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REVIEW

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History and evolution of immunotherapeutic effect of cryoablation of solid tumors

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Abstract

Cryoablation as a locoregional treatment for cancer has seen great advances over the past century. As a local thermal ablative method, cryoablation is unique in that it preserves tumor associated antigens, releasing them into the circulation and allowing the immune system to mount a targeted, systemic anti-cancer response in a phenomenon known as the abscopal effect. Advances in our understanding of tumor biology and cancer immunology have simultaneously led to the development of various anti-cancer immunotherapies. Whether through stimulating an immune response or inhibiting immunosuppression, these therapies have demonstrated efficacy in the treatment of numerous cancers and represent a new frontier in cancer treatment. In recent years, several pre-clinical and clinical studies have sought to elucidate the potential synergy between combination immunotherapy and cryoablation with the hope of augmenting the abscopal effect. This emerging field of "cryoimmune" therapy, although still in its infancy, represents an exciting new direction in cancer treatment.

Keywords: *immunotherapy; cryoablation; abscopal effect*

Introduction

Cancer is becoming increasingly prevalent and in 2018, there were 18.1 million new cases of cancer and 9.6 million cancer-related deaths worldwide [1]. In recent years, the complex role that the immune system plays in cancer prevention, development and progression has been elucidated, bringing to light the concept of cancer immunoediting. Cancer immunoediting posits that the immune system can both constrain and promote tumor development through three phases termed elimination, equilibrium and escape (Figure 1) [2]. In the elimination phase, the innate and adaptive immune system can recognize and eliminate transformed cells or developing tumors, potentially halting tumor progression. Subclones of cells that are able to escape the elimination phase can then progress to the equilibrium phase in which tumors remain dormant and overall growth is limited due to competing immune mechanisms. Over time, however, as the tumor cells undergo constant immune selection with increasing genetic instability, they may enter the escape phase, leading to unrestrained growth and overt clinical disease. Factors that contribute to the escape phase transition include (but are not limited to): 1. loss of antigen processing or presentation mechanisms including the major histocompatibility complex (MHC) I pathway, among others, 2. an increasingly suppressive tumor microenvironment mediated by CD4+CD25+FoxP3+ T cells (Tregs) and other suppressive cells or cytokines, and 3. T cell anergy or tolerance through failure to express costimulatory molecules or through the upregulation of inhibitory molecules such as programmed death ligand (PD)-L1, among others [2-4]. In recent years, our understanding of these inhibitory pathways (PD-1/PD-L1 and CTLA-4), in addition to our understanding of tumor biology and immunology in general, have led to the development of immune checkpoint inhibitors and other anti-cancer immunotherapies that have significantly altered treatment paradigms [5,6].

This transition largely occurs because of activation of immunosuppressive or immunoevasive pathways. Patients in the escape phase undergoing immunotherapy can undergo a complete response, in which case the immune state would revert back to the elimination phase, or patients may experience a partial response where immunotherapy may fail to completely overcome immunosuppressive/immunoevasive mechanisms, resulting in reversion to the equilibrium phase. Conversely, patients may experience no response or progressive disease, in which case they are considered to have innate resistance. DC=dendritic cell; MDSC=myeloid-derived suppressor cell; MHCI=major histocompatibility class I; NKT=natural killer T cell; PD-L1=programmed cell death ligand I; TAM=tumor associated macrophage. Reproduced with permission from [4].

Cryoablation, or the freezing of diseased tissue as a treatment for a variety of cancers, has also seen great advances with time. Since the discovery of the effects of cold temperatures on cancer cell viability by English physician Dr. James Arnott in the 1850's [7], the field of cryoablation has made great strides. It has been touted as a minimally-invasive and cost-effective local ablative method for a variety of cancers. Cryoablation, sometimes referred to as cryosurgery or cryotherapy, involves the repeated freezing and thawing of tumor cells, which leads to their destruction through several mechanisms including intracellular and extracellular ice formation, shear and osmotic stress and microvascular thrombosis. One of the potential benefits of tumor cryoablation, when compared to other local thermal ablative methods such as microwave or radio frequency ablation, is the preservation of the many heterogeneous tumor associated antigens after the procedure [8]. These intact antigens have been observed to act like a vaccine or "cryo-immunization", allowing the adaptive immune system to mount a targeted, tumor-specific response towards any distant metastases or future recurrences in a phenomenon known as the abscopal effect. Significantly elevated levels of immune mediators including interleukin (IL)-1, IL-6, nuclear factor kappa-light-chain-enhancer of activated B cells (NfκB) and tumor necrosis factor (TNF)-α have been noted after cryoablation [8-10]. Interestingly, the immunostimulatory response to cryoablation is not always seen—in certain studies, no immune response has been observed while in other cases, immunosuppression with increased tumor burden following cryoablation has been observed [11,12]. Over the years, investigators have sought to elucidate the factors that may influence an immunostimulatory versus immunosuppressive response. Current hypotheses center on the technical factors of the cryoablation procedure itself, including duration of therapy, rate of freezing, minimum temperature achieved, and number of freeze/thaw cycles [13,14]. Additionally, the proportion of cells undergoing apoptosis versus necrosis may also play a role in the ensuing immune response or lack thereof. Given the advances in our understanding of tumor biology, immunology and anti-cancer immunotherapies in recent years, there has been increased interest in examining the potential synergistic effects of combining various immunotherapies with cryoablation. Here, we briefly analyze the mechanisms of immune response to cryoablation and then review various pre-clinical and clinical studies that examine the synergistic effects of cryoablation and immunotherapy for a variety of cancers.

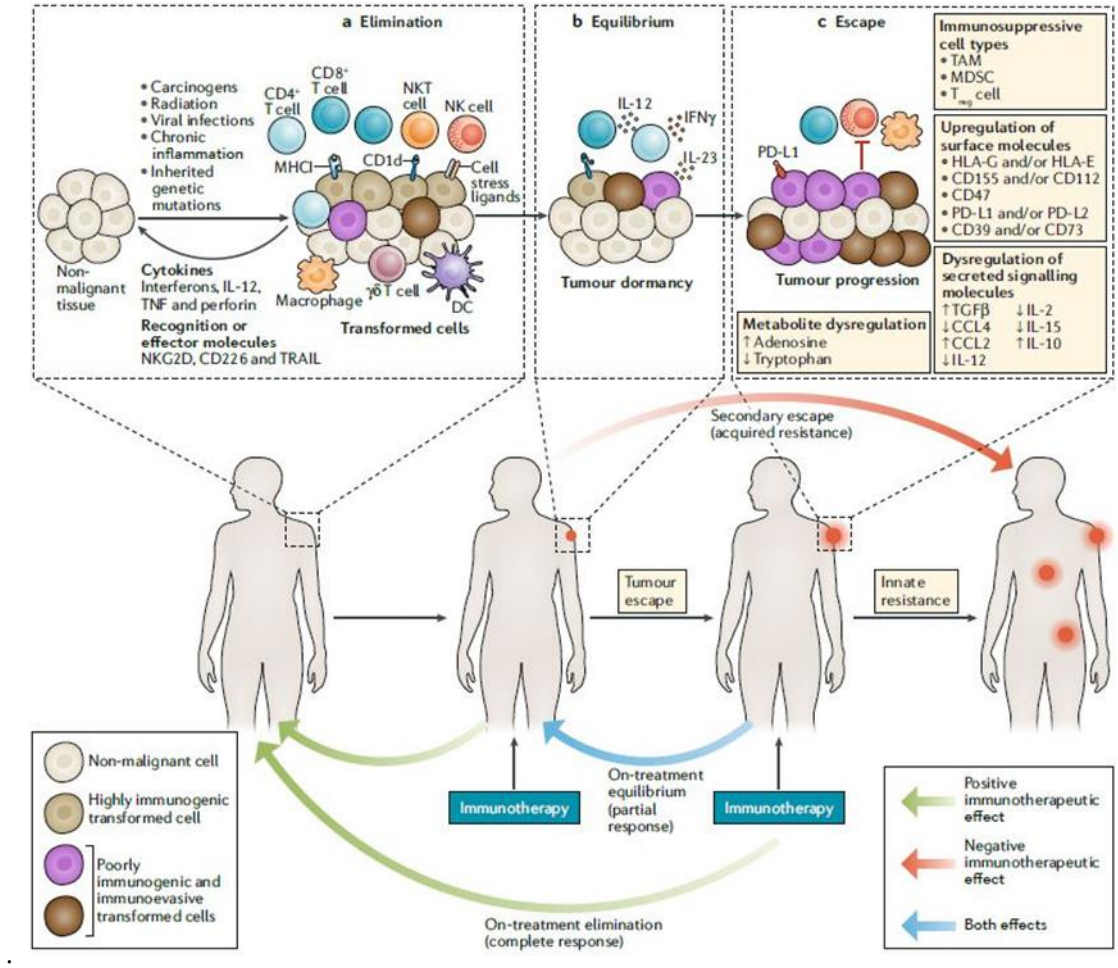

Figure 1. The process of cancer immunoediting and immunotherapy response.

a. In the elimination phase, highly immunogenic transformed cells can be recognized and eliminated by the innate and adaptive immune system. b. In the equilibrium phase, subclones of cells that were able to escape the elimination phase can remain dormant, with overall growth being limited by the adaptive immune system. c. In the escape phase, cells undergo uncontrolled growth leading to a clinically detectable tumor.

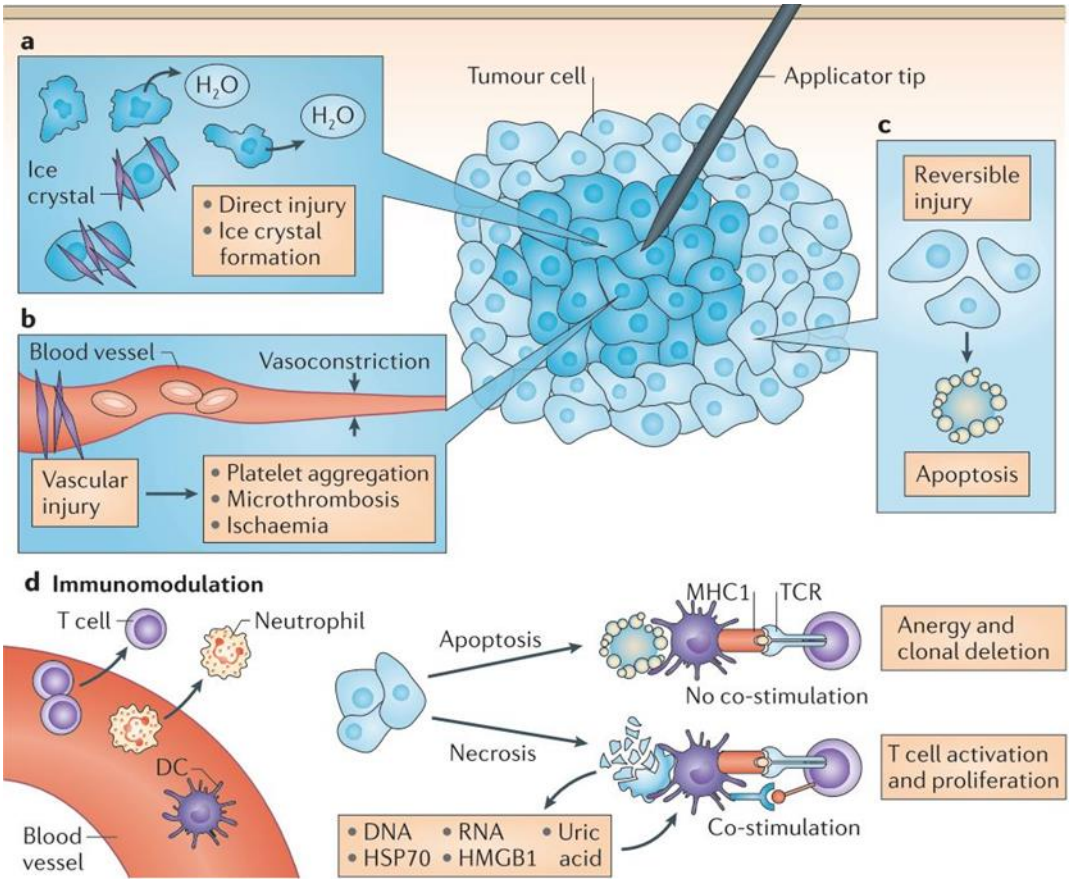

Figure 2. Cell death and immunomodulation in cryoablated tumors.

a. Within the central zone of a cryoablated lesion, cells predominantly undergo necrosis as a result of ice crystal formation which directly injures cells, or through osmotic shrinkage that results from extracellular ice formation. b. Alternatively, cells in the central zone are subject to ischemia and eventual coagulative necrosis resulting from cold-induced vascular injury, microthombosis and/or vasoconstriction. c. Within the peripheral zone, cells undergo apoptosis as a result of sub-lethal cold temperature given their distance from the probe. d. The resulting anti-tumor immune response from cryoablation is dependent on the mechanism of cell death.

Mechanisms of Immune Response to Cryoablation

An understanding of the mechanism of cell death, antigen uptake and presentation by antigen presenting cells (APC's), and types of cytokines released from cryoablated tumor cells and their micro-environment is crucial to understanding the immune response to cryoablation. Cryoablated cells undergo either necrosis or apoptosis and the mechanism of cell death depends on several factors including proximity to the cryotherapy probe, temperature and rate of freezing, number of freeze-thaw cycles, and the size of the lesion being treated [15]. Generally, cells further from the probe (in the peripheral zone) freeze at a slower rate and undergo apoptosis, while cells closer to the probe (central zone) undergo a coagulative necrosis as a result of rapid freezing, direct cell injury, or vascular injury (Figure 2) [16]. Cells undergoing necrosis rapidly release intracellular contents such as pro-inflammatory cytokines and heat shock proteins, in addition to tumor associated antigens. Antigen uptake and subsequent activation of APC's, in addition to their expression of co-stimulatory "danger signals" induced from intracellular content release, leads to a release of immunostimulatory cytokines including IL-2, interferon (IFN)-γ, TNF-α, and granulocyte macrophage colony-stimulating factor (GM-CSF). This ultimately results in cytotoxic T cell activation, B cell proliferation and differentiation, and an increase in both CD4+ and CD8+ cell counts and Th1/Th2 ratios [15]. The release of these cytokines has been associated with the abscopal effect in tumors and the subsequent T cell response has been demonstrated to be increased in a variety of cryoablated tumors when compared to surgical resection, highlighting the ability of cryoablation to promote a robust immune response [17]. Tumor cells undergoing apoptosis, however, often do not cause an immune response as there is no release of intracellular contents or cytokines. APC's that present antigens of apoptotic tumor cells do so without T cell co-stimulation, leading to no T cell response. Moreover, studies have also illustrated that tumor cells undergoing apoptosis secrete IL-10 and transforming growth factor (TGF)- β, which induce T cell anergy and clonal deletion, with a concomitant increase in regulatory T cells (Tregs), which ultimately suppresses the immune response [15].

Cells undergoing necrosis release intracellular contents that allow dendritic cells to express danger signals via co-stimulatory molecules, leading to T cell activation and proliferation. Cells undergoing apoptosis do not release intracellular contents and are

taken up by antigen presenting cells resulting in no danger signals or co-stimulation of T cells. This process results in anergy and clonal deletion. MHC1=Major histocompatibility complex 1; HSP70=heat shock protein 70; HMGB1=high mobility group protein B1; TCR=T cell receptor. Reproduced with permission from [18].

A number of studies have attempted to further elucidate the mechanisms, extent, and timing of immune response in a variety of cryoablated cancers. Kato et al. [19], collected tumor tissue from 22 kidney cancer patients prior to cryoablation and 3 months after ablation, in addition to peripheral blood samples for a subset of patients. Ribonucleic acid was extracted from these samples and next generation sequencing was used to determine the diversity of the T cell receptor beta chain repertoire. Expression analysis of immune related genes was also performed in the tissues. Within the treated tumor tissues, the investigators discovered oligoclonal expansion of certain T cell subclones and increased levels of CD11c-positive cells (markers for macrophages and dendritic cells) that were not present in untreated tissue, indicating a strong immune response generated by cryoablation. Some of these clonally expanded cells were also found in peripheral circulation, highlighting a robust immune response and potential for an abscopal effect. Immune and cellular response mechanisms within cryoablated tumors have also been studied using 3-dimensional tissue-engineered models of prostate and renal cancers in an attempt to better replicate *in vivo* conditions [20]. In a 2013 study, Robilotto et al. utilized this approach to demonstrate the differential activation of apoptotic pathways in prostate cancer cells when exposed to varying temperatures during cryoablation [21]. The investigators demonstrated that when cryoablation occurs at temperatures ≤ 30°C, prostate cancer cells undergo rapid apoptosis via a membrane mediated (caspase-dependent) mechanism, while cryoablation at temperatures ≥ 30°C leads to apoptosis through the slower mitochondrial pathways. At 6 hours post thaw, complete necrosis was observed in the cells cryoablated to ≤ 30°C, while 75-85% of cells remained in the group cryoablated to temperatures ≥ 30°C. Furthermore, when solubilized TRAIL (a known initiator of the membrane mediated apoptotic pathway) was added to cells that were cryoablated to temperatures ≥ 30°C, nearly all cells were destroyed, highlighting the potential utility of TRAIL in sensitizing cancer cells to cryoablation. These results support the use of 3D tissue models to study *in vivo* molecular mechanisms for apoptosis and necrosis, and also support their use to better predict response to cryotherapy.

Cryoablation plus immunotherapy

Given the competing immunostimulatory and immunosuppressant responses that can be initiated by cryoablation via apoptosis and necrosis, additional therapies to modulate these immune responses may prove fruitful. The utility of neoadjuvant or adjuvant chemo- and/or radiation therapy in the setting of cryoablation has been well studied, and through various pre-clinical and clinical studies, the role of combination cryotherapy and immunotherapy is being elucidated. Whether through administration of cytokines that augment the innate immune response or through monoclonal antibodies that deactivate the inhibition of the adaptive immune response, advances in the field of immunotherapy show great promise in enhancing the tumoricidal effect of cryoablation.

Stimulating immune response

A In a study of mice with subcutaneous GL216 glioma, Xu et al. demonstrated the utility of combining cryoablation with GM-CSF, a cytokine that stimulates granulocyte and monocyte production [22]. The mice were divided into four groups including those receiving no treatment, GM-CSF only, cryoablation only, or the combination of GM-CSF and cryoablation. The investigators discovered that mice in the combination therapy group had increased numbers and activity of dendritic cells (DCs) on immunohistochemistry and flow cytometry when compared to mice in other groups. Increased cytotoxic CD8+ activity and IFN-γ secretion in the combination group was also noted, further demonstrating the immunological synergism between these two therapies.

In addition to GM-CSF, several studies have examined the role of cytidyl guanosyl oligodeoxynucleotide (CpG-ODN) in augmenting the cryoimmune response. CpG-ODN is a toll-like receptor 9 agonist and activation of this receptor on DC's leads to their maturation and eventual Th-1 immune response. In a study on Lewis lung cancer mice that underwent cryoablation with subsequent peritumoral injection of immature DC's, Zhang et al. [23], examined the effect of differential timing of CpG-ODN administration on tumor cell survival. Mice were administered CpG-ODN either concurrently, 6 hours, 12 hours, or 24 hours after DC cell injection, and their immune response was measured. The researchers demonstrated significantly increased expression levels of CD4+ and CD8+ T cells, IL-12, IFN-γ, and TNF-α, all potent immune modulators, in the 12 hour group. Furthermore, mice in this group demonstrated superior survival and decreased metastasis when compared to mice in other groups. The investigators concluded that a 12 hour delay in CpG-ODN administration gave DC's sufficient time to uptake antigen and mount a targeted response results which could potentially impact outcomes in cryoimmune therapy. The results of this study were mirrored in those of a study by Alteber et al. [24], who also examined cryoablation plus CpG-ODN in Lewis lung cancer in mice. The investigators followed a similar protocol, yet found that concurrent CpG-ODN administration with the injection of immature DC's induced the more robust anti-tumor response. Nevertheless, this study also demonstrated that combined cryoimmune therapy with immature DC's and CpG-ODN results in decreased tumor growth and metastases, prolonged survival, and a lasting anti-tumoral immunity that protects mice from rechallenge proof that CpG-ODN can facilitate an abscopal effect. The role of CpG-ODN in cryoimmune therapy has also been examined in human epidermal growth factor receptor 2 (HER2) positive mice undergoing cryoablation with positive results [25]. In this study, researchers examined immune responses of cryoablation in immunocompetent mice and also those with severe combined immunodeficiency (SCID). Cryoablation resulted in strong innate and adaptive immune responses in the immunocompetent mice and CpG-ODN administration eliminated or delayed local tumor recurrence in immunocompetent and SCID mice, respectively. These results allude to the role CpG-ODN plays in stimulating innate immunity and in controlling local tumor recurrence, lending support to CpG-ODN use with cryoablation in future cryoimmune investigations.

Investigations into the combination of cryoablation and immunostimulatory natural killer (NK) cell therapy have been performed in human subjects with promising results. A study to understand the effect of Herceptin, an anti-HER2 antibody, and allogenic NK cell therapy in women with recurrent HER2+ breast cancer who underwent cryoablation was performed. Women receiving all three therapies were found to have significantly increased progression-free survival (PFS) at 12 months, with median PFS not reached at study conclusion, when compared to women only receiving cryoablation or cryoablation plus Herceptin [26]. Additionally, women in the triple therapy group were noted to have significantly lower levels of carcino-embryonic antigen (CEA), cancer antigen 15-3 and circulating tumor cells. Similar studies at the same institution examining allogenic NK immunotherapy combined with cryoablation in advanced and metastatic hepatocellular carcinoma, advanced renal cell carcinoma, advanced non-small cell lung cancer, and metastatic pancreatic cancer were also performed with promising results [27-31]. It should be noted, however, that the statistical power of these studies was limited and that caution should be used when interpreting the efficacy and survival outcomes for patients treated with combination allogenic NK cell therapy and cryoablation. Nevertheless, the combination therapy was tolerated well for the majority of patients in these studies. These results warrant larger cohort investigations to determine the efficacy of this combination treatment. The results of various studies examining the combination of cryotherapy and immunotherapy are summarized in Figure 3.

Author	Year	Tumor Model	Co-adminstered therapy	Tumor response	Reference
Xu et al.	2015	GL261 mice (Glioma)	GM-CSF	Increased IFN-γ, TH1 cells and CD8+ cell counts	22
Zhang et al.	2016	LLC mice (Lung)	CpG-ODN and DC	Increased CD4+, CD8+, IFN-γ, TNF-α, survival rates	23
Alteber et al.	2014	LLC mice (Lung)	CpG-ODN and DC	Increased survival rate, decreased metastases	24
Veenstra et al.	2014	HER2+ mice (Breast)	CpG-ODN	Decrease or elimination of local recurrence	25
Liang et al.	2017	HER2+ patients (Breast)	NK and Herceptin	Increased total T cell cound, TNF-β and IFN-γ	26
Lin et al.	2017	Advanced NSCLC patients	NK	Increased response rate, disease control rate	27
Lin et al.	2017	Advanced HCC patients	NK	Increased PFS, RR, disease control rate	28
Lin et al.	2017	Advanced RCC patients	NK	Increased RR, decreased tumor size	29
Niu et al.	2013	Metastatic Pancreatic patients	DC and CIK	Increased median OS	30
Niu et al.	2013	Metastatic HCC patients	DC and CIK	Increased median OS	31
Zhang et al.	2015	GL261 mice (Glioma)	DC and anti-Treg antibody	Increased survival rates	32
Kudo-Saito et al.	2016	B160-F10 (Melanoma) / CT26 (Colon) mice	Anti-Alcam (mAb) and anti-CTLA-4	Increased CD8+ counts, suppressed metastases and tumor growth	33
Waitz et al.	2012	TRAMP C2 mice (Prostate)	anti-CTLA-4	Increased CD4+, CD8+ and CD8+/Treg ratios	34
Li et al.	2014	RM-1 mice (Prostate)	anti-CTLA-4	Decreased Treg functionality	35
Page et al.	2016	HER2+ patients	anti-CTLA-4	Increased and diversified T cell clonal repertoire	36

Figure 3. Pre-clinical and clinical studies examining cryoablation plus immunotherapy. LLC: Lewis Lung Cancer; NSCLC: Non-Small Cell Lung Cancer; HCC: Hepatocellular Carcinoma; RCC: Renal Cell Carcinoma; CIK: Cytokine-Induced Killer Cell; PFS: Progression-Free Survival; RR: Response Rate; OS: Overall Survival.

Cancer	Co-administered Therapy	Phase	Number of Participants	Primary End Point	Secondary End Point	Estimated Study Completion Date	Status	Location	Clinical Trial Identifier
Metastatic CRC	AlloStim®	II/III	450	OS	Safety, health-related QOL	10/1/2020	Not yet recruiting	Thailand	NCT01741038
Oligo-metastatic Prostate cancer	Pembrolizumab	II	12	Assess feasibility via proportion of men with PSA <0.6 ng/ml at 1 year	Efficacy of combination therapy on PD-1/PD-L1 expression as assessed by biopsy 6 months post-treatment	11/1/2018	Active	USA	NCT02489357
Metastatic Lung cancer	Immune checkpoint inhibitors	II	20	Cumulative incidence of TRAE	Radiologic response of cryoablation	3/31/2025	Recruiting	USA	NCT03290677
Non-Hodgkin Lymphoma	DC therapy, Pembrolizumab	I/II	44	Safety, tolerability and dose schedule	Feasibility of combination therapy, QOL, response rate, survival	2/15/2021	Recruiting	USA	NCT03035331
Unresectable stage III-IV Melanoma	DC therapy, Pembrolizumab	Ib/II	39	Objective response rate	Safety, survival	10/31/2022	Recruiting	USA	NCT03325101

Figure 4. Ongoing studies investigating combination cryoablation and immunotherapy. CRC: Colorectal Cancer; DC: Dendritic Cell; OS: Overall Survival; PSA: Prostate-Specific Antigen; Ng/Ml: Nanogram/Milliliter; TRAE: Treatment-Related Adverse Events; QOL: Quality Of Life; PD-L1: Programmed Death Ligand 1.

Overcoming the Immunosuppressive State

Regulatory T cells aid in keeping the immune system in check by suppressing the activation of helper T cells and cytotoxic T cells against self-antigens, yet they have also been found to suppress anti-tumor responses. The presence of high levels of Tregs in tumor tissue has been associated with increase recurrence and poor prognosis, and suppression of their function has been the subject of intense research [32]. Recently a study was conducted to elucidate Treg response to cryoablation in prostate cancer patients [33]. The investigators measured the circulating Treg levels, specifically CD4+CD25+CD127- cells, before and after cryosurgery. Compared to healthy volunteers, prostate cancer patients were noted to have increased levels of circulating Tregs. For prostate cancer patients undergoing cryoablation, Treg levels were decreased for up to 8 weeks after surgery, however their suppressive function, as noted by immunosuppressive assays, was increased in two-thirds of patients and cancer recurrence was noted in 17% of patients. The investigators hypothesized that cytokine release from ablated tumor cells not only attracted Tregs into the tumor microenvironment, but also stimulated their suppressive function. In another study examining cryoablation of GL261 subcutaneous glioma mouse models, DC's from tumor draining lymph nodes that were isolated demonstrated an increased ability to activate T cells in vitro [34]. In vivo, however, DC function was limited and there was weak anti-tumor immunity overall, indicating a possible dampening of the immune response due to the presence of Tregs. Upon administration of anti-Treg antibodies and tumor re-challenge, anti-tumor immunity and mouse survival rates significantly improved. The results of these studies highlight the suppressive role Tregs play in anti-cancer immunity and warrant further investigation into Treg activity in cryoablated tumors.

A 2016 study identified a new molecular marker involved in immunosuppression following cryoablation called activated leukocyte cell adhesion molecule (ALCAM/CD116) [35]. ALCAM+ mesenchymal stem-like cells have previously been found to induce metastases and prevent cytotoxic T cell generation, while simultaneously limiting their activity. In this study, metastatic murine melanoma cells were subcutaneously and intravenously implanted into C57BL/6 mice. The subcutaneous tumors were subsequently treated with cryoablation and an increase in ALCAM+ cell populations including CD4+Foxp3+Tregs, CD45- mesenchymal stem/stromal cells, and CD11b+Gr1+myeloid derived suppressor cells was noted. Importantly, these cells significantly suppressed production of IFN-γ and decreased cytotoxic T cell activity, leading to increased metastasis and tumor growth, suggesting that ALCAM+ cells play a significant role in immune inhibition. The investigators subsequently injected a monoclonal anti-ALCAM antibody intratumorally into a subset of cryoablated tumors and ALCAM inhibition was noted to increase the number and function of cytotoxic T cells, suppressing tumor growth and metastasis, while also elucidating a mechanism that limits cryoablation efficacy [35]. Additionally, one group of mice received an intraperitoneal injection of anti-CTLA-4 antibody, an immune checkpoint inhibitor, in addition to the anti-ALCAM antibody. In this triple therapy group, cytotoxic T cell activity was greatly enhanced, resulting in complete remission in 6 out of 7mice. Furthermore, this anti-tumor effect persisted even after tumor re-challenge [35]. The efficacy of this triple therapy was then replicated in a murine colon cancer model, highlighting the promise of ALCAM inhibition in overcoming immune suppression following cryoablation.

A number of other studies have also examined the efficacy of Ipilimumab, an anti-CTLA-4 antibody, in combination with cryoablation. In a TRAMP C2 mouse model of prostate cancer, Waitz et al. demonstrated that the administration of anti-CTLA-4 antibodies after cryoablation of a primary tumor led to decreased growth of secondary tumors seeded by challenge at distant sites, when compared to cryotherapy of these secondary tumors alone [36]. Moreover, analysis of the secondary tumors in the combination therapy group revealed higher levels of intratumoral CD4+ and CD8+ T cells and also increases in the ratio of CD8+ T cells to Tregs when compared to the monotherapy group, indicating inhibition of the Treg mediated immunosuppression through Ipilimumab. The results of a similar study using a prostate cancer murine model mirrored these results,

demonstrating increased regression of metastases in a group receiving combination therapy [37]. In this study, investigators also noted a time-dependent decrease in the number of Tregs in the cryoablation monotherapy group, while Tregs levels remained stable in the combination therapy group, albeit with decreased functionality [37]. The role of Ipilimumab in combination with cryotherapy was also examined in a pilot study of 18 women with early-stage breast cancer [38]. Through T cell receptor DNA sequencing of cryoablated breast tumors, the investigators found the T cell clonal repertoire to be significantly expanded, both in the peripheral blood and intratumorally, in the combination group when compared to monotherapy (cryoablation or Ipilimumab only) groups, indicating a robust immune response from combination therapy. Collectively, the results of the aforementioned studies support further investigation into the efficacy of combination cryoablation and immunotherapy, and a number of trials are currently ongoing (Figure 4).

Conclusions

Advances in our understanding of cancer immunology, anti-cancer immunotherapies and mechanisms of immune response to cryoablation are rapidly expanding the pool of potential cancer therapies. Immunotherapies, particularly immune checkpoint inhibitors, have already demonstrated survival benefits for a variety of cancers. The combination of these therapies with minimally-invasive local therapies including cryoablation represents the logical next step for cancer treatment. The synergism between cryotherapy and immunotherapy has been demonstrated in a number of pre-clinical and clinical studies, exemplifying the promise of these combined modalities. Although larger, randomized, multicenter studies are needed to validate these preliminary findings, and to determine the optimal mode of treatment, the initial results are encouraging and represent a significant step towards targeted cancer treatment.

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