Postablation Immune Microenvironment: Synergy between Interventional Oncology and Immuno-oncology

DaeHee Kim, MD[1] Joseph P. Erinjeri, MD, PhD[1]

[1] Interventional Radiology Service, Department of Radiology, Memorial Sloan Kettering Cancer Center, New York, New York

Semin Intervent Radiol 2019;36:334–342

Address for correspondence Joseph P. Erinjeri, MD, PhD, Interventional Radiology Service, Department of Radiology, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, H-118, New York, NY 10065 (e-mail: erinjerj@mskcc.org).

Abstract

Current tumor thermal ablation techniques rely on extreme temperatures to induce irreversible cellular injury and coagulative tissue necrosis. Ablation-induced cellular injury or death releases cancer neoantigens and activates the cancer-immunity cycle, potentially generating tumor-specific immune effectors. However, multiple negative regulatory modulators exist at each step of the cycle, mitigating meaningful and therapeutic anticancer effect provided by the immune system. Recent studies have focused on the introduction and testing of adjuvant immunotherapy combined with ablation to synergistically shift the equilibrium out of inhibitory immune modulation. This article reviews the immune microenvironment in relation to image-guided ablation techniques and discusses current and upcoming novel strategies to take advantage of antitumor immunity.

Keywords

- immune microenvironment
- ablation
- interventional oncology
- immunotherapy

Interventional oncology (IO) has seen remarkable progress over the last two decades to now offer a variety of locoregional cancer treatments. The minimally invasive nature of IO was the main driver for the specialty's emergence and growth, particularly as an alternative to traditional surgical and systemic treatments. With the degree of image resolution offered by current clinical imaging technologies, the efficacy of IO therapies is now well established at the tissue or organ level.

Recent advancements in immunooncology research have brought a new "magnifying glass" to IO, namely, one focusing on the immune microenvironment. The effect of IO therapeutics is now investigated at the cellular and molecular level with a focus on the immune system. A unique feature of IO therapy is particularly pertinent in these investigations: IO achieves in situ destruction, during which tumor-specific neoantigens are released and potentially inducing the so-called abscopal effect or in vivo antitumor vaccination.[1,2] In addition, the spontaneous remission of distant malignant disease after focal thermal ablation has been reported occasionally, but the exact mechanism remains elusive.[3–6]

Current tumor ablation techniques rely on extreme temperatures to induce irreversible cellular injury and result in tissue coagulative necrosis. The commonly used techniques include radiofrequency ablation (RFA), microwave ablation (MWA), and cryoablation, while the newer techniques such as laser and high intensity focused ultrasound (HIFU) have demonstrated promising results. While not based on thermal energy, irreversible electroporation (IRE) is also employed along with the aforementioned thermal ablation methods.

The aim of this article is to provide a review on the immune microenvironment in relation to current image-guided ablation techniques and to discuss current and upcoming developments of novel IO strategies to take advantage of antitumor immunity.

Cancer-Immunity Cycle

Antitumor immunity involves a series of events referred to as the cancer-immunity cycle[7] (►**Fig. 1**). Oncogenic neoantigens expressed by tumor cells are processed by antigen-presenting cells (APCs) to prime and activate antigen-specific CD8+

Issue Theme Ablative Therapy: Part 1; Guest Editor, Beau B. Toskich, MD

DOI https://doi.org/10.1055/s-0039-1696704.
ISSN 0739-9529.

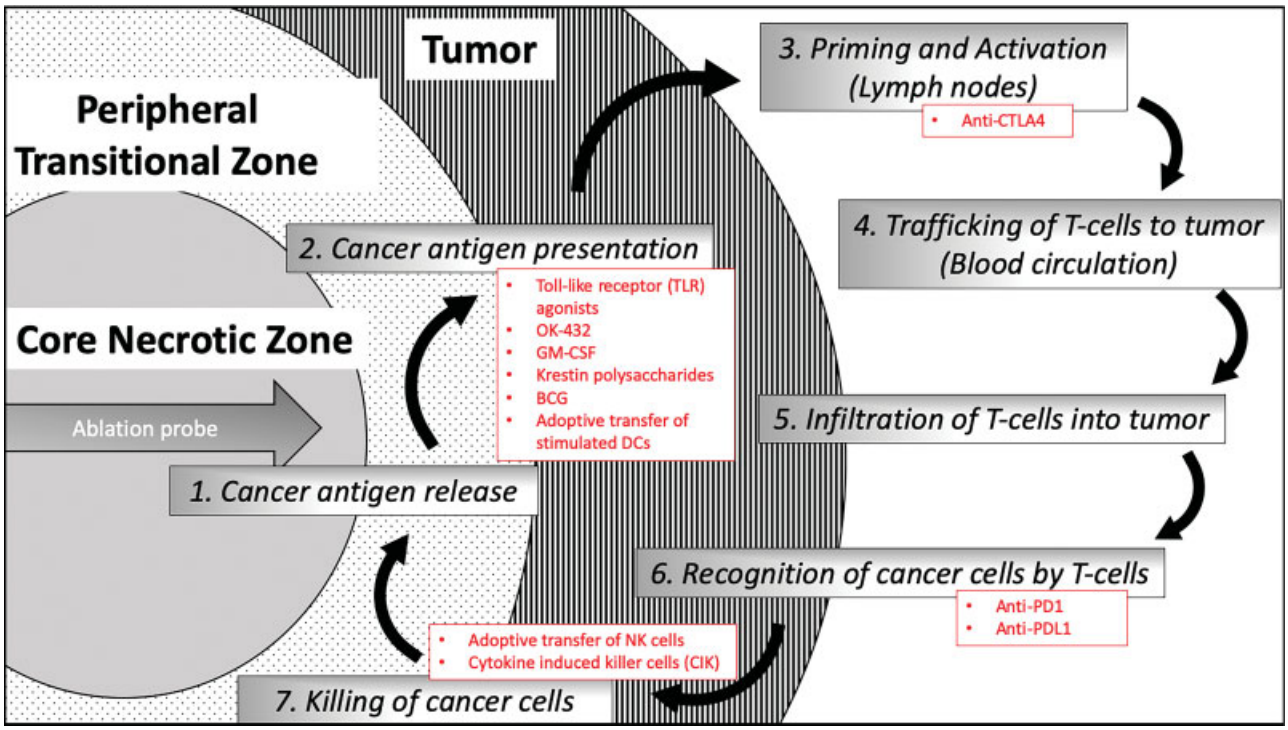

Fig. 1 Postablation immune microenvironment: cancer-immunity cycle. The area of thermal ablation can be divided into three different zones: central necrotic, peripheral transitional, and normal surrounding tissues. Cancer neoantigens released from cellular injury or death will activate the cancer-immunity cycle, generating tumor-specific immune effectors. These activated effector cells travel via systemic circulation and carry out their tumor-killing function when they encounter their specific antigen expressed within the tumor beds. However, there are multiple negative regulatory mechanisms at each step of the cancer-immunity cycle, preventing meaningful and therapeutic anticancer effect provided solely by the immune system. Multiple immunomodulatory strategies have been combined with image-guided locoregional ablation to synergistically shift the equilibrium out of inhibitory immune modulation (red boxes).

cytotoxic T-lymphocytes (CTLs) at tumor-draining lymph nodes (TDLNs). These activated effector cells travel via systemic circulation and carry out their tumor-killing function when they encounter their specific antigen expressed within the tumor beds.

Despite successful initial priming of CTLs,[8,9] subsequent clonal expansion leading to an effector population rarely comes to completion.[7] There are multiple negative regulatory mechanisms at each step of the cancer immunity cycle, preventing meaningful and therapeutic anticancer effects provided solely by the immune system. This can explain anecdotal reports of effective immunotherapy throughout the history of immuno-oncology including original observations by Fehleisen, Busch, and Coley.[3–6,10–12] Additionally, the tumor cell population can escape the immune system's immunoediting, where tumor cells evade immune surveillance, by promoting the selective survival of the immune-resistant antigen cohort.[13,14,22] The goal of cancer immunotherapy is to modulate negative regulatory mechanisms and induce an effective cancer elimination process without creating an overt autoimmune reaction or accelerating the immunoediting process. Recent successes in systemic immune checkpoint inhibitors such as anti-PD1 (programmed cell death protein 1), anti-PDL1 (programmed death ligand 1), and anti-CTLA4 (cytotoxic T-lymphocyte associated protein 4) therapies are examples of these efforts.

Cellular Injury and Immunogenicity of Thermal Ablative Therapy

Currently available ablative therapies, with the exception of IRE, rely on heat-based cellular destruction methods. RFA, MWA, HIFU, and laser ablation induce hyperthermic cellular damage, while cryoablation induces cellular injury by freezing. Regardless of the technique, thermal ablation can be divided into three zones: central necrotic, peripheral transitional, and normal tissue[15–17] (►**Fig. 1**). The central area is characterized by direct and immediate cellular injury achieved by lethal temperatures, that is, greater than 60°C in hyperthermic methods and below −20°C in cryoablation.[15,16] The peripheral or transitional zone is a band-like area of thermal conduction between the central necrotic zone and surrounding normal tissue, characterized by a steep temperature gradient toward normal tissue temperature. Within the peripheral zone, different degrees of cellular injury will result in a mixture of necrotic, delayed/indirect, and reversible injury. This is also the area where most of the immune and inflammatory cellular infiltrates are found.[15,18] Delayed or indirect cellular injury can manifest via many different mechanisms including apoptosis, ischemia–reperfusion, and innate and adaptive immune response.[16]

Cellular necrosis immediately releases immunogenic intracellular contents (such as DNA, RNA, and heat shock proteins [HSPs]) directly into the extracellular matrix. Unlike necrosis,

apoptosis is a much more intrinsic process of cell death and is less immunogenic or inflammatory.[19] The ratio between postablation apoptosis and necrosis may be a key modulator to achieving a maximally immunogenic microenvironment.[15] Some authors also suggest that different immunogenic qualities can be achieved by modifying ablation protocols or parameters.[18,20]

Postablation Inflammation: Immunogenic versus Prooncogenic

All image-guided ablative therapies create in situ tissue damage which induces not only an immunogenic response but also local wound healing and a systemic inflammatory response. Postablation inflammation is well established clinically, with symptomatic treatment generally being applied in a conservative manner. At the molecular level, tissue remodeling upregulates and releases many prooncogenic growth factors and cytokines.[21] There is experimental and clinical evidence of worse outcomes of ablation therapy with accelerated local or off-target tumor progression,[18,22–27] suggesting that a postablation microenvironment was potentially prooncogenic. In animal HCC model, RFA was associated with increased tumor burden and decreased survival compared with partial hepatectomy or sham controls.[27] Furthermore, this prooncogenic effect of RFA was attenuated by postablation systemic inhibition of c-met signaling.[27] In another animal study with breast cancer, RFA of the normal liver was associated with the growth of a distant tumor, which could also be suppressed by c-met inhibition.[22] In a separate study, the same group also reported a tumorigenic effect observed with MWA which was attenuated by high-power, faster MWA protocol.[18]

Several efforts have been made to unfold the enigma of postablation inflammation by characterizing peripheral blood mononuclear cell (PBMC) samples. In a study with 193 patients with CRLM (colorectal liver metastases) treated with RFA, elevated lymphocyte–monocyte ratio (LMR) was identified as a significant negative predictor for recurrence and mortality.[28] Decreased post-RFA neutrophil–lymphocyte ratio (NLR) was associated with improved disease-free survival in 178 small hepatocellular carcinoma (HCC) patients,[29] while increased postablation NLR was associated with local recurrence and metastatic disease in patients with 185 renal cell carcinoma (RCC) patients treated with RFA.[30] Additionally, an increase in the number of interferon gamma (IFN-γ) producing cytotoxic natural killer (NK) cells was associated with longer disease-free survival after RFA in 37 subjects with HCC.[31]

Methods of Ablation and Immune Stimulation

As RFA is the oldest technique, there are many studies in the literature regarding the postablation immune reaction of this technique. Within hours or days after RFA, there is a local and systemic release of proinflammatory cytokines, including IL (interleukin)-1β, IL-1α, IL-6, IL-8, IL-18, and TNFα (tumor necrosis factor α).[21,32–35] Intriguingly, anti-inflammatory cytokines such as IL-10 and transforming growth factor-beta are also shown to be released and upregulated after RFA.[35,36] Some studies have reported contradictory findings regarding the regulation of specific cytokines, reflecting the complex and dynamic nature of the process.[33,36,37] Apart from cytokines, an important immune modulator found in the post-RFA microenvironment is the extracellular heat shock protein (HSP).[38–41] HSP belongs to a group of endogenous molecules and is known as a "danger signal" released by necrotic cells. Extracellular HSP70 is a chaperone molecule facilitating the antigen presentation of APCs to T-cells[42,43] and is upregulated in the tumor microenvironment.[38–41] In a study with 22 posthepatic RFA subjects, serum HSP70 level was significantly elevated within 24 hours and was positively associated with survival benefits and time to disease progression.[44] Although it was first discovered in a post-RFA setting, HSP upregulation and release have also been identified in other ablative modalities.[45–48] Additional danger signal molecules such as high-morbidity group box-1 (HMGB-1) are under investigation regarding their role in postablation immune reaction.[49,50]

Cryoablation has been shown to induce a more robust systemic immune reaction compared with hyperthermic ablative methods in multiple experimental and clinical studies.[21,32,51,52] Furthermore, postablation systemic or remote inflammatory organ injuries, the so-called cryoshock, have been reported after large-volume cryoablation.[53–56] The hyper-immunogenicity of cryoablation is hypothesized to be due to the relative preservation of tumor neoantigens during freezing cell injury compared with hyperthermal ablation techniques. In cryoablation, osmotic changes across intra- and extracellular compartments induce cellular injury without affecting the potential intracellular tumor antigens including DNA, RNA, or proteins. Conversely, hyperthermal ablation denatures and degrades proteins, erasing part of the critical tumor immunogenic footprints.

MWA appears to induce the least magnitude of immune or inflammatory reaction. In the animal liver, a significantly lower level of inflammatory cytokines (IL-1β and IL-6) and HSP70 in peripheral blood samples was observed after MWA compared with RFA or cryoablation.[32,48] In another animal study, the level of proinflammatory cytokines after MWA was similar to that of the surgical resection group that did not have any ablation.[23] Thus, eventually, the low inflammatory characteristic of MWA enables it to achieve a successful larger volume hepatic ablation without collateral inflammatory remote organ injuries.[48,57] Nevertheless, despite these reproducible observations, the exact mechanism for minimal immune or inflammatory response after MWA remains unknown.

HIFU destroys cells mechanically by acoustic cavitation and histotripsy.[58–60] Acoustic cavitation results from the repeated growth and collapse of micro gas bubbles within the tissue under the influence of high-pressure sound waves. Mechanical fractionation combined with a boiling process enables histotripsy which eventually generates an emulsified acellular homogenate surrounded by very thin, less than 1 mm, margins, with minimal fibrosis.[58–60] This liquefied lesion is absorbed rapidly by the surrounding tissue and easily infiltrated by immune cells. In a clinical study with 48

breast cancer subjects, HIFU-treated lesions were removed by mastectomy and demonstrated markedly increased immune cell infiltrates compared with surgical specimens not treated with HIFU.[61]

Laser ablation has had limited use in tumors and little data are available on its immune stimulation. Similar to other hyperthermal techniques, a postablative increase of proinflammatory cytokines in peripheral blood has been observed.[62] In preclinical animal experiments, tumor-specific immune cell generation has also been observed after laser ablation.[63,64]

IRE is the newest ablation technique and does not incorporate thermal energy. IRE selectively disrupts the lipid bilayer cellular membrane via the electrical induction of permanent nanopores. Preliminary microscopic studies in animal samples treated with IRE demonstrated minimal damage in extracellular structures as well as minimal fibrotic or inflammatory margins.[65] In an osteosarcoma animal model, IRE generated increased peripheral T-lymphocytes compared with surgical resection.[66] In a recent study employing an animal pancreatic cancer model, IRE exhibited a twofold increase in CD3+ T cell infiltrates compared with cryoablation.[67]

In summary, non-hyperthermal ablation modalities such as cryoablation, IRE, and HIFU appear to generate a more robust immune response compared with hyperthermal modalities, possibly to the greatest degree with IRE. Regardless of ablative techniques, it is clear that proinflammatory cytokines and the danger molecule signaling pathway are activated in the immediate postablative period. The remaining and important question is if this translates into a therapeutically immunogenic or prooncogenic environment and which factors modulate the balance between them.

Investigating the postablation immune response is a challenging process. With varying ablation devices, heterogeneous parameters and protocols at each center, and various types of tumors treated, it is difficult to perform a standardized multicenter trial or to compare studies across centers or countries. Investigations are further challenged by the varying baseline individual immune function at the time of treatment. No optimal time point for sampling or quantifying the immune response has been determined. Finally, investigations may require additional invasive sampling of the treated area. Hence, to date, most investigators have chosen to look at the systemic immune response based on PBMCs as discussed earlier,[28–31] and immunopositron emission tomography has been investigated for the possible noninvasive monitoring of immune response.[68,69]

Combination Therapy: Ablation and Immunotherapy

It is established that a tumor-specific immune-stimulatory effect is generated after image-guided locoregional ablation. However, given that this rarely translates into robust effector clonal expansion or tumor killing, many recent studies have focused on the introduction and testing of adjuvant immunotherapy combined with ablation to synergistically shift the equilibrium out of inhibitory immune modulation (►**Fig. 1**).

Ablation combined with immune checkpoint inhibitors has demonstrated a potential positive synergistic effect on tumors elsewhere in the body in multiple studies (►**Table 1**). In animal studies, the combination of anti-CTLA-4 and cryoablation[70] or anti-PD1 and RFA[71] slowed tumor growth and increased effector T cell infiltration of distant tumors. In a pilot clinical study in 18 metastatic breast cancer patients, cryoablation combined with anti-CTLA-4 (ipilimumab) revealed a sustained increase of IFN-γ, effector T cells, and the ratio of effector T cells to regulatory T cells (Treg) in peripheral blood samples.[72] In another pilot study,[73] 12 Barcelona clinic liver cancer stage C patients with metastatic HCC who failed sorafenib treatment underwent percutaneous RFA and chemoablation therapy combined with anti-CTLA-4 (tremelimumab). The investigators of that study reported that the patients had 7.4 months median time to progression and 10.1 months median overall survival without any severe treatment-related toxicity. The patients also had favorable objective treatment responses with increased active CD8+ CTL infiltration in the distant untreated lesion. Additionally, case reports have described the successful treatment of colorectal lung metastasis using MWA/anti-PD-1 (pembrolizumab)[74] and metastatic clear cell RCC using cryoablation/anti-PD-1 (nivolumab) combinations.[75]

Positive modulation of the antigen-presentation process by dendritic cells (DCs) has been combined with ablation, showing promising results. In animal studies combining intratumoral injection of activated DC with RFA[76] or cryoablation,[77] significant growth inhibition on distal untreated tumors and increased effector immune cell infiltrates were demonstrated. Similarly, in animal studies combining RFA or cryoablation with intratumoral stimulation of native DC by using toll-like receptor (TLR) agonists,[78–80] OK-432,[81] granulocyte-macrophage colony-stimulating factor (GM-CSF),[82] krestin polysaccharide,[83] and BCG,[84] a favorable response in untreated tumor burden was demonstrated. Some of these preclinical models were successfully reproduced in a few clinical studies. Thakur et al[85] and Si et al[86] confirmed robust tumor-specific CTL function in untreated tumors in patients with metastatic RCC and prostate cancer when treated with cryoablation and GM-CSF injection. Niu et al retrospectively reviewed 106 patients with metastatic pancreatic cancer[87] and demonstrated significantly improved median survival when cryoablation was combined with adoptive transfer of GM-CSF stimulated DCs compared with cryoablation only, immunotherapy only, or chemotherapy only (13, 7, 5, and 3.5 months, respectively). The authors applied the same strategy in 45 patients with metastatic HCC,[88] revealing significantly improved overall survival with cryo-immunotherapy compared with cryoablation only (32 vs. 17.5 months).[88]

NK cells are cytotoxic effector lymphocytes within the innate immune system. They play an important role in cancer immune surveillance and are able to induce rapid immune responses against malignant cells in an antigen-independent manner.[89] Adoptive transfer of allogenic NK cells has been combined with ablative therapies in preclinical and clinical studies, demonstrating potential efficacy and safety. In a pilot clinical study of cryoablation and NK cell therapy in 48 patients with treatment-resistant metastatic breast cancer,

Table 1 Currently available human clinical data on the combination of immunomodulation and ablation as of June 2019

Methods of immunomodulation	Studies	Phase	Immunomodulators	Ablative methods	Disease (no. of subjects)	Impact of combinatory therapy
Immune checkpoint inhibitors	McArthur et al[72]	I, II	Ipilimumab (anti-CTLA4)	Cryoablation	Breast cancer (19)	↑ IFN-γ, ↑ effector T cells,↑ effector T cells/regulatory T cells ratio in peripheral blood
	Duffy et al[73]	I, II	Tremelimumab (anti-CTLA4)	RFA + chemoablation	HCC (12)	7.4-mo median time to disease progression, 10.1-mo median overall survival, ↑ in-situ CD8+ cells in treatment responders
	Bäcklund and Freedman[74]	Case report	Pembrolizumab (anti-PD1)	MWA	Colorectal lung metastasis (1)	Complete response at 8 mo
	Soule et al[75]	Case report	Nivolumab (anti-PD1)	Cryoablation	Renal cell carcinoma (1)	Decreased size and FDG uptake in osseous metastatic disease in 1 mo
Intratumoral injection of GM-CSF	Thakur et al[85]	I, II	GM-CSF	Cryoablation	Renal cell carcinoma (6)	↑ Tumor-specific IFN-γ (+) effector T cells, ↑ Th1/Th2 ratio in peripheral blood of treatment responders
	Si et al[86]	I, II			Prostate cancer (12)	↑ Tumor-specific effector T cells in peripheral blood
Adoptive transfer of activated allogenic immune cells	Niu et al[87]	Observational	GM-CSF stimulated allogenic DCs	Cryoablation	Pancreatic cancer (106)	↑ Median overall survival with combinatory treatment
	Niu et al[88]	Observational			HCC (45)	↑ Median overall survival with combinatory treatment
	Liang et al[90]	I, II	Stimulated allogenic NK cells	Cryoablation	Breast cancer (48)	↑ IFN-γ, ↑ effector T cells in peripheral blood with combinatory treatment
	Lin et al[91]	I, II		Cryoablation	Renal cell cancer (60)	↑ Th1 cytokines, ↑ effector T cells in peripheral blood with combinatory treatment
	Lin et al[92]	I, II		Cryoablation	NSCLC (60)	↑ Th1 cytokines, ↑ effector T cells in peripheral blood with combinatory treatment
	Yang et al[93]	I, II		IRE	Primary liver cancer (40)	↑ Overall survival, ↑ progression-free survival, ↑ Th1 cytokines, ↑ effector T cells in peripheral blood with combinatory treatment

Abbreviations: DCs, dendritic cells; FDG, fluorodeoxyglucose; GM-CSF, granulocyte-macrophage colony-stimulating factor; HCC, hepatocellular carcinoma; IFN, interferon; IRE, irreversible electroporation; MWA, microwave ablation; NK, natural killer; NSCLC, non-small cell lung cancer; RFA, radiofrequency ablation.

increased immune effector T cells and Th-1-type cytokines in the peripheral blood was demonstrated in the combinatory treatment group ($n = 16$), although the increment in progression-free survival was not statistically significant compared with the cryoablation-only group ($n = 16$).[90] Of note, this study also included a triple-combination group ($n = 16$, cryoablation, allogenic NK cells, trastuzumab) demonstrating a significant increase in immune response and progression-free survival compared with the aforementioned two groups. Two prospective clinical studies combining cryoablation with allogenic NK cell immunotherapy of metastatic RCC ($n = 60$)[91] and non-small cell lung cancer ($n = 60$)[92] also revealed enhanced effector immune cell quantity as well as Th-1–type cytokines in the peripheral blood. However, these studies did not report any clinical outcomes regarding progression-free survival or regression of distant untreated tumor. Lastly, one study using a combination of IRE and allogenic NK cell therapy for primary liver cancer patients demonstrated survival benefits and increased immune response compared with IRE therapy only.[93]

Future Directions

As of June 2019, there are 22 ongoing or planned clinical trials for various combinatory regimens of locoregional ablative therapy and immunotherapy registered in the *clinicaltrials.gov* database (►**Table 2**). Additionally, there are also trials investigating the efficacy and safety of immunotherapy combined with other locoregional IO therapies such as chemoembolization or radioembolization. In addition to these preliminary outcome-based studies, there are many important questions to be answered.[94] Most importantly, the timing, sequence, number, and type of combinatory therapy should be determined to optimize the synergistic efficacy.

There is a great potential in the combination of locoregional therapy and immunotherapy. The synergy between interventional radiology and immunooncology with their different philosophies and approaches will enhance our understanding of cancer biology in general. Many innovative, minimally invasive locoregional IO therapies have been developed, demonstrating similar safety and efficacy profiles between them albeit the great heterogeneity in techniques and application.

Table 2 Locoregional ablative therapy combined with immunotherapy: ongoing and planned registered trials as of June 2019

Trial no.	Ablative modality	Immunotherapy	Disease (no. of subjects)	Phase	Expected study completion dates (mm/yyyy)
NCT02851784	MWA	CIK cells	Hepatocellular carcinoma (50)	II, III	12/2017
NCT03101475	SBRT or RFA	Durvalumab (anti-PDL1) + Tremelimumab (anti-CTLA4)	Colorectal liver metastasis (70)	II	01/2023
NCT03695835	Cryoablation or RFA	Anti-PD1 + anti-CTLA4 + GM-CSF	Prostate adenocarcinoma (18)	Observational	12/2025
NCT03864211	MWA or RFA	Toriplimab (anti-PD1)	Hepatocellular carcinoma (120)	I, II	03/2021
NCT03753659	MWA or RFA	Pembrolizumab (anit-PD1)	Hepatocellular carcinoma (30)	II	09/2022
NCT02678013	RFA	CIK cells	Hepatocellular carcinoma (210)	III	01/2022
NCT02849366	Cryoablation	NK cells	Soft-tissue sarcoma (30)	I, II	07/2019
NCT02849379	Cryoablation	NK cells	Tongue cancer (30)	I, II	07/2019
NCT02849353	Cryoablation	NK cells	Ovarian cancer (30)	I, II	07/2019
NCT02849340	Cryoablation	NK cells	Cervical cancer (30)	I, II	07/2019
NCT02849314	Cryoablation	NK cells	Laryngeal cancer (30)	I, II	07/2019
NCT02849327	Cryoablation	NK cells	Hepatic metastatic disease (30)	I, II	07/2019
NCT02844335	Cryoablation	NK cells	Breast cancer (30)	I, II	07/2019
NCT02843607	Cryoablation	NK cells	Renal cell carcinoma (30)	I, II	07/2019
NCT02843815	Cryoablation	NK cells	Non-small cell lung cancer (30)	I, II	07/2019
NCT02849015	Cryoablation	NK cells	Primary liver tumor (10)	I, II	07/2018
NCT01853618	Cryoablation or RFA or TACE	Tremelimumab (anti-CTLA4)	Primary liver tumor (61)	I, II	12/2020
NCT03008343	IRE	NK cells	Primary liver tumor (20)	I, II	12/2019
NCT00891475	RFA	Sunitinib (tyrosine kinase inhibitor) or IFN-α	Renal cell carcinoma (114)	I, II	01/2011
NCT03949153	Cryoablation	Ipilimumab (anti-CTLA4)	Cutaneous melanoma (15)	I, II	12/2021
NCT03939975	RFA or MWA	Pembrolizumab or nivolumab or JS001 (anti-PD1)	Hepatocellular carcinoma (50)	II	05/2023
NCT03237572	HIFU	Pembrolizumab (anti-PD1)	Breast cancer (15)	I	11/2021

Abbreviations: CIK, cytokine-induced killer; HIFU, high intensity focused ultrasound; IFN, interferon; IRE, irreversible electroporation; MWA, microwave ablation; NK, natural killer; RFA, radiofrequency ablation; TACE, transarterial chemoembolization.

Further understanding of the tumor immune microenvironment may also shed light on mechanisms to differentiate and tailor IO therapies toward more personalized cancer therapy.

Conclusion

Locoregional tumor ablative techniques induce tumor-derived immunogenic activation at local and systemic levels, which may ultimately translate into beneficial therapeutic effects in local and remote tumors. To overcome the body's innate negative immune modulation in the cancer-immunity cycle and to optimize the immunogenic response, ablative therapy has been combined with various immunotherapy regimens, resulting in promising outcomes, although these findings remain preliminary. Understanding the tumor microenvironment will help advance our knowledge of existing IO procedures, as well as aid in the development of innovative, personalized oncologic care.

Disclosure Statement
The authors declare that they have no conflicts of interest.

Source of Funding
This article was funded in part through the NIH/NCI Cancer Center Support Grant P30 CA008748.

References

1 Mole RH. Whole body irradiation; radiobiology or medicine? Br J Radiol 1953;26(305):234–241
2 Waitz R, Solomon SB. Can local radiofrequency ablation of tumors generate systemic immunity against metastatic disease? Radiology 2009;251(01):1–2
3 Kim H, Park BK, Kim CK. Spontaneous regression of pulmonary and adrenal metastases following percutaneous radiofrequency ablation of a recurrent renal cell carcinoma. Korean J Radiol 2008; 9(05):470–472
4 Rao P, Escudier B, de Baere T. Spontaneous regression of multiple pulmonary metastases after radiofrequency ablation of a single metastasis. Cardiovasc Intervent Radiol 2011;34(02):424–430
5 Sánchez-Ortiz RF, Tannir N, Ahrar K, Wood CG. Spontaneous regression of pulmonary metastases from renal cell carcinoma after radio frequency ablation of primary tumor: an in situ tumor vaccine? J Urol 2003;170(01):178–179
6 Soanes WA, Ablin RJ, Gonder MJ. Remission of metastatic lesions following cryosurgery in prostatic cancer: immunologic considerations. J Urol 1970;104(01):154–159
7 Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. Immunity 2013;39(01):1–10
8 Zerbini A, Pilli M, Penna A, et al. Radiofrequency thermal ablation of hepatocellular carcinoma liver nodules can activate and enhance tumor-specific T-cell responses. Cancer Res 2006;66 (02):1139–1146
9 Hansler J, Wissniowski TT, Schuppan D, et al. Activation and dramatically increased cytolytic activity of tumor specific T lymphocytes after radio-frequency ablation in patients with hepatocellular carcinoma and colorectal liver metastases. World J Gastroenterol 2006;12(23):3716–3721
10 Fehleisen F. Ueber die Züchtung der Erysipelkokken auf künstlichem Nährboden und ihre Uebertragbarkeit auf den Menschen. Dtsch Med Wochenschr 1882;8(31):553–554
11 Busch W. Aus der Sitzung der medicinischen Section vom 13 November 1867. Berlin Klin Wochenschr. 1868;5:137
12 Coley WB. The treatment of malignant tumors by repeated inoculations of erysipelas. With a report of ten original cases. 1893. Clin Orthop Relat Res 1991;(262):3–11
13 Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 2002;3(11):991–998
14 Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science 2011;331(6024):1565–1570
15 Chu KF, Dupuy DE. Thermal ablation of tumours: biological mechanisms and advances in therapy. Nat Rev Cancer 2014;14 (03):199–208
16 Nikfarjam M, Muralidharan V, Christophi C. Mechanisms of focal heat destruction of liver tumors. J Surg Res 2005;127(02):208–223
17 Ahmed M, Brace CL, Lee FT Jr, Goldberg SN. Principles of and advances in percutaneous ablation. Radiology 2011;258(02):351–369
18 Velez E, Goldberg SN, Kumar G, et al. Hepatic thermal ablation: effect of device and heating parameters on local tissue reactions and distant tumor growth. Radiology 2016;281(03):782–792
19 Basu S, Binder RJ, Suto R, Anderson KM, Srivastava PK. Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-kappa B pathway. Int Immunol 2000;12(11):1539–1546
20 Sabel MS, Su G, Griffith KA, Chang AE. Rate of freeze alters the immunologic response after cryoablation of breast cancer. Ann Surg Oncol 2010;17(04):1187–1193
21 Erinjeri JP, Thomas CT, Samoilia A, et al. Image-guided thermal ablation of tumors increases the plasma level of interleukin-6 and interleukin-10. J Vasc Interv Radiol 2013;24(08):1105–1112
22 Ahmed M, Kumar G, Moussa M, et al. Hepatic radiofrequency ablation-induced stimulation of distant tumor growth is suppressed by c-Met inhibition. Radiology 2016;279(01):103–117
23 Kang TW, Lim HK, Lee MW, et al. Aggressive intrasegmental recurrence of hepatocellular carcinoma after radiofrequency ablation: risk factors and clinical significance. Radiology 2015; 276(01):274–285
24 Nikfarjam M, Muralidharan V, Christophi C. Altered growth patterns of colorectal liver metastases after thermal ablation. Surgery 2006;139(01):73–81
25 Kang TW, Kim JM, Rhim H, et al. Small hepatocellular carcinoma: radiofrequency ablation versus nonanatomic resection–propensity score analyses of long-term outcomes. Radiology 2015;275 (03):908–919
26 Lee DH, Lee JM, Lee JY, Kim SH, Han JK, Choi BI. Radiofrequency ablation for intrahepatic recurrent hepatocellular carcinoma: long-term results and prognostic factors in 168 patients with cirrhosis. Cardiovasc Intervent Radiol 2014;37(03):705–715
27 Rozenblum N, Zeira E, Scaiewicz V, et al. Oncogenesis: an "off-target" effect of radiofrequency ablation. Radiology 2015;276(02): 426–432
28 Facciorusso A, Del Prete V, Crucinio N, Serviddio G, Vendemiale G, Muscatiello N. Lymphocyte-to-monocyte ratio predicts survival after radiofrequency ablation for colorectal liver metastases. World J Gastroenterol 2016;22(16):4211–4218
29 Dan J, Zhang Y, Peng Z, et al. Postoperative neutrophil-to-lymphocyte ratio change predicts survival of patients with small hepatocellular carcinoma undergoing radiofrequency ablation. PLoS One 2013;8(03):e58184
30 Chang X, Zhang F, Liu T, Wang W, Guo H. Neutrophil-to-lymphocyte ratio as an independent predictor for survival in patients with localized clear cell renal cell carcinoma after radiofrequency ablation: a propensity score matching analysis. Int Urol Nephrol 2017;49(06):967–974
31 Zerbini A, Pilli M, Laccabue D, et al. Radiofrequency thermal ablation for hepatocellular carcinoma stimulates autologous NK-cell response. Gastroenterology 2010;138(05):1931–1942
32 Ahmad F, Gravante G, Bhardwaj N, et al. Changes in interleukin-1β and 6 after hepatic microwave tissue ablation compared with

radiofrequency, cryotherapy and surgical resections. Am J Surg 2010;200(04):500–506
33 Rozenblum N, Zeira E, Bulvik B, et al. Radiofrequency ablation: inflammatory changes in the periablative zone can induce global organ effects, including liver regeneration. Radiology 2015;276 (02):416–425
34 Evrard S, Menetrier-Caux C, Biota C, et al. Cytokines pattern after surgical radiofrequency ablation of liver colorectal metastases. Gastroenterol Clin Biol 2007;31(02):141–145
35 Fietta AM, Morosini M, Passadore I, et al. Systemic inflammatory response and downmodulation of peripheral CD25+Foxp3+ T-regulatory cells in patients undergoing radiofrequency thermal ablation for lung cancer. Hum Immunol 2009;70(07):477–486
36 Iwahashi S, Shimada M, Utsunomiya T, et al. Epithelial-mesenchymal transition-related genes are linked to aggressive local recurrence of hepatocellular carcinoma after radiofrequency ablation. Cancer Lett 2016;375(01):47–50
37 Schell SR, Wessels FJ, Abouhamze A, Moldawer LL, Copeland EM III. Pro- and antiinflammatory cytokine production after radiofrequency ablation of unresectable hepatic tumors. J Am Coll Surg 2002;195(06):774–781
38 Schueller G, Kettenbach J, Sedivy R, et al. Heat shock protein expression induced by percutaneous radiofrequency ablation of hepatocellular carcinoma in vivo. Int J Oncol 2004;24(03):609–613
39 Rai R, Richardson C, Flecknell P, Robertson H, Burt A, Manas DM. Study of apoptosis and heat shock protein (HSP) expression in hepatocytes following radiofrequency ablation (RFA). J Surg Res 2005;129(01):147–151
40 Yang WL, Nair DG, Makizumi R, et al. Heat shock protein 70 is induced in mouse human colon tumor xenografts after sublethal radiofrequency ablation. Ann Surg Oncol 2004;11(04):399–406
41 Figueiredo C, Wittmann M, Wang D, et al. Heat shock protein 70 (HSP70) induces cytotoxicity of T-helper cells. Blood 2009;113 (13):3008–3016
42 Chen T, Guo J, Han C, Yang M, Cao X. Heat shock protein 70, released from heat-stressed tumor cells, initiates antitumor immunity by inducing tumor cell chemokine production and activating dendritic cells via TLR4 pathway. J Immunol 2009;182(03):1449–1459
43 den Brok MH, Sutmuller RP, Nierkens S, et al. Efficient loading of dendritic cells following cryo and radiofrequency ablation in combination with immune modulation induces anti-tumour immunity. Br J Cancer 2006;95(07):896–905
44 Haen SP, Gouttefangeas C, Schmidt D, et al. Elevated serum levels of heat shock protein 70 can be detected after radiofrequency ablation. Cell Stress Chaperones 2011;16(05):495–504
45 Nikfarjam M, Muralidharan V, Su K, Malcontenti-Wilson C, Christophi C. Patterns of heat shock protein (HSP70) expression and Kupffer cell activity following thermal ablation of liver and colorectal liver metastases. Int J Hyperthermia 2005;21(04):319–332
46 Kroeze SG, van Melick HH, Nijkamp MW, et al. Incomplete thermal ablation stimulates proliferation of residual renal carcinoma cells in a translational murine model. BJU Int 2012;110(6, Pt B):E281–E286
47 Hundt W, O'Connell-Rodwell CE, Bednarski MD, Steinbach S, Guccione S. In vitro effect of focused ultrasound or thermal stress on HSP70 expression and cell viability in three tumor cell lines. Acad Radiol 2007;14(07):859–870
48 Ahmad F, Gravante G, Bhardwaj N, et al. Renal effects of microwave ablation compared with radiofrequency, cryotherapy and surgical resection at different volumes of the liver treated. Liver Int 2010;30(09):1305–1314
49 Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature 2002;418 (6894):191–195
50 Klune JR, Dhupar R, Cardinal J, Billiar TR, Tsung A. HMGB1: endogenous danger signaling. Mol Med 2008;14(7-8):476–484
51 Ravindranath MH, Wood TF, Soh D, et al. Cryosurgical ablation of liver tumors in colon cancer patients increases the serum total ganglioside level and then selectively augments antiganglioside IgM. Cryobiology 2002;45(01):10–21
52 Jansen MC, van Hillegersberg R, Schoots IG, et al. Cryoablation induces greater inflammatory and coagulative responses than radiofrequency ablation or laser induced thermotherapy in a rat liver model. Surgery 2010;147(05):686–695
53 Seifert JK, Morris DL. World survey on the complications of hepatic and prostate cryotherapy. World J Surg 1999;23(02): 109–113, discussion 113–114
54 Washington K, Debelak JP, Gobbell C, et al. Hepatic cryoablation-induced acute lung injury: histopathologic findings. J Surg Res 2001;95(01):1–7
55 Chapman WC, Debelak JP, Wright Pinson C, et al. Hepatic cryoablation, but not radiofrequency ablation, results in lung inflammation. Ann Surg 2000;231(05):752–761
56 Seifert JK, France MP, Zhao J, et al. Large volume hepatic freezing: association with significant release of the cytokines interleukin-6 and tumor necrosis factor a in a rat model. World J Surg 2002;26 (11):1333–1341
57 Ahmad F, Gravante G, Bhardwaj N, et al. Large volume hepatic microwave ablation elicits fewer pulmonary changes than radiofrequency or cryotherapy. J Gastrointest Surg 2010;14(12): 1963–1968
58 Mauri G, Nicosia L, Xu Z, et al. Focused ultrasound: tumour ablation and its potential to enhance immunological therapy to cancer. Br J Radiol 2018;91(1083):20170641
59 Roberts WW, Hall TL, Ives K, Wolf JS Jr, Fowlkes JB, Cain CA. Pulsed cavitational ultrasound: a noninvasive technology for controlled tissue ablation (histotripsy) in the rabbit kidney. J Urol 2006;175 (02):734–738
60 Copelan A, Hartman J, Chehab M, Venkatesan AM. High-intensity focused ultrasound: current status for image-guided therapy. Semin Intervent Radiol 2015;32(04):398–415
61 Lu P, Zhu XQ, Xu ZL, Zhou Q, Zhang J, Wu F. Increased infiltration of activated tumor-infiltrating lymphocytes after high intensity focused ultrasound ablation of human breast cancer. Surgery 2009;145(03):286–293
62 Kallio R, Sequeiros R, Surcel HM, Ohtonen P, Kiviniemi H, Syrjälä H. Early cytokine responses after percutaneous magnetic resonance imaging guided laser thermoablation of malignant liver tumors. Cytokine 2006;34(5-6):278–283
63 Lin WX, Fifis T, Malcontenti-Wilson C, et al. Induction of Th1 immune responses following laser ablation in a murine model of colorectal liver metastases. J Transl Med 2011;9:83
64 Vogl TJ, Wissniowski TT, Naguib NN, et al. Activation of tumor-specific T lymphocytes after laser-induced thermotherapy in patients with colorectal liver metastases. Cancer Immunol Immunother 2009;58(10):1557–1563
65 Rubinsky B, Onik G, Mikus P. Irreversible electroporation: a new ablation modality–clinical implications. Technol Cancer Res Treat 2007;6(01):37–48
66 Li X, Xu K, Li W, et al. Immunologic response to tumor ablation with irreversible electroporation. PLoS One 2012;7(11):e48749
67 White SB, Zhang Z, Chen J, Gogineni VR, Larson AC. Early immunologic response of irreversible electroporation versus cryoablation in a rodent model of pancreatic cancer. J Vasc Interv Radiol 2018;29(12):1764–1769
68 Wei W, Jiang D, Ehlerding EB, Luo Q, Cai W. Noninvasive PET imaging of T cells. Trends Cancer 2018;4(05):359–373
69 Larimer BM, Wehrenberg-Klee E, Dubois F, et al. Granzyme B PET imaging as a predictive biomarker of immunotherapy response. Cancer Res 2017;77(09):2318–2327
70 Waitz R, Solomon SB, Petre EN, et al. Potent induction of tumor immunity by combining tumor cryoablation with anti-CTLA-4 therapy. Cancer Res 2012;72(02):430–439
71 Shi L, Chen L, Wu C, et al. PD-1 blockade boosts radiofrequency ablation-elicited adaptive immune responses against tumor. Clin Cancer Res 2016;22(05):1173–1184

72 McArthur HL, Diab A, Page DB, et al. A pilot study of preoperative single-dose ipilimumab and/or cryoablation in women with early-stage breast cancer with comprehensive immune profiling. Clin Cancer Res 2016;22(23):5729–5737
73 Duffy AG, Ulahannan SV, Makorova-Rusher O, et al. Tremelimumab in combination with ablation in patients with advanced hepatocellular carcinoma. J Hepatol 2017;66(03):545–551
74 Bäcklund M, Freedman J. Microwave ablation and immune activation in the treatment of recurrent colorectal lung metastases: a case report. Case Rep Oncol 2017;10(01):383–387
75 Soule E, Bandyk M, Matteo J. Percutaneous ablative cryoimmunotherapy for micrometastatic abscopal effect: no complications. Cryobiology 2018;82:22–26
76 Nakagawa H, Mizukoshi E, Iida N, et al. In vivo immunological antitumor effect of OK-432-stimulated dendritic cell transfer after radiofrequency ablation. Cancer Immunol Immunother 2014;63(04):347–356
77 Machlenkin A, Goldberger O, Tirosh B, et al. Combined dendritic cell cryotherapy of tumor induces systemic antimetastatic immunity. Clin Cancer Res 2005;11(13):4955–4961
78 Chavez M, Silvestrini MT, Ingham ES, et al. Distinct immune signatures in directly treated and distant tumors result from TLR adjuvants and focal ablation. Theranostics 2018;8(13):3611–3628
79 den Brok MH, Sutmuller RP, Nierkens S, et al. Synergy between in situ cryoablation and TLR9 stimulation results in a highly effective in vivo dendritic cell vaccine. Cancer Res 2006;66(14):7285–7292
80 Redondo P, del Olmo J, López-Diaz de Cerio A, et al. Imiquimod enhances the systemic immunity attained by local cryosurgery destruction of melanoma lesions. J Invest Dermatol 2007;127 (07):1673–1680
81 Hamamoto S, Okuma T, Yamamoto A, et al. Radiofrequency ablation and immunostimulant OK-432: combination therapy enhances systemic antitumor immunity for treatment of VX2 lung tumors in rabbits. Radiology 2013;267(02):405–413
82 Xu H, Wang Q, Lin C, et al. Synergism between cryoablation and GM-CSF: enhanced immune function of splenic dendritic cells in mice with glioma. Neuroreport 2015;26(06):346–353
83 Urano M, Tanaka C, Sugiyama Y, Miya K, Saji S. Antitumor effects of residual tumor after cryoablation: the combined effect of residual tumor and a protein-bound polysaccharide on multiple liver metastases in a murine model. Cryobiology 2003;46(03):238–245
84 Hamamoto S, Okuma T, Yamamoto A, et al. Combination radiofrequency ablation and local injection of the immunostimulant bacillus Calmette-Guérin induces antitumor immunity in the lung and at a distant VX2 tumor in a rabbit model. J Vasc Interv Radiol 2015;26(02):271–278
85 Thakur A, Littrup P, Paul EN, Adam B, Heilbrun LK, Lum LG. Induction of specific cellular and humoral responses against renal cell carcinoma after combination therapy with cryoablation and granulocyte-macrophage colony stimulating factor: a pilot study. J Immunother 2011;34(05):457–467
86 Si T, Guo Z, Hao X. Combined cryoablation and GM-CSF treatment for metastatic hormone refractory prostate cancer. J Immunother 2009;32(01):86–91
87 Niu L, Chen J, He L, et al. Combination treatment with comprehensive cryoablation and immunotherapy in metastatic pancreatic cancer. Pancreas 2013;42(07):1143–1149
88 Niu LZ, Li JL, Zeng JY, et al. Combination treatment with comprehensive cryoablation and immunotherapy in metastatic hepatocellular cancer. World J Gastroenterol 2013;19(22):3473–3480
89 Fang F, Xiao W, Tian Z. NK cell-based immunotherapy for cancer. Semin Immunol 2017;31:37–54
90 Liang S, Niu L, Xu K, et al. Tumor cryoablation in combination with natural killer cells therapy and Herceptin in patients with HER2-overexpressing recurrent breast cancer. Mol Immunol 2017; 92:45–53
91 Lin M, Xu K, Liang S, et al. Prospective study of percutaneous cryoablation combined with allogenic NK cell immunotherapy for advanced renal cell cancer. Immunol Lett 2017;184:98–104
92 Lin M, Liang SZ, Wang XH, et al. Clinical efficacy of percutaneous cryoablation combined with allogenic NK cell immunotherapy for advanced non-small cell lung cancer. Immunol Res 2017;65(04): 880–887
93 Yang Y, Qin Z, Du D, et al. Safety and short-term efficacy of irreversible electroporation and allogenic natural killer cell immunotherapy combination in the treatment of patients with unresectable primary liver cancer. Cardiovasc Intervent Radiol 2019;42(01):48–59
94 Erinjeri JP, Fine GC, Adema GJ, et al. Immunotherapy and the interventional oncologist: challenges and opportunities—a society of interventional oncology white paper. Radiology 2019;292 (01):25–34