Summary & conclusions

Chapter 1 outlines cancer as a complex disease characterized by uncontrolled cell growth, tumor formation, and metastasis. Key hallmarks include evading cell death and immune suppression, driven by genetic mutations and interactions with the TME. While conventional treatments like surgery, chemotherapy, and radiation remain essential, they face challenges such as tumor heterogeneity and resistance. As a result, there is growing interest in personalized treatments and combination therapies. Cancer immunotherapy, ever since William Coley's early work to today's breakthroughs like immune checkpoint inhibitors, CAR-T cell therapy, and cancer vaccines, have revolutionized modern oncology. Despite issues like resistance and toxicity, innovations in immunotherapy and combinatorial strategies hold promise for better therapeutic outcomes. Additionally, therapeutic ultrasound has shown diverse potential in a variety of medical applications. This dissertation focuses on the application of focused ultrasound in oncology, specifically softer mechanical therapies, and its implications in immune modulating effects, such as inducing ICD.

The concept of ICD and its pivotal role in shaping future immunotherapeutic strategies are discussed in **chapter 2**. The chapter delves into how various chemical and physical therapies, such as anthracyclines and HIFU, can induce ICD, and how these therapies might fully exploit the biological effects they trigger in the context of immunotherapy. With the fast evolving insights in cell death process, it is evident that ICD will be integral to the development of novel or repurposed cancer therapies. This includes enhancing immune system activation and improving therapeutic efficacy through combination approaches, making ICD a cornerstone of future cancer treatment strategies. This chapter also explores the immune-modulating effects of various physical therapies, including cryoablation, hyperthermia, and ultrasound. Although these therapies were originally developed in oncology with the primary aim of achieving tumor ablation and destruction, it has since become clear that they also induce ICD and modulate immune responses. Ongoing research continues to investigate how these therapies can be optimized to harness their immune-modulating properties, showing promising potential for enhancing therapeutic outcomes.

Chapter 3 explores the design and validation of a custom-built setup, demonstrating that reproducible *in vitro* HIFU treatments can be achieved. The study confirmes the efficacy of the acoustic system in delivering controlled HIFU exposure to cancer cell monolayers, while simultaneously monitoring and quantifying cavitation events through PCD. The cavitation detection technique shows promise for future translation of this ultrasound setup from in vitro to in vivo or clinical settings. In addition to efficiently destroying and ablating cancer cells, HIFU treatment induced the expression of several key

hallmarks of ICD within the remaining intact cell population. Notable markers such as ATP release and CALR exposure were observed, indicating the initiation of an immune-modulatory response. Furthermore, a significant release of tumor-specific antigens was detected in the SN following distinct HIFU treatments, including gp100 for B16F10 melanoma cells and gp70 for CT26 colon carcinoma cells. These findings highlight the dual impact of HIFU, both in direct tumor cell ablation and in promoting immune activation through antigen release. Overall, the development of the HIFU setup demonstrated promising outcomes, not only in effective treatment delivery but also in the real-time monitoring of the treatment.

Chapter 4 evaluates the potential of HIFU-treated cancer cells to function as a vaccine by assessing their ability to induce protective immune responses. This approach was tested using an abscopal model, yielding promising results in terms of tumor growth inhibition and survival rates, particularly within the CT26 model. Notably, significant protective immunity against tumor growth was observed, leading to markedly increased survival rates in CT26 mice, although similar effects were not seen in the B16F10 model. The chapter also explores the therapeutic immune responses elicited by the HIFU vaccine in both tumor-bearing and healthy mice, focusing on the specific immune cell populations involved in the response. These findings, despite being varying, offer valuable insights into the broader potential of combining HIFU with immunotherapy to enhance anti-tumor immunity. Despite the limitations of the HIFU-treated cancer cell vaccine—such as potential loss of antigenicity and adjuvanticity, which may inhibit the full exploitation of ICD—the significant immune responses observed are promising. When HIFU is delivered directly to the tumor in vivo, we can anticipate even more beneficial outcomes, including enhanced and improved immune responses. This chapter underscores the potential of HIFU-treated cancer cells as a viable vaccine strategy and highlights the importance of further research to optimize this approach in the context of cancer immunotherapy.

Chapter 5 explores the future of HIFU in cancer treatment, emphasizing advancements in imaging and monitoring technologies to enhance accuracy and safety. Integrating modalities such as MRI, ultrasound, and CT is crucial for effective treatment planning and real-time monitoring. Despite progress, challenges remain in precise tumor targeting and avoiding damage to healthy tissues. Future developments in adaptive therapies, supported by AI and machine learning, aim to optimize treatment parameters in real time. Emerging technologies like nanoparticles and microbubbles enhance ultrasound energy absorption, while HIFU's evolving role in inducing ICD highlights its potential as a complementary approach to immunotherapy. The chapter emphasizes the necessity for personalized cancer treatments due to tumor heterogeneity, which affects patient outcomes. It discusses the limitations of traditional therapies that do not consider individual tumor profiles, as seen in models like B16F10 and CT26. Advances in genomics, proteomics, and bioinformatics are transforming

treatment through molecular profiling, enabling tailored therapies that improve efficacy and reduce toxicity. Additionally, the chapter explores combinatorial therapeutic strategies, emphasizing that standalone therapies often fail in advanced cancers due to resistance and recurrence. By targeting multiple pathways simultaneously, combinatorial therapies can reduce treatment resistance and enhance immune recognition. This multi-pronged, personalized approach positions combinatorial strategies as essential for future cancer treatment paradigms