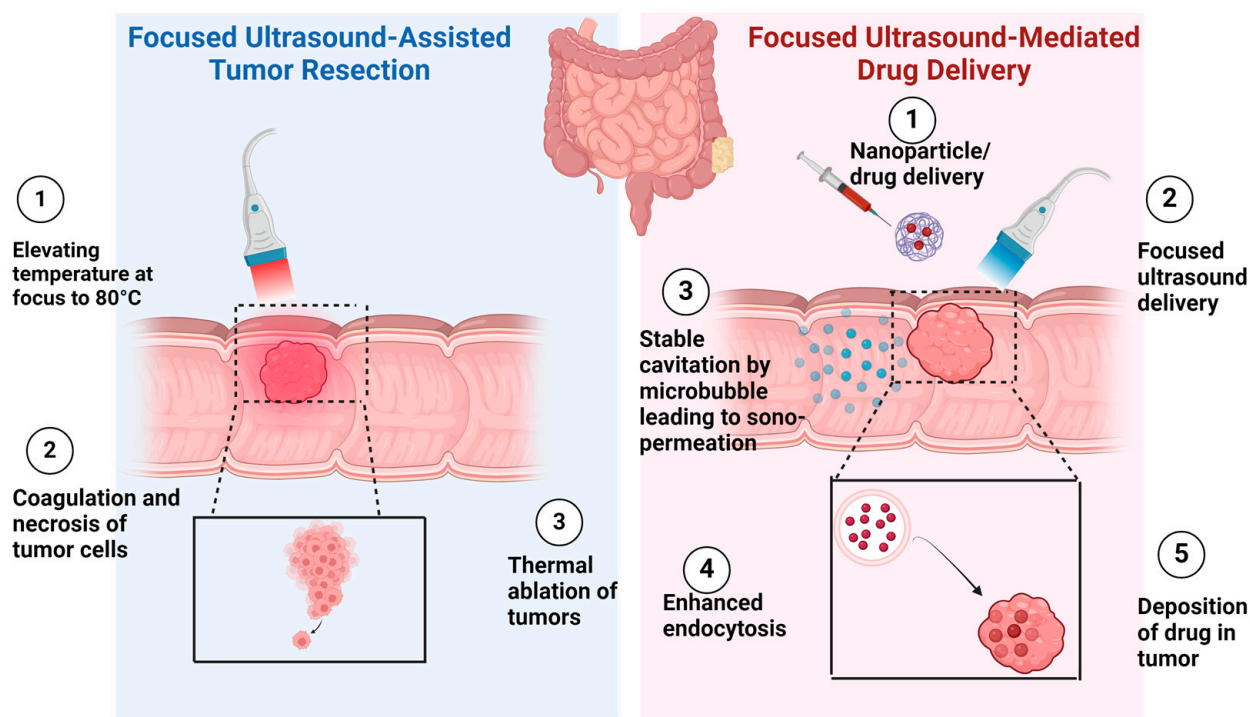


Figure 4. Clinical trial landscape for ultrasound-mediated therapeutics in colorectal cancer.

FUS presents specific applications in the treatment of colorectal cancer, including precise targeting and the delivery of therapeutic agents to primary tumors in the colon or rectum [85]. This targeted approach allows for localized treatment, minimizing harm to surrounding healthy tissue, thereby reducing side effects while simultaneously enhancing the effectiveness of the treatment [63]. Additionally, in the case of metastatic colorectal cancer, focused ultrasound offers a non-invasive option to target and treat metastatic lesions. This intervention can slow disease progression and enhance patient survival [86]. Moreover, FUS can strengthen the efficacy of chemotherapy by improving drug delivery to tumor sites. This optimization could potentially allow for the administration of lower drug doses or reduction in treatment frequency, ultimately improving patient outcomes and ensuring a better quality of life [19].

5. Emerging Trends in High-Intensity Focused Ultrasound Research for Colon Cancer Treatment

HIFUS is increasingly recognized as a minimally invasive treatment for gastrointestinal malignancies. Clinical studies, primarily focused on liver and pancreatic cancers, demonstrate HIFU's safety and tolerability, with notable survival advantages observed in hepatocellular carcinoma (HCC) compared to transarterial chemoembolization (TACE) and comparable outcomes to radiofrequency (RF) therapies [20]. HIFU also provides consistent pain relief in pancreatic cancer patients. However, further research is needed to enhance targeting precision and efficacy monitoring, potentially expanding into endoscopic-

based therapies [21]. One research study investigated the potential synergistic effects of combining pulsed HIFU therapy with cetuximab, an epidermal growth factor receptor (EGFR)-targeted chemotherapeutic drug, in treating human colon cancer xenografts in mice. Mice were divided into control, pulsed HIFU alone, cetuximab monotherapy, and combined therapy groups. The combination therapy group exhibited significantly suppressed tumor growth compared to all other groups, with reduced final tumor volumes. These results suggest that combining pulsed HIFU therapy with cetuximab enhances the antitumor effect of cetuximab in human colon cancer xenograft models in mice [87]. Another study aimed to investigate the potential of combining FUS with microbubbles (MBs) to augment the efficacy of chemotherapy in patients with liver metastases from colorectal cancer. Seventeen patients were included, with two liver lesions randomized for treatment with either FUS + MBs or standard chemotherapy alone. After chemotherapy, FUS treatment was administered using specific parameters, and changes in metastasis size were monitored via computed tomography. The results indicated the safety and feasibility of FUS+MB treatment, although response variations were observed between lesions. While there was a tendency towards more considerable volume reduction in lesions treated with FUS+MBs, the mixed response to chemotherapy and lesion heterogeneity complicated interpretation. The study concluded that FUS+MB combination therapy holds promise but warrants further investigation through multicenter trials with standardized protocols [68]. One research group explored the immunological effects of HIFUS on mouse colon adenocarcinoma tumors. The results revealed that thermal and mechanical HIFUS treatments led to increased dendritic cell activity and accumulation in lymph nodes. Moreover, HIFU significantly reduced primary tumor growth and conferred protection against tumor re-challenge. Immunological assays indicated enhanced cytotoxic T lymphocyte activity and increased tumor-specific IFN- γ -secreting cells in mice treated with HIFU, mainly mechanical HIFU. These findings suggest that HIFU treatment can elicit a systemic antitumor immune response, with mechanical HIFU potentially enhancing dendritic cell activation and overall treatment efficacy. Another investigation evaluated the impact of FUS on pyroptosis in a mouse colon cancer (CC) model. After establishing an orthotopic CC mouse model, mice were divided into groups receiving FUS alone or in combination with a pyroptosis inhibitor (BAY11-7082). The results showed that FUS reduced tumor fluorescence intensity and intestinal tissue injury in CC mice, with an increased expression of pyroptosis-related markers. The addition of BAY11-7082 reversed some effects of FUS, suggesting that FUS exhibits antitumor activity in CC models, possibly through promoting pyroptosis [65]. Some researchers explored the cytotoxic effects of heat on colon cancer cells and the involvement of different cell death processes such as autophagy, apoptosis, and necroptosis. Human colon cancer cell lines, HCT116 and HT29, were subjected to ablative temperatures, and cell viability and protein regulation were assessed. The results showed that exposure to ablative thermal doses decreased cell viability, with the induction of apoptosis and autophagy depending on the thermal dose and the time that elapsed after treatment. Autophagy induction mainly occurred in live cells, while necroptosis did not significantly affect cell viability. Overall, autophagy, apoptosis, and necroptosis contributed to the response of colon cancer cells to supra-normal temperatures [66]. Similarly, another study evaluated the safety and efficacy of HIFU therapy for hepatic metastases from colorectal cancer in 18 patients. Following treatment, patients maintained stable vital signs and normal organ function, with a median survival rate of 16 months. Among the lesions followed up, 17 showed significant shrinkage, while 5 developed new metastases. The study concluded that HIFU is a safe and effective non-invasive option for treating hepatic metastases from colorectal cancer [71]. Recently, one study investigated the effects of HIFU on HT-29 colon cancer cell lines, focusing on viability, death, and apoptosis rates. The results showed that increasing thermal doses decreased cell viability and increased death rates, both in HIFUS-treated and adjacent cells. Apoptosis levels also rose with higher thermal doses, particularly in neighboring cells. Notably, adjacent cells exhibited higher viability rates but more significant apoptosis than cells directly exposed to HIFU, indicating

HIFUS-induced apoptosis even at lower doses. These findings underscore the importance of precise dose selection in HIFU treatment for optimal efficacy while minimizing damage to surrounding tissues. Another retrospective study assessed the effectiveness and safety of HIFUS as an adjuvant therapy for systemic chemotherapy in patients with unresectable colorectal cancer liver metastasis (CRLM). Comparing a HIFUS group to a non-HIFUS group, the study found higher disease control and objective remission rates in the HIFUS group. The median progression-free survival was significantly longer in the HIFUS group, particularly for patients with smaller lesions (<5.0 cm). Adverse events were mostly mild, with few cases of skin burns in the HIFUS group. These findings suggest that HIFUS could be valuable to systemic chemotherapy for managing unresectable CRLM, especially for smaller lesions with acceptable safety profiles [70].

Some clinical studies evaluated the efficacy and safety of ultrasound-guided HIFUS ablation in patients with colorectal liver metastases (CRLMs) ineligible for hepatectomy. Among 43 enrolled patients, a high objective response rate of 97.7% was achieved, with complete response observed in 21 patients and partial response in 21. The median overall survival (OS) was estimated at 31 months, with favorable 1-year and 18-month OS rates. Adverse events were primarily mild, including pain and local skin edema, with no severe complications reported. These findings suggest that ultrasound-guided HIFU ablation presents a safe and effective therapeutic option for patients with colorectal liver metastases (CRLM), particularly those who are not suitable candidates for surgical resection [71].

One preclinical research aimed to assess the efficacy of targeted endostatin-loaded microbubbles in combination with FUS for inhibiting angiogenesis in colon tumors. BALB/c nude mice with these tumors were divided into five groups receiving different treatments. The results showed that the group treated with endostatin microbubbles and ultrasound had the most minor tumors and the lowest blood flow parameters. Additionally, this group exhibited increased tumor necrosis and reduced microvascular density compared to other groups. These findings suggest that the combined therapy effectively damages tumor microvasculature and inhibits angiogenesis in colon cancer. HIFUS is a versatile tool that merges surgery, oncology, and biomedical engineering with its precise yet non-invasive nature [63].

Oxford has been at the forefront of HIFUS research for the past two decades, building on earlier work led by Professor Gail ter Haar and her team at the Institute of Cancer Research in Surrey. Various applications have been explored, including tissue ablation and drug delivery, particularly in the treatment of solid abdominal tumors such as those affecting the liver, kidney, uterus, pancreas, pelvis, and prostate. Oxford's clinical studies demonstrate the safety, tolerability, and effectiveness of HIFU, laying the foundation for its integration into clinical management strategies [75].

Additionally, ongoing preclinical and translational research at the Institute of Biomedical Engineering, University of Oxford, further advances the understanding and application of this promising therapy. Another study aimed to assess the efficacy of HIFU with toroid-shaped emitters in treating unresectable colorectal liver metastases. Surgical resection is typically the only curative option, but many patients are ineligible for surgery due to various limitations. The study utilized a HIFU device with 256 toroid-shaped emitters and integrated ultrasound imaging to create lesions in pigs under real-time guidance. The results showed that HIFUS lesions were immediately hypoechoic on ultrasound, with an average coagulated volume of 7.0 cm³ and an average diameter of 19.5 mm [72]. Real-time visualization allowed for the easy juxtaposition of lesions, creating larger lesions without significant complications. The toroid HIFUS device demonstrated shorter treatment times, non-invasiveness, and improved reliability compared to current ablative methods, suggesting its potential as a promising treatment modality for unresectable colorectal liver metastases, including those near large vessels [75].

Another prominent research group developed a combinational therapy using attenuated *Salmonella* to deliver low-temperature-sensitive liposomes (LTSLs) loaded with doxorubicin into colon cancer cells. These thermobots efficiently transported LTSLs and induced the nuclear localization of doxorubicin within cancer cells. Additionally, when combined with HIFU heating, the robots promoted the polarization of macrophages to the M1 phenotype and enhanced therapeutic efficacy in

murine colon tumors. The results suggest that this combination therapy approach has promising potential for improving colon cancer treatment [77]. One study investigated the effects of sub-ablative heat and HSP90 inhibition on colon cancer cells, aiming to enhance cell death and induce a pro-immune phenotype. Thermal doses decreased cell viability and increased intracellular HSP70 levels, ATP release, and Calreticulin/HSP70/HSP90 exposure in the plasma membrane while downregulating CD47. Combined with HSP90 inhibition, thermal doses led to synergistic decreases in cell viability and the induction of apoptosis, along with increased Calreticulin exposure, CD47 downregulation, and HSP70 release. These findings suggest that sub-ablative heating, in combination with HSP90 inhibition, can promote a pro-immunogenic form of cell death in colon cancer cells [78]. Some research studies evaluated the immunological response triggered by HIFU treatment in mice with colon adenocarcinoma tumors. Thermal and mechanical HIFU exposure increased DC activity and accumulation in draining lymph nodes, significantly reducing tumor growth and protecting against tumor re-challenge. Immunological assays confirmed enhanced cytotoxic T lymphocyte activity and increased tumor-specific IFN- γ -secreting cells in mice treated with FUS, particularly with mechanical HIFU, inducing higher cytotoxicity. These findings suggest that FUS treatment elicits a systemic antitumor immune response, with mechanical HIFU showing potential for enhancing DC activation and antitumor immunity, thereby increasing the efficacy of HIFU cancer treatment [64]. Another research group investigated the potential of low-pressure, pulsed-mode HIFU in the presence of MBs to induce an antitumor immune response and inhibit tumor growth. They conducted experiments on tumor-bearing animals using sonographically guided FUS, and a suppression of tumor progression was observed with both low-pressure FUS exposures. The treatment led to a transient increase in non-T regulatory tumor-infiltrating lymphocytes (TILs) and the continual infiltration of CD8⁺ cytotoxic T-lymphocytes, resulting in an increased ratio of CD8⁺/Treg and inhibited tumor growth. These findings suggest that low-pressure FUS exposure with MBs could be a potential tool for cancer immunotherapy by triggering an anticancer immune response [68]. A Chinese research group evaluated the effectiveness and safety of combining low-dose HIFU with SOX chemotherapy in treating metastatic colorectal cancer patients with pelvic masses. The results showed that the HIFU+SOX group had a significantly longer median progressive-free survival (PFS) than the SOX group (11.2 months vs. 7.1 months). However, the overall survival was longer in the HIFU+SOX group (21.9 months vs. 16.9 months). The combination therapy of HIFU and SOX was effective and well tolerated for late-stage colorectal cancer patients with pelvic masses [79].

6. Conclusions and Future Directions

Although therapeutic FUS treatments have shown promising clinical outcomes and a lack of severe adverse effects, the survival rate of patients undergoing therapy with FUS with colorectal cancer cannot be predicted due to the absence of enough experimental data. However, there is some hope for colorectal cancer patients undergoing treatment with FUS that FUS can transform immunologically cold tumors into hot ones after FDA approval of ICI. Furthermore, data suggested that merging chemoradiotherapy treatments with immune-based therapies does not compromise immune responses, thereby easing the process of integrating treatment strategies with therapeutic FUS. Some experiments are being carried out to investigate the synergy between immunotherapy and various oncology interventions to evaluate their efficacy in treating colorectal cancer patients, such as CD40, MHC-II, ICI, and Toll-like receptor (TLR) agonists, adoptive T-cell therapies, oncolytic viruses, and vaccines. Colorectal tumors, particularly those with heightened stiffness, show promise for treatment using physical techniques like therapeutic FUS. Extensive preclinical and clinical evidence underscores the significant advantages of therapeutic FUS in colorectal cancer management. Nonetheless, the substantial costs of specific immunotherapies challenge advancements in this domain. The anticipated expiration of ICI antibody patents may offer relief regarding this concern shortly. Moreover, continuous technological advancements, such as advancements in thermometry, enhanced software user interfaces,

motion compensation, and the innovation of endoscopic transducers, are poised to refine the precision and effectiveness of FUS therapies. Preclinical investigations show that implementing mechanical HIFU in clinical practice will yield more pronounced pro-inflammatory responses. Standardizing treatment protocols achieved through cavitation and thermal ultrasound doses across various facilities will deepen our understanding of the bioeffects associated with therapeutic FUS. It will empower clinicians to administer more uniform and efficacious treatments. Consequently, therapeutic FUS in clinical settings is poised to continue growing, with the increased adoption of combination treatment strategies for colorectal cancer patients in the foreseeable future.

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