How Sound Waves Could Revolutionize Cancer (Immuno)therapy

Natasha D. Sheybani

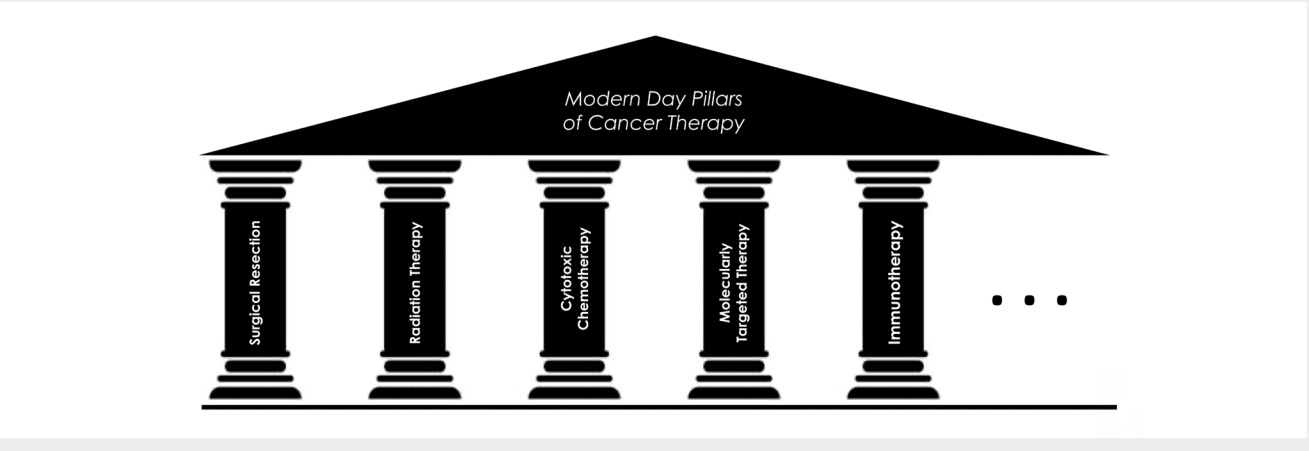
The Existential Threat of Cancer

In *The Emperor of All Maladies*, Siddhartha Mukherjee (2010) conveys the intimate and inextricable connection between cancer and the human body: "Cancer's life is a recapitulation of the body's life, its existence a pathological mirror of our own." Indeed, cancer, most simply defined as the uncontrolled growth and spread of abnormal cells in the body, represents a distorted reflection of our own biological processes. It is a formidable disease that touches every life, directly or indirectly, and the continued quest to conquer this common adversary binds us all in a profound way. Indeed, cancer affects millions of people each year, and according to the American Cancer Society, it is the leading cause of death in the United States for the population below age 85 (Siegel et al., 2024). Alarmingly, the incidence of several common cancers is rising worldwide, and, despite age historically being among the strongest risk factors for diagnosis, cancer patients are getting younger (Siegel et al., 2024). These trends underscore a dire need for safer, more effective cancer treatments. As this article highlights, sound waves are poised to play a transformative role in fulfilling this need.

Cancer Treatment 101

Before diving into the role of acoustics, however, it is necessary to introduce the current state of cancer therapy. Standard cancer therapies encompass a versatile array of treatments designed to target and eliminate cancer cells, ultimately aiming to cure the patient, prolong life, or reduce symptoms (**Figure 1**). The therapeutic mainstays for cancers vary across anatomical region, subtype, and stage but generally include surgery, chemotherapy, and radiation therapy. Surgery involves highly invasive physical removal of the tumor and, in some cases, surrounding tissue to prevent further cancer spread. Chemotherapy, discovered in the early 1940s, uses potent small-molecule drugs to destroy rapidly dividing cells, a downside being that this can also affect healthy cells and harbor significant side effects. Radiation therapy (i.e., radiotherapy) found its origins in the 1890s and employs high-energy ionizing radiation to target and

Figure 1. *Modern-day pillars of cancer treatment. These pillars include the mainstays of surgery, radiotherapy, chemotherapy, molecularly targeted therapy, and immunotherapy.*



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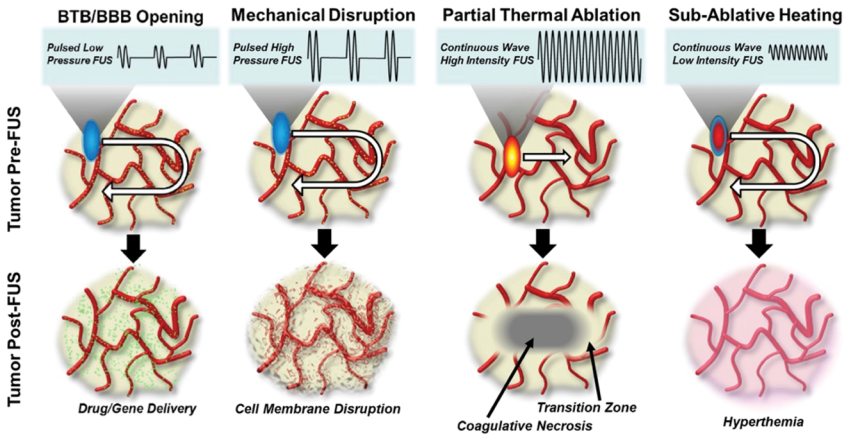
kill tumors by damaging the DNA of cancer cells but with off-target toxicity risks similar to chemotherapy (Sudhakar, 2009). Collectively, these treatment modalities have borne a profound impact on cancer outcomes. However, the safety risks and toxicities of these treatments remain a significant disadvantage for patients (Shanholtz, 2001). Although surmountable in some cases, these risks can fundamentally impact both short- and long-term quality of life, causing physical discomfort, emotional and psychological distress, and disruption of normal activities.

The late twentieth century has ushered in a transformative shift in cancer treatment with the introduction of molecularly targeted therapies and immunotherapies that are revolutionizing the way we combat malignancies with more specific targeting of cancer cells or by harnessing the eradicative power of the immune system. By honing in on specific molecular targets, these therapeutic categories offer radical potential for more precise and effective treatments with reduced damage to normal cells, leading to improved outcomes and fewer side effects for cancer patients (Min and Lee, 2022). Specifically with the advent of immunotherapies, a class of drugs designed to engage the body's own surveillance system, the immune system, to recognize and attack cancer cells (discussed in greater detail in **Cancer Immunotherapy: Promise and Problems**), there is newfound promise not just for combatting primary tumors, but also for elaborating immunological memory to drive effective responses against the chief cause of mortality in most advanced cancer settings, metastases (Davis, 2000). Despite the marked impact of these advancements on cancer care, challenges remain. The success of both molecularly targeted therapies and immunotherapies has been limited by such factors as tumor heterogeneity, physical barriers to delivery, complex mechanisms of tumor evolution, and off-target effects (Huang et al., 2014; Whiteside et al., 2016).

Despite innumerable advances in cancer care, a clear need remains for therapeutic options that balance the need for improved efficacy with prioritization of minimal risk to patients. If we peer into the "constellation" of cancer therapy options, a particular "star" shines bright as being a powerful asset to both conventional and novel cancer treatment paradigms due to its uniquely nontoxic and versatile nature, therapeutic ultrasound.

Figure 2. *Examples of thermal or mechanical therapeutic ultrasound regimens applied in tumors. Focused ultrasound (FUS) waves (**top, light blue:** representative sound wave patterns) can be tuned to ablative or subablative exposure levels to result in a broad range of bioeffects to tumor tissue (**tan; red:** blood vessels). These include (**left to right**) blood-brain/tumor-barrier (BBB/BTB) opening with microbubbles for drug or gene (**green dots**) delivery; mechanical disruption (i.e., mechanical ablation) resulting in cell membrane disruption and tissue fractionation; thermal ablation resulting in coagulative necrosis, i.e., "burning" of tissue (**gray oval**); and subablative heating resulting in hyperthermia, i.e., "warming" of tissue (**pink**). Sonications can be applied in a variety of patterns to achieve total or subtotal tumor coverage (**white arrows**). Not pictured are other known mechanisms of action such as radiosensitization and sonodynamic therapy. Adapted from Curley et al. (2017), copyright Ivyspring International Publisher; licensed under a Creative Commons Attribution-Noncommercial 4.0 International (CC BY NC 4.0) license (creativecommons.org/licenses/by-nc/4.0).*



The Multifaceted Marvel of Therapeutic Ultrasound

Not to be confused with ultrasound for biomedical imaging applications, therapeutic ultrasound (TUS) has emerged as a promising modality for transforming cancer treatment, offering a nonionizing, noninvasive, precisely targeted, and highly tunable approach for acoustic energy deposition that can be leveraged to destroy cancer cells, facilitate delivery of cancer therapeutics, and even modulate the tumor-immune landscape (**Figure 2**). Utilizing high-energy ultrasound waves, this technique generates thermal and/or mechanical bioeffects that can be targeted to tumor tissues with submillimeter precision while sparing intervening or surrounding healthy tissue. In addition to its precise and minimally toxic nature, perhaps one of the greatest strengths of TUS is its versatility, herein described.

Thermal Disruption of Tumors

TUS can yield controlled and localized heating that induces thermal ablation, where continuously applied acoustic energy is concentrated to confer rapid temperature elevations >55°C on average. This results in destructive "burning" of the targeted area, characterized by protein denaturation and cell death. Often, this ablated zone is surrounded by a transitional zone of irreversible heat-mediated cell damage, termed the "peri-ablative margin." By carefully controlling the duration, intensity, and location of the ultrasound energy, clinicians can achieve precise and customizable ablation zones while minimizing damage to surrounding healthy tissues. Real-time imaging guidance, often involving ultrasound or magnetic resonance imaging (MRI), ensures accurate targeting and monitoring of the ablation process. Continuous sound waves can also be tuned to lower exposure conditions to yield hyperthermic temperatures, typically between 40° and 45°C. This moderate "warming" can have several biological effects, including increasing blood flow, enhancing local drug delivery, and sensitizing tumor cells to radiation therapy and chemotherapy.

Mechanical Disruption of Tumors

TUS can also achieve tissue disruption through mechanical forces rather than thermal affects. At high amplitudes, the interaction of pulsed ultrasonic waves with endogenous microscopic gas bubbles in the targeted tissue microenvironment can result in localized acoustic cavitation activity through the oscillation and rapid collapse of bubbles, generating shock waves and microstreaming forces that exert mechanical stresses on the surrounding tissue (see Maxwell et al., 2012). The mechanical forces produced by cavitation can cause physical fragmentation of cells, tissues, and even subcellular organelles, yielding yet a different mechanism of tumor ablation.

At lower intensities, TUS can also be applied in tandem with the administration of exogenous cavitation nuclei such as nano- or microbubbles (e.g., Matula and Chen, 2013). The amplification of mechanical exposures in this case originates within the blood vessels and exerts forces on the surrounding tissue via induction of shear stresses, microjets, and localized pressure changes. The mechanical disruption brought about by micro- or nanobubble-assisted TUS can have various effects, including enhancing drug delivery through increased vascular permeability (e.g., transient opening of the blood-brain barrier; highlighted by Konofagou, 2017), promoting tissue permeabilization for targeted therapies and even facilitating tissue ablation.

Physical Sensitization of Tumors

Aside from classically thermal or mechanical perturbations, TUS offers numerous other mechanisms of action with an emerging promise for cancer therapy. Sonodynamic therapy (SDT) leverages small molecule agents (e.g., 5-aminolevulinic acid) that are preferentially taken up by metabolically active cancer cells. The activation of these agents by low-intensity ultrasound waves can induce tumor killing through oxidative damage and eventual cell death in targeted cancer cells, enabling a modality for tumor cell-specific killing with TUS.

Alternatively, efforts are underway to use TUS as a strategy for making tumors more sensitive to radiotherapy. Known as "radiosensitization," this involves leveraging the mechanical or thermal bioeffects of TUS to enhance susceptibility of cancer cells to killing via ionizing radiation energy. Through mechanisms that are still being uncovered, TUS can heighten the susceptibility of cancer cells to radiation-induced damage, thereby offering promise to improve "standard of care" outcomes or mitigate toxicities by enabling titration of radiation doses.

The versatile mechanisms of TUS have given way to numerous advancements in the treatment of cancer, including nonsurgical tumor debulking, cancer-associated pain palliation, and combinatorial treatment with radiotherapy, chemotherapies, molecularly targeted therapies,

and immunotherapies. However, to cover all these topics in meaningful depth would be infeasible within the scope of this article. The remainder of this article thus focuses on TUS for enabling the strategy that is submitted as having the most curative potential in the fight against cancer, *immunotherapy*.

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Cancer Immunotherapy: Promise and Problems

Heralded by *Science Magazine* as "Breakthrough of the Year" in 2013 and the topic of the 2018 Nobel Prize in Physiology or Medicine awarded to James Allison and Tasuku Honjo, cancer immunotherapy represents a revolutionary frontier in cancer treatment (Loontz, 2013). The origins of cancer immunotherapy can be traced much farther back than the early 2000s, however, specifically to around 1891 with the pioneering work of William Coley, often referred to as the "Father of Cancer Immunotherapy" (Dobosz and Dzieciątkowski, 2019).

Inspired by observations that some cancer patients who developed infections unexpectedly experienced tumor regression, Coley experimented with intentionally infecting patients with bacteria to stimulate an immune response against their tumors. He developed a mixture known as "Coley's toxins," which demonstrated some success in treating certain cancers.

Despite initial skepticism and subsequent decline in use of his methods with the advent of radiation and chemotherapy, Coley's work laid the foundation for modern cancer immunotherapy. The resurgence of the immunotherapy field in the late twentieth century has brought with it an accelerated pace of discovery yielding novel immune targets and the development of various immunotherapy drugs including antibodies, engineered immune cells, and cancer vaccines, all uniformly aimed at unleashing the immune response for precise recognition, targeting, and elimination of cancer cells throughout the body. With its many modes of action, immunotherapy holds great potential for achieving durable and long-lasting remissions, even cures, across various cancer types (Harris et al., 2016).

Although the impact of cancer immunotherapies has been transformative in select cancers, an unfortunate reality remains: they are ineffective against most tumors. Indeed, most solid cancers create a particularly daunting set of diverse and complex challenges, which still need to be addressed for the promise of immunotherapy to be fully realized. Recent statistics suggest only ~20-40% of patients, depending on solid cancer type, respond to immunotherapy drugs (Pilard et al., 2021). This excludes a significant proportion of patients and motivates the continued quest to extend the benefits of immunotherapy to the broader cancer patient population.

One of the primary hurdles to effective immunotherapy is the heterogeneity of tumors, where genetic and molecular differences within and between tumors can result in variable responses. Additionally, many tumors leverage sophisticated mechanisms to evade the immune system before, or even after, the ramping up of a response that can limit the effectiveness of immune-based treatments. The risk of immune-related adverse effects is another challenge, because activating the immune system can sometimes lead to unintended inflammation and damage to healthy tissues, known as immune-mediated toxicities. Moreover, identifying reliable biomarkers to monitor treatment progress or predict which patients will benefit from immunotherapy remains a critical need. Finally, manufacturing and delivering specific immunotherapeutic agents, such as engineered immune

cells, also present logistical and cost barriers due to their complexity. Overcoming these obstacles is paramount, requiring innovative strategies to enhance immune response specificity and durability as well as development of combination therapies that prioritize both efficacy and safety profiles (P. Sharma et al., 2017).

Potentiating Immunotherapies with Therapeutic Ultrasound

It has been widely posited that rational combinatorial strategies hold the key to advancing immunotherapy outcomes (Yap et al., 2021). TUS is remarkably well-suited to be a promising combinatorial strategy due to the numerous ways in which it can be leveraged to potentiate different immunotherapy classes. As discussed in this section, TUS is capable of evoking immunogenic signatures that intersect with the classical "cancer immunity cycle" (Sheybani and Price, 2019), which is a conceptual framework describing the steps required for the immune system to effectively recognize and eradicate cancer cells, fully explained in Chen and Mellman (2013). The earliest observations supporting the immunogenicity of TUS date back to the early 1990s when local hyperthermia or thermal ablation exposures unexpectedly mobilized host immune responses in preclinical or clinical cancer settings. Since then, we have also learned that TUS can augment immunotherapy delivery and even remotely control immune cells. Taken together, these capabilities hold profound implications for improving the effectiveness and precision of immunotherapy paradigms.

Inducing Immunogenic Cell Death

Many cells in our bodies are constantly dying and turning over. It would be a major problem if that natural homeostatic process elicited an immune response every time. As such, our bodies have evolved special mechanisms for alerting the immune system to cell death that warrants a response. We call this immunogenic cell death (ICD). ICD describes a specific mode of cell death that can be recognized by the immune system. ICD is essentially one of the first crucial steps in the cancer immunity cycle because it sets the stage for an effective immune response coordinated to recognize and attack cancer cells.

Unlike conventional cell death, which can occur without eliciting an immune response, ICD is characterized by the release or exposure of specific "danger signals" (otherwise known as alarmins) and the exposure of antigens (discussed in ***Priming Innate Immunity***) that enhance the visibility of dying cancer cells to the immune system (Hayashi et al., 2020). To this end, the concept of ICD is thus critical for cancer immunotherapy because it converts the tumor into its own vaccine, thereby enhancing the effectiveness of immune-based treatments. Some of the ablative modes of TUS, such as thermal and mechanical ablation, have been shown to result in the widespread translocation of key alarmins and heat shock proteins within and surrounding the ablation zone (Hu et al., 2005). Other approaches have also utilized nanoparticles decorated with alarmins (Sethuraman et al., 2020) or the help of agents like chemotherapy (Ya et al., 2023) to elaborate more robust ICD signatures in TUS applications.

Priming Innate Immunity

The "first responders" of the immune system are known as innate immune cells. The innate immune response plays a crucial role in the body's initial defense against cancer, serving as the first line of immune surveillance and attack. Central to this response are innate immune cells, known as dendritic cells (DCs), which are key antigen-presenting cells capable of bridging the innate and adaptive immune systems. On encountering cancer cells, DCs ingest tumor antigens (molecules produced by cancer cells that can be recognized by the immune system), and these antigens are then processed and presented on the surface of DCs in conjunction with major histocompatibility complex (MHC) molecules. The mature antigen-loaded DCs migrate to distal sites like the lymph nodes or spleen, where they cross-pollinate with naive T cells and present them with specific tumor antigens. This "priming" event activates T cells to become tumor specific, rendering them capable of recognizing and killing cancer cells bearing the specific antigens. Unfortunately, tumors have sophisticated ways of rendering innate immunity aberrant by limiting antigen availability or repertoire, downregulating MHC molecules, or conferring immunosuppressive signals that limit productive DC function. This is excitingly where TUS has again demonstrated promise for overcoming these critical barriers.

For example, thermal ablation has been demonstrated across numerous solid tumor settings to promote DC maturity and function by elevating DC representation in draining lymph nodes, upregulating MHC and activation molecules, and promoting intratumoral antigen cross-presentation (Chavez et al., 2018). Mechanical TUS regimens have displayed a similar capacity across

tumor settings within (Curley et al., 2020) and outside (Hendricks-Wenger et al., 2021) the brain. Recent work has even demonstrated that mechanical ablation shapes innate immune responses through marked improvement of tumor-associated antigen availability and promotion of antigen acquisition by conventional DCs (Thim et al., 2024). Several approaches have strategically coupled TUS with innate immune adjuvants such as CD40 agonists, toll-like receptor (TLR) agonists, and cancer vaccines to more robustly invigorate the early stages of the "cancer immunity cycle" (Singh et al., 2019).

Improving Immunotherapy Delivery and Persistence

The transport of systemically administered immunotherapies to and within solid tumors faces several significant barriers that directly limit therapeutic efficacy. One primary obstacle is the abnormal and often poorly organized blood vessel network within solid tumors, known as the blood-tumor barrier, which impairs effective drug delivery and distribution. In brain tumors, an additional layer of complexity is introduced by the protective blood-brain barrier (Arvanitis et al., 2019). Solid tumors also typically exhibit high interstitial fluid pressures and possess dense extracellular matrices that further hinder the penetration of therapeutic agents into the tumor core. Hypoxia and acidic conditions within the tumor can also negatively affect immune cell function and survival. TUS has been variably utilized as a strategy to overcome these barriers.

For example, thermal ablation has been demonstrated to reduce interstitial fluid pressure and improve macromolecule penetrance into tumor tissues (Sassaroli and O'Neill, 2014). Mechanical perturbations, with or without microbubbles, have been exploited for breaking down dense extracellular matrix, as in the particularly challenging setting of pancreatic cancer (Maloney et al., 2017). Meanwhile, both thermal and nonthermal bioeffects of TUS have been shown to increase tissue oxygenation, holding critical implications for the treatment of hypoxic tumors (D. Sharma et al., 2022). A known effect of microbubble-assisted TUS is transiently overcoming the blood-brain and/or blood-tumor barriers, which has given way to a rich tapestry of studies demonstrating improved delivery of immunomodulatory chemotherapies (Arrieta et al., 2024), immune checkpoint inhibitors (Lee et al., 2023), adoptive cellular therapies (Sabbagh et al., 2021), gene therapies (Ilovitsh et al., 2020), and more. Early-phase clinical trials have also demonstrated the capacity of TUS-mediated blood-brain/-tumor barrier opening to augment delivery of adjuvant chemotherapies and immunotherapies into brain tumors and even improve the local persistence of the latter (Meng et al., 2021).

Chasing the Abscopal Effect

In cancer treatment, there is a phenomenon known as the abscopal effect (from the Latin words "ab," meaning "away from," and "scopos," meaning "target"). This describes a situation where localized therapy, such as TUS, leads to the regression of cancerous lesions at sites distant from the primary treatment area; put another way, the abscopal effect refers to the systemic impact of a localized intervention. To this end, the abscopal effect is widely regarded as the "holy grail" for locoregional therapies, and TUS is no exception in this regard.

The abscopal effect has been reported sporadically in clinical cases implementing TUS for ablation, and research is ongoing to better understand the mechanisms behind abscopal responses and how they can be reliably induced for more comprehensive metastatic disease control and improved patient outcomes. Combining localized treatments like TUS with systemic immunotherapies is proving to be a promising strategy for elaborating abscopal effects.

Numerous preclinical studies deploying thermal or mechanical TUS have reported elaboration of productive T cell responses, and even abscopal responses, across varying immunophenotypes including melanoma, breast cancer, and other settings (van den Bijgaart et al., 2017). Promisingly, anecdotal observations of the abscopal effect have been made in the clinic, as in the example of a recent hallmark case report that reported abscopal control following mechanical ablation of liver cancer (**Figure 3**) (Vidal-Jove et al., 2021). The number of these observations is continuing to grow as global clinical adoption of TUS technology expands.

Sonogenetics

When peering into the future of TUS and cancer therapy, a new area on the horizon, sonogenetics, can be appreciated. Sonogenetics is an emerging field that involves the use of TUS to modulate cellular function and activity through the introduction of genetically encoded ultrasound-sensitive proteins. This technique holds significant potential for cancer immunotherapy by providing a noninvasive and highly precise method to control and enhance the action of

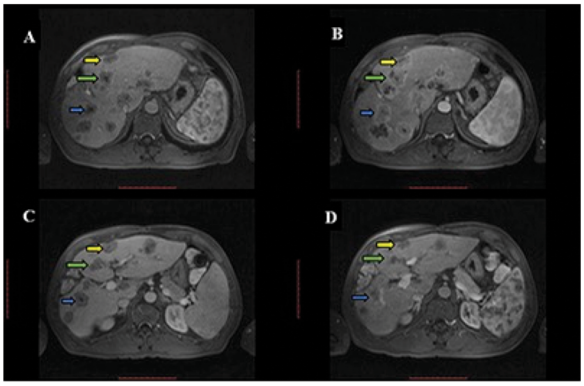


Figure 3. *Representative magnetic resonance imaging (MRI) images illustrating abscopal effect following mechanical ablation in liver cancer. Images depict one week prior to treatment (**A**), one day posttreatment (**B**), one week posttreatment (**C**), and eight weeks posttreatment (**D**). **Colored arrows:** three different untreated liver tumors tracked over time, displaying apparent volume shrinkage after mechanical ablation of a separate mass. Originally adapted from Vidal-Jove et al. (2021), with permission of IEEE; reproduced from shorturl.at/KL5Uw.*

immune cells against tumors. The basic concept of sonogenetics involves genetically engineering immune cells, such as T cells or dendritic cells, to express ultrasound-sensitive ion channels or mechanoreceptors that enable direct response to an acoustic stimulus. A compelling example of this was the recent engineering of "remotely controllable" chimeric antigen receptor (CAR) T cells, wherein CAR domains could be switched on or off via ultrasound exposure, thereby offering a novel strategy for localized T cell engagement and cytotoxicity as well as mitigation of on-target, off-tumor toxicities (**Figure 4**) (Y. Wu et al., 2021). The convergent innovations in TUS and synthetic biology that are giving way to sonogenetics offer the potential to enhance the specificity, efficacy, and safety of immunotherapies, paving the way for more effective treatments for various cancer types.

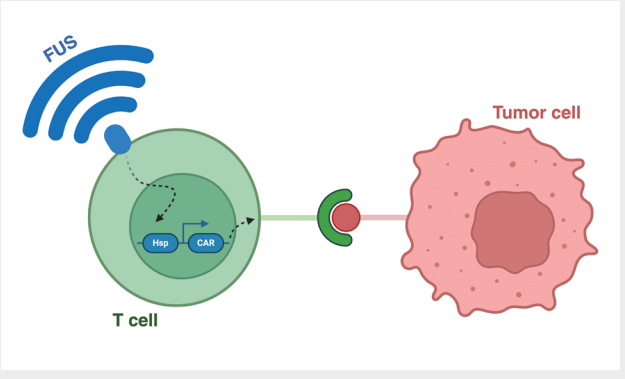
Clinical Advancement

TUS has been steadily advancing over decades as a clinical modality for oncology applications, with some of the earliest clinical observations of immunomodulation dating back to the mid-1990s and early 2000s (Rosberger et al., 1994; F. Wu et al., 2004). Despite the disruptive intersection with immuno-oncology having come online in just the last several years, clinical investigations have accelerated at an unprecedented pace. The "first-in-human" trial to combine TUS with immunotherapy (now completed) was launched at the University of Virginia, Charlottesville, in 2017; this trial assessed the combination of thermal ablation and pembrolizumab (Keytruda) in metastatic breast cancer patients. Presently, multiple clinical trials across the globe are taking aim at solid cancers, including breast, brain, skin, pancreatic, prostate, and others, to assess the safety, feasibility, and preliminary efficacy of combining TUS with immunotherapies. Furthermore, numerous ongoing early-phase clinical trials have demonstrated a commitment to further knowledge by integrating immunological assessments into primary or exploratory endpoints. Importantly, this has given rise to timely consensus dialogues addressing the critical need for field-wide standardization of immunology analyses (Padilla et al., 2023). Given the accelerated pace of advancement in this field, the coming years are certain to see clinical evidence build rapidly and the portfolio of novel immunotherapy combinations with TUS continue to grow.

Conclusions and Future Directions

A twisted echo of our cellular functions gone awry, cancer is a stark and persistent reminder of our shared vulnerability and mortality as human beings. There is a growing recognition of the need for precision and individualization in the management of this formidable disease.

Figure 4. *Mechanism of action for sonogenetically controllable chimeric antigen receptor (CAR) T cells. The T cell (**green**) is engineered with a genetic circuit linking expression of the T cell receptor (CAR) to heat shock protein (HSP), which can be induced through remote heating of the tumor via ultrasound (FUS). With FUS exposure, the CAR is expressed on the surface of the T cell, enabling tumor antigen recognition and targeted tumor cell (**pink**) killing. Adapted from Y. Wu et al. (2021), with permission of Springer Nature.*



Fortunately, the promise of delivering on this need has never been greater than it is today. TUS is positioned to play an exciting role therein, as a readily accessible, non-invasive, non-ionizing focal therapy modality and the only of its kind to converge tunability, spatial precision, and marked versatility in the treatment of cancer. In an emerging scientific era where physics is being used to control and manipulate biology, we are rapidly appreciating the depths of TUS' capabilities in allyship to immunotherapies. While this is already transforming hope for the more effective, if not someday curative, treatment of primary and disseminated cancers (in particular, anatomically challenging or advanced metastatic tumors), the hard work is not yet complete.

More studies are needed to optimize treatment protocols and advance control and prediction of the many mechanisms of action elaborated by TUS. Furthermore, systematic investigations are needed to determine the favorability of thermal versus mechanical immunostimulation across cancer immunotherapy applications. Finally, better response metrics will be needed to enable rational decision-making for combinations and improved real-time adaptation of treatment paradigms where needed. That said, the momentum in this field is nothing short of inspiring, and the next decade will surely deliver on these needs with the advent of new TUS technologies and discoveries. TUS stands at the cutting edge of next-generation cancer therapies, with tremendous promise for improving patient outcomes and markedly expanding the horizons of cancer immunotherapy.

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About the Author

Natasha D. Sheybani
nds3sa@virginia.edu
Department of
Biomedical Engineering
University of Virginia
415 Lane Road
Charlottesville, Virginia 22908, USA

Natasha D. Sheybani is an assistant professor of biomedical engineering and research director of the Focused Ultrasound Cancer Immunotherapy Center at the University of Virginia (UVA), Charlottesville, where she directs a laboratory focused on developing next-generation cancer management strategies with therapeutic ultrasound. She received her BS from Virginia Commonwealth University, Richmond, and her PhD from UVA, followed by a postdoctoral fellowship at Stanford University, Stanford, California. She has received awards from the National Institutes of Health Director's Office, National Cancer Institute, and National Science Foundation. Her research and translational efforts have been recognized by STAT News (see bit.ly/3y2cNvW) and Forbes Magazine's "30 Under 30" List in Science (see bit.ly/3LvkIVL).