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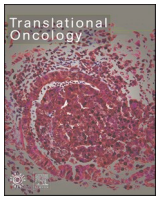

Review Article

Contribution of immune cells in synergistic anti-tumor effect of ablation and immunotherapy

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ABSTRACT

Thermal ablation results in the damage of tumor tissue, which leads to localized necrosis and incites a significant inflammatory response, accompanied by the infiltration of numerous immune cells. Nevertheless, depending solely on the singular approach of thermal ablation frequently is difficult in eliciting a robust anti-tumor response. Research suggests that integrating immune modulators into conventional ablation techniques has the potential to enhance the elicited immune response, finally initiating synergistic effect without significantly elevated risk profiles. This article comprehensively analyses the immunological effects resulting from post-ablation alone and its synergy with immunotherapies, and accentuates the heterogeneous alterations noted in immune cells across distinct malignancies. Collectively, the article delves into the theoretical framework and advancements in clinical trials concerning the combined thermal ablation and immunotherapy for treating malignant tumors.

1. Introduction

Thermal or energy-based tumor ablation involves the localized application of extreme temperatures, either high or low, to induce irreversible cellular injury, ultimately resulting in apoptosis and coagulative necrosis of tumor cells. The method of tumor ablation includes radiofrequency ablation (RFA) and microwave ablation (MWA), cryoablation, irreversible electroporation, as well as less studied high-intensity focused ultrasound and laser ablation. Ablation therapy serves not only physical treatment but also bears relevance to immunotherapy. During the ablation process, intracellular components and tumor-associated antigens were released, which can be recognized and attacked by immune cells, thus triggering an immune response. Following ablation, the levels of cytokines such as Interleukin-6 (IL-6) and Interleukin-10 (IL-10) may be increased [1], thereby influencing the activation and regulation of immune cells. Additionally, ablative therapy may trigger the immune system to develop a memory of tumor antigens, which prevents tumor recurrence and metastasis.

However, the specific mechanisms underlying the immunomodulatory effects of ablation remain unclear. Relying solely on the immune response initiated by ablation proves inadequate for achieving complete tumor eradication. Common complications arising from incomplete ablation encompass residual local tumors and marginal recurrences, which substantially impede clinical outcomes and prognosis [2]. Both preclinical and clinical investigations have provided evidence suggesting that the combination of ablation with suitable immunotherapy regimens can elicit potent systemic anti-tumor immune responses [3]. Therefore, this article aims to provide a comprehensive summary of recent advancements in immune cell dynamics within the tumor microenvironment following ablation treatments, whether administered in isolation or in conjunction with immunotherapy. Additionally, it seeks to undertake an exhaustive examination of the potential mechanisms underpinning ablation procedures, with the overarching goal of offering enhanced and more efficacious clinical treatment strategies. Fig. 1 illustrates the crucial immune cell response in thermal ablation alone and combination therapy. Table 1 and Table 2 summaries T cell and other immune cells response of current available clinical studies on ablation alone or combined with immunotherapies, respectively.

2. T cells

2.1. Peripheral blood

Ablative therapy not only destroys tumors in situ to create an

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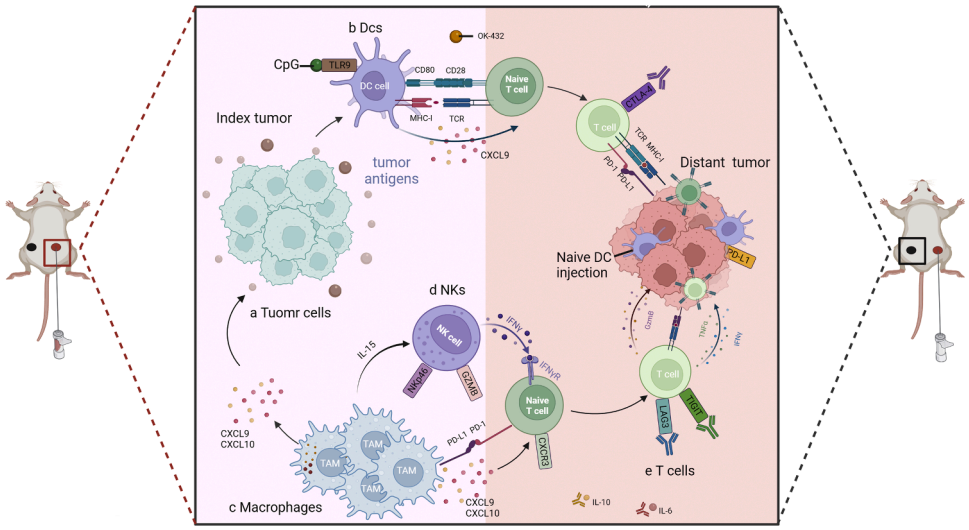

Fig. 1. The immune system is activated by thermal ablation. a, Thermal ablation can destroy local tumor tissue, thus leading to the release of tumor antigens. b, Thermal ablation induces co-stimulator and CXCL9 expression in DCs, thereby potentially promoting the activation and proliferation of T cells. c, Thermal ablation upregulates the expression of CXCL9 and CXCL10 in tumor-associated macrophages, as well as the expression of their receptor CXCR3 on T cells. d, The macrophages activated by MWA generate IL-15, which activates NK cells to suppress the occurrence of metastasis. e, Activation of tumor specific T cell by thermal ablation.

Table 1
Summary of T cell immune response on ablation alone and combining ablation with immunotherapies.

Disease	Technique	Immunotherapy Agent	Immune Response	Reference
NSCLC	RFA	/	↑CD4+T cells, ↑CD8+T cells, ↑pro-inflammatory cytokines	12
NSCLC	MWA	AXL-targeted CAR T-cells	↑central memory T cells, ↓Tregs	14
NSCLC	RFA	Toll-like receptor 9 (TLR9) agonists	↑CTLs	15
HCC	RFA	PD-1 blockade	↑OS and RFS	17
HCC	MWA	/	↑CD3+T cells, no significant correlation with CD8+ cells and Tregs	18
Melanoma, liver metastases	Cryoablation	Pembrolizumab	↑serum IL-6, ↑NK cells, ↓Tregs	21
Recurrent HCC	RFA	/	↑METTL1, ↑ CD11b+CD15+PMN-MDSCs, ↓CD8+T cells	22
HCC	MWA	GM-CSF/anti-CTLA-4	↑CD8+T cells, ↑IFN-γ, ↑Granzyme B, ↓ immunosuppressive cells	25
HCC	MWA	DC, CTL, and CIK	improved immune suppression	28
Breast cancer	RFA	/	↓Tregs	31
Breast cancer	MWA	/	↑ICOS+CD4+T cells, ↑CD8+T cells, ↑IFN-γ	32
Breast cancer	MWA	/	↑NK cells, ↑CD8+T cells, ↑ the interactions among CD4+T cells	33
Breast cancer	MWA	OK-432	boosted peripheral T cell responses	34
Breast cancer	Cryoablation	anti-CTLA-4 antibody (ipilimumab)	activated proliferating peripheral T cells	35
CRC, live metastases	RFA	PD-1 blockade	↑CD8+T cells, ↑IFN-γ and TNF-α, amplified the ratio of tumor-infiltrating effector T cells to Tregs	31
CRC	MWA	PD-1 blockade	↓IFN-γ+CD8+T cell numbers were reduced in CXCL10 knockout mice	36

RFA: radiofrequency ablation; MWA: microwave ablation; HCC: hepatocellular carcinoma; CRC: Colorectal cancer; NSCLC: non-small cell lung cancer; MDSC: myeloid-derived suppressor cell; CTL: cytotoxic T lymphocyte; NK: Natural Killer.

Table 2
Summary of other cells immune response on ablation alone and combining ablation with immunotherapies.

Disease	Technique	Immunotherapy Agent	Immune Response	Reference
CRC	MWA	TIGIT/LAG3 blockade	↑CD8+T cells, ↑CXCL9 and CXCL10 in TAMs	37,38
CRC	iRFA	/	↑CCL2, ↑TNF-α	40
HCC	RFA	/	↓CD169+macrophages, ↓MDSCs/Tregs	41
Melanoma	cryoablation	CpGB treatment	↑the expression of CD80 and MHC I and II on DCs	43
HCC	cryoablation	PD-L1 blockade	↑the secretion of CXCL9 from cDC1 cells, ↑CD8+T cells, ↑NK cells, ↓PD-L1highCD11b+ MDSC	44
HCC	RFA	OK432-stimulated dendritic cell	↑TAA-related T cell responses	45
HCC	MWA	Dendritic cell-derived exosomes (Dex) or DCs	↑CD8+T cells, ↓Tregs	48
Breast cancer	Cryoablation	NK cells	↑effector T cells, ↑ Th1-type cytokines	56
Breast cancer	MWA	/	↑IL-15 from macrophages, ↑ NK cells	58

RFA: radiofrequency ablation; MWA: microwave ablation; HCC: hepatocellular carcinoma; CRC: Colorectal cancer; MDSC: myeloid-derived suppressor cell; TAM: Tumor-Associated Antigens; DC: dendritic cell; NK: Natural Killer.

antigenic environment necessary for anti-tumor immunity but also induces the production of cytokines, thereby altering both local and systemic immune environment. Serum levels of IL-6 and IL-10 are shown to be elevated after RFA [4]. A modest increase in IL-6 levels can facilitate immune cell activation and contribute to tumor control. However, excessive elevation of IL-6 can induce inflammation and foster treatment resistance. IL-10, on the other hand, is an immunosuppressive cytokine known to promote Treg differentiation [5]. Furthermore, IL-10 can indirectly inhibit CTL activation by diminishing the production of IL-2 and IFN-γ from Th1 cells. However, Wu et al indicate a significant increase in the levels of peripheral blood cytokines such as IFN-γ, IL-2, and IL-12 in patients post-ablation, while the levels of anti-inflammatory cytokines IL-4 and IL-10 were notably decreased [6]. Furthermore, sustained increase in IL-6 and IL-10 during Pembrolizumab therapy could be a negative biomarker of prognosis in BRAF wild type metastatic melanoma patients [7]. Together, these findings collectively indicate that thermal ablation has the potential to enhance tumor-specific T cell responses and modify the cytokine secretion profile.

2.2. Lung cancer

Non-small cell lung cancer (NSCLC), a prevalent type with a better 5-year survival rate than other lung cancers, often presents at advanced stages [8]. Identified gene variations, both germline and somatic, link some common lung cancer-related mutations to the epidermal growth factor receptor gene (EGFR). Blocking EGFR involves anti-EGFR monoclonal antibodies or small molecule tyrosine kinase inhibitors (TKIs). TKI-based therapy, including gefitinib and erlotinib, has notably enhanced survival and quality of life for NSCLC patients, particularly those with EGFR driver mutations. Despite these advancements, the majority still face an unfavorable prognosis due to intrinsic or acquired TKI resistance, often tied to secondary EGFR mutations or inherited EGFR single-nucleotide polymorphisms (SNPs) [9,10]. Furthermore, common TKI side effects like rash and diarrhea might correlate with EGFR gene polymorphisms [11]. For EGFR-negative NSCLC patients or previous TKI blockers, it remains a valuable area for further investigation.

RFA therapy is commonly used for both non-small cell lung cancer (NSCLC) and locally advanced lung cancer. The necrotic tumor tissue resulting from RFA can act as an in-situ source of antigens, which triggers in-situ immunity and induces the upregulation of dendritic cells to mediate sustained activation of T cells. Schneider et al. investigated the area that was ablated in patients with NSCLC, revealing an increase in CD4+ and CD8+ lymphocytes in the outer region, along with a heightened presence of pro-inflammatory cytokines [12]. Furthermore, the combination of MWA and Camrelizumab improved the objective response rate to 29.9% for advanced NSCLC patients [13]. In the context of combination therapy, pre-clinical studies have demonstrated anti-tumor activity of AXL-targeted CAR T-cells in combination with tumor-targeted MWA. Specifically, the activation, invasion, persistence, and tumor-suppressive characteristics of AXL-CAR-T cells in AXL-positive NSCLC xenografts are enhanced through TME remodeling by MWA. Simultaneously, MWA combined with AXL-targeted CAR T-cells triggers the activation and functionality of central memory T cells, while reducing the proportion of Tregs [14]. Furthermore, the combination of Toll-like receptor 9 (TLR9) agonists with RFA not only controls the primary lesion but also enhances the ability of CTLs to eliminate tumor cells, thereby further fortifying the potent inhibition of primary tumor growth and lung metastasis [15]. In summary, whether ablation alone or in conjunction with other immune therapy can promote the number and function of T cells, thus offering novel therapeutic strategies and directions in lung cancer.

2.3. Hepatocellular carcinoma

In recent years, RFA and MWA has been extensively used in the management of unresectable hepatocellular carcinoma (HCC) and recurrent HCC [16]. The enhanced efficacy of anti-PD-1 combined with RFA was confirmed, where patients who underwent combined therapy demonstrated a longer OS and a higher recurrence-free survival (RFS) compared to RFA alone [17]. To investigate the impact of MWA on tumor-infiltrating lymphocytes in liver cancer patients, Katharina et al. conducted an analysis of peripheral blood mononuclear cells in patients who experienced early recurrence and long-term remission after MWA treatment [18]. Following MWA, a high density of CD3+T cells within the lesion was linked to a favorable prognosis, while there was no significant correlation with CD8+ cells. On the other hand, individuals undergoing prolonged remission exhibited a greater ratio of circulating effector memory T cells. Conversely, there is no disparity in Tregs post MWA, as corroborated by preceding investigations [19]. Notably, further experimental results demonstrate that effective inhibition of c-Met can suppress the growth stimulation of distant tumors caused by RFA, providing a potential strategy for the comprehensive treatment of liver cancer [20]. In a separate pilot study, 15 patients diagnosed with liver metastases from melanoma underwent cryoablation and pembrolizumab treatment. The study observed consistently elevated serum IL-6 levels immediately following cryoablation [21]. Three weeks after the treatment, a noteworthy rise in Natural Killer (NK) cells and a decline in Tregs were observed. Additionally, both immunohistochemistry and multiplex immunofluorescence staining demonstrated a significant upregulation of METTL1 in recurrent HCC following RFA [22]. This response was accompanied by increased CD11b+CD15+polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) and decreased CD8+T cells. Mechanistically, the heat-mediated upregulation of METTL1 results in increased translation of TGF-β2, which, in turn, creates an immunosuppressive microenvironment. Various interventions, including the blockade of the Mettl1-TGF-β2-PMN-MDSC axis using anti-Ly6G antibody, genetic knockdown of intrinsic Mettl1 or Tgfb2 in liver cancer, or inhibition of the TGF-β signaling pathway, significantly attenuated tumor progression and restored CD8+T cell populations.

A series of studies have investigated the potential synergistic effects of thermal ablation combined with immunotherapy. Firstly, there was a notable increase in the proportion of CD4+ and CD8+T cells between combined therapy and MWA or anti-PD-1/CTLA-4 blockade alone [23,24]. Moreover, the Th1/Th2 cell ratio in the combination treatment group was significantly higher than other groups, suggesting that the prevalence of Th1-type immune response may contribute to the tumor suppression. Additionally, the MWA/GM-CSF/anti-CTLA-4 triple combination therapy promoted the infiltration of CD8+T cells into the tumor tissue, enhanced the production of anti-tumor effector factors such as IFN-γ and Granzyme B, and reduced the number of immunosuppressive cells within the tumor microenvironment [25]. Moreover, the combined application of active CCL3/MIP-1α with RFA also markedly enhanced the anti-tumor effect, leading to reduced tumor volume and prolonged survival of mice, thereby bolstering the anti-tumor immune response [26]. Thus, the administration of intra-tumoral vaccines through specific stimulation of GM-CSF/CCL3/MIP-1α combined with local tumor ablation may represent a pivotal component of future multimodal anti-tumor strategies. In the context of neoadjuvant treatment for liver cancer, pre-treatment with G47Δ virus before radiofrequency ablation leads to increased infiltration of CD8+T cells within the tumor microenvironment [27]. Moreover, the sequential administration of G47Δ, RFA, and immune checkpoint inhibitors (ICIs) exhibits a synergistic effect, further enhancing the overall anti-tumor efficacy.

Available data strongly support the potential benefits of Cytokine-induced killer (CIK) cell therapy in conjunction with other immunomodulatory approaches for cancer treatment, with clinical studies primarily concentrating on its implications in the treatment of HCC. The research conducted in ten individuals diagnosed with HCC has examined the effects of MWA combined with adoptive immunotherapy. This approach involved the infusion of DC, CTL, and CIK. The primary

objective of this phase I study was to assess the safety of this sequential treatment combination. Subsequently, peripheral blood analyses indicated promising indications of improved immune suppression [28]. In addition, in a recent single-arm prospective study, 7 patients diagnosed with HCC underwent RFA followed by autologous T lymphocytes stimulation. This stimulation involved a combination of immobilized RetroNectin and anti-CD3 monoclonal antibody, a method believed to induce more significant T cell expansion than other established approaches [29]. The study not only affirmed the safety of this treatment strategy but also showed enhancements in circulating lymphocyte profiles. Taken together, thermal ablation alone or combined with immunotherapies could promote the infiltration of CD8+T cells into the tumor tissue and enhance the production of anti-tumor effector factors to prevent hepatocellular carcinoma growth.

2.4. Breast cancer

In early-stage breast cancer, the majority of cases are successfully treated through a combination of primary tumor resection and systemic therapy. However, some patients still experience recurrence, which could be linked to circulating tumor cells and the immunosuppressive microenvironment [30]. Furthermore, an upregulation in the expression of heat shock protein 70 and heightened caspase-3 enzyme activity are observed, accompanied by a significant reduction in the circulating population of Tregs following RFA [31]. In the investigation of blood samples extracted from individuals diagnosed with early-stage breast cancer, it was observed that MWA led to a notable elevation in the levels of CD4+T cells and serum IFN-γ, instead of causing an increase in CD8+T cells. Moreover, the proportion of ICOS+ activated CD4+T cells was significantly elevated. Collectively, the finding indicates MWA induces a Th1-type immune response in early-stage breast cancer patients by activating the ICOS pathway [32].

To investigate cell interactions after ablation, Zhou et al. characterized the peripheral immune response of 6 patients with early-stage breast cancer induced by MWA via using scRNA-seq [33]. The data indicate systemic NK cells and CD8+T cells were activated following MWA, resulting in heightened cytotoxic and chemokine activities. After MWA, there was an augmentation in the interactions among CD4+T cells, including interactions with B cells and other CD4+T cells. Furthermore, activated dendritic cells displayed enhanced interactions with CD4+T cells in peripheral blood. Additionally, there was a notable expansion of T-cell clones along with an increase in T-cell receptor diversities, indicating MWA-induced antigen release. In addition to ablation alone, the combination of OK-432 and MWA not only boosted peripheral T cell responses but also synergistically induced systemic tumor-specific immunity, potentially resulting in tumor rejection during rechallenge experiments [34]. Moreover, a preliminary study evaluating the viability of neoadjuvant therapy using the anti-CTLA-4 antibody (ipilimumab) in conjunction with cryoablation for operable breast cancer patients indicated the tolerability of the combined treatment without postponing surgical intervention. Furthermore, the study revealed elevated levels of activated proliferating peripheral T cells following the combination therapy in comparison to either treatment administered individually [35,36].Taken together, these clinical data inspire us that the elevated temperature may reorganize tumor immune microenvironment.

2.5. Colorectal cancer

Local RFA resulted in an increase in T cell infiltration and PD-L1 expression in distant tumors of both synchronous colorectal liver metastasis patients and tumor-bearing mice [37]. Initially, the ratio of CD8+ tumor-infiltrating lymphocytes (TILs) producing IFN-γ and TNF-α showed an early increase but gradually decreased over time. However, the combination of local RFA with anti-PD-1 antibody significantly augmented tumor antigen-specific T cell responses and amplified the ratio of tumor-infiltrating effector T cells to Tregs, resulting in a cooperative suppression of distant tumor growth. Furthermore, there was an increase in the expression of TIGIT after MWA in MC38 mouse model [38]. Adding TIGIT blockade into MWA can upregulate the expression of CXCL9 and CXCL10 in tumor-associated macrophages (TAMs), as well as the expression of their receptor CXCR3 on T cells, which inhibiting tumor growth and enhancing anti-tumor immunity [38]. Furthermore, MWA combined with LAG3 blockade alleviates CD8+T cell immunosuppression [39]. When MWA combined with anti-PD-1 therapy, CD8+tumor-infiltrating lymphocytes (TILs) and IFN-γ+CD8+T cell numbers were reduced in CXCL10 knockout mice compared to wild-type mice, which suggests that CXCL10 regulates the synergistic effects of thermal ablation and anti-PD-1 therapy [40].

3. Macrophages

Macrophages play a crucial role in ablative therapy due to their inflammation-regulating abilities, which can significantly impact the therapeutic efficacy and prognosis of tumors. Furthermore, macrophages interact with other immune cells, such as T cells and natural killer cells, thereby modulating immune reactions and anti-tumor immune responses. Consequently, conducting comprehensive research on the interplay between macrophages and ablative therapy holds the potential to further optimize strategies for treating tumors.

The proportion of CD169+ macrophages in the local liver was reduced due to RFA, which was accompanied by a decrease in the expression of immune inhibitory molecules, including Tim-3 and PD-L1 [41]. Additional investigation utilizing RNA-sequencing and flow cytometry revealed that the liver's CD169+ macrophages played a crucial part in reconstructing the tumor microenvironment. These cells recruited CD8+ T cells and NK cells while inhibiting the accumulation of MDSCs and Tregs. Additionally, iRFA of residual tumors results in accelerated progression due to macrophage infiltration, inducing resistance to anti-PD-1 treatment [42]. Tumor cells secrete increased levels of CCL2 following iRFA, which subsequently promotes the activation and aggregation of macrophages. The activated macrophages, in turn, stimulate the production of more CCL2 from residual tumor cells by releasing tumor necrosis factor-alpha (TNF-α). This process further facilitates the recruitment and persistence of myeloid cells infiltration within the tumor microenvironment, concomitantly suppressing T cell activity. Furthermore, scRNA-seq reveals enhanced immune-suppressive function in macrophages after iRFA. Macrophages use LC3-associated phagocytosis to phagocytose heat-treated cells and enhance IL-4-mediated macrophage programming through the PI3Kγ/AKT pathway, thus suppressing T cell proliferation. Collectively, the transfer of CD169+macrophages synergistically enhanced the RFA-induced suppression of distant tumors by recruiting T cells and NK, while iRFA induced IL-4-mediated macrophage programming to suppress T cell proliferation.

4. Dendritic cells

Dendritic cells (DCs) are vital antigen-presenting cells in the immune system, possessing a high capacity for antigen capture and processing. Studies have demonstrated that thermal ablation not only eradicates tumors but also reduces the immune-suppressive condition by exposing tumor-associated antigens, which could potentially overcome tumor immune tolerance [37]. In the B16OVA mouse melanoma model, compared to the untreated group and monotherapy, cryoablation combined with CpG B treatment induced mature and efficient cross-presentation of DCs in tumor-bearing mice [43], which significantly increased the expression of CD80 and MHC I and II on DCs. Furthermore, the effectiveness of autologous dendritic cell tumor injection was compared to that of RFA combined with OK432-stimulated dendritic cell tumor injection in 30 patients with HCC [44]. The results of the study revealed that patients who exhibited significantly enhanced

immune responses specific to tumor-associated antigens (TAAs) had much higher rates of 5-year RFS compared to other patients. These findings are consistent with previous research conducted on mouse models of HCC, which demonstrated that RFA enhances TAA-related T cell responses [45]. However, a different phase II investigation revealed that the use of a dendritic cell vaccine pulsed with TAA as adjuvant treatment extended the RFS of patients who attained complete remission in non-RFA therapy cohorts, in contrast to those in the RFA-received cohort [46]. Consequently, the optimal combinations of dendritic cell treatments remain an unresolved question.

In addition to stimulating endogenous dendritic cells, several investigations have delved into the benefits of merging ablative therapy with direct injection of ex vivo DCs into the tumor environment. A study using the MB49 mouse model of urothelial carcinoma demonstrated that RFA combined with immature ex vivo DCs could enhance anti-tumor T cell responses and tumor regression [47]. However, in vivo, DC vaccines may undergo conversion to tolerogenic DCs through the action of immune-suppressive cytokines secreted by tumor cells or exosomes. Zhong et al. compared the anti-tumor effects of MWA combined with either dendritic cell-derived exosomes (Dex) or DCs in the treatment of liver cancer [48]. The combination of MWA with Dex treatment significantly enhanced infiltration of CD8+T cells into the tumor and reduced the number of Tregs. Furthermore, the concentration of plasma IFN-γ was increased, while plasma IL-10 was decreased compared to MWA monotherapy. In conclusion, MWA combined with Dex effectively suppressed tumor growth and improved the immune microenvironment.

5. Natural killer cells

Besides DCs, other cell types have exhibited indications of antitumor response when introduced through adoptive transfer. Researchers have extensively explored the malignant cell-recognizing ability of NK cells, which are innate immune effector lymphocytes utilizing self-ligand recognition to swiftly employ destructive mechanisms against stressed cells [49]. NK cells induce tumor cell apoptosis through various functional mechanisms, including the production of cytotoxic granules containing enzymes and perforin, death receptor-mediated apoptosis, cytoplasmic granule secretion, and antibody-dependent cellular cytotoxicity [50]. Standard radioactive and calibrated colorimetric assays demonstrated an association between reduced NK cell activity and increased spontaneous LDH-releasing activity of peripheral blood lymphocytes in patients with advanced breast cancer, indicating an altered profile of cell membrane damage [51]. According to the data, thermal ablation increased the production of activation signals by NK cells, outweighing the inhibition signals. Consequently, this led to the activation of NK cells and subsequent killing of target cells. The results indicate that the release of tumor-associated antigens by RFA leads to a substantial enhancement in both the frequency and functionality of CD3-CD56+NK cells among individuals diagnosed with hepatocellular carcinoma [52,53]. The expression levels of NK cell receptors NKp46, perforin, and granzyme B are associated with Relapse free survival [53]. Furthermore, the persistent presence of NK cells one month after RFA is associated with a higher incidence of HCC recurrence [54]. Remarkably, the infiltration of NK cells for eradicating PD-L1highCD11b+ myeloid cells through the antibody-dependent cell-mediated cytotoxicity (ADCC) effect is synergistically heightened by the anti-PD-L1 antibody following Cryoablation therapy [55]. Mechanistically, the administration of an anti-PD-L1 antibody stimulated the infiltration of CD8+ T cells by augmenting the secretion of CXCL9 from cDC1 cells. Additionally, it enhanced the ability of NK cells to eliminate PDL1highCD11b+ cells through an ADCC mechanism.

In an initial pilot clinical trial involving 48 patients with treatment-resistant breast cancer, the combination of NK cell transfer and cryoablation led to an increase in effector T cells and Th1-type cytokines in the peripheral blood of treated patients [56]. Furthermore, the clinical impact of NK cell immunotherapy was investigated in combination with IRE in patients with primary liver cancer. The study indicated an enhancement in survival rates and a stronger immune response compared to IRE treatment alone, suggesting potential therapeutic benefits [57]. Finally, MWA not only stimulates NK cells to produce an anti-tumor effect against primary breast cancer but also influences on the progression of metastatic tumors. Studies in two murine models of stage IV breast cancer demonstrated that MWA can suppress the progression of lung metastasis and improve the survival rate of mice with primary breast cancer [58]. Depletion experiments indicate that the key factor prolonging survival lies in NK cells rather than CD4+ or CD8+T cells. Additionally, MWA activates macrophages and induces the production of IL-15, thereby activating NK cells to suppress the occurrence of metastasis. Together, MWA inhibits breast cancer metastasis through the macrophage/IL-15/NK cell axis.

6. Discussion

The interaction between the immune system and cancer is complex and dynamic. The immune system assumes a crucial position in maintaining an equilibrium between promoting cancer growth and suppressing it. Localized tumor ablation induces cell death directly or indirectly, significantly altering the physical environment and functionality of the tumor. Each type of ablation leads to different types of cellular stress and tissue damage, resulting in diverse immune outcomes [59]. The motivation behind combining thermal ablation and ICIs in cancer treatment is rooted in the synergistic effects between these two distinct therapies, each with its unique mechanisms of action. After thermal ablation, damage and inflammatory responses, along with wound healing processes, lead to the recruitment of diverse cell types to the ablation site. These cellular activities have the potential to either enhance or inhibit the immune response, depending on their specific functional phenotype. Thermal ablation can either enhance or inhibit anti-tumor immune responses, depending on the composition and phenotypes of the recruited immune cells. A significant biomarker for predicting treatment outcomes and effectiveness is the crucial group of immune effectors, namely tumor-infiltrating lymphocytes (TILs), which comprises cytotoxic T cells, helper T cells, B cells, and NK cells [60]. There is evidence indicating a positive correlation between tumor-infiltrating CD8+ T cells and enhanced cancer prognosis following diverse types of thermal ablation methods [61]. Most thermal ablations also increase the number and cytotoxic function of NK cells [62,63]. Enhanced immune responses have been linked to the rise of infiltrating Th1 CD4+ T cells and CD4+ CTLs following localized ablation [64]. Besides TILs, DCs and M1 macrophages are additional significant immune effectors acknowledged for their role in enhancing TIL activation [65,66]. There is significant evidence indicating that thermal ablation has the potential to stimulate the activation and maturation of DCs, while also inducing the polarization of macrophages towards the M1 phenotype [67].

Multiple cell types play a role in creating an immune-suppressing microenvironment within tumors. These cell types include cancer-associated fibroblasts, MDSCs, Tregs, and TAMs, which often exhibit properties that suppress the immune system and promote tumor growth [68]. Theoretically, the removal or reduction of immunosuppressive cell populations existing within the tumor microenvironment and/or the disruption of tissue barriers may stimulate the infiltration of cytotoxic T lymphocytes into the tumor, boost anti-tumor immune responses, and foster the development of immunologic memory [59]. The research results concerning how thermal ablation modulates these immune-suppressive cell types present conflicting outcomes. A number of studies demonstrate a reduction in the frequency of Treg cells within tumors and peripheral blood after RFA, thus enhancing anti-tumor immunity [63,69]. In addition, the immune response typically resulting from independent thermal ablation is often insufficient to produce the required systemic and enduring anti-cancer impact essential for eradicating distant metastases. In combination therapy, Immune checkpoint

Table 3
Clinical trails that investigated the effect of ablation and immunotherapy.

Disease	Ablation therapy	Immunotherapy	Phase	No. patients	End date	Trial No.
Recurrent HCC	Thermal ablation	Toripalimab	II	116	2024/7/31	NCT05240404
Melanoma and Soft Tissue Sarcoma	Thermal ablation	IP-001 (1 % N-dihydro-galacto-chitosan, Immunophotonics Inc.)	I/II	39	2025/12/31	NCT03993678
HCC	Incomplete Thermal Ablation	Pembrolizumab or Nivolumab or JS001	II	50	2019/7/31	NCT03939975
Advanced Pancreatic Adenocarcinoma	Irreversible Electroporation	Nivolumab	II	10	2026/4/1	NCT03080974
Intrahepatic Recurrent Early Stage HCC	Thermal Ablation	Tislelizumab	II	125	2025/12/18	NCT04663035
CRC, Non-small Cell Lung Cancer, and Soft Tissue Sarcoma	Thermal Ablation	1.0% IP-001 for Injection	I/II	44	2024/5/1	NCT05688280
Advanced Non-Small Cell Lung Cancer	Ablation	Nivolumab	II	10	2018/3/1	NCT02469701
Advanced HCC	Thermal Therapy	PD-1 antibody SHR-1210	II	90	2023/11/1	NCT04204577
Advanced HCC	Thermal Ablation	TQB2450 Solution	III	80	2023/12/31	NCT04665609
Advanced HCC	Cryoablation	NK Immunotherapy	I/II	30	2019/7/1	NCT02843802
Solid Tumors	Cryoablation	Tislelizumab	II	25	2025/12/30	NCT06032845
Metastatic Cancer	Cryoablation	Immunotherapeutic Agent	/	25	2025/8/31	NCT04150939
High Risk Breast Cancers	Cryoablation	PD-1 Inhibitor	I/II	36	2027/6/1	NCT05806385
Relapsed/Refractory Solid Tumors	Cryoablation	Nivolumab, Ipilimumab	II	5	2025/7/1	NCT05302921
Metastatic Lung Cancer and Metastatic Melanoma	Cryoablation	Immune Checkpoint Inhibitor Therapy	/	20	2025/3/31	NCT03290677
Breast Cancer	Cryoablation	Ipilimumab	I	19	2014/12/1	NCT01502592
Metastatic Urothelial Carcinoma	Cryoablation	Pembrolizumab	II	30	2024/12/1	NCT04701918
Metastatic CRC	Cryoablation	AlloStim® Immunotherapy	II	12	2018/9/1	NCT02380443
Advanced BTC	Cryoablation	Sintilimab, Lenvatinib	II	25	2025/12/30	NCT05781074
Advanced HCC	Cryoablation	Dendric Cell/CIK	I/II	15	2024/12/1	NCT05622825
Advanced Melanoma	Cryoablation	Ipilimumab, Nivolumab	II	37	2028/1/1	NCT05779423
Early Stage/ Resectable Breast Cancer	Cryoablation	Ipilimumab	/	5	2024/6/1	NCT02833233
Gastric Cancer Liver Metastasis	Cryoablation	Tislelizumab	II	25	2025/12/30	NCT05893056
Unresectable Liver Metastasis from Solid Tumors	Cryoablation	Sintilimab	II	25	2024/10/30	NCT05098847
Melanoma Liver Metastasis	Cryoablation	Tislelizumab	II	25	2024/8/16	NCT05406466
Advanced breast cancer	Cryoablation	NK immunotherapy	I/II	60	2019/7/1	NCT02844335
Advanced esophageal cancer	Cryoablation	NK immunotherapy	I/II	60	2019/7/1	NCT02843581
Advanced kidney cancer	Cryoablation	NK immunotherapy	I/II	30	2019/7/1	NCT02843607
Recurrent laryngeal cancer	Cryoablation	NK immunotherapy	I/II	30	2019/7/1	NCT02849314
Recurrent tongue cancer	Cryoablation	NK immunotherapy	I/II	30	2019/7/1	NCT02849379
Recurrent pharyngeal cancer	Cryoablation	NK immunotherapy	I/II	30	2019/7/1	NCT02849327
Recurrent ovarian cancer	Cryoablation	NK immunotherapy	I/II	30	2019/7/1	NCT02849353
Recurrent cervical cancer	Cryoablation	NK immunotherapy	I/II	30	2019/7/1	NCT02849340
Unresectable Mesotheliomas	Cryoablation	Pembrolizumab	I	10	2024/10/1	NCT05071014
Multi-primary Lung Cancer	Cryoablation	Camrelizumab	I/II	20	2022/12/1	NCT04201990
NSCLC	Cryoablation	NK Immunotherapy	I/II	30	2019/7/1	NCT02843815
HCC or BTC	RFA/Cryoablation	Tremelimumab	I/II	61	2017/6/7	NCT01853618
Lung Cancer	Cryosurgery or IRE surgery	γδ T Cell Immunotherapy	I/II	30	2019/6/15	NCT03183232
Liver Cancer	Cryosurgery or IRE surgery	γδ T Cell Immunotherapy	I/II	30	2019/6/15	NCT03183219
Advanced HCC	Cryoablation	Tislelizumab	II	25	2025/12/30	NCT05897268
Advanced HCC	Cryoablation	Camrelizumab	II	34	2024/8/31	NCT04724226
Advanced HCC	Cryoablation	Tislelizumab	II	25	2024/9/20	NCT05057845
Triple Negative Breast Cancer	Cryoablation	Atezolizumab	I	0	2021/11/17	NCT04249167
Recurrent sarcoma	Cryoablation	NK immunotherapy	I/II	30	2019/7/1	NCT02849366
liver metastatic triple-negative breast cancer	Cryoablation	Tirelizumab	II	15	2024/4/1	NCT05303038
Metastatic Lung Adenocarcinoma	Cryoablation	Pembrolizumab	III	214	2025/8/1	NCT04339218

(*continued on next page*)

Table 3 (*continued*)

Disease	Ablation therapy	Immunotherapy	Phase	No. patients	End date	Trial No.
Metastatic or Locally Advanced Soft Tissue Sarcoma	Cryoablation	Ipilimumab, Nivolumab	II	30	2023/3/1	NCT04118166
Locally Advanced and Metastatic Renal Cell Carcinomas	Cryoablation	Pembrolizumab	I	0	2018/10/17	NCT03189186
Refractory or Metastatic Cancer	Cryoablation	AlloStim-7, AlloStim8, AlloStim-9	I/II	50	2011/5/1	NCT00861107
Triple-negative Breast Cancer	Cryoablation	Pembrolizumab	II	80	2026/6/1	NCT03546686
Stage II-Stage IV Cancer	Cryoablation	AlloStim	I/II	9	2013/7/1	NCT01065441
Newly Diagnosed Oligo-metastatic Prostate Cancer	Cryoablation	Pembrolizumab	/	12	2017/11/30	NCT02489357
HCC or BTC	Cryoablation/RFA	Durvalumab, Tremelimumab	II	54	2022/12/31	NCT02821754
Lung Metastases or Primary Lung Cancer	Cryoablation	GM-CSF	II	8	2010/3/1	NCT00514215
Advanced Intrahepatic Cholangiocarcinoma	Cryoablation	Camrelizumab	II	25	2024/5/10	NCT04299581
Metastatic or Unresectable Melanoma	Cryoablation	Pembrolizumab, Therapeutic Autologous DCs	I/II	7	2024/10/31	NCT03325101
Metastatic Renal Cell Carcinoma Conditions	Cryoablation	Tremelimumab	I	29	2022/6/20	NCT02626130
Breast Cancer	Cryosurgery or IRE surgery	γδ T cells	I/II	100	2019/6/15	NCT03183206
Non-Hodgkin Lymphoma	Cryoablation	DC Therapy, Pembrolizumab	I/II	11	2024/8/1	NCT03035331
Liver Metastases of CRC	RFA	CIK cells	II/III	60	2014/12/1	NCT02419677
HCC or BTC	Cryoablation/RFA	Tremelimumab	I/II	61	2017/6/7	NCT01853618
Cervical Cancer	RFA	CIK cells	II	10	2040/6/1	NCT02490748
Ovarian carcinoma	RFA	CIK cells	II	50	2040/6/1	NCT02487693
Cholangiocarcinoma	RFA	CIK cells	II/III	30	2033/7/1	NCT02482454
HCC or BTC	RFA and cryoablation	Tremelimumab,Durvalumab	II	54	2022/12/31	NCT02821754
Liver Metastases of CRC	RFA	Camrelizumab	I/II	23	2021/11/1	NCT04202978
HCC	RFA/MWA	Pembrolizumab	II	30	2024/6/1	NCT03753659
Advanced Solid Tumors	RFA	Anti-PD-1	I	30	2025/8/3	NCT04864379
HCC at Intermediate Stage	Ablation	Durvalumab	/	30	2025/10/1	NCT04517227
Short-term Recurrent HCC	RFA	Toripalimab	/	90	2025/12/1	NCT05162898
Small HCC	RFA	Atezolizumab	II	202	2027/7/1	NCT04727307
Metastatic Colorectal Cancer	RFA	Pembrolizumab	II	34	2024/4/1	NCT02437071
HCC	MWA	CIK cells	II/III	40	2021/3/1	NCT02851784
Unresectable Locally Advanced Pancreatic Cancer	MWA	Tremelimumab, Durvalumab	II	12	2023/12/31	NCT04156087
HCC	RFA/MWA	Pembrolizumab	II	30	2024/6/1	NCT03753659
Advanced NSCLC	MWA	Pembrolizumab	/	100	2029/11/1	NCT03769129
Metastatic NSCLC	MWA	Camrelizumab	II	200	2022/2/28	NCT04102982
HCC	RFA/MWA	Toripalimab	I/II	145	2023/5/30	NCT03864211

HCC: hepatocellular carcinoma; NSCLC: non-small cell lung cancer; CRC: colon rectal cancer; BTC: biliary tract cancer; RFA: radiofrequency ablation; MWA: microwave ablation; CIK: cytokine-induced killer; /: Not applicable.

inhibitors might induce a durable and effective anti-tumor immunity, countering local recurrence and metastasis.

As of September 2022, there are 79 clinical trials registered or planned on clinicaltrials.gov, investigating combinations of different ablation techniques and immunotherapy methods (Table 3). In addition to investigating the interactions between ablation and immunotherapeutic agents, various clinical trials and studies have been undertaken to validate the potential clinical benefits of combination therapy. In spite of the considerable body of research indicating that hyperthermia has the potential to elicit robust immune responses and establish a tumor-friendly environment that increases susceptibility to immunotherapy, the efficacy of combination therapies is influenced by several variables, encompassing tumor position, dimension, and stage of disease [70]. Moreover, a comprehensive assessment of the stimulating or inhibitory signals on immune cells within the tumor microenvironment is essential for predicting the efficacy of combination treatments and facilitating the personalized design of coordinated protocols.

7. Conclusion

The treatment of thermal ablation is not only efficient in promoting localized tumor control but also in the release of neoantigens within the tumor site. Nonetheless, its clinical advantages are often hindered due to the inherent negative modulation of the tumor microenvironment on the immune system. The merging of ablation and immunotherapy has resulted in a synergistic impact by adjusting the functionality of immune cells, consequently deepening our comprehension of holistic cancer management.

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