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Interventional Oncology in Immuno-Oncology Part 1: Thermal Ablation

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Abstract

Thermal ablation occupies a unique position among the various modalities available to treat malignancies. Initially utilized as a minimally invasive form of palliation, ablative techniques are increasingly being recognized for their role in activating an immune response. Locally destructive, but not thoroughly extirpative, thermal ablation function to generate an in situ tumor vaccine capable of stimulating and enhancing both innate and adaptive immune responses. As monotherapy, the response engendered remains therapeutically insufficient, but newer data suggests that when used as an adjuvant or neoadjuvant, ablation may synergistically boost the anticancer immune response produced by other, sequentially acting immunotherapies. The purpose of this review is to discuss the local and systemic immunological effects induced by thermal ablation. Radio frequency, microwave, and cryoablation will all be considered in addition to focused ultrasound ablation.

Keywords
- immuno-oncology
- cryoablation
- vaccine
- radio frequency ablation

Striking a balance between eradicating a malignancy and minimizing collateral damage has proven to be a persistently elusive goal. As a result, patients undergoing traditional oncologic treatment have often been plagued by side effects and tumor recurrences. Treatments such as external beam radiation or systemic chemotherapy are often not sufficiently tumor specific and the resulting collateral damage may result in significant morbidity.[1–4] On the other hand, the inability to comprehensively detect and physically remove micrometastases is a limitation of surgical excision that may set the stage for early recurrence.[5–8]

Another option to consider for these patients is interventional oncology. With the use of image-guided percutaneous and catheter-based procedures, interventional oncologists can deliver therapy directly to the tissue in question. Much like surgical excision, many interventional techniques favor precision over systemic efficacy; however, a key difference between these two schools of thought is that interventional oncologists do not remove the cancerous tissue; the tumors are instead destroyed in situ.[9] Thermal ablation in particular disrupts malignant cells, causing the release of antigens and inducing the expression of proinflammatory cytokines that can go on to activate and prime the immune system to attack occult malignant cells.[10] While ablation alone may stop short of enacting a complete antitumor immune response, it may help to set the stage for other, more systemically acting immunotherapies by functioning as an in situ tumor vaccine.[11–13]

Though each thermal ablation modality may differ in respect to their exact mechanisms of action, they all induce

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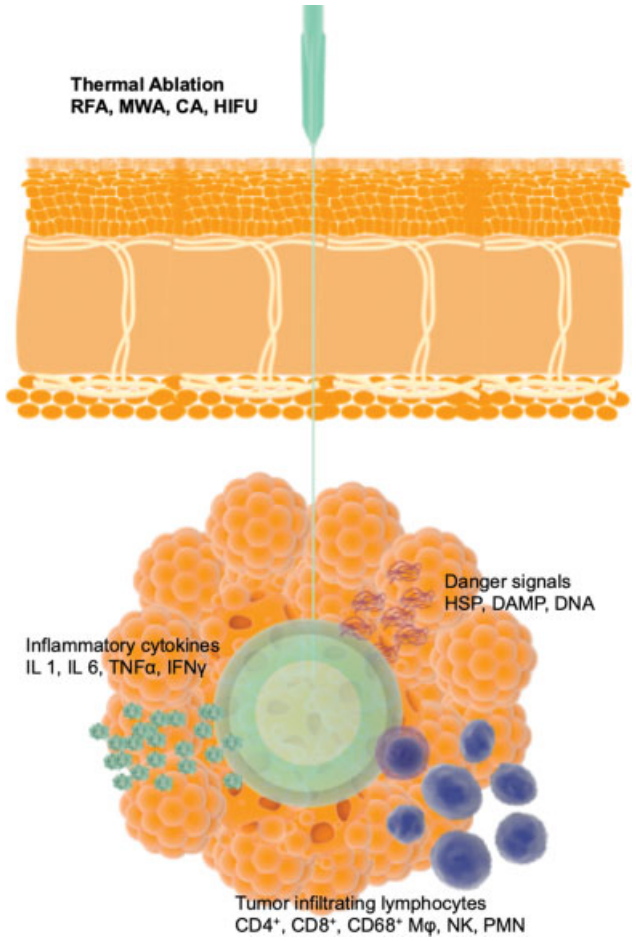

Fig. 1 A general depiction of the cellular disruption and immunostimulation that results from the various forms of thermal ablation. Abbreviations: CA, cryoablation; DAMP, damage-associated molecular protein; DNA, deoxyribonucleic acid; HIFU, high-intensity focused ultrasound; HSP, heat shock protein; IFN-γ; interferon gamma; IL-6, interleukin 6; MWA, microwave ablation; NK, natural killer; RFA, radio frequency ablation; TNF-α, tumor necrosis factor alpha.

necrosis or apoptosis of malignant cells thus exposing antigens that would otherwise escape detection.[9,14,15] Antigens unveiled by thermal destruction can be taken up by antigen presenting cells that may then go on to prime cytotoxic cells for an antitumor immune response[9,16] (►**Fig. 1**). This ability of ablation to expose the antigenic components of malignant cells to the immune system has been referred to as generating an in situ cancer vaccine.[12,16,17] Although not the focus of this discussion, it is worth noting that many investigators have sought to capitalize on and enhance this immunogenic effect by combining forms of ablation with medical therapies such as granulocyte-macrophage colony-stimulating factor (Perothech, Rocky Hill, NJ), bacterial-derived immunostimulants, or immune checkpoint inhibitors.[18–21] The current review will cover the immunological effects that have been observed when thermal ablation is used as a monotherapy.

Radio Frequency Ablation

Radio frequency ablation (RFA) utilizes applicator probes in conjunction with a grounding pad to generate a high-frequency, alternating current. Through frictional heating, this form of ablation is capable of producing temperatures in excess of 60°C, thereby causing coagulative necrosis of the nearby cells.[9] When the temperature rises above 100°C, tissue can char and the impendence to RF current increases drastically, thus limiting the effect of the ablation probe.[22] Additionally, RFA's mechanism of heating with electromagnetic current is particularly susceptible to the heat-sink effect, where nearby blood vessels siphon away heat and mitigate thermal damage[23] (►**Fig. 2A**).

Thus far, the results of studies seeking to characterize the immune response to RFA have been somewhat variable (►**Table 1**). For example, Matuszewski et al found in a study of renal cell carcinoma (RCC) that RFA decreased the circulating concentration of $CD56^{+}CD16^{+}$ ($CD56^{dim}$) natural killer (NK) cells in four out of six patients. Of the remaining two patients in the study, both had an increase in proportion and number of $CD56^{+}CD16^{+}$ ($CD56^{dim}$) NK cells and one maintained a significant increase over the entire 2 months follow-up period.[24] In contrast, Zerbini et al reported that RFA of hepatocellular carcinoma (HCC) lesions increases the absolute number of peripheral NK cells along with promoting their activation and functional activity at the 4-week post-ablation mark.[25] Notably, Matuszeski's group evaluated a single subtype of NK cells ($CD56^{dim}$), whereas Zerbini et al made efforts to distinguish between $CD56^{dim}$ and $CD56^{bright}$ populations. In doing so, Zerbini et al identified a significant increase in the $CD56^{dim}$ subset that corresponded to a significant decrease in the $CD56^{bright}$ subset. They further showed that there was no associated significant increase in K_i-67, suggesting that RFA had caused an increase in $CD56^{dim}$ cells by inducing a differentiation of NK cells from the $CD56^{bright}$ subset to $CD56^{dim}$, thereby shifting NK cells to a more cytotoxic phenotype.[25,26] Given the variation among Matuszewski's own patients, it is possible that the differing NK cell responses could be due to variables in RFA application or patient biomarker expression that have not yet been fully elucidated.

Similar variations in T cell response have also been observed after RFA. In the same study as above, Matuszeski's group also identified an increase in the numbers of $CD4^{+}$ and $CD8^{+}$ T cells in one out of six patients as well as an increase in the percentage of activated, DR^{+} T cells in five out of six patients two weeks after RCC ablation. The numbers of $CD4^{+}$ and $CD8^{+}$ T cells remained significantly elevated throughout the study period, while the proportion of activated T cells declined at week 4, before rising again at week 8.[24] Conversely, in a clinical study of RFA for liver cancer, patients with colorectal metastases, but not those with primary disease, demonstrated a transient decrease in $CD3^{+}CD4^{+}$-T cells 2 days after ablation.[27] Likewise, a study by Giardino et al demonstrated that the amount of circulating $CD4^{+}$, $CD8^{+}$ T, and effector memory T cells exhibited an initial reduction in numbers that they attributed to surgically induced immune suppression followed by a significant rebound indicative of an activated immune response.[28] Together these results suggest that the immune response induced by RFA changes over time.

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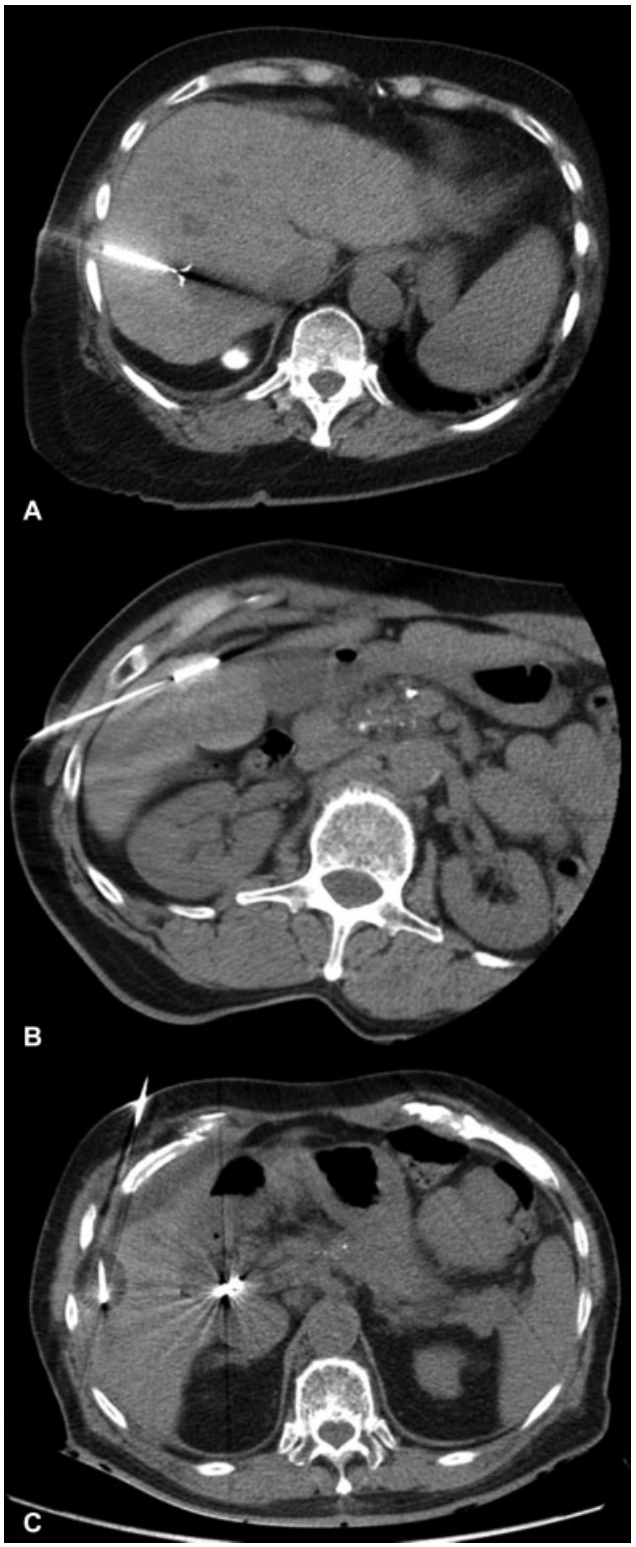

Fig. 2 Computed tomography images obtained during ablation procedures at Yale New Haven Hospital. (**A**) Radio frequency ablation, (**B**) microwave ablation, (**C**) cryoablation.

Significant modulations in other inflammatory markers have also been reported after RFA. In addition to its effects on T cells, Giardino et al also found that RFA was associated with a significant induction of the pro-inflammatory cytokine interleukin 6 (IL-6) and antigen presenting dendritic cells (DC).[28] Other studies have shown that RFA increases IL-6 as well as IL-1β, hepatocyte growth factor, vascular endothelial growth factor (VEGF), and tumor necrosis factor alpha (TNF-α).[29–32] However, at least one group has reported that there was no effect on IL-1β or IL-6 up to 48 hours after RFA treatment of hepatic metastases.[33] One possible explanation for this outlier is that levels of these inflammatory cytokines vary over time after RFA, just as T cell populations do, but more data are necessary to make that assertion. Numerous studies have also reported on the effect that RFA has on heat shock proteins (HSP), with results showing that RFA significantly induces cellular expression of HSP70 and HSP90, as well as increasing the serum levels of HSP70.[34–39] Concomitant decreases in nuclear expression of HSP90 suggest that RFA promotes its translocation to the cell surface.[34]

One group that looked specifically at the immune response in intestinal mucosa induced by RFA of the liver in rats found that after ablation, expression of CD4+ T cells, $CD8^+$ T cells, $CD68^+$ macrophages, and MAdCAM-1 all increased significantly as compared with control. Furthermore, the proinflammatory cytokines TNF-α, IL-6, and nuclear factor kappa beta (NF-κβ) also increased significantly.[40] RFA of hepatic tumors has also been shown to induce systemic inflammatory symptoms, elevating temperature, mean arterial pressure, IL-1, adrenaline, and noradrenaline up to 24 hours after the procedure.[41] Along the same lines, another study showed that RFA produced fever and increased neutrophils only in patients with metastatic liver cancer, not those with primary disease.[42]

Notably, the inflammatory response after RFA has been consistently reported as being less significant than what is seen after cryoablation (CA).[30] Similarly, while the in situ destruction of tumoral tissue by RFA has been shown to promote DC infiltration, loading, and maturation, it is reportedly far less efficient than in CA.[43–45]

In regard to the effects of RFA on immunosuppression, Giardino et al showed that RFA does not induce expansion or activation of regulatory T cells.[28] Other investigators have reported that RFA does not have an impact on the immunosuppressive cytokines transforming growth factor-beta (TGF-β) or IL-10.[32,33] However, it has also been reported in lung cancer patients that RFA produces a significant reduction in circulating $CD25^+FoxP3^+$ regulatory T cells that corresponds to increase in CD4+ T cells and interferon gamma (IFN-γ) secreting cells between 30 and 90 days after ablation.[46] Another group found that while RFA significantly reduced the volume of circulating regulatory T cells (T_{regs}), it was less profound than surgical excision.[38]

Several authors have studied the specificity of the antitumor immune response. In a rabbit model of VX2 hepatoma, RFA was able to generate a significant tumor-specific T cell response as well as dense T cell infiltration.[47] Clinical studies of primary and secondary liver cancer have shown that RFA is capable of generating tumor-specific antibodies as well as tumor-specific $CD4^+/CD8^+$ T cells that may persist for months after treatment.[48,49] Dromi et al demonstrated the tumor-specific memory response by showing that RFA

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provided protection against tumoral rechallenge.[44] Another group showed that after RFA treatment, the tumoral antigen mucin 1 (MUC1) was able to stimulate T cells to release IFN-γ and increase the amount of circulating B cells.[27] Mizukoshi et al used an IFN-γ enzyme-linked immunospot assay (ELISPOT) assay to analyze the immune response to 11 tumor-associated antigen (TAA) peptides after RFA in HCC patients. They found that an increase in the number of TAA-specific T cells occurred in 62.3% of patients. Likewise, the increase in tumor-specific T cells was associated with protection from HCC recurrence, although this protection was not sufficient to completely prevent recurrent disease.[50] Hiroishi et al found similar results, with 80% of the HCC patients showing a significant increase in TAA-specific CD8+ T cells. They also showed that the magnitude of the specific T cell response was a significant prognosticator for protection against recurrence.[51] An additional study investigated the ability of RFA to induce a tumor-specific T cell response in patients with HCC. By assessing T cell reactivity to HCC-derived protein lysates via an IFN-γ ELISPOT, as well as via intracellular IFN-γ staining, they found that RFA significantly increased anti-tumor-specific T cell responsiveness, a higher frequency of circulating tumor-specific T cells, and increased expression of activation and cytotoxic surface markers. However, they also found that this immune response was not sufficient to protect against HCC recurrence. Evidence of eventual immune escape came in the form of a new HCC nodule that was not recognized by the T cells obtained at the time of ablation.[52] Other groups have specifically examined the immunological effect that RFA has on distal, untreated lesions. They have found that RFA inhibits distal lesion growth and increases the distal infiltration of neutrophils and $CD4^+$ T cells, but not of $CD8^+$ T cells.[53,54]

Microwave Ablation

Microwave ablation (MWA) is a newer form of hyperthermic ablation that differs from RFA in that it utilizes an electromagnetic field to generate heat via dipole agitation and hysteresis, instead of frictional heating via an alternating current. Unlike the current of RFA, the fields generated by MWA are able to pass through tissues with high impedance, and as such it remains effective when tissues char at temperatures above 100°. The size of MWA zones has also been shown to be less affected by local blood vessels (the heatsink effect) than RFA[55](►**Fig. 2B**).

Compared with the research on RFA, relatively little work has gone into investigating the immunological effects of MWA in isolation (►**Table 2**). Much of the existing research has examined the effect that MWA has in combination with immunotherapy. In terms of MWA alone, the existing body of evidence seems to suggest that the immune response stimulated by MWA is substantially less than other forms of ablation. One clinical study of MWA for the treatment of HCC found that after ablation, the numbers of T cells, NK cells, and macrophages increased significantly in both the treated and untreated lesions. The same study also found a significant correlation between the level of immune cell infiltration and survival.[56] Another study that compared forms of ablation found that the levels of IL-1β and IL-6 induced after ablation were significantly less than after RFA or CA.[31] Similarly, a different study found that while MWA did induce significant expression of HSP70, the upregulation was less than RFA and CA.[57]

Several studies have compared the immunological effects of MWA alone versus MWA in combination with an immunotherapy. In these papers, valuable data exists on the effect that MWA monotherapy has on the immune system. For example, one study performed on murine model of breast cancer found that after MWA alone there was a significant increase in the tumoral infiltration of $CD4^+$ and $CD8^+$ T cells along with significant increases in their splenic equivalents when compared with control. The same study also identified a statistically significant increase in tumor-specific IFN-γ secreting cells as well as an increase in serum levels of IL-12 after treatment. The authors further enhanced these responses with the addition of the immunostimulant, OK-432 (Lukang Pharmaceutical, Shandong, China).[21]

Cryoablation

CA is a unique hypothermic method of ablation; destroying cells by producing local temperatures as low as −160°C. These temperatures are achieved via the release of liquid argon gas that rapidly expands and evaporates, thereby extracting the heat out of nearby tissues and generating an ice ball.[58,59] The alternation of argon and helium gas allows for a rapid freeze-thaw cycle. During the freezing phase, higher intracellular osmolarity initially causes ice crystals to form outside the cell. The resultant change in the osmotic gradient pulls water out of the cell, causing injury via cellular dehydration. When helium is released and the cells thaw, the osmotic gradient reverses, and water rushes back into the cells causing swelling and membrane rupture. As the influx of extracellular fluid lowers intracellular osmolarity, intracellular ice crystals may form, further damaging cellular membranes and organelles. Ice crystals can also form in the walls of nearby blood vessels and cause endothelial damage that initially increases vascular permeability and eventually leads to thrombosis. Occlusion of blood flow causes tissue ischemia that further potentiates tumor cell death[9,15,59,60](►**Fig. 2C**).

There is evidence to show that CA is capable of generating both immunostimulatory and immunosuppressive effects (►**Table 3**). The prevailing response is believed to be dictated by the ratio of necrotic versus apoptotic cells, with a combination of the two seeming to be ideal. The cells closest to the applicator probe die by necrosis and release "danger signals" in the form of damage associated molecular proteins (DAMPs) such as DNA, RNA, and HSP.[61,62] Phagocytosis of DAMPs by DC promotes the expression of co-stimulatory molecules necessary for the activation of a cytotoxic T cell response such as CD80 and CD86.[63,64] On the other hand, physiologic apoptosis is a highly regulated process that does not allow for the release of DAMPs. When DCs process apoptotic cells, the expression of co-stimulatory molecules

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Table 1 A concise description of the significant immunological effects induced by radiofrequency ablation

Radiofrequency ablation			
Humans	Renal cell carcinoma	↓ $CD56^{dim}$ NK cells	24
		↑DR^+ T cells	
		↑$CD4^+$ T cells	
		↑$CD8^+$ T cells	
		↑Tumor-specific antibodies	48
		↑HSP70 expression	36
	HCC	↑ $CD56^{dim}$ NK cells	25
		↓$CD56^{bright}$ NK cells	
		↑Tumor-specific $CD4^+$ T cells	48–50,52
		↑Tumor-specific $CD8^+$ T cells	48–52
		↑Activation of myeloid DCs	45
		↑TNF-α	
		↑IL-1 β	
		↑IL-6	41
		↑HSP70 expression	34,36
		↑HSP90 expression	34
	Metastatic liver cancer	↑Tumor-specific antibodies	48
		↑Tumor-specific $CD4^+$ T cells	48,49
		↑Tumor-specific $CD8^+$ T cells	
		↑IL-6	32,41
		↑HGF	32
		↑VEGF	
		↑HSP70 expression	36
		↑Neutrophils	42
		↑Neutrophils in distal lesions	53
		↑$CD4^+$ T cells in distal lesions	
	Pancreatic cancer	↑$CD4^+$ T cells	28
		↑$CD8^+$ T cells	
		↑Effector memory T cells	
		↑DCs	
		↑IL-6	
	Primary or metastatic lung cancer	↓$CD25^+FoxP3^+$ T cells ↑$CD4^+$ T cells	46
		↑IFN-γ secreting cells	
Animals	Sprague Dawley rats	↑TNF-α	29
		↑IL-6	29,31
		↑Il-1β	31
		↑HSP70 expression	57
	Wistar rats (effects in intestinal mucosa)	↑$CD4^+$ T cells ↑$CD8^+$ T cells	40
		↑$CD68^+$ macrophages	
		↑MadCAM-1	

(*Continued*)

Table 1 (*Continued*)

Radiofrequency ablation			
		↑TNF-α	
		↑IL-6	
		↑NF-κβ	
	Fisher 344 rats with murine breast cancer (MatBIII)	↑NK cell infiltration ↑HSP70 expression	38
		↓T_{Reg} cells	
	Nude rats with human HCC (SK-HEP-1)	↑HSP70 expression ↑HSP90 expression	35
	Domestic swine	↑TNF-α	30
		↑IL-1β	
	C57BL/6N mice with melanoma (B16-OVA or B16-luc)	↑DC loading ↑HSP70 expression	43 39
	C57BL/6 mice with urothelial cancer (MB49)	↑Systemic antitumor T cell response	44
		↑Tumor regression	
		↑DC infiltration	
	BALB/c mice with murine HCC (BNL)	↑T cell infiltration of distal lesions ↓Growth of distal lesions	54
	Nude mice with human CRC (HT29)	↑HSP70 expression	37
	Rabbit VX hepatoma	↑Tumor-specific T cell response	47
		↑T cell infiltration	

Abbreviations: CRC, colorectal cancer; DC, dendritic cell; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; HSP70, heat shock protein 70; IFN-γ, interferon gamma; IL-6, interleukin 6; NF-κβ, nuclear factor kappa beta; NK, natural killer; SK-HEP-1,—; TNF-α, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.

is inhibited and the immune system is suppressed.[65–67] If the volume of apoptotic cells exceeds the body's ability to clear it, the cells may become secondarily necrotic. Danger signals released from secondarily necrotic cells or nearby necrotic cells might be sufficient to trigger the maturation of DCs that have taken up apoptotic remnants.[68] In fact, CA has been shown to rescue the function of tumor-induced tolerogenic DC, downregulating their expression of IL-10 and allowing them to again act in an immunostimulatory manner.[69] Newer studies suggest that apoptotic cells themselves may be capable of releasing DAMPs and stimulating DC maturation in a process referred to as immunogenic cell death.[66,67]

As an example of how the ratio of necrotic to apoptotic cells can shift the immunologic response to CA, the rate of freeze has an effect on the extent of necrosis as well as the magnitude of the immunostimulatory response induced by CA. Faster rates are associated with a significant increase in tumor-specific T cells in draining lymph nodes as well as decreased pulmonary metastases and prolonged survival when compared with slower rates. Interestingly, a low rate

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Table 2 A concise description of the significant immunological effects induced by microwave ablation

Microwave ablation			
Humans	Hepatocellular carcinoma	↑T cell infiltration	56
		↑NK cells infiltration	
		↑Macrophage infiltration	
		↑T cells in distal lesions	
		↑Macrophages in distal lesions	
		↑NK cells in distal lesions	
Animals	Sprague Dawley rats	↑HSP70 expression	57
	BALB/c mice w/ murine breast cancer (4T1)	↑$CD4^+$ T cells infiltration ↑$CD8^+$ T cells infiltration	21
		↑Splenic $CD4^+$ T cells	
		↑Splenic $CD8^+$ T cells	
		↑Tumor-specific IFN-γ secreting cells	
		↑IL-12	

Abbreviations: HSP70, heat shock protein 70; IFN-γ, interferon gamma; IL-12, interleukin 12; NK, natural killer.

of freezing was actually associated with an increase in T_{reg} cells and a resulting suppression of $CD8^+$ T cell stimulation.[70]

There is some data to suggest that the immune response to CA changes over time from immunosuppression to immunostimulation. In a series of papers published between 1982 and 1998, one group observed an initial increase in immunosuppression that they suggested could be due to the induction of splenic suppressor T cells (now known as T_{reg} cells).[71–74] Another group identified an increase in the population of splenic suppressor T cells (T_{regs}) after CA only between 1 and 10 days after treatment.[75] Using a rat model of breast adenocarcinoma, Misao et al found that while mice treated with surgical excision had a superior response to re-challenge 1 to 3 weeks after treatment, the mice that had been cryoablated experienced a significantly higher rejection rate than surgery at week 10. At this time point, only 18% of the surgically treated rats rejected the re-challenge, compared with 80% in cryoablated mice.[76] An additional study concluded that after CA there is early immunosuppression followed by increasing tumor draining lymph node cellularity and resistance to rechallenge that peaks at ten weeks.[77] It is possible that early after CA, the immunosuppressive signals from the apoptotic cells are dominant, but as the body fails to clear those cells and they become secondarily necrotic, the response shifts to an immunostimulatory one.

There is a robust body of evidence to support the immunostimulatory response induced by CA. Samples of tumor tissue taken after ablation in kidney cancer patients have demonstrated elevated transcriptional levels of $CD8^+$, $CD4^+$, CD11c, and granzyme A along with a high $CD8^+/FoxP3^+$ ratio.[78] In melanoma patients, CA has been associated with a significant increase in the number of circulating total and helper T cells, as well as in HLA-DR^+ antigen presenting cells, and in the ratio of helper to suppressor T cells.[79] Several animal studies have also highlighted a postablative increase in tumor-specific cytotoxicity as well as a significant increase in infiltrating neutrophils, macrophages, $CD4^+$, and $CD8^+$ T cells after CA, particularly at the periphery of the ablation zone, where the hypothermic injury is sublethal and apoptotic cell death occurs.[80,81] A murine model was also used to demonstrate that there is an increase in the ratio of IFN-γ to IL-4 after CA, suggesting that there is a postablative shift in the ratio of Th1 to Th2 effector cells.[80] In a trial conducted on patients with primary and metastatic liver cancer, a post-CA increase in the ratio of IFN-γ to IL-4 as well as an increase in TNF-α and a decrease in IL-10 were all associated with tumor necrosis outside of the treatment area.[82] Another study that sought to investigate the prognostic ability of postablative T_{reg} cells found that after CA, there was an overall decreased frequency of peripheral T_{reg} cells and a reduction in the $FoxP3^+/CD8^+$ T cell ratio.[83] Sabel et al used a murine model of breast cancer to demonstrate significant increases in IFN-γ and IL-12 as well as in tumor-specific T cell responses in tumor draining lymph nodes after CA.[84] Several other authors have also shown that CA confers significant resistance to rechallenge with tumoral antigens.[11,84,85] An abscopal effect has even been seen, with significant postablation protection from metastases in the liver and lungs in addition to inhibition of secondary tumor growth.[86–88]

The immune response after CA has been shown to be more substantial than after other forms of ablation. Compared with RFA or MWA, circulating levels of IL-1, IL-6, NF-κβ, and TNF-α are all significantly more elevated after CA.[89,90] Loading of in vivo DCs with tumoral antigens is also vastly more efficient after CA than after RFA.[43] One potential explanation for the enhanced immune response after CA could be the mechanism of cell injury that it causes: hyperthermic ablative modalities like RFA and MWA destroy cellular antigens, whereas the osmotic shifts that occur with CA do not. Unlike hyperthermic techniques, the immunostimulation after CA can be so significant as to cause systemic inflammatory syndromes[91–94]

Focused Ultrasound Ablation

High-intensity focused ultrasound (HIFU) differs from other forms of thermal ablative therapies in that it is truly non-invasive. Traditionally, HIFU damages cells thermally by using the acoustic energy of several highly concentrated ultrasound beams to produce internal temperatures over

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Table 3 A concise description of the significant immunological effects induced by cryoablation

Cryoablation			
Humans	RCC	↑$CD4^+$ transcriptional levels	78
		↑$CD8^+$ transcriptional levels	
		↑CD11c transcriptional levels	
		↑Granzyme A transcriptional levels	
		↑$CD8^+$/$FoxP3^+$ ratio	
		↑IL-10	90
		↑IL-6	
	HCC	↓$FoxP3^+$ T cells	83
		↑$CD8^+$/$FoxP3^+$ ratio	
		↑IL-10	90
		↑IL-6	
	Metastatic liver cancer	↑TNF-α	82
		↓IL-10	
		↑IFN-γ/IL-4 ratio	
	Lung cancer	↑IL-10	90
		↑IL-6	
	Cholangiocarcinoma	↑TNF-α	82
		↓IL-10	
		↑IFN-γ/IL-4 ratio	
	Melanoma	↑Total T cells	79
		↑$CD4^+$ T cells	
		↑$HLA\text{-}DR^+$ cells	
		↑Helper/regulatory T cell ratio	
		↑IL-10	90
		↑IL-6	
Animals	Sprague Dawley rats	↑HSP70 expression	57
	BALB/c mice with murine breast cancer (4T1)	↑Tumor-specific T cells in TDLN ↓Pulmonary metastases	70
		↑Survival	
	BALB/c mice with murine breast cancer (MT-901)	↑IL-12 ↑IFN-γ	84
		↑Protection vs rechallenge	
	BALB/c mice with murine RCC (Renca)	↑$CD4^+$ T cell infiltration ↑$CD8^+$ T cell infiltration	80
		↑Neutrophil infiltration	
		↑Macrophage infiltration	
		↑IFN-γ/IL-4 ratio	
	Nude mice with human melanoma (IIB-MEL-J)	↑Neutrophil infiltration ↑Macrophage infiltration	81
	C57BL/6n mice with melanoma (B16OVA)	↑Protection vs rechallenge ↑DC loading	11 43
	BALB/c mice with murine colon cancer (26-B)	↑IL-1α ↑TNF-α	86 86,87
		↑IFN-γ	
		↑IL-4	
		↑IL-10	
		↓Secondary tumor growth	

Abbreviations: DC, dendritic cell; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; HSP70, heat shock protein 70; IFN-γ, interferon gamma; IL-6, interleukin 6; RCC, renal cell carcinoma; TDLN, tumor draining lymph nodes; TNF-α, tumor necrosis factor alpha.

60°C. With the appropriate settings, HIFU can become mechanical high intensity focused ultrasound (M-HIFU) and cause nonthermal, mechanical damage via a process called acoustic cavitation. As the ultrasonic waves collide with the cells, the alterations in pressure cause gaseous nuclei within the cells to expand and contract, eventually leading to collapse of organelles and lysis of cellular membranes.[10,58]

Much like CA, HIFU has been shown to be highly immunogenic (►**Table 4**). Wu et al demonstrated that HIFU preserves tumor antigens and increases surface expression of HSP70, indicating that tumors treated by HIFU can stimulate an immune response.[95] Multiple studies corroborating the increased expression of HSP70 have been documented, in addition to research showing that HIFU also induces HSP72 and HSP73.[96,97] Several authors have shown that HIFU-ablated tumors significantly promote the maturation of DCs by upregulating the expression of costimulatory molecules.[98,99] In particular, Xu et al used a clinical study of human breast cancer to show that HIFU significantly increased the expression of CD80 and CD86 along with HLA-DR on DCs and macrophages. Additionally, their study found that the numbers of DCs, macrophages, and B cells infiltrating the margins of ablated zones increased significantly after HIFU.[100] A related study performed by Lu et al showed that HIFU also significantly increases marginal infiltration of $CD3^+$, $CD4^+$, $CD8^+$, B Cells, and NK cells. They further characterized these tumor-infiltrating lymphocytes as cytotoxic by showing a significant increase in fas^+, $perforin^+$, and $granzyme^+$ cells postablation.[101] Knowing the lymphocytes tend to infiltrate at the periphery of ablated lesions, one group of investigators set out to maximize the peripheral area created by HIFU treatment by using a "sparse scan" strategy that generated clusters of well-separated lesions. They found that this technique was significantly more effective at increasing DC infiltration and promoting their maturation than traditional dense scanning.[102]

In terms of cell-mediated immunity, a significant increase in $CD4^+$ cells has been associated with HIFU.[103] Several groups have documented cases where patients with solid malignancies had abnormal $CD4^+/CD8^+$ ratios before treatment and then subsequently had T cell ratios normalize after

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Table 4 A concise description of the significant immunological effects induced by high-intensity focused ultrasound

High-intensity focused ultrasound			
Humans	Breast cancer	↑Infiltration of DCs	100
		↑Maturation of DCs	
		↑Infiltration of macrophages	
		↑Maturation of macrophages	
		↑Infiltration of B lymphocytes	100,101
		↑Infiltration of $CD3^+$ T cells	101
		↑Infiltration of $CD4^+$ T cells	
		↑Infiltration of $CD8^+$ T cells	
		↑Infiltration of NK cells	
		↑$Granzyme^+$ TILs	
		↑$Perforin^+$ TILs	
		↑$FasL^+$ TILs	
		↑HSP70 expression	95
	Prostate cancer	↑HSP72 expression	97
		↑HSP73 expression	
	Choroidal melanoma	↑$CD4^+/CD8^+$ T cell ratio	104
	Pancreatic cancer	↑NK cell activity	107
	Renal cell carcinoma	↑$CD4^+$ T cells	103
		↑$CD4^+/CD8^+$ T cell ratio	
	Hepatocellular carcinoma	↑$CD4^+$ T cells	103
		↑$CD4^+/CD8^+$ T cell ratio	
		↓VEGF	108
		↓TGF-β1	
		↓TGF-β2	
	Metastatic liver cancer	↓VEGF	108
		↓TGF-β1	
		↓TGF-β2	
	Osteosarcoma	↑$CD4^+$ T cells	103
		↑$CD4^+/CD8^+$ T cell ratio	
		↓VEGF	108
		↓TGF-β1	
		↓TGF-β2	
	Cholangiocarcinoma	↓VEGF	108
		↓TGF-β1	
		↓TGF-β2	
Animals	FVB mice transgenic for HSP70-luc2A-eGFP	↑HSP70 expression	96
	C57BL/6J mice with murine hepatoma (H22)	↑Maturation of DCs ↑TNF-α secretion	98,99 99,105
		↑IFN-γ secretion	105
		↑$MHC\ 1^+/CD8^+$ T cells	
		↑Inhibition of tumor progression	
	C57BL/6 mice with murine colon cancer (MC38)	↑Tumor-specific IFN-γ secreting cells ↑DC accumulation in TDLN	106
		↑Protection vs rechallenge	
		↑CD11c+ cells	
	C57BL/6 & BALB/c mice with murine glioma (GL261)	↓IL-10 expression from DCs ↑Functional recovery of tolerogenic DCs	69
		↑Protection vs rechallenge	

Abbreviations: DC, dendritic cell; HGF, hepatocyte growth factor; HSP70, heat shock protein 70; IFN-γ, interferon gamma; IL-6, interleukin 6; MHC-1, major histocompatibility complex 1; NK, natural killer; TDLN, —; TGF-β1, transforming growth factor-beta 1; TIL, tumor-infiltrating lymphocytes; TNF-α, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.

HIFU.[103,104] Additional studies have shown that HIFU increases the number of cytotoxic T cells as well as the amount of IFN-γ and TNF-α secretion.[105] Protection against a tumoral rechallenge has also been proven to result from HIFU. Hu et al used a murine model of colon adenocarcinoma to show that thermal HIFU can protect against rechallenge in addition to increasing the accumulation of DCs into tumor draining lymph nodes as well as the number of tumor-specific cytotoxic T cells.[106] Another clinical study that examined the use of HIFU for the treatment of late stage pancreatic carcinoma found that while HIFU did lead to a significant increase in NK activity, the increase in $CD3^+$ and $CD4^+$ cells was non-significant.[107] One study found that in patients with various solid malignancies, HIFU decreased the circulating levels of the immunosuppressive cytokines, VEGF, TGF-β1, and TGF-β2. Notably they were able to further clarify that the levels of these cytokines were significantly lower after HIFU in patients with nonmetastatic disease as compared with those with metastases.[108] Given the range of responses seen with differing applications of HIFU, it is possible that this variation in postablative cell mediated immunity is due to variations in technique.

Several investigators have looked specifically into the immune effects of M-HIFU. They found over several studies that M-HIFU induces a more robust release of danger signals, enhances DC and cytotoxic T cell function, and diminishes metastatic burden when compared with thermal HIFU.[106,109,110] Furthermore, when used as neoadjuvant therapy for prostate cancer, M-HIFU was found to inhibit the growth of rechallenged tumors, increase the number of cytotoxic T cells, and prolong host survival. The authors attributed the decrease in immunosuppression to a down-regulation of the tumor suppressor molecule STAT3.[111]

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Conclusion

In situ modulation of malignant cells by thermal ablation may function as an in situ cancer vaccine, enhancing immunogenicity and promoting a tumor-specific immune response capable of exerting abscopal effects. The various forms of ablation have all shown this ability to some extent, though CA seems to be the most potent immunostimulant. Current and future studies investigating the incorporation of ablation into comprehensive immunotherapeutic regimens hold great potential for the treatment of solid tumors.

Conflict of Interest Statement
The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript. Hyun S. Kim served on Advisory boards for Boston Scientific and SIRTex.

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