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The Impact of Histotripsy Technique in Tumor Ablation - a Systematic Review

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Abstract

According to a study conducted in the U.S., cancer affects more than 700,000 individuals worldwide each year, with 35,660 new cases diagnosed in the U.S. in 2015 alone. However, there are only a few standard treatment choices, which include radiation, chemotherapy, and surgery. This systematic review report on the impact of histotripsy in tumor ablation. In this paper, the effectiveness, safety and advantages of histotripsy compared to other methods are explored. A search was done on ScienceDirect, PubMed, Google Scholar, and Scopus databases for articles published from the date of inception of the databases to 21st March 2023. Reference lists of identified studies were also screened. Non-duplicate articles were identified, and 12 articles were included for thematic analysis. During the analysis, different effects of histotripsy were categorized. This categorization led to the realization of the effectiveness, immunological effects, safety and advantages of histotripsy compared to the therapeutic options. Histotripsy is effective and safe treatment for various types of cancer, including cholangiocarcinoma, pancreatic cancer, breast cancer, colorectal cancer, osteosarcoma, and hepatocellular carcinoma (HCC).

Keywords: Histotripsy; Tumor ablation; Immune system activation; Preclinical models; Adverse events.

1. Introduction

According to a study conducted in the U.S., cancer affects more than 700,000 individuals worldwide each year, with 35,660 new cases diagnosed in the U.S. in 2015 alone [1]. There are only a few standard treatment choices, which include radiation, chemotherapy, and surgery [2]. Less than 25% of patients with medically treatable tumors are cured, and only 20% of the patients have these tumors [3]. A recent shift has been toward less invasive yet successful medical interventions.

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Today, numerous minimally invasive and non-invasive treatments are available for various diseases, and many of these are carried out with the aid of increasingly complex image guidance. One of them is the development of stereotactic body radiation therapy (SBRT) from planar radiation therapy, but toxicity still places restrictions on treatment quantities and sites [4]. Cryoablation, microwave ablation, and radiofrequency ablation are among the thermal-based ablations that are most commonly administered percutaneously while being guided by images [5,6]. Targeted tissue is heated or frozen using these technologies to cause necrosis. The heat sink effect brought on by blood flow, which restricts the ablation zone and makes margins unpredictable, is one of the limitations of thermal modalities. Another limitation is the crucial dependence on physician expertise [7,8]. Treatment of tumors close to sensitive organs is hampered by thermal spread. High-intensity focused ultrasound (HIFU) is a non-invasive thermal ablation method employing externally applied ultrasound energy to produce thermal necrosis [9]. Although uterine fibroids, neurological disorders, and tumors of the prostate, breast, liver, and pancreas have all been successfully treated with HIFU in clinical settings, its use is still uncommon because of anatomical difficulties and lengthy treatment periods [10].

Histotripsy uses carefully controlled, high-amplitude ultrasound pulses to produce acoustic cavitation bubble clouds and mechanically dissolve tissue. It is a non-thermal, non-invasive, and non-ionizing focused ultrasound ablation method [11]. Histotripsy may circumvent the problems of thermal ablation by physically destroying the target tissue [12]. Furthermore, histotripsy has been shown to have tissue-selective characteristics, sparing tissues with higher mechanical strength and density compared to the target tissue, like large blood vessels, nerves, and ducts [13]. High-precision ablation is another benefit of histotripsy. Since the histotripsy treatment's cavitation bubble cloud is usually visible on ultrasound imaging and is only a few millimeters, ultrasound can be used for both the therapy and real-time image guidance and treatment monitoring [14]. Histotripsy is a hopeful procedure for precise, non-invasive tissue ablation due to all these characteristics.

Numerous preclinical applications of histotripsy have been studied, such as the treatment of liver [15], kidney [16], prostate tumors [17], neurological diseases [18], thrombosis [19], and biofilms [20]. However, to our knowledge, no systematic study on the effect of histotripsy in tumor ablation has been done. This study provides a systematic review of the literature on the impact, effectiveness, and safety of histotripsy in tumor ablation.

1.1. Research Question

What is the impact, efficacy and or safety of histotripsy technique in tumor ablation?

2. Materials and Methods

2.1. Eligibility Criteria

Only articles published from 1st January 2000 to 21st March 2023 and in English were eligible. Published studies were the only type of publication considered eligible; thus, there was no consideration for grey literature.

2.2. Literature Search and Information Sources

This research paper was prepared following the Preferred Reporting Items for Systematic Review and Meta-

Analysis (PRISMA) guidelines [21]. A search for articles was conducted using ScienceDirect, PubMed, Google Scholar, and Scopus databases for articles published from 1st January 2000 to 21st March 2023. The reference lists of identified studies were also screened to identify relevant articles not captured by the electronic searches. Similar keywords were used to generate search strings for the index databases. The search strategy for PubMed was as follows: ("histotripsy") AND ("tumor ablation" OR "cancer ablation") AND (effect OR impact OR evaluation). A similar logic was followed while conducting literature searches in other databases.

2.3. Inclusion criteria

Articles had to be published in peer-reviewed journals and written in the English language. All scientific original human and animal papers reporting on the impact of histotripsy in tumor ablation.

2.4. Exclusion criteria

Systematic reviews, meta-analyses, non-journal papers, conference proceedings, letters to authors, and comments on published articles were excluded. Studies that did not report on the impact of histotripsy in tumor ablation.

2.5. Assessment of methodological quality

The critical appraisal tool used in this systematic review is an adaptation and miniaturization of the qualitative studies checklist in the Best Bets Critical Appraisal Worksheets [22]. The checklist was primarily developed for qualitative studies and thus had to be modified to accommodate other study types. The assessment tools used are: clarity of study objective(s), suitability of study design, sample size justification, clarity in data collection, clarity of data analysis, clearly presented results, the validity of results, and literature value of results.

2.6. Data Extraction.

Data from the included studies was extracted to a predefined study descriptor table. The extracted information was: lead author, publication year, study design, study region, name or type of tumor, study population, type of histotripsy, and the objective or the theme of the study.

3. Results

3.1. Search results

The database search yielded a total of 643 articles. 551 articles were identified from Google Scholar, 51 articles from ScienceDirect, 17 articles from PubMed, and 24 articles from Scopus. Ten articles were identified from the screening of reference lists of identified studies. 98 duplicates were removed. 531 articles were excluded during the screening of titles and abstracts. They were of the study methodology stated in the exclusion criteria or did not report about did not report on the effect of histotripsy ablation. The remaining 24 articles were read in full; only 12 completely agreed with the inclusion criteria. Figure 1 below shows the process of study selection presented in a flowchart.

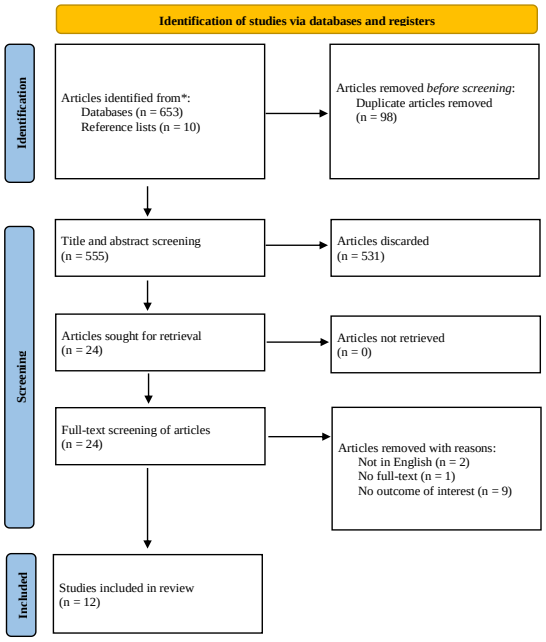

Figure 1: Study selection process

3.2. Results of quality appraisal

Table 1: Results of critical appraisal. Filled circles indicates the assessed item is clear, empty unbroken circles indicate cannot tell, and empty broken circles indicate not clear

Author and year	Assessment items							
	Study objectives	Study design	Sample size justification	Data collection	Data analysis	Presentation of results	Validity of results	Literature value
(Hendricks-Wenger et al., 2022)	●	●	○	●	●	○	●	○
Hendricks-Wenger et al. (2021)	●	○	●	○	●	●	●	●
(Hoogenboom et al., 2017)	●	○	○	●	●	○	●	○
(Nam et al., 2020)	○	●	○	●	●	●	○	●
(Pepple et al., 2023)	●	○	●	○	●	○	●	○
(Qu et al., 2020)	●	●	○	●	○	●	●	○
(Ruger et al., 2023)	○	○	○	○	●	○	●	○
(Schade et al., 2019)	●	●	●	○	●	○	○	●
(Vlaisavljevich et al., 2016)	●	○	○	●	○	○	●	●
(Vlaisavljevich et al., 2017)	●	●	●	○	●	○	●	○
(Worlikar et al., 2018)	●	●	○	○	○	●	●	●
(Worlikar et al., 2022)	●	●	●	○	○	○	●	●

3.3.Results of Data Extraction

Table 2: The twelve articles which met the inclusion criteria to be involved in this review

Author and Year	**Study Design**	**Study Region**	**Type of Tumor**	**Study Participants**	**Type of Histotripsy**	**Theme /Objective**
(Hendricks-Wenger et al., 2022)	Research article	USA	Cholangiocarcinoma	Seventeen female mice	Standard histotripsy	Safety and efficacy of histotripsy for treating Cholangiocarcinoma tumors
Hendricks-Wenger et al. (2021)	Research article	USA	Pancreatic cancer	Male and female mice aged between 7-10weks	Standard histotripsy	Impact of histotripsy on tumor microenvironment and immune system
(Hoogenboom et al., 2017)	Research article	Netherlands	Breast cancer, pancreatic cancer and melanoma	Female mice (9–11 weeks old)	Boiling histotripsy	Impact of boiling histotripsy on tumor reduction in murine tumor models
(Nam et al., 2020)	Research article	USA	Colorectal and breast cancer	Colorectal cancer and breast cancer mouse models	Boiling histotripsy	The potential immunological benefits of boiling histotripsy over thermal ablation
(Pepple et al., 2023)	Research article	USA	Melanoma and liver tumors	Male and female mice aged 6–8 weeks old	Standard histotripsy	The effects of histotripsy on tumor ablation
(Qu et al., 2020)	Research article	USA	Not specified	Mice (*Mus musculus*) aged 6–8 weeks old	Standard histotripsy	The effects of histotripsy adaptive immune responses
(Ruger et al., 2023)	Research article	USA	Osteosarcoma(OS)	Dogs with appendicula	Standard histotripsy	Safety and effect of ablating primary

Author and Year	Study Design	Study Region	Type of Tumor	Study Participants	Type of Histotripsy	Theme /Objective
				r bone lesions		bone tumors with histotripsy in canine O.S. patients
(Schade et al., 2019)	Research article	USA	Renal tumor	Eker rat Renal tumor model	Boiling histotripsy	Effects of histotripsy on renal tumor ablation
(Vlaisavljevich et al., 2016)	Research article	USA	Liver tumor	20 male Sprague Dawley rats	Standard histotripsy	Response of liver tissue to histotripsy therapy
(Vlaisavljevich et al., 2017)	Research article	USA	Liver tumor	22 healthy mixed-breed pigs	Standard histotripsy	Histotripsy therapy in liver ablation
(Worlikar et al., 2018)	Research article	USA	Liver tumor	21 mice weighing 20-30 gm	Standard histotripsy	Effects of histotripsy on liver tumor ablation
(Worlikar et al., 2022)	Research article	USA	Liver tumor	Sprague-Dawley rats weighing 125–175 g	Standard histotripsy	Effects of histotripsy on the risk of recurrence and metastases

3.4.Characteristics of individual studies: summary

This systematic review included twelve research articles published between 2016 and 2023. While one investigation originated in the Netherlands, the remaining studies were conducted within the United States. The studies covered a spectrum of malignancies, with five specifically examining the effects of histotripsy on liver tumors, two on melanoma, two on pancreatic cancer, and one each on renal tumor, breast cancer, colorectal cancer, cholangiocarcinoma, and osteosarcoma.

Within the scope of histotripsy modalities, three studies delved into the effects of boiling histotripsy, while the remaining nine concentrated on standard histotripsy. It is noteworthy that all investigations were conducted using animal models, with the species encompassing mice, pigs, and dogs.

3.5.Analysis and consistent themes

Thematic analysis, guided by Braun and Clarke's methodology [23], was employed to discern and analyze

recurring themes within the studies included in our investigation. We scrutinized reports from these studies alongside the findings articulated in the research articles to assess the effectiveness, safety, and feasibility of utilizing histotripsy for tumor ablation.

The discerned themes have been systematically categorized and subcategorized, presenting a structured overview of the principal patterns and insights derived from the collective body of literature.

Tumor Size Reduction

Cholangiocarcinoma tumor

In the investigation conducted by Hendricks-Wenger et al., the impact of histotripsy on Cholangiocarcinoma ablation was explored using a patient-derived xenograft mouse model [24]. The study revealed that both low- and high-dose histotripsy treatments resulted in a significant reduction in tumor size compared to the control group with a maximum reduction in tumor size of 73% ($p < 0.05$). Specifically, the mean tumor volume reduction in the low-dose histotripsy group was 40%, while the mean tumor volume reduction in the high-dose histotripsy group was 67% [24].

Pancreatic cancer

Hendricks-Wenger et al. explored the impact of histotripsy on pancreatic tumor ablation in male and female mice aged between seven to ten weeks old [25]. Their study revealed a significant reduction in tumor size for the majority of mice subjected to histotripsy ablation. Specifically, the mean tumor size decreased from 497.9 mm^3 before ablation to 11.7 mm^3 after ablation, indicating an average reduction of 97.6%. Additionally, Hoogenboom et al. delved into the effects of magnetic resonance-guided boiling histotripsy on pancreatic tumor models [26]. Their findings demonstrated a substantial tumor reduction (57%) in pancreatic cancer models utilizing magnetic resonance-guided boiling histotripsy.

Breast cancer

Studies investigating the impact of histotripsy on breast cancer ablation and tumor size reduction include those conducted by Hoogenboom et al. (2017) and Nam et al. (2020). In their respective findings, both studies revealed that boiling histotripsy led to a noteworthy reduction in tumor size. The treated mice exhibited an average reduction ranging from 79% to 85% [26,27].

Melanoma

Two studies, documented by Hoogenboom et al. (2017) and Pepple et al. (2023) respectively, investigated the impact of histotripsy in Melanoma tumor ablation [26,28]. The application of magnetic resonance-guided boiling histotripsy demonstrated a substantial reduction in tumor size by 69% in melanoma cancer [26]. Pepple et al.'s study observed a significant decrease in tumor size following histotripsy treatment, with an average reduction of around 70%. This reduction was coupled with a notable decrease in tumor growth rate, indicating

the potential efficacy of histotripsy in effectively mitigating tumor burden [28].

Colorectal cancer

As per the investigation conducted by Nam et al., boiling histotripsy demonstrated a substantial impact on tumor size reduction. The average reduction in tumor size among the treated mice was reported to be 85% [27].

Liver cancer

Three studies explored the impact of histotripsy in tumor ablation [28,29,30]. According to Pepple et al., histotripsy treatment demonstrated a substantial reduction in tumor size, with an average decrease of approximately 70%. This reduction coincided with a significant decrease in the tumor growth rate [28]. In the investigations led by Worlikar and colleagues, utilizing a subcutaneous xenograft murine model and rodent model, histotripsy exhibited effectiveness in reducing the size of liver tumors. The studies reported a noteworthy decrease in tumor volume within the histotripsy-treated group compared to the control group [29,30],

Osteosarcoma

Ruger et al. investigated the impact of histotripsy treatment in osteosarcoma tumor ablation among dogs with appendicular bone lesions [31]. The study revealed a significant reduction in both tumor size and volume in the treated tumors, as observed in both in vitro and in vivo studies.

Renal tumor

Schade et al. investigated the impact of histotripsy on tumor reduction in renal tumor patients using the Eker rat renal tumor model. Their study demonstrated that histotripsy treatment resulted in a significant reduction in both tumor size and volume among the treated rats. This effect was observed immediately after treatment and persisted in the long-term follow-up period [16].

Overall Survival

According to Hendricks-Wenger et al., both low- and high-dose histotripsy treatments improved the overall survival of the mice compared to the control group. Specifically, the median survival time of the control group was 30 days, while the median survival time of the low-dose histotripsy group was 40 days, and the high-dose histotripsy group was 60 days [24]. In colorectal cancer and breast cancer mouse models, boiling histotripsy resulted in a significant increase in overall survival, with treated mice surviving an average of 55 days compared to 25 days for untreated mice [27].

Tumor appearance

The ablation zone induced by boiling histotripsy exhibits a well-defined demarcation, clearly distinguishing the treated tissue from the surrounding healthy tissue [26]. Boiling histotripsy, characterized by high-intensity ultrasound waves inducing localized boiling, causes extensive tissue damage, rendering the treated tumors as

necrotic debris. This aligns with the established mechanism of boiling histotripsy, leading to rapid tissue destruction [27]. Ruger et al. noted alterations in the appearance of treated tumors following histotripsy, demonstrating increased necrosis or cell death in both in vitro and in vivo studies [31].

Lesions formation and lesion progression

Histotripsy demonstrates efficacy in forming well-defined lesions in liver tissue, featuring a distinct demarcation between the treated and untreated areas. These lesions exhibit a gradual decrease in size over time, as evidenced in multiple studies [15,29,32]. Vlaisavljevich et al.'s investigation revealed that histotripsy-treated lesions, marked by tissue damage and inflammation, exhibited progression over time. This was evident through the expansion of cavitation bubbles within the treated tissue [32].

Post-treatment Complications

According to Hendricks-Wenger et al., none of the mice in the control group encountered post-treatment complications. In the low-dose histotripsy group, one mouse experienced a liver rupture, while in the high-dose histotripsy group, two mice experienced liver ruptures, and an additional mouse faced a subcapsular hematoma reference[24].

In the vivo study by Ruger et al., minimal complications and no adverse effects were observed during the treatment [31].

According to Vlaisavljevich et al., histotripsy demonstrated safety in liver ablation within the preclinical porcine model, with no indications of major complications such as bleeding, perforation, or bile leakage. The study did note minor complications like fever and transient elevations in liver enzymes, but these issues resolved without the need for intervention [15].

In the study by Worlikar et al., there were no major complications, such as bleeding or tissue damage, observed in the histotripsy-treated group. However, the researchers did note some minor complications, including skin burns and muscle contractions, which resolved within a few days without requiring any intervention [29].

In a separate study, Worlikar and colleagues noted that histotripsy did not facilitate the formation of intrahepatic metastases in the rodent liver tumor model. There was no significant difference observed in the incidence or number of intrahepatic metastases between the histotripsy-treated and control groups [30].

Acute Immune Cellular Response and Immune Cell Composition

According to the research by Hendricks-Wenger et al., histotripsy ablation promotes immune system activation and alters the tumor microenvironment in a way that increases the infiltration of immune cells [25]. Specifically, the ablation increases the infiltration of macrophages, natural killer cells, T cells, and dendritic cells into the tumor microenvironment [25]. Additionally, the researchers found that histotripsy ablation causes changes in the expression of immune regulatory genes in the tumor microenvironment, suggesting a shift towards an anti-tumor

immune response [25].

Nam et al. reported that boiling histotripsy results in a significant increase in the number of immune cells infiltrating the tumor, including CD4+ and CD8+ T cells, natural killer cells, and macrophages. The researchers also observed an increase in the expression of several pro-inflammatory cytokines and chemokines, suggesting that boiling histotripsy may have immunostimulatory effects that could enhance the anti-tumor immune response [27].

Pepple et al. observed that histotripsy treatment led to an increase in the infiltration of immune cells, specifically T cells and macrophages, within the treated tumors. This immune cell infiltration was associated with an increase in the expression of several immune checkpoint markers, suggesting that histotripsy may enhance the efficacy of immune checkpoint inhibitor therapy. Additionally, the researchers found evidence of systemic immune activation, with an increase in the number of immune cells within the spleen and blood, indicating that histotripsy may have an abscopal effect, meaning it could stimulate an immune response not only in the treated tumor but also in distant, untreated tumors [28].

Qu et al. reported that histotripsy treatment in a preclinical model led to a notable increase in immune cell infiltration not only in the treated tumor but also in distant, untreated tumors, indicating a potential abscopal effect [33]. This treatment also resulted in an elevated expression of various immune checkpoint markers, suggesting that histotripsy could enhance the effectiveness of immune checkpoint inhibitor therapy. Combining histotripsy treatment with immune checkpoint inhibitor therapy demonstrated a significant reduction in tumor growth rate and increased survival in preclinical models. Importantly, the study found that histotripsy treatment alone or in combination with immune checkpoint inhibitor therapy did not induce any significant toxicity or adverse effects in the treated animals [33].

In the in vivo study conducted by Ruger et al., researchers observed evidence of immune cell infiltration within the treated tumors, suggesting that histotripsy may elicit an immune-stimulatory effect [31].

Schade et al. reported that histotripsy treatment promotes a systemic inflammatory response, with increased levels of circulating cytokines and chemokines, as observed in the treated rats [16]. This indicates that histotripsy may have an immune-stimulatory effect.

According to Worlikar et al., histotripsy treatment induced an immune response in the rodent model. The researchers observed an increase in the infiltration of immune cells in the histotripsy-treated group compared to the control group [30].

4. Discussion

The thematic analysis of the included studies resulted in the categorization of various effects of histotripsy in tumor ablation. This categorization has subsequently yielded outcomes and indicators that offer insights into the effectiveness and safety of histotripsy in tumor ablation. The established order derived from this review is as follows.Firstly, this systematic review underscores the effectiveness of histotripsy in reducing tumor size across

diverse preclinical models of cancers, encompassing cholangiocarcinoma, pancreatic cancer, osteosarcoma, and hepatocellular carcinoma [16,24,25,26,27,28,29,30,31]. For instance, a study focusing on cholangiocarcinoma in a patient-derived xenograft mouse model demonstrated a significant reduction in tumor size with histotripsy [24]. Another investigation exploring histotripsy ablation for pancreatic cancer reported alterations in the tumor microenvironment and a decrease in tumor size in a subcutaneous model [24]. Similarly, a study characterizing histotripsy's ablation effects on osteosarcoma in dogs found it to effectively reduce tumor size [31]. Furthermore, research examining histotripsy for the non-invasive ablation of hepatocellular carcinoma (HCC) tumors in a subcutaneous xenograft murine model revealed a significant reduction in tumor size [28]. These findings hold promise, suggesting histotripsy as a potentially safe and effective non-invasive treatment option for various cancers. The capacity of histotripsy to reduce tumor size may prove valuable in minimizing the necessity for invasive surgeries or radiation therapy, thereby mitigating potential significant side effects for patients.

Secondly, the evidence from this study indicates that histotripsy can effectively modify the tumor microenvironment and trigger immune system activation, instigating acute immune cellular responses and alterations in immune cell composition across preclinical models of various cancers. For example, an investigation into histotripsy for pancreatic cancer revealed its capacity to alter the tumor microenvironment, prompting immune system activation, and inducing changes in immune cell composition along with an acute immune cellular response [25]. Another study delved into the immunological effects of boiling histotripsy for cancer treatment, unveiling its ability to elicit an acute immune response marked by the activation of natural killer cells and dendritic cells [27]. Similarly, research exploring spatiotemporal local and abscopal cell death and immune responses to histotripsy-focused ultrasound tumor ablation reported a robust acute inflammatory response and alterations in immune cell composition, including the activation of macrophages and natural killer cells [28]. Moreover, a separate article noted that non-thermal histotripsy tumor ablation stimulated abscopal immune responses, potentially enhancing cancer immunotherapy [30]. These findings hold significance, suggesting that histotripsy may augment the body's immune response to cancer, potentially leading to improved clinical outcomes. The induced acute immune cellular response and changes in immune cell composition by histotripsy could enhance the effectiveness of other cancer treatments, notably immunotherapy. Furthermore, histotripsy's ability to alter the tumor microenvironment and promote immune system activation may render previously resistant tumors more amenable to treatment.

Thirdly, the current study reveals that the post-histotripsy appearance of tumors may vary, contingent on the specific tumor model and treatment parameters. Some studies depict complete tumor destruction, devoid of residual tissue or discernible fluid collections on imaging studies [26,27]. Conversely, other studies note the presence of residual fluid collections, necrotic tissue, or areas displaying reduced enhancement on imaging studies, indicative of potential incomplete tumor destruction [31,32]. Interestingly, certain cases exhibit an improvement in the appearance of treated tumors over time, featuring reduced fluid collections and heightened enhancement on follow-up imaging studies [15,29]. These findings underscore the complexity of interpreting tumor appearance post-histotripsy treatment, emphasizing that it may not always be straightforward. The variability observed in tumor appearance could stem from differences in the extent and nature of tumor destruction, coupled with variations in inflammatory and healing responses post-treatment. Additionally, factors

such as diverse imaging modalities, timing of imaging studies, and the interpretation of imaging findings may contribute to the observed variability in tumor appearance following histotripsy treatment.

Lastly, this review established that histotripsy treatment is associated with several complications and adverse events. In a study focusing on cholangiocarcinoma in a mouse model, the authors reported perioperative mortality in some mice, likely attributed to hepatic bleeding and bile leakage. Moreover, signs of liver dysfunction, such as elevated liver enzymes, were observed in some mice after treatment [24]. The osteosarcoma study in dogs noted delayed wound healing in one dog, possibly linked to the substantial size of the treatment site [31]. In the study on non-invasive liver ablation in a porcine model, post-treatment complications, including fever, lethargy, and abdominal discomfort, were reported in some pigs [15]. The investigation on non-invasive ablation of hepatocellular carcinoma in a mouse model observed signs of liver dysfunction, including elevated liver enzymes, in some mice after treatment [29]. Lastly, the study on the impact of histotripsy on the development of intrahepatic metastases in a rodent liver tumor model reported the occurrence of intrahepatic abscesses in some rats post-treatment [30]. These findings underscore that, while histotripsy shows promise in treating various tumors, it is not without associated risks and complications. Careful patient selection, monitoring, and follow-up are crucial to minimize the potential for adverse events. Further research is warranted to enhance our understanding of factors contributing to complications and adverse events associated with histotripsy treatment, allowing the development of strategies to mitigate these risks.

4.1. Limitations of the study

The inherent constraints and limitations of the study should be considered for a nuanced interpretation of its findings. The basis being animal model studies, while offering valuable preclinical insights, may not consistently mirror human responses. The absence of human trials limits the direct extrapolation of this study's outcomes to clinical contexts, underscoring the need for future human-centered investigations. Incorporating Randomized Controlled Trials (RCTs), acknowledged as a gold standard in clinical research, would enhance the robustness and reliability of the presented evidence. This highlights an avenue for future research.The observed variability in tumor appearance post-histotripsy treatment introduces complexity in result interpretation. Factors such as imaging modalities, timing of studies, and result interpretation contribute to this variability, emphasizing the multifaceted nature of assessing tumor response.The identification of complications and adverse events associated with histotripsy, including hepatic bleeding and intrahepatic abscesses, accentuates the necessity for cautious patient selection and vigilant monitoring. While these findings underscore the importance of patient safety, further research is warranted to comprehend and effectively mitigate these adverse events.Thorough investigation into the safety aspects of histotripsy is crucial for its successful translation into clinical practice, ensuring not only efficacy but also sustained patient well-being.

5. Conclusion

The systematic review found that histotripsy can effectively reduce tumor size and alter the tumor microenvironment, promoting immune system activation in various cancer types, including cholangiocarcinoma, pancreatic cancer, osteosarcoma, and hepatocellular carcinoma. Additionally, histotripsy was shown to induce

both local and abscopal cell death, leading to immune responses that can target distant tumor sites beyond the targeted ablation zone. However, some studies also identified potential complications and adverse events associated with histotripsy treatment, including the development of intrahepatic metastases in a rodent liver tumor model, indicating the need for further investigation of the long-term effects of histotripsy. The findings suggest that histotripsy may be a promising non-invasive method for tumor ablation and immune system activation in cancer treatment. However, further research is needed to fully understand its long-term effects and potential adverse events.

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